Postprint of: Rutkowska M., Falandysz J., Saba M., Szefer P., Misztal-Szkudlińska M., Konieczka P., A method for the analysis of methylmercury and total Hg in fungal matrices, APPLIED MICROBIOLOGY AND BIOTECHNOLOGY Vol. 106 (2022), pp. 5261-5272

A method for the analysis of methylmercury and total Hg in fungal matrices

Małgorzata Rutkowska[1], Jerzy Falandysz[2*], Martyna Saba[3], Piotr Szefer[4], Małgorzata Misztal-Szkudlińska[4], Piotr Konieczka[1]

[1]Gdańsk University of Technology, Department of Analytical Chemistry, 80-233 Gdańsk, 11/12 G. Narutowicza Street, Poland

[2]Medical University of Lodz, Department of Toxicology, 1 Muszyńskiego Street, 90-151 Lódź, Poland

[3]Główny Inspektorat Jakości Handlowej Artykułów Rolno-Spożywczych, Laboratorium Specjalistyczne w Gdyni, Al. Marszałka Piłsudskiego 8/12, 81-378 Gdynia, Poland

[4]Medical University of Gdańsk, Department of Food Sciences, Al. Gen. J. Hallera 107, 80-416 Gdańsk, Poland

[*]Correspondoing autor

Abstract

The aim of the study was to develop an efficient method for the determination of monomethyl-mercury (MeHg) and total mercury (THg) content in materials such as fungal sporocarps and sclerotia. Certified Reference Materials (CRMs) with the assigned values of MeHg and THg as well as the control materials (dried mushrooms) with known content of THg were evaluated for method validation. Recovery of MeHg from reference materials was at the following levels: from tuna fish at 87.0 ± 2.3% (THg at 101.9 ± 1.2%), from fish protein at 99.4 ± 1.3% (THg at 92.70 ± 0.41%) and from dogfish liver at 96.45 ± 0.73%. Recovery of THg from the fungal control material CS-M-5 was at 104.01 ± 0.60% (contribution of MeHg in THg content was at 6.2%), from CS-M-4 at 101.1 ± 2.0% (contribution at 3.2%), from CS-M-3 at 100.55 ± 0.67% (contribution at 0.6%) and from CS-M-2 at 101.5 ± 2.7% (contribution at 3.7%). The content of MeHg in randomly selected wild fungi and their morphological parts was in the range from 0.006 to 0.173 mg kg^{-1} dry weight (dw). In the case of THg, the concentration values were in the range from 0.0108 to 10.27 mg kg^{-1} dw. The MeHg content in the control materials with the assigned THg values was determined. Since the control materials play an important role in all elements of the quality assurance system of measurement results, they can be used to analyse MeHg as the first control material for fungi.

Key points

- An extraction procedure for MeHg analysis in fungi was developed and optimized
- Recovery of MeHg from the certified reference non-fungal materials was > 87%
- Fungal control materials with assigned THg concentration can serve also for MeHg analysis

Keywords: environment, fungi, macromycetes, mushrooms, food toxicology, sclerotium, trace elements

Introduction

Mercury (Hg) is a common, toxic trace constituent in the earth's upper crust that typically contaminates all kinds of foods in ultra-trace amounts (Antunović et al., 2020; Nawrocka et al., 2020). Mono-methylmercury (MeHg) is a highly toxic derivative of Hg that is biosynthesized in minute amounts by lower food web organisms in the environment (Villar et al., 2020; Xu et al., 2019). MeHg binds to amino acid cysteine (MeHg-Cys) and as cysteine adduct it mimics amino acid methionine and hence is readily absorbed, bioaccumulated and biomagnified from the bottom to the top of food webs (Le Croizier et al., 2020; Ralston and Raymond, 2018 and 2010). MeHg has high neurotoxicity and passes through the placenta (Manhães et al., 2021). Neurotoxic MeHg caused severe poisonings and fatalities in humans who ate the contaminated fish and shellfish in Japan during the early 1950s ((Falandysz et al., 2020c; Yokoyama, 2018). Thus, the occurrence of MeHg in foods in parallel to inorganic Hg(II) is of special concern not only for the sake of human health, but also from the point of view of analytical and environmental chemistry (Fernandes et al., 2020; Sulimanec Grgec et al., 2020; Médieu et al., 2021).

At present, a number of different analytical methods are used to determine Hg and MeHg in samples with a complex matrix composition. The determination of THg is usually carried out using a vapour generation technique in combination with atomic absorption spectrometry (CV-AAS) (Ferreira et al., 2015; Rieder et al., 2011; Peregrino et al., 2011), or fluorescence spectrometry (CV-AFS) (Wang et al., 2017). Speciation of mercury is usually carried out with the use of high-performance liquid chromatography (HPLC) (Zhang et al., 2019; Zhu et al., 2017), gas chromatography (GC) coupled with AFS, AAS, AES or inductively coupled plasma mass spectrometry (ICP-MS) (Ferreira et al., 2015; Zhu et al., 2017; Gorecki et al., 2013; Houserová et al., 2006; Leopold et al., 2010; Suvarapu and Baek, 2017; Gao et al.,

2010; Reyes et al., 2009; Fischer et al., 1995). Non-chromatographic speciation of mercury has also been applied (Vieira et al., 2009; Ruiz-De-Cenzano et al., 2016).

Soil macromycetes, including edible species, are organisms that accumulate more Hg than other land creatures, and their fruiting bodies (mushrooms) are characterized by high values of bioconcentration factor (BCF) for this element (Árvay et al., 2014, 2015, 2017, 2022; Drewnowska et al., 2012; Falandysz, 2014, 2016 and 2017; Falandysz et al., 2022; Fischer et al., 1995; Kojta and Falandysz, 2016; Širić and Falandysz, 2020; Melgar et al., 2009). Indeed, in the terrestrial environment mushrooms accumulate the largest amount of Hg among all living organisms. A range of mushrooms of several genera from families such as *Agaricaceae* and *Boletaceae* or species such as *Leccinum extremiorientale,* that grow in unpolluted soils in forests of the Yunnan province in China and elsewhere showed THg in high concentrations, exceeding 5.0 mg kg^{-1} dw and up to 22 mg kg^{-1} dw (Ostos et al., 2015; Bergin et al., 2021; Falandysz et al., 2015, 2019 and 2020a).

Edible mushrooms that grow in the regions of cinnabar ore (HgS) mining and processing, or other industrial activities, can accumulate Hg in the amount of several tens of milligrams per kilogram of dried biomass (Árvay et al., 2017; Bargagli and Baldi, 1984; Kavčič et al., 2019). Because of its toxicodynamic features, MeHg is environmentally the most relevant Hg compound contaminating foodstuffs, including mushrooms (Nawrocka *et al.*, 2020; Ralston and Raymond, 2018; Médieu et al., 2021; Fernandes et al., 2020; Sulimanec Grgec et al., 2020; Stijve and Roschnik, 1974). All types of chemical analysis that are required for the control of food contamination with toxic substances as well as environmental protection, are of great importance and are activities that require new analytical methodologies, where certified reference materials are used to ensure the quality control of the determined results in addition to other quality assurance measures. It is extremely important to continuously enrich the range of available reference and control materials so that they are as "identical" as possible in terms

of chemical composition and physical form to the content of the test samples. However, there are currently no commercially available certified MeHg reference materials with a mushroom matrix.

The aim of the study was to adapt and validate a efficient method for the determination of both THg and MeHg in materials such as sporocarps (fruiting bodies) and sclerotia of fungi. The quantification of MeHg in vegetation was investigated with the use of a toluene and L-cysteine extraction which until now has only been used for sediment or animal tissues (Maggi et al., 2009; Rutkowska et al., 2019). The entire analytical procedure has been validated and its applicability studied by using a series of randomly selected fungal materials from our repository.

Material and methods

Reagents, standards and certified reference materials and control fungal materials

A mercury standard – MSHG, at a concentration 100.10 ± 0.43 μg mL^{-1} in 10% HCl was purchased from Inorganic Ventures, INC (USA). N-Acetyl-L-cysteine was obtained from Sigma Aldrich (Germany). Sodium sulfate anhydrous pure (99% purity) was purchased from POCh (Poland). Hydrobromic acid (47% purity) and toluene were obtained from Merck (Germany). Sodium acetate was purchased from Stanlab (Poland). Certified reference materials BCR-463 (tuna fish), DORM-2 (fish protein) and DOLT-4 (dogfish liver) were supplied by IRMM (Belgium) and NRC (Canada). The control materials such as CS-M-5 (*Suillus bovinus*), CS-M-4 (*Leccinum scabrum*), CS-M-3 (*Boletus edulis*) and CS-M-2 (*Agaricus campestris*) were purchased from LGC STANDARDS Sp. z o.o. (Poland).

Mushrooms (fruiting bodies) and sclerotia

The samples of mushrooms and fungal sclerotia that were selected to examine the applicability of the methods, consisted of a range of species previously collected in Europe, Asia and North America, and were either taken from the authors' repositories or were donations. All the previously collected mushroom samples were correctly treated - cleaned/sectioned/dried and then processed to a powder and in this form they were stored in tightly closed plastic containers in a dry and clean condition. Before the analysis the samples were prophylactically dehydrated again by lyophilisation for 48 hours. Most of the fungal materials studied contained THg in several studies that were carried out by different analysts (Falandysz, 2014; Falandysz et al., 2007; Falandysz et al., 2007; Jarzyńska and Falandysz, 2011; Maćkiewicz and Falandysz, 2012; Wiejak et al., 2016), but MeHg has not yet been investigated. For the present study all sample IDs were coded, so that they were unknown to the analysts involved in the determinations.

Instrumentation

A Mercury/MA-3000 mercury analyser supplied by Nippon Instruments Corporation (NIC, Japan) was used to analyse mercury with the cold vapour technique, and purified dry air was used as the carrier gas. Millipore's Milli-Q® water purification system (USA), IKA KS501 digital laboratory shaker (Merck, Germany) and Centrifuge 5702 (Eppendorf, Germany) were used for the MeHg extraction.

Sample preparation for MeHg determination

The organic forms of mercury were extracted from the fungi samples using the method described by Maggi et al. (2009), with small changes (Fig. 1). The authors of this manuscript have tried to provide a kind of guide that will make it possible to follow the proposed extraction procedure accurately. Preparation of samples for analysis must precede the stage where the samples are dried (freeze-dried) and homogenized to obtain the most homogeneous material possible. For a single experienced chemist, it is possible to prepare 8 mushroom samples for analysis in one working day. This time includes the preparation of both laboratory facilities (glassware, plasticware and reagents) and all stages of sample preparation (weighing, centrifuging, shaking and separation). Thus, the effect of 8 hours of work would result in 8 extracts, which can be directly transferred for measurement of the MeHg content using atomic absorption spectroscopy coupled with the cold vapour technique. The proposed procedure (Figure S1, Supplementary Material) can be successfully applied in laboratories engaged in environmental or food contamination analysis and consists of the following steps of sample preparation:

1. Weighing of approx. 250 mg of the sample for the plastic falcon centrifuge tubes with screw caps.
2. Adding 4 mL of 48% HBr to each sample, then shaking for 5 minutes/4.8 g using a mechanical vertical shaker.
3. Adding 10 mL of toluene, then vigorously mixing for 20 minutes/4.8 g and centrifuging for 10 minutes/3000 g.
4. Transferring the organic layer (with the use of a pipette) to the previously prepared plastic falcon tubes. It is important to transfer the toluene layer quantitatively into the second vessel, and special care should be taken not to contaminate the extract.
5. Repeating toluene extraction. Adding of 5 mL of toluene to the falcon tubes containing HBr and the sample. Shaking for 20 minutes/4.8 g and centrifuging for 10 minutes/3000 g.

6. Quantitatively transferring the supernatant containing organomercury species to the second vial again. This should provide about 15 mL of pure extract.

7. Subjecting the combined organic extracts twice to back extraction with 1% (v/v) L-cysteine to extract MeHg from toluene. The L-cysteine solution should be prepared by dissolving 1% L-cysteine chloride in 12.5% anhydrous sodium sulphate and 0.775% sodium acetate. 3 mL of this solution are added to each of the toluene extracts.

8. Shaking for 20 minutes/4.8 g and centrifuging for 5 minutes/3000 g.

9. Transferring the organic layer (with the use of a pipette) to the previously prepared plastic falcon tubes. Transferring the L-cysteine layer to the previously prepared glass vials. This stage may be the most difficult due to the fact that it is necessary to quantitatively transfer the "lower" layer, over which a cloudy sediment may additionally form.

10. Repeating the whole procedure: L-cysteine (2 mL) is added again to the falcon tubes containing toluene supernatant, followed by shaking for 20 minutes/4.8 g and centrifuging for 5 minutes/3000 g and transferring the L-cysteine layer to the glass vials with extreme precision and accuracy.

11. After completing the last stage, 5 mL aliquot of L-cysteine extract is obtained, which can be immediately measured using the mercury analyser.

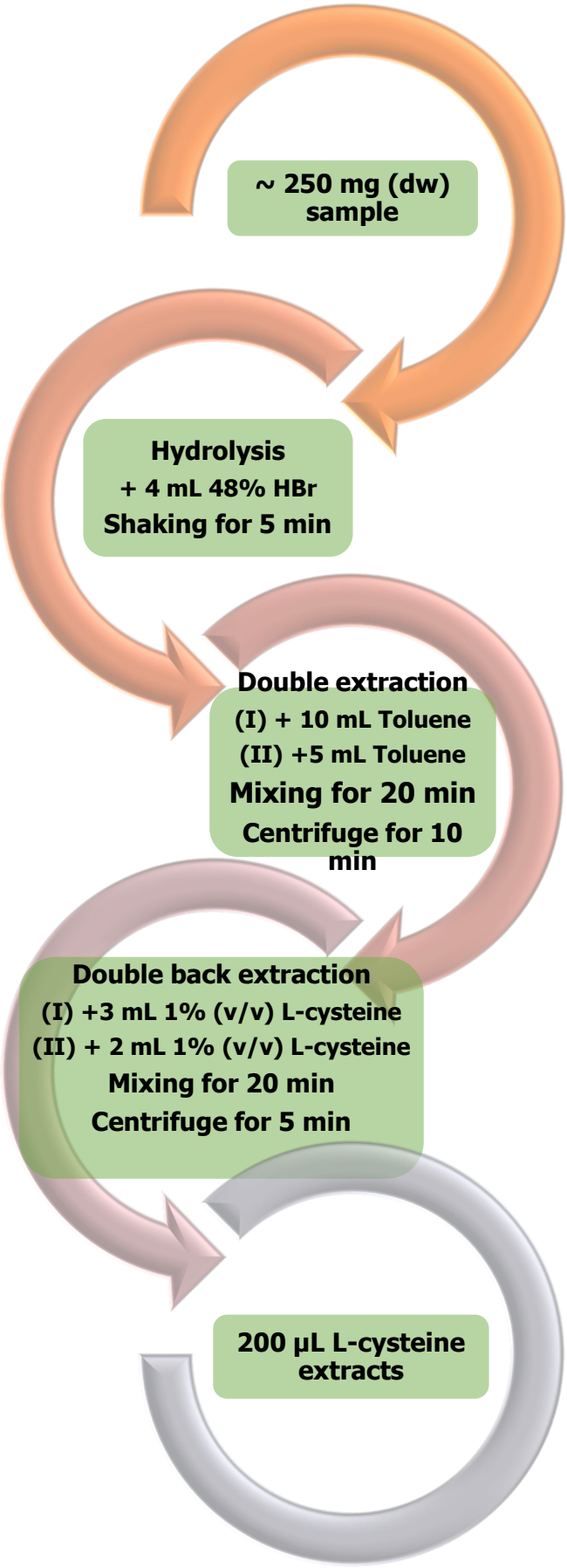


Figure 1. Scheme of an analytical procedure for the extraction of methylmercury from fungal materials.

Determination procedure

Blanks (n = 32), samples, certified and control materials for THg and MeHg (CRMs: BCR-463, DOLT-4 and DORM-2; Control materials: CS-M-4, CS-M-3 and CS-M-2) were analyzed as well. The THg and MeHg contents (pre-extraction analysis) were determined in six replicate measurements for each sample.

Method validation

Due to the fact that attempts have been made to extend the applicability of the extraction method to another type of matrix – fungal products – it was necessary to optimize the extraction conditions and to validate the analytical procedure. The validation process involved a series of measurements of the CRM samples. The volumes of solvents and other reagents as well as the centrifugation and shaking time were varied (Table 1). Additionally, the time of the extraction process itself was an important parameter taken into account. The measurements were carried out in six replicates. The acceptable recovery for this type of analysis should be in the range of 80% to 120%. Satisfactory results were obtained for each of the CRMs analyzed, except for the methodology presented in point V. The recovery values for BCR 463 material are the lowest, possibly due to the fact that it is the sample with the highest fat content (tuna fish). There were no statistically significant differences for the inter- and intra-day values obtained during the validation process. With reference to the principles of green analytical chemistry, a

methodology was selected in which the lowest amount of toxic solvents was used while maintaining high recovery factors. Method IV was used for further analyses.

Table 1. Extraction process parameters used in process optimisation

No.	Hydrolysis HBr [mL]/ Shaking [min]/[g]	Double extraction Toluene [mL]/ Shaking [min]/ Centrifuging [min]/[g]	Double back-extraction L-Cysteine [mL]/ Shaking [min]/ Centrifuging [min]/[g]	Recovery BCR 463 DOLT-4 DORM-2 [%]
I	*10 mL/5 min/ 4.8 g*	*20 mL/20 min/20 min/2400 g* *15 mL/20 min/20 min/2400 g*	*6 mL/20 min/20 min/2400 g* *6 mL/20 min/20 min/2400 g*	*92.7 ± 2.1* *99.5 ± 2.4* *97.0 ± 1.3*
II	*8 mL/5 min/5.0 g*	*10 mL/20 min/10 min/3000 g* *10 mL/20 min/10 min/3000 g*	*5 mL/20 min/10 min/3000 g* *5 mL/20 min/10 min/3000 g*	*93.1± 1.1* *98.1 ± 1.4* *98.0 ± 2.3*
III	*5 mL/5 min/ 4.8 g*	*10 mL/20 min/10 min/3000 g* *10 mL/20 min/10 min/3000 g*	*3 mL/20 min/5 min/3000g* *2 mL/20 min/5 min/3000g*	*90.9 ± 2.5* *97.5 ± 2.7* *98.1 ± 1.7*
IV	***4 mL/5 min/4.8 g***	***10 mL/20 min/10 min/3000 g*** ***5 mL/20 min/10 min/3000 g***	***3 mL/20 min/5 min/3000 g*** ***2 mL/20 min/5 min/3000 g***	***87.0 ± 2.3*** ***99.4 ± 1.3*** ***96.45 ± 0.73***
V	*4 mL/5 min/5 g*	*5 mL/20 min/10 min/3000 g* *5 mL/20 min/10 min/3000 g*	*3 mL/20 min/5 min/300 0g* *2 mL/20 min/5 min/3000 g*	*42.8±3.7* *48.6±1.9* *51.2±2.8*

The International Conference on Harmonization (ICH) and the United States Pharmacopeia (USP), have recommended the following validation parameters: linearity, measuring range, limit of detection (LOD), limit of quantification (LOQ), method limit of detection (MDL), method limit of quantification (MQL), repeatability, indirect precision.

Repeatability and indirect precision were determined based on the calculated value of the standard deviation (SD) of the measurement series. The SD values were calculated from the results obtained from 6 independent determinations of the analyte in standard samples. Intermediate precision values exceeded the repeatability values, which was in line with expectations. The linearity was estimated by carrying out measurements, in three repetitions, of 10 and 6 samples of standard solutions, for low and high concentration levels respectively. Then, regression parameters and the measuring range were determined. The numerical values of the regression parameters of the calibration curves formed the basis for estimating the value of the LOD and LOQ of the analytical method used. The LOD value was determined using the following equation (Konieczka and Namieśnik, 2007):

$$LOD = \frac{3.3 s_a}{b} \qquad (2.1)$$

S_a – standard deviation of the intercept,

b – slope.

When calculating the numerical value of LOQ, the dependence described by the following equation was used (Konieczka and Namieśnik, 2007):

$$LOQ = 3 \cdot LOD \qquad (2.2)$$

The obtained numerical values of LOD and LOQ parameters were converted into corresponding MDL and MQL values, assuming that the weight of the analysed sample was 100 mg.

Results

The extraction is relatively fast and consumes small amounts of solvents. The values of coefficients of variation (CV) and estimated uncertainties for both test and CRM samples were low (CV < 10%), which confirms good repeatability and precision of the described method (Table 2 and 3).

Table 2. Validation parameters of the procedure for the determination of THg and MeHg in the control and certified reference material aliquots (assuming the weight = 100 mg)

Parameter		Value	
		THg	MeHg
Linearity	• 10 measurement points, • 3 repetitions, • concentration range: 10.25 (ng) ÷103 (ng) y = 1.0006x – 0.15	0.999	
	• 6 measurement points, • 3 repetitions, • concentration range: 103 (ng) ÷1025 (ng) y = 0.9987x + 1.1	1.000	
LOD (ng)		0.096	
LOQ (ng)		0.29	
MDL (ng g^{-1})		0.96	1.0
MQL (ng g^{-1})		2.9	3.1
Measuring range (ng g^{-1})		2.9 ÷ 102.5	3.1 ÷ 110.0
Repeatability CV (%)		2.8	1.4
Indirect precision CV (%)		4.7	3.9
Recovery (%)			
CS-M-5		104.0 ± 0.6	Not applicable
CS-M-4		101.1 ± 2.0	Not applicable
CS-M-3		100.6 ± 0.7	Not applicable
CS-M-2		101.5 ± 2.7	Not applicable
BCR 463		101.9 ± 1.2	87.0 ± 2.3
DOLT-4		92.7 ± 0.4	99.4 ± 1.3
DORM-2		Not applicable	96.5 ± 0.7

The percentage contribution of MeHg in THg in the control fungal materials studied varied from 0.6 to 6.2%, which is in the lower range of values determined for various mushroom species reported by other authors (Fischer et al., 1995; Bargagli and Baldi, 1984; Stijve and Roschnik, 1974). The reason for this is probably the low biodiversity of the species.

The accuracy of the extraction procedure used and of the Hg determination method was assessed on the basis of the results of the analysis of the CRM samples and laboratory control materials. The obtained results show that the recovery of the analytical procedure was satisfactory. The acceptable recovery for this type of analysis should fall within the range of 80 to 120 %.

No fungal control or reference materials with known values for MeHg are available commercially so far. However, BCR-463, DOLT-4 and DORM-2 were certified for MeHg content. The concise data on the validation parameters and the results of analyses of THg and MeHg in the control materials such as CS-M-4, CS-M-3 and CS-M-2 and in the CRMs: BCR-463, DOLT-4 and DORM-2 are presented in Tables 2 and 3, respectively. Known (certified) values of concentration were used to evaluate the determinations of MeHg in these CRMs and our results were in good agreement (Tables 2 and 3).

As mentioned, in order to get an insight into the applicability of the method over a wide range of MeHg concentrations and varying contributions to THg concentration, a range of fungal products of different origin have been studied, including the morphological parts (caps and stipes) and the whole sporocarps of species such as *Agaricus arvensis* Schaeff., *Albatrellopsis ellisii* (Berk. ex Cooke & Ellis) Teixeira (previous name: *Albatrellus ellisii*), *Boletus aereus* Bull., *Boletus bainiugan* Dentinger, *Boletus edulis* Bull., *Calvatia gigantea*, (Batsch) Lloyd, *Chlorophyllum rhacodes* (Vittad.) Vellinga (previous name: *Macrolepiota rhacodes*), *Cortinarius caperatus* (Pers.) Fr., *Jahnoporus hirtus* (Cooke) Nuss, *Lactarius controversus* Pers., *Lactarius vellereus* (Fr.) Kuntze, *Macrolepiota procera* (Scop.) Singer,

Retiboletus griseus (Frost) Manfr. Binder & Bresinsky (previous name: *Boletus griseus*), *Tricholoma equestre* (L.) P. Kumm., *Xerocomus subtomentosus* (L.) Quél., and sclerotia of *Pachyma hoelen* Fr., (syn. *Wolfiporia cocos* sensu auct.) (Table 4). The multi-step sample preparation process consisting of multiple-extractions was applied to these samples. The obtained experimental data for selected fungal materials are shown as the means of all results with estimated expanded uncertainties (k = 2) in Table 4. The range of THg concentrations determined in these fungal materials was relatively wide, i.e. from 0.011 to 10 mg kg^{-1} dw, in comparison to commonly available retail, non-fungal foods including fish and animal meats (Nawrocka et al., 2020; Médieu et al., 2021; Donadt et al., 2021). In the case of MeHg, concentrations ranged from 0.0091 to 0.173 mg kg^{-1} dw in the caps, stipes and whole carpophores, from 0.0062 to 0.0091 mg kg^{-1} dw in the polypore samples and from 0.0092 to 0.011 mg kg^{-1} dw in sclerotia (Table 4).

The percentage contribution of MeHg to THg in the studied fungal materials ranged from 0.43 to 11.6% in the caps, stipes and the whole carpophores of typical mushrooms (in that order), from 9.6 to 23% in the polypore fungi and at 84.8 to 85.2% in sclerotia samples.

Discussion

The aim was to find a procedure for which the analyte recovery was high, and the toxic solvent use was as low as possible – in accordance with the principles of green analytical chemistry. This method apart from a simple course of analytical steps has documented relevant analytical parameters that characterize its performance, such as linearity, good detection sensitivity (LOD, LOQ, MDL, MQL) and measuring range, repeatability, indirect precision, percentage recovery from reference (certified and control) materials and applicability for fungal materials, and work productivity with reference to the principles of green analytical chemistry while maintaining

high recovery. The proposed extraction procedure is efficient, precise and suitable for the analysis of MeHg together with THg in fungal materials and including control materials using the same instrumentation - Mercury/MA-3000 mercury analyser. Thus, by extension, the fungal control materials (CS-M-5, CS-M-4, CS-M-3 and CS-M-2), used for THg and certain other elements, could serve as the first fungal control materials for MeHg in mushrooms, provided that more results are collected under interim precision conditions for comparison and verification.

Heavy metal contamination of foodstuffs is of a serious concern by health authorities worldwide. The environmental mobility of evaporated elemental mercury along with the existing and ongoing anthropogenic and geogenic sources of its emission still continues. Thus, the presence of mercury and especially of its highly toxic derivative like methylmercury in foods receives special attention. WHO has approved US EPA levels for mercury and recommend that foods with Hg levels of 0.5 mg kg^{-1} or higher cannot be sold for consumption by humans (US EPA (United States Environmental Protection Agency), 2004). The same maximum level for Hg in food products (0.5 mg kg^{-1}) has been set by the European Commission in the Official Journal of the European Union (EU, 2006). The government in Japan, due to high fish consumption, has recommended that fish with Hg levels of 0.3 mg kg^{-1} (WW) or higher should not be sold (Ikem and Egiebor, 2005). Mercury levels in mushrooms, obtained during the study, in some cases exceeded the standards applicable in various regions of the world.

Sclerotia of *Pachyma hoelen* is an edible and popular medicinal product in Asia that is very low in THg (Falandysz et al., 2020b; Wiejak et al., 2016; Zhang et al., 2022), while interestingly most of it is in the form of MeHg (Table 4). A much higher range of MeHg contribution in THg in the edible fungal products – as has been determined in this study, with contributions consistently exceeding the values reported previously (Stijve and Roschnik, 1974;

Bargagli and Baldi, 1984; Fischer *et al.*, 1995; Ruiz-De-Cenzano et al., 2016), is worth of further studies.

Mercury both from the geogenic source at the sites with mercuriferous belt and from places with legacy (anthropogenic) pollution is well accumulated by various soil mushrooms (Falandysz, 2016 and 2017; Falandysz et al., 2005a, 2019b and 2020a; Ostos et al., 2017), and this is also confirmed with results from this study. The species such as *Boletus aereus* and *Boletus bainiugan* from Yunnan, which is region diverse in types of soils and topography and extremely biodiverse and where soil is naturally enriched in Hg, shows THg in the range from 2.4 to 10 mg kg^{-1} dw, while *Retiboletus griseus* with 0.36 to 0.56 mg kg^{-1} dw is substantially lower (Table 4). On the other side, the specimens of *Xerocomus subtomentosus* and *Calvatia gigantea* from the Sobowidz site also showed elevated THg in the range from 1.4 to 6.2 mg kg^{-1} dw (Table 4). The Sobowidz site was earlier identified as suspicious place because of an elevated THg in mushroom *Lactarius volemus* and adjacent soil substrate – possibly because of legacy of the nonferrous metal foundry there (Falandysz, 2017).

Nevertheless, in order to accurately distinguish how much Hg accumulated in the mushroom comes from geogenic or anthropogenic sources (at least of a local or regional origin), further studies would require the measurement of stable Hg isotopes (^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg and ^{202}Hg) for the isotope index analysis and determining their proportion in relation to the composition in a possible source material, e.g. vermilion (cinnabar; HgS) ore. In many places, due to existing and ongoing anthropogenic sources of Hg emissions and also from the volcanic eruptions and evaporation from mercuriferous belts, absorption of this element by mycelium (developing in the topsoil) from the environmental background and accumulation in the reproductive part (fruit body) will be roughly the resultant of such sources. As mentioned, all soil mushrooms compared to vegetables and plants or terrestrial animals show much higher concentration of accumulated Hg, while some, e.g. *Boletus edulis* or *Macrolepiota procera*

(Table 4) from a "pristine" sites (without local/regional geogenic or anthropogenic sources of pollution) are also enriched. In the case of the results obtained for MeHg in mushrooms, apart from the very high share of MeHg in TH in sclerotia of *Pachyma hoelen*, another distinguish feature is a small (0.43 - 0.85%) share of MeHg in THg in *Boletus aereus* from Yunnan, which is known as the species well accumulating Hg in regions with geogenic background (Falandysz et al., 2005a, 2017; Ostos et al., 2017). For other species, the proportion of MeHg to THg varies to some extent (Table 4), while for this type of environmental study, large collections of species and specimens are required to get a clear picture. There are numerous Hg methylating microbial communities in forest soils (Xu et al., 2019), thus absorption and accumulation of MeHg and share in relation to THg in mushroom can be more site specific than species-specific for individuals of the same species or genera?

Previous authors studying MeHg (also THg) in mushrooms used toluene as the extracting agent (Stijve and Roschnik, 1974). Dried mushroom samples of about 5 g (rarely more) were soaked overnight with hydrochloric acid and purified extracts were examined by gas chromatography (GC) (5 ft x $^{1}/_{8}$ inch glass column with 5% Carbowax 20 M on Chromosorb W) with electron capture detector. After re-extraction of MeHg with aqueous cysteine quantification limit was at ppb level, and confirmation procedure was by using silica-gel chromatography (MDL was 0.02 μg) (Stijve and Roschnik, 1974). Ten years later, the authors extracted MeHg from 5 g of dehydrated mushrooms with benzene-cysteine and after digestion of the cysteine extracts with hot (70 °C) solution of concentrated nitric acid, the MeHg was determined (as THg) by cold-vapour atomic absorption spectroscopy (AAS). The MeHgCl standard solution was used in replicates for control of extraction efficiency (CV for 5 replicates was 9.4%) (Bargagli and Baldi, 1984). In a study later for another 10 years, the dried mushrooms of 0.15-0.5 g were dissolute with 25% (w/v) KOH solution in methanol with aid of an ultrasonic bath (3 h), and extracted MeHg was derivatized with sodium boron tetraethyl and

then cryogenic trapped on a packed GC column and as volatile species separated and determined by AAS (Fischer et al., 1995). Under the recommended conditions, the MQL for MeHg was typically around 4 μg kg^{-1} dw, and for labile Hg^{2+} was 75 μg kg^{-1} dw (increasing the sample mass to 0.5 g and further optimization of the derivatization reaction conditions may result in the MeHg MQL at 1 μg kg^{-1} dw (Fischer et al., 1995).

The more laborious procedure involves treating 0.1 g of the dried mushrooms with a mixture of 5% H_2SO_4 and 18% KBr solutions, and 1 M $CuSO_4$ solution, double extraction with CH_2Cl_2, centrifugation, treatment of the combined extracts with Milli-Q water, evaporation of the organic solvent at about 90 °C (also purging with N_2 to remove solvent completely), adjustment of aliquot pH to 4.6 with 100 mL of acetate buffer, derivatization of Hg compounds with 1% NaBEt4 (50 mL), purging ethylated methyl-Hg as ethylmethyl-Hg onto Tenax trap (Hg free N_2), attachment of traps to Ar flow, thermal desorption (180 °C) of MeHg on GC column, conversion of the Hg species to HgO by pyