**Reference Measurements for Priority and Essential Trace Elements and
Methyl Mercury with Isotope Dilution Inductively Coupled Plasma-Mass
Spectrometry for Seafood Safety Assessment and CRM Production**

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Abstract

The continuous release of anthropogenic pollutants into marine environment increase the needs for the development of analytical procedures for their accurate determination in many sample types. Sound strategies for seafood safety monitoring call for measurement systems capable of producing comparable analytical results with demonstrated quality. Method validation, traceability and uncertainty of analytical results are the three milestones to assess data quality. Some trace elements are essential for biological structures, but they can become toxic at concentrations beyond those necessary for their functions; others, are toxic even at very low levels. Their accurate determination in reference samples serves as an important step in seafood safety control and pollution monitoring.

Isotope Dilution Inductively Coupled Plasma Mass Spectrometry (ID ICP-MS) has been applied for the determination of the total mass fractions of five trace elements (Cd, Cu, Hg, methyl mercury (MeHg), Pb, and Zn) in marine biota candidate reference material IAEA-476. Because of the complex matrix of the sample and the expected spectral interferences, special care was taken for the validation of the applied methodology, particularly for its measurement step. Reference isotopic measurements were carried out by Sector Field Inductively Coupled Plasma Mass Spectrometer (ICP-SFMS).

The entire ID ICP-SFMS measurement procedure was described by mathematical modelling and the combined uncertainty of measurement results estimated. All factors influencing the final results and isotopic equilibrium were systematically investigated. This included the procedural blank, the moisture content in biota samples and all factors affecting the blend ratio measurements (*i.e.*, instrumental background, spectral interferences, dead time, mass discrimination effects as well as the repeatability of measured isotope ratios). Modeling of the entire measurement procedure and the use of appropriate certified reference materials (CRM) enable to assure the traceability of obtained values to the International System of Units (SI).

Finally, the developed procedure has been successfully applied in the process of the certification of the International Atomic Energy Agency (IAEA) certified reference material for trace elements and MeHg mass fractions in fish homogenate sample. The excellent agreement between obtained results in the present study and those derived from the IAEA certification campaign for trace elements and MeHg in the same sample matrix further validated the reference methods developed in the IAEA.

Keywords: *Isotope ratios, ID ICP-SFMS, fish biota sample, CRM, reference measurements, seafood safety*

1. Introduction

In the recent years, food science has advanced rapidly prompted by the continuous emerging technology and the development of the analytical instrumental techniques. The increased incidence of diverse foodborne diseases combined with the media distribution has promoted a public awareness of the relationship between diet and health. Global population is more concerned for the food quality and safety: nutritional content, presence of additives and contaminants (King et al. 2017). Accordingly, a precise knowledge of the chemical composition of the food is requested for consumers' health and safety. In fact, food safety and security are included in the 17 Sustainable Development Goals for 2030 recently published by the United Nations (United Nations 2015). Currently, developed and developing nations are making efforts to ensure food and minimize population health risks, especially to children, old people and pregnant women, which are the main vulnerable groups.

In this context, special attention should be paid to the risks of fish consumption. As it is known, fish is fundamental part in a balanced diet since it is an exceptional source of quality proteins and essential fatty acids, offering many health benefits (Malakootian et al. 2016; Merciai et al. 2018; Anual et al. 2018). Nevertheless, trace elements have been found in fish tissues as a result of bioaccumulation which may persist in the environment. The problem is also exacerbated by the fact that inorganic contaminant concentrations tend to be bioaccumulated and biomagnified to the higher concentrations in species occupying a higher level in the trophic chain, including humans (Burger et al. 2014; Ourgaud et al. 2017; Laird et al. 2017; Merciai et al. 2018; Anandkumar et al. 2018). Hence, besides its benefits, fish consumption has associated human health risks.

In nutrition field, there is a need to distinguish between essential (*e.g.*, Cu, Zn) and non-essential (*e.g.*, Cd, Hg, MeHg, Pb) trace elements. The former are indispensable components of biological structures and consequently, the human organism needs them, but just in limited amounts, becoming damaging at concentrations higher than necessary; in contrast, the exposure of the latter results in toxic effects even at trace levels (Mieiro et al. 2012; Lemos et al. 2013; Gu et al. 2015; Jayaprakash et al. 2015; Pedron et al. 2016; Bay et al. 2017; Makedonski et al. 2017; Moura et al. 2018; Zoroddu et al. 2019). Normally, toxic

effects are not a result of a single exposure, but from a continuous contact with the elements. Therefore, short- and long-term exposure to trace elements can be both dangerous for human health.

In the last decades, European Commission designed and implemented a series of regulations and guidelines establishing the maximum allowable levels of some contaminants in foodstuff by Regulation (EC) No 1881/2006 (Commission Regulation of European Commission 2014) in order to protect humans from the effects of chemical contaminants and to guarantee worldwide health. For the important decisions concerning regulatory compliance reliable measurement results and high-quality reference values are of high importance, the latter being those that are reliable, have sufficiently small uncertainty values (so that others can refer to them) and a demonstrated traceability to the common system of references and whose measurement procedure is validated (Magnusson et al. 2005).

The selection of proper analytical procedures and their validation is therefore essential to produce reference results of demonstrated quality. Likewise, method validation requires the use of appropriate CRMs and, owing to the noticeable lack of environmental matrix CRMs, marine food origin CRMs characterization is of great importance. Considering the low levels of contaminant elements in fish samples, developing sensitive analytical procedures able to accurately determine trace elements mass fractions is essential in order to ensure an effective seafood safety control. To date, many analytical techniques for trace elements determination in marine biota have been reported, including: Atomic Absorption Spectrometry (Metian et al. 2013; Bandowe et al. 2014; Burger et al. 2014; Goutte et al. 2015; Bay et al. 2017; Chouvelon et al. 2017; Łuczyńska et al. 2018; Bilandžić et al. 2018; Zuliani et al. 2019; Plessl et al. 2019), Inductively Coupled Plasma Optical Emission Spectrometry (Jooste et al. 2015; Goutte et al. 2015; Ourgaud et al. 2017; Milošković et al. 2018) and Inductively Coupled Plasma-Mass Spectrometry (Goutte et al. 2015; Squadrone et al. 2016; Chouvelon et al. 2017; Ourgaud et al. 2017; Bilandžić et al. 2018; Sofoulaki et al. 2019; Zuliani et al. 2019).

The Comité Consultative de Qualité de Matières (CCQM) recognized ID ICP-MS as a potentially primary method of measurement (García Alonso and Rodríguez-González 2013). The ID methodology is based on an intended change of an element isotopic composition by adding an isotopic standard containing the same element but with a distinct isotopic composition (*i.e.*, enriched in the less naturally abundant) and measuring the magnitude of the change induced. One of the significant advantages over other approaches is that the analyte recovery does not have to be quantitative, assuring that isotopic equilibration between the analyte of

interest and added spike material has been achieved (Krata et al. 2018). The use of ID ICP-MS enables the highest metrological quality of results which are directly traceable to the International System of Units. Therefore, this methodology is able to provide results with sufficiently small uncertainties and superior accuracy. The ID ICP-MS is often used to produce reference values and in the CRMs certification processes.

The aim of this study was to develop one reliable reference procedure, based on the application of ID ICP-SFMS methodology, for the quantification of trace elements in fish samples, which can be applied for the characterisation of CRMs with marine origin as well as for the regulation compliance and food safety controls.

The modelling of the entire measurement procedure and the use of appropriate CRMs together with the systematic assessment of all factors influencing measurement results and their uncertainties (*e.g.*, sample-spike isotopic equilibrium, blend ratio measurements, isotopic standard ratio measurements, instrumental background, dead time, mass discrimination effects, repeatability of measured isotopic ratios as well as efficiency of the sample preparation procedure, procedural blank and moisture content of the sample) as well as enable to assure the traceability of measurement results to the SI units.

The final purpose of this development was the use of obtained with the proposed reference procedure results for the characterisation of trace elements and MeHg mass fractions in the future CRM - IAEA-476.

2. Experimental part

2.1. Chemicals and materials

High quality deionised water (resistivity > 18 MΩ) obtained from Milli-Q Element system (Millipore, Bedford, MA, USA) was used throughout this work.

Ultra-pure HNO₃ (Ultrex[®], J. T. Baker, Phillipsburg, NJ, USA) and H₂O₂ (Merck, Darmstadt, Germany) were used for sample digestion. HCl (Merck, Darmstadt, Germany) was also used for sample digestion prior mercury and MeHg determination.

New Teflon[®] and polyethylene labware (*e.g.*, vessels, bottles, tips, syringes, columns, etc.) employed was exhaustively cleaned with an acid cleaner system. In order to avoid memory effects from previous experiments,

Teflon[®] vessels employed for sample digestion were cleaned twice using a microwave procedure consisting in the addition of 7 g of concentrated HNO₃ to each vessel followed by a microwave treatment at 350 W and 100 °C during 20 min (*i.e.*, 10 min ramp, 10 min hold) and a final rinse with Milli-Q water.

To reduce the risk of airborne contamination all sample processing steps were performed in a laminar clean hood.

The following certified isotope reference materials and their appropriate dilutions were used in this study: IRMM 622 (¹¹¹Cd), IRMM 632 (⁶⁵Cu), IRMM 654 (⁶⁸Zn), ERM-AE 640 (²⁰²Hg) from the European Commission, Institute for Reference Materials and Measurements, Geel, Belgium; NIST SRM-991 (²⁰⁶Pb) from National Institute for Standards and Technologies, Maryland, USA. ²⁰¹Hg enriched monomethylmercury standard in acetic acid/methanol (3:1) was purchased from the Innovative Solutions in Chemistry, Oviedo, Spain.

Single element standard solutions for cadmium and copper with natural isotopic composition (Merck, Darmstadt, Germany) or certified isotopic reference materials ERM-AE639 and IRMM-3702 for Hg and Zn respectively from the European Commission, Institute for Reference Materials and Measurements, Geel, Belgium were used for mass discrimination corrections during ICP-SFMS measurements, NIST SRM-981 from National Institute for Standards and Technologies, USA was employed for mass discrimination correction when Pb isotope ratios were measured.

Working standard solutions were prepared gravimetrically by appropriate dilution of stock standard solutions with a solution containing 2 % (w w⁻¹) nitric acid in precleaned Teflon[®] vials. The natural isotopic compositions for Cd, Hg, Cu and Zn were taken from IUPAC tables (Meija et al. 2016).

The anion exchanger AG1X-8 (Bio-Rad, Hercules, USA) was used for matrix separation. AG1X-8 was converted from the chloride form to the nitrate form by shaking it with 2% (w w⁻¹) nitric acid.

2.2. Instrumentation

All isotope ratio measurements were carried out with an ICP-SFMS (Attom, Nu Instrument Ltd., Wrexham, UK). The liquid sample introduction system used for this purpose was composed by a MicroMist nebulizer (Glass Expansion, Australia) coupled to a Peltier-cooled cyclonic spray chamber (Glass Expansion, Australia). Servo operating mode and fast scan ion optics were used for all isotope ratio measurements carried out with ICP-

SFMS. The fast scan ion optics was used for fast peak jumping between selected masses across a mass range of total width of 40 % of the static mass at which the magnet is parked.

Although this approach is the most efficient for isotope ratio measurements, mass calibration was systematically performed at both low and medium mass resolution levels.

The measurement methods and system parameters were previously optimised for the best isotope ratio measurement repeatability and they are listed in Table 2.

All the weight measurements were carried out with an electronic analytical balance XP205 – (Mettler Toledo, Switzerland) with a maximum and minimum load of 220 g and 1 mg, respectively, and an uncertainty of ± 0.01 mg. The balance was coupled with an antistatic device in order to eliminate or reduce electro-static charges.

Sample decomposition was carried out in a closed system assisted with microwave radiation using a Mars X-press Microwave System (CEM, Matthews, NC, USA), equipped with 12 Teflon[®] vessels.

Moisture determination was performed by a Karl Fischer titrator system 852 KFT Titrand and 885 Compact Oven SC (Metrohm, Switzerland).

Mass fractions measurements for homogeneity and stability studies for Zn were carried out using a High-Resolution Continuum Source Atomic Absorption Spectrometer (HR-CS AAS), ContrAA 700, equipped with a flame system, which is commercially available from Analytik Jena AG (Jena, Germany). The total mercury determination in the homogeneity study was carried out using an Advanced Mercury Analyser (AMA-254, Altech, Czech Republic). The determination of MeHg for homogeneity study was performed by Gas Chromatography- Atomic Fluorescence Spectrometry (GC-AFS, MERX-M, Brooks Rand, USA).

Mass fractions measurements for homogeneity and stability studies for Cu, Pb and Cd were performed with a quadrupole inductively coupled plasma mass spectrometer (XSeries 2 Thermo Scientific, Germany) equipped with a cyclonic spray chamber cooled by Peltier system (Elemental Scientific Inc., USA) to the temperature of 5° C.

2.3. Sampling and sample treatment of the fish homogenate sample

About 350 kg of mixed fish was collected in the eastern Irish Sea. After removal of the skin, the fish filleted was freeze-dried, ground to powder and sieved at 250 μ m. The portion above 250 μ m was reprocessed by

micronisation. The obtained sample < 180 µm was further homogenized and after checking for the homogeneity of the sample material, aliquots of about 10 g were packed into pre-cleaned plastic containers and then sealed in plastic bags.

2.3.1. Homogeneity study

. The within-bottle homogeneity was assessed by 15 replicate determinations of the content of the investigated trace elements and MeHg in one bottle. For all analytes except Hg and CH₃Hg, subsamples of 0.2 g were mineralized with 5 ml concentrated HNO₃ in a microwave oven. The final measurements for Cd, Cu and Pb were performed by ICP-SFMS and for Zn by Flame AAS under repeatability conditions, and in a randomized way. The determination of the total Hg was done in solid subsamples (50 mg) with an AMA. MeHg was determined by GC AFS after alkaline digestion and room temperature (Carrasco and Vassileva 2015). The obtained values for within bottle homogeneity in the present study were as follows: 1.2% for Cd and Cu; 2.4% for Pb; 2.2% for Zn; 3.9% for Hg and 3.2% for MeHg.

2.3.2. Stability study

Three sets of five bottles each were stored in the dark at different temperatures (-20°C, +20°C and +60°C) just after the bottling process and kept at described conditions over a period of 2 years. One isochronous study over 6 weeks was applied in order to evaluate the short-term stability of the materials during transport, and one isochronous study over 24 months, to evaluate the stability during the storage period. The obtained results were compared with the results from samples kept at -20°C (considered as the reference temperature) during this period.

All analytical methods used for homogeneity and stability studies were previously validated.

The obtained result showed sufficient stability of the raw fish sample homogenate. More details concerning the preparation of candidate reference material, homogeneity and stability testing are given elsewhere (International Atomic Energy Agency 2018).

2.3.3. Moisture determination

Moisture is operationally dependent parameter and its content in the investigated sample was performed with Karl Fischer method (Margolis and Huang 2005), which is a primary method for moisture content. Correction for dry-mass was obtained from separate portions of sample of mass between 0.2 - 0.3 g taken from three different bottles. The average moisture content was calculated and used for element mass fraction correction.

Moisture was determined in parallel with each sample analysis, to account also for the hygroscopicity of the material since the bottle was first opened.

2.4. Preparation of blend samples

For the isotopic ratio measurements, 3 to 6 blend solutions (*i.e.*, sample and isotopically enriched solution-spike) were generally prepared for each analyte, using subsamples from one bottle. Weighing of the subsamples and addition of the spike aliquots were performed exclusively according to metrological gravimetric principles in the clean and humidity-controlled area using substitution measurements against operational mass standards. The evaluation of the added amount of the reference solution was a compromise between several factors including: results from preliminary semi-quantitative measurements, characteristics of the spike materials, achieving a sufficiently high counting rate, dead time effects and the final total uncertainty.

The sample preparation procedure for all analytes except for CH_3Hg was as follows: ~0.2 g of the biota sample was put into a microwave vessel and spiked directly with one of the following spike solutions: ~0.14 g of the IRMM-622 ^{111}Cd (15 times gravimetrically diluted), ~0.34 g of the IRMM 632 ^{65}Cu , ~0.4 g of the ERM-AE 640 (^{202}Hg), ~0.24 g of the NIST SRM-991 ^{206}Pb (15 times gravimetrically diluted) or ~1.04 g of the IRMM 654 ^{68}Zn spike solution. A volume of 5 mL concentrated HNO_3 was subsequently added into the digestion vessels, which were kept for 24 hours to allow a cold pre-digestion of the biota sample. Then 2 mL of 30 % (w w^{-1}) H_2O_2 were further added into the microwave vessels prior to the microwave digestion step.

Microwave digestion programme for the investigated elements with exception of MeHg is shown in Table 1. The obtained blend solutions were placed on a ceramic hot plate and evaporated to near dryness. The final digests were taken up in 2 % (w w^{-1}) HNO_3 , transferred quantitatively to 50 mL precleaned polyethylene tubes

and then stored at 4 °C. Blend solutions were further diluted in 2 % (w w⁻¹) HNO₃ prior to ICP-SFMS measurements.

For extraction and determination of MeHg content in biota sample the procedure was as follows: ~0.2 g biota sample, ~1.122 g of the metrologically diluted MeHg²⁰¹ (in a mixture of 0.5 % CH₃COOH and 0.2 % HCl acids) and 10 mL of 15 % HCl were subsequently added into the Teflon[®] vessels. The programme for microwave assisted extraction of MeHg was a power/time control programme, set at 800 W for 30 min. After the microwave extraction, sample solutions were centrifugated for 10 min at 5000 rpm to promote phase separation. The supernatant was removed by careful pipetting.

Six procedural blanks were also prepared and subjected to the entire sample preparation procedure together with the fish homogenate sub-samples.

2.5. Matrix-separation by anion exchange chromatography for Cd and MeHg

In order to minimise the polyatomic interferences coming from the presence of Mo and Zr in the biota sample, matrix separation was applied in the blend solutions before Cd measurements. In chlorine containing solutions, Cd²⁺ forms anionic chloride complexes and, for this reason, when the solution is passed through an anion exchange column in dilute hydrochloric acid, the matrix cations Na⁺, K⁺, Mg²⁺, Ca²⁺ and Ni²⁺ pass the column without interaction with the anion exchanger, whereas the Cd chloride complexes [CdCl₄]²⁻ are retained and can be eluted from the column with 2 % (w w⁻¹) HNO₃. Using this separation technique, both the effect of the high saline matrix and spectral interferences can be minimised.

Matrix separation was performed after microwave digestion. For this purpose, 0.5 mL of 35.5 % HCl was added to blend solutions and, after that, the solutions were poured into separate ion exchange columns (3 x 0.8 cm), previously filled with the anion exchanger AG1X-8 in the nitrate form. Before use the ion exchanger was washed intensively several times with 2% HNO₃, left to settle, and the acid was decanted off. This procedure was repeated six times until the cleaning process of the ion-exchange resin was completed.

After loading the blend sample the column was rinsed first with 10 mL 2.5 % HCl, then with 10 mL 0.25 % HCl. The elution of the samples was done with 12 mL 2 % (w w⁻¹) HNO₃. The first eluted fraction (5 mL) was discarded, and the last eluted fraction (7 mL) was collected. After that, the Cd, Mo and Pd isotopes were measured by ICP-SFMS, as isotopes of interest and interferences, respectively.

The same anion exchange column was used for the separation of Hg and MeHg. The supernatant, obtained after applying the microwave extraction procedure for MeHg with 15 % HCl, was passed through the column, where inorganic Hg and MeHg were retained. The rest of major matrix elements passed through the column without interaction with the anion exchanger.

Elution of MeHg was performed with two fractions (5 mL) of 0.2 % HCl followed by three fractions (5 mL) of 0.05 % HCl. Fractions of interest were fractions 3 to 5 (*i.e.*, fractions between 10-25 mL HCl added), where the eluted MeHg was collected. The inorganic Hg was bound on the resin and was not eluted in the applied conditions. The ID ICP-SFMS measurement for MeHg was performed in the mixture of the three fractions (*i.e.*, fractions 3, 4 and 5 in a 15 mL final volume). The separation procedure used was preliminary validated and described in details elsewhere (Vassileva et al. 2014).

2.6. IDMS calculation and uncertainty estimation

The set of equations described in Table 3, representing the ID ICP-SFMS mathematical model, was used to calculate Cd, Cu, Hg, MeHg, Pb and Zn mass fractions and to estimate their expanded uncertainties. The value for each parameter in the described equations was obtained either by a measurement, mathematical calculation or from the certificates and had an associated standard uncertainty.

The individual uncertainty components of all identified experimental parameters involved in the analytical protocol were combined according to the ISO guidelines (Joint Committee for Guides in Metrology 2008). All uncertainties indicated are expanded uncertainty $U_c = k u_c$; where u_c is a combined standard uncertainty and k is a coverage factor equal to 2. Combined standard uncertainties on the results were obtained according to the uncertainty propagation law. In practice, a dedicated software programme (GUM Workbench[®] Version Pro 2.4, MetroData GmbH, Grenzach-Wyhlen, Germany) was used, based on the numerical method of differentiation described by Kragten (Kragten 1994).

The uncertainties for the spike materials were given on their corresponding certificates. The biota sample was assumed to contain natural isotopic composition of Cd, Cu, Hg and Zn and isotopic composition with associated uncertainties was taken from IUPAC (Meija et al. 2016). The lead isotopic composition was determined experimentally by ICP-SFMS measurements.

3. Results and discussion

3.1. Sample preparation

Sample digestion and ion exchange chromatography, both included in the sample preparation procedure, were investigated as critical points for establishing the isotopic equilibrium in the spiked samples. The major advantage of IDMS is that the analytes in the spike and sample solutions behave approximately similarly, which is largely different in conventional internal standard calibration strategy. Another important advantage is that once the isotopic equilibration is achieved, there is no need to consider the analyte losses during sample treatment. Complete digestion of the sample ensures isotope equilibration and drastically reduces the matrix effects during the isotope ratio measurements. This is advantageous for samples which induce strong matrix effects, such as marine samples. However, the procedure is not independent from other possible sources of uncertainty, such as random contamination.

In the present study several digestion mixtures were evaluated, and it was found that the digestion with the $\text{HNO}_3\text{--H}_2\text{O}_2$ mixture gives clear and colourless solutions.

The addition of HCl has shown a negative influence on the digestion efficiency due to the formation of precipitates. However, the addition of small volumes of HCl to the $\text{HNO}_3\text{--H}_2\text{O}_2$ mixture improved recovery for Hg, due to the formation of a less volatile mercury compound HgCl_2 . Therefore, a mixture of 5 mL concentrated HNO_3 , 2 mL H_2O_2 with addition of 0.1 mL HCl was further applied in the present study.

The evaporation to dryness and dissolution of the residues in 2 % (w w^{-1}) HNO_3 also results into less solid residues in the digests, which is advantageous for the following ICP-SFMS measurements. In this way the solutions used for the correction of the instrumental mass bias matched the matrix of the sample.

3.2. Selection of the isotopes and isotope ratios measurements

The selection of the isotopes to be measured in the blend samples was done with respect to availability of spike materials, abundance of the isotopes and possible spectral interferences during ICP-SFMS measurements.

The signal intensities used for isotope ratio measurements were corrected for dead time, instrumental background and possible spectral interferences. The dead time value and its associated standard uncertainty were determined according to a method described by Nelms et al. (2001).

After a preliminary semiquantitative analysis, blend solutions were diluted to achieve intensities ranging between 100 000 and 300 000 counts s⁻¹ with the purpose to minimize the uncertainty contribution associated with the correction for dead time and instrumental background. Careful dual rinsing followed by a check of the instrumental background was performed prior every blend solution measurement in order to monitor sample to sample memory effects and correct for them, if necessary.

Mass discrimination during the measurement process was calibrated by applying the standard-sample bracketing method (Bolea-Fernández et al. 2016). For this method, analyte and sample matrix matching was applied and every two blend solutions a standard solution with known isotopic composition was analysed. Furthermore, standard concentrations were appropriately adjusted in order to achieve similar intensities to the intensities obtained from blend solutions. Hence, a multiplicative mass discrimination factor, K-factor, was obtained by comparing the measurements of the isotope ratio of the standard solution to its certified value (Meija et al. 2016). The blend isotope ratios, r_{blend} , were corrected after measuring the isotopic ratio of interest in the standard as a reference. K-factors and corrected isotope ratios were calculated using eq. 3 from Table 3.

Cd measurements by ICP-SFMS can easily suffer from isobaric and polyatomic spectral interferences. The main isobaric interferences arise from tin, as the isotopes ¹¹²Sn, ¹¹⁴Sn and ¹¹⁶Sn interfere with ¹¹²Cd, ¹¹⁴Cd and ¹¹⁶Cd. Pd also interferes with ¹⁰⁶Cd, ¹⁰⁸Cd and ¹¹⁰Cd, being the latter interference the most inconvenient. The polyatomic spectral interferences come from the formation of MoO⁺ and ZrO, which affects all Cd isotopes except ¹⁰⁶Cd. However, this isotope has a too low natural abundance (1.25 %) to be suitable as a reference isotope for IDMS. The $n(^{110}\text{Cd})/n(^{111}\text{Cd})$ ratio was chosen for Cd determination as ¹¹⁰Cd and ¹¹¹Cd s are the two less affected by isobaric interferences Cd isotopes.

Cd measurements in biota sample were performed by ICP-SFMS after matrix separation as described in the Chapter 2.5. Together with Cd isotopes the intensities of ⁹⁰Zr, ⁹⁵Mo and ¹¹⁷Sn were also monitored. After the matrix separation the signal of the ⁹⁰Zr decreased to the level of the instrumental background and the intensities of the ⁹⁵Mo were reduced with 80 %. An interference correction on ¹¹⁰Cd and ¹¹¹Cd for the remaining polyatomic interference coming from MoO⁺ was further applied. The correction was performed by using an oxide formation ratio of 0.04 % based on results from separate experiment for the evaluation of MoO⁺ formation at the measurement conditions applied in this study.

There was no change in Sn intensity since Sn is also transformed in chloride complex $[\text{SnCl}_4]^{2-}$ and retained with the Cd in the column.

One interference study was carried out to evaluate the influence of the ArNa^+ formation ($m/z = 63$) in the plasma on the $n(^{63}\text{Cu})/n(^{65}\text{Cu})$ isotope ratio and the copper content determined by IDMS. The overall effect of Na present in the blend sample on the $n(^{63}\text{Cu})/n(^{65}\text{Cu})$ blend ratio was investigated according to the method described elsewhere (Diemer et al. 2002). Several solutions with increasing amounts of Na to a fixed amount of Cu (1 ng g^{-1}) were used to evaluate the degree of ArNa^+ formation. Fig. 1 illustrates the regression line observed for the apparent $n(^{63}\text{Cu})/n(^{65}\text{Cu})$ ratio as a function of the amount of Na added to the model solution. The regression equation was used as an attempt to quantify the interference effect.

Taking into consideration the concentration of Na found in the sample digest ($\sim 26.8 \text{ } \mu\text{g g}^{-1}$ from the external quantitative analysis), and the slope obtained from the experimental regression line, it was estimated that at the dilution level used for the measurements (5-fold dilution for Cu determination), this effect would produce an increase of the $n(^{63}\text{Cu})/n(^{65}\text{Cu})$ ratio by 0.27, or 12 %, to the finally calculated Cu mass fraction. Hence, an additive correction factor to the final Cu mass fraction was implemented. The fish homogenate sample was measured also at medium mass resolution and results were compared with the corrected mass fraction obtained at low resolution. The good agreement found was the evidence for the appropriate correction of the polyatomic interferences, when the ICP-SFMS measurements are performed at low mass resolution mode.

Mercury was quantified by using the $n(^{200}\text{Hg})/n(^{202}\text{Hg})$ isotopic ratio. All selected isotopes were free of spectral interferences. The main problem that arise when using ICP-SFMS for mercury determination was the memory effect for mercury, which may result in a background increase with time. To overcome this limitation, prolonged washing with 2% HNO_3 was applied. Even with longer washing time, the increase of blank signal intensities over the few hours measurement time was observed. This effect was properly accounted using additive correction factor on the uncertainties, coming from the correction for the background and the memory effects, during the ICP-SFMS measurements. HCl was not added for reducing the memory effect because HCl addition to the plasma might arise changes in the plasma properties or interferences due to formation of polyatomic Cl^- species.

The Pb natural isotopic composition in the sample was determined by independently measuring in unspiked subsamples different pairs of Pb isotopes ratios: $n(^{204}\text{Pb})/n(^{206}\text{Pb})$, $n(^{207}\text{Pb})/n(^{206}\text{Pb})$ and $n(^{208}\text{Pb})/n(^{206}\text{Pb})$. As the

isotope ^{204}Pb suffers from isobaric interference caused by mercury isotopes, ^{202}Hg isotope was also monitored during the determination of the Pb isotopic composition and used for the interference correction. The lead natural isotopic composition in the sample is shown in Table 4. From this data, the atomic weight of lead in the biota sample was calculated to be $M_{\text{Pb, sample}} = 207.21 \text{ g mol}^{-1}$.

The spike IRMM 654 is enriched on ^{68}Zn isotope and to avoid interferences from ^{64}Ni on mass ^{64}Zn , the isotope ^{66}Zn was selected as the reference isotope for $n(^{66}\text{Zn})/n(^{68}\text{Zn})$ isotope ratio determination.

Procedural blanks were submitted to the same sample digestion and dilution procedure together with the blend solutions. Analysis of the procedural blank solutions was performed by ICP-SFMS using external calibration.

3.3. Moisture determination

Moisture determination is fundamental for an effective comparison of the results between laboratories, especially when working with hygroscopic samples or in a humid environment. Hygroscopicity of samples can be important because it generates relatively high additional uncertainty contribution (around 20 % relative). Furthermore, dry mass correction might depend on the method used and hence, for the comparison being acceptable, the moisture content in different laboratories needs to be determined with the same method.

In this case the measurand (*i.e.*, moisture content in the sample) was to some extent “operationally defined” (Grobecker et al. 2001; Rückold et al. 2001). In parallel with the dry oven method at 85°C , total water content in the fish homogenate sample was also determined with Karl Fischer method, considered as primary method for water content. Results obtained with dry oven method, which was the prescribed by the organisers of the certification campaign method for moisture content determination, and Karl Fisher method were further compared. The excellent agreement between results for moisture content obtained with both methods ($7.6 \pm 0.2\%$ for the dry oven method and $7.5 \pm 0.2\%$ for Karl Fisher method) additionally validated the moisture determination step of the analytical procedure used in this study (*i.e.*, dry oven at $85 \pm 2^{\circ}\text{C}$) for bottles kept at 20°C and showed that the moisture content cannot be a source of bias for these reference measurements.

3.4. Content and uncertainty

In this study ID ICP-SFMS was applied as a primary method of measurement, where the measurement process is well understood, and a model equation can be written down, permitting the calculation of the mass fractions of investigated trace elements and MeHg and the estimation of their uncertainties. The complete protocol deployed for the determination of the Cd, Hg, MeHg, Cu, Pb and Zn mass fractions in the marine biota sample combined a sample decomposition stage carried out in closed microwave system with ID ICP-SFMS measurements. The mass fractions of the investigated analytes were calculated with the set of equations shown in Table 3, representing the mathematical model of the applied analytical procedure.

The individual uncertainty components associated to the corrections for moisture content, procedural blank, homogeneity of the marine biota sample, but also to those of the isotope ratio measurement results (*i.e.*, instrumental background, spectral interferences, dead time and mass discrimination effects, as well as the repeatability of isotopic ratios measurements) were propagated together. The isotope ratios repeatability in the blend and K factor solutions were varying from 0.1% to 0.15% for the selected in this study $n(^{63}\text{Cu})/n(^{65}\text{Cu})$ and $n(^{66}\text{Zn})/n(^{68}\text{Zn})$ isotope ratios, 0.2% -0.3% for the $n(^{111}\text{Cd})/n(^{110}\text{Cd})$ and $n(^{208}\text{Pb})/n(^{206}\text{Pb})$ isotope ratios and 0.32% -0.45 % for the $n(^{200}\text{Hg})/n(^{202}\text{Hg})$ isotope ratio.

Beside the correction for mass discrimination effects on the measured ratios (K factor in eq. 3 from Table 3) the isotope signal intensities were also corrected individually for “additive” effects (*i.e.*, instrumental background and memory effect, isobaric interferences and dead time effect). These factors cannot be neglected and can even have contribution to the combined uncertainty, as it will be shown later. However, propagating these uncertainties directly with the repeatability of the measurements of the individual isotope signal intensities can lead to a gross overestimation of the resulting combined uncertainty. To avoid this risk and for the combined uncertainty calculations only, “additive” corrections on intensities were translated into multiplicative correction factors on ratios following a method described elsewhere (Quétel et al. 2001) and illustrated in eq. 3 and 5 (δ factors).

Additionally, it was important that uncertainty on the submitted for the characterisation of the IAEA-476 candidate CRM mass fraction covers for possible lack of within bottle homogeneity. Therefore, the combined uncertainty on obtained in this study mass fractions included contribution from uncertainty on within bottle homogeneity. Each IDMS result submitted for characterisation of the candidate CRM was multiplied by a unity factor caring uncertainty equal to the within bottle variance (Vassileva et al. 2014).

The obtained expanded uncertainties ($k=2$) for the mass fractions of the investigated analytes were as follow: on the Cd ($U = 1.7 \%$), Cu ($U = 1.6 \%$), Zn ($U = 3.4 \%$), Pb ($U = 3.60 \%$), Hg ($U = 4.2 \%$) and MeHg ($U = 4.5 \%$) The main contribution to the uncertainty was in most of the cases (Zn, Pb, Hg) the amount of the element in the procedural blank (~ 10 -50%). Secondly, the uncertainty contribution coming from the within-bottle homogeneity of the fish homogenate sample was significant particularly for the determinations involving Hg and MeHg mass fractions (~ 40 -60%), followed by the uncertainty of the spike solution concentrations (~ 2 -10%), isotope ratios for blends and K factors (~ 2 -15%), IUPAC data (~ 5 -40%) etc. The other sources of uncertainty (*i.e.*, moisture content, corrections for the spectral interferences, dead time, etc.) were below the 5 % from the total combined uncertainty. Detailed information about the main contributors to the combined uncertainties of the determined mass fractions with the applied analytical procedure are presented in Table 5.

In that way, the combined uncertainty statements went far beyond the simple repeatability calculations and reflected the understanding of the measurement process. It is evident that the total uncertainty budget directly enables the analyst to source the main contributors of uncertainty and figure the contribution of the single parameters to the total uncertainty of the adequate element mass fraction in the marine biota sample.

3.5. Validation of the measurement process and SI traceability of the obtained results.

According to the ISO/IEC 17025 standard, validation is “the confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled”(International Organization for Standardization and International Electrotechnical Commission 2017). A set of ISO/IEC 17025 recommendations for validation of the measurement process were followed during present investigation. Our validation strategy was based on the following steps:

- A systematic assessment of all factors influencing the final measurement results.
- Mathematically modelling of the entire measurement process.
- Estimation of measurement uncertainties.
- Use of reference standard materials for the calibration and mass discrimination correction, during ICP-SFMS measurement.

- Participation in the certification campaign of the IAEA-476 candidate CRM.

The entire ID ICP-SFMS measurement process was described by mathematical modelling presented in Table 3 and the combined uncertainty budget was calculated for each element mass fraction result. All factors influencing the final results and isotopic equilibrium were systematically investigated, which included the procedural blank (*i.e.*, contamination), the moisture content and all factors affecting the blend ratio measurements (*i.e.*, instrumental background, spectral interferences, the dead time effect, the mass discrimination and isotope ratios repeatability). The individual uncertainty contributions attached to all identified experimental steps involved in the measurements were combined together according to ISO guidelines.

As it is shown in the Table 6, the mass fraction values obtained in the present study were not significantly different within stated uncertainty from the certified values for Cd, Cu, Hg, MeHg, Pb, Zn achieved in the IAEA-476 certification process (International Atomic Energy Agency 2018). The good agreement between the results additionally validated the analytical procedure which was developed and applied for the determination of the element mass fractions in the fish homogenate candidate reference material.

An additional requirement of the ISO/IEC 17025 (ch. 5.6.2.2.) (International Organization for Standardization and International Electrotechnical Commission 2017) is for measurement results is their traceability to the common reference from the international system of units, SI system.

Metrological traceability is defined as the “property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty” (Joint Committee for Guides in Metrology 2012). When establishing an unbroken chain of calibrations and thus SI traceability, the measurement results can be considered as comparable.

Traceability in this study was achieved by applying the IDMS as a primary method of measurement, having the highest metrological qualities, whose operation were completely described and understood, for which a complete uncertainty statement was written down in terms of SI units. In addition, all standard solutions and blend samples were prepared gravimetrically.

The traceability chain, linking the element mass fraction to the SI units is evidenced in the ID ICP-SFMS mathematical model given in Table 3. This model, together with the associated equations, sub-calculations or the references to the certified values, relates each calculation parameter to the SI units of the mole and the kilogram.

4. Conclusions

This work was focused on the analytical performance of IDMS for the accurate and SI traceable measurements of priority and essential trace element mass fractions in food of marine origin, namely, fish homogenate, for food safety control and CRMs characterization purposes. SI-traceable certification results for Cd, Cu, Hg, CH₃Hg, Pb and Zn mass fractions in marine biota sample within 1-5 % combined uncertainty were achieved using the proposed ID ICP-SFMS methodology. The measurement procedure was described by a series of model equations that reflected the reality of our experiments, including all the parameters influencing measurement result.

The modeling of the analytical process allowed to achieve adequate validation of analytical procedure, to establish traceability of the measurement results and to estimate final expanded uncertainty. The demonstrated traceability of the obtained results and the low combined uncertainty values obtained further confirmed the suitability of the proposed methodology for reference purposes.

Obtained results for Cd, Cu, Hg, MeHg, Pb and Zn were used as the IAEA contribution in the characterization process of the IAEA-476 Certified Reference Material for trace elements and MeHg. The excellent agreement of the values obtained in this study with the IAEA-476 CRM certified values further validated the proposed analytical protocol.

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Compliance with Ethical Standards

491 **Declaration of interest:** Miriam Garcia has obtained fellowship from the International Atomic Energy Agency.
492 Emilia Vassileva declared no conflicts of interest. Sabine Azemard declared no conflicts of interest. Antonio
493 Canals declared no conflicts of interest.

494 **Ethical statement:** *All institutional and national guidelines for the care and use of fish samples were followed.*

495 **Informed consent:** *Not applicable.*
496

References

- Anandkumar A, Nagarajan R, Prabakaran K, Bing CH, Rajaram R (2018) Human health risk assessment and bioaccumulation of trace metals in fish species collected from the Miri coast, Sarawak, Borneo. *Mar Pollut Bull* 133:655–663. doi: 10.1016/J.MARPOLBUL.2018.06.033
- Anual ZF, Maher W, Krikowa F, Hakim L, Ahmad NI, Foster S (2018) Mercury and risk assessment from consumption of crustaceans, cephalopods and fish from West Peninsular Malaysia. *Microchem J* 140:214–221. doi: 10.1016/J.MICROC.2018.04.024
- Bandowe BAM, Bigalke M, Boamah L, Nyarko E, Saalia FK, Wilcke W (2014) Polycyclic aromatic compounds (PAHs and oxygenated PAHs) and trace metals in fish species from Ghana (West Africa): Bioaccumulation and health risk assessment. *Environ Int* 65:135–146. doi: 10.1016/J.ENVINT.2013.12.018
- Bay P, Arulkumar A, Paramasivam S, Rajaram R (2017) Toxic heavy metals in commercially important food fishes collected from Palk Bay, Southeastern India. *Mar Pollut Bull* 119:454–459. doi: 10.1016/j.marpolbul.2017.03.045
- Bilandžić N, Sedak M, Čalopek B, Đokić M, Varenina I, Kolanović BS, Luburić DB, Varga I, Benić M, Roncarati A (2018) Element contents in commercial fish species from the Croatian market. *J Food Compos Anal* 71:77–86. doi: 10.1016/J.JFCA.2018.02.014
- Bolea-Fernández E, Balcaen L, Resano M, Vanhaecke F (2016) Tandem ICP-Mass Spectrometry for Sr isotopic analysis without prior Rb/Sr separation. *J Anal At Spectrom* 31:303–310. doi: 10.1039/c5ja00157a
- Burger J, Gochfeld M, Jeitner C, Pittfield T, Donio M (2014) Heavy metals in fish from the Aleutians: Interspecific and locational differences. *Environ Res* 131:119–130. doi: 10.1016/J.ENVRES.2014.02.016
- Carrasco L, Vassileva E (2015) Determination of methylmercury in marine sediment samples: Method validation and occurrence data. *Anal Chim Acta* 853:167–178. doi: 10.1016/J.ACA.2014.10.026
- Chouvelon T, Brach-Papa C, Auger D, Bodin N, Bruzac S, Crochet S, Degroote M, Hollanda SJ, Hubert C, Knoery J, Munsch C, Puech A, Rozuel E, Thomas B, West W, Bourjea J, Nikolic N (2017) Chemical

contaminants (trace metals, persistent organic pollutants) in albacore tuna from western Indian and south-eastern Atlantic Oceans: Trophic influence and potential as tracers of populations. *Sci Total Environ* 596–597:481–495. doi: 10.1016/J.SCITOTENV.2017.04.048

Commission Regulation of European Commission (2014) Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. 1–36

Diemer J, Que CR, Taylor PDP (2002) Comparison of the performance of different ICP-MS instruments on the measurement of Cu in a water sample by ICP-IDMS. *J Anal At Spectrom* 17:1137–1142. doi: 10.1039/b201443b

García Alonso JI, Rodríguez-González P (2013) Introduction to Isotope Dilution Mass Spectrometry. In: *Isotope Dilution Mass Spectrometry*. Royal Society of Chemistry, pp 1–39

Goutte A, Cherel Y, Churlaud C, Ponthus J-P, Massé G, Bustamante P (2015) Trace elements in Antarctic fish species and the influence of foraging habitats and dietary habits on mercury levels. *Sci Total Environ* 538:743–749. doi: 10.1016/J.SCITOTENV.2015.08.103

Grobeck KH, Isengard H, Rückold S (2001) The effects of drying on biological matrices and the consequences for reference materials. *Food Control* 12:401–407

Gu Y, Lin Q, Wang X, Du F, Yu Z, Huang H (2015) Heavy metal concentrations in wild fishes captured from the South China Sea and associated health risks. *Mar Pollut Bull* 96:508–512. doi: 10.1016/j.marpolbul.2015.04.022

International Atomic Energy Agency (2018) Certification of Trace Elements and Methylmercury Mass Fractions in Fish Homogenate IAEA-476. International Atomic Energy Agency, Vienna

International Organization for Standardization, International Electrotechnical Commission (2017) General requirements for the competence of testing and calibration laboratories

Jayaprakash M, Kumar RS, Giridharan L, Sujitha SB, Sarkar SK, Jonathan MP (2015) Bioaccumulation of metals in fish species from water and sediments in macrotidal Ennore creek, Chennai, SE coast of India: A metropolitan city effect. *Ecotoxicol Environ Saf* 120:243–255. doi: 10.1016/j.ecoenv.2015.05.042

Joint Committee for Guides in Metrology (2008) Evaluation of measurement data — Guide to the expression of

- 548 uncertainty in measurement. Joint Committee for Guides in Metrology
- 549 Joint Committee for Guides in Metrology (2012) International vocabulary of metrology - Basic and general
550 concepts and associated terms (VIM) 3rd edition. Joint Committee for Guides in Metrology
- 551 Jooste A, Marr SM, Addo-Bediako A, Luus-Powell WJ (2015) Sharptooth catfish shows its metal: A case study
552 of metal contamination at two impoundments in the Olifants River, Limpopo river system, South Africa.
553 *Ecotoxicol Environ Saf* 112:96–104. doi: 10.1016/J.ECOENV.2014.10.033
- 554 King T, Cole M, Farber JM, Eisenbrand G, Zabarar D, Fox EM, Hill JP (2017) Food safety for food security:
555 Relationship between global megatrends and developments in food safety. *Trends Food Sci Technol*
556 68:160–175. doi: 10.1016/j.tifs.2017.08.014
- 557 Kragten J (1994) Tutorial review. Calculating standard deviations and confidence intervals with a universally
558 applicable spreadsheet technique. *Analyst* 119:2161–2165. doi: 10.1039/AN9941902161
- 559 Krata AA, Wojciechowski M, Kalabun M, Bulska E (2018) Reference measurements of cadmium and lead
560 contents in candidates for new environmental certified materials by isotope dilution inductively coupled
561 plasma mass spectrometry. *Microchem J* 142:36–42. doi: 10.1016/J.MICROC.2018.06.013
- 562 Laird B, Chan HM, Kannan K, Husain A, Al-Amiri H, Dashti B, Sultan A, Al-Othman A, Al-Mutawa F (2017)
563 Exposure and risk characterization for dietary methylmercury from seafood consumption in Kuwait. *Sci*
564 *Total Environ* 607–608:375–380. doi: 10.1016/J.SCITOTENV.2017.07.033
- 565 Lemos LS, de Moura JF, Hauser-Davis RA, de Campos RC, Siciliano S (2013) Small cetaceans found stranded
566 or accidentally captured in southeastern Brazil: Bioindicators of essential and non-essential trace elements
567 in the environment. *Ecotoxicol Environ Saf* 97:166–175. doi: 10.1016/j.ecoenv.2013.07.025
- 568 Łuczyńska J, Paszczyk B, Łuczyński MJ (2018) Fish as a bioindicator of heavy metals pollution in aquatic
569 ecosystem of Pluszne Lake, Poland, and risk assessment for consumer's health. *Ecotoxicol Environ Saf*
570 153:60–67. doi: 10.1016/J.ECOENV.2018.01.057
- 571 Magnusson B, Tressl I, Haraldsson C (2005) Isotope dilution with ICP-MS — simplified uncertainty estimation
572 using a robust procedure based on a higher target value of uncertainty. *J Anal At Spectrom* 20:1024–1029

- 573 Makedonski L, Peycheva K, Stancheva M (2017) Determination of heavy metals in selected black sea fish
574 species. Food Control 72:313–318. doi: 10.1016/j.foodcont.2015.08.024
- 575 Malakootian M, Mortazavi MS, Ahmadi A (2016) Heavy metals bioaccumulation in fish of southern Iran and
576 risk assessment of fish consumption. Environ Heal Eng Manag 3:61–68. doi: 10.15171/EHEM.2016.02
- 577 Margolis SA, Huang PH (2005) Water determination. Encycl. Anal. Sci. 61
- 578 Meija J, B Coplen T, Berglund M, Brand W, De Bièvre P, Gröning M, Holden N, Irrgeher J, D Loss R, Walczyk
579 T, Prohaska T (2016) Isotopic compositions of the elements 2013 (IUPAC Technical Report). Pure Appl
580 Chem 88:293–306
- 581 Merciai R, Rodríguez-Prieto C, Torres J, Casadevall M (2018) Bioaccumulation of mercury and other trace
582 elements in bottom-dwelling omnivorous fishes: The case of *Diplodus sargus* (L.) (Osteichthyes:
583 Sparidae). Mar Pollut Bull 136:10–21. doi: 10.1016/j.marpolbul.2018.08.061
- 584 Metian M, Warnau M, Chouvelon T, Pedraza F, Rodriguez y Baena AM, Bustamante P (2013) Trace element
585 bioaccumulation in reef fish from New Caledonia: Influence of trophic groups and risk assessment for
586 consumers. Mar Environ Res 87–88:26–36. doi: 10.1016/J.MARENVRES.2013.03.001
- 587 Mieiro CL, Coelho JP, Pacheco M, Duarte AC, Pereira ME (2012) Trace elements in two marine fish species
588 during estuarine residency: Non-essential versus essential. Mar Pollut Bull 64:2844–2848. doi:
589 10.1016/J.MARPOLBUL.2012.08.033
- 590 Milošković A, Milošević Đ, Radojković N, Radenković M, Đuretanović S, Veličković T, Simić V (2018)
591 Potentially toxic elements in freshwater (*Alburnus* spp.) and marine (*Sardina pilchardus*) sardines from the
592 Western Balkan Peninsula: An assessment of human health risk and management. Sci Total Environ
593 644:899–906. doi: 10.1016/J.SCITOTENV.2018.07.041
- 594 Moura JF, Tavares DC, Lemos LS, Acevedo-Trejos E, Saint’Pierre TD, Siciliano S, Merico A (2018)
595 Interspecific variation of essential and non-essential trace elements in sympatric seabirds. Environ Pollut
596 242:470–479. doi: 10.1016/J.ENVPOL.2018.06.092
- 597 Nelms SM, Quérel CR, Prohaska T, Vogl J, Taylor PDP (2001) Evaluation of detector dead time calculation
598 models for ICP-MS. J Anal At Spectrom 16:333–338. doi: 10.1039/b007913h

- 599 Ourgaud M, Ruitton S, Bourgogne H, Bustamante P, Churlaud C, Guillou G, Lebreton B, Harmelin-Vivien ML
600 (2017) Trace elements in a Mediterranean scorpaenid fish: Bioaccumulation processes and spatial
601 variations. *Prog Oceanogr*. doi: 10.1016/J.POCEAN.2017.11.008
- 602 Pedron T, Segura FR, da Silva FF, de Souza AL, Maltez HF, Batista BL (2016) Essential and non-essential
603 elements in Brazilian infant food and other rice-based products frequently consumed by children and celiac
604 population. *J Food Compos Anal* 49:78–86. doi: 10.1016/J.JFCA.2016.04.005
- 605 Plessl C, Gilbert BM, Sigmund MF, Theiner S, Avenant-Oldewage A, Keppler BK, Jirsa F (2019) Mercury,
606 silver, selenium and other trace elements in three cyprinid fish species from the Vaal Dam, South Africa,
607 including implications for fish consumers. *Sci Total Environ* 659:1158–1167. doi:
608 10.1016/J.SCITOTENV.2018.12.442
- 609 Quétel CR, Prohaska T, Nelms SM, Diemer J, Taylor PDP (2001) ICP-MS applied to isotope abundance ratio
610 measurements: Performance study and development of a method for combining uncertainty contributions
611 from measurement correction factors. In: Holland G, Tanner SD (eds) *Plasma Source Mass Spectrometry:*
612 *The New Millennium*. The Royal Society of Chemistry, pp 257–269
- 613 Rückold S, Grobecker KH, Isengard H-D (2001) Water as a source of errors in reference materials. *Fresenius J*
614 *Anal Chem* 370:189–190
- 615 Sofoulaki K, Kalantzi I, Machias A, Pergantis SA, Tsapakis M (2019) Metals in sardine and anchovy from
616 Greek coastal areas: Public health risk and nutritional benefits assessment. *Food Chem Toxicol* 123:113–
617 124. doi: 10.1016/J.FCT.2018.10.053
- 618 Squadrone S, Burioli E, Monaco G, Koya MK, Prearo M, Gennero S, Dominici A, Abete MC (2016) Human
619 exposure to metals due to consumption of fish from an artificial lake basin close to an active mining area
620 in Katanga (D.R. Congo). *Sci Total Environ* 568:679–684. doi: 10.1016/J.SCITOTENV.2016.02.167
- 621 United Nations (2015) Transforming our world: the 2030 agenda for sustainable development. 16301:1–36
- 622 Vassileva E, Wysocka I, Betti M (2014) Reference measurements for cadmium, copper, mercury, lead, zinc and
623 methyl mercury mass fractions in scallop sample by isotope dilution inductively coupled plasma mass

- 624 spectrometry. *Microchem J* 116:197–205. doi: 10.1016/j.microc.2014.05.011
- 625 Zoroddu MA, Aaseth J, Crisponi G, Medici S, Peana M, Nurchi VM (2019) The essential metals for humans: a
626 brief overview. *J Inorg Biochem* 195:120–129. doi: 10.1016/J.JINORGBIO.2019.03.013
- 627 Zuliani T, Vidmar J, Drinčić A, Ščančar J, Horvat M, Nečemer M, Piria M, Simonović P, Paunović M, Milačić
628 R (2019) Potentially toxic elements in muscle tissue of different fish species from the Sava River and risk
629 assessment for consumers. *Sci Total Environ* 650:958–969. doi: 10.1016/J.SCITOTENV.2018.09.083
- 630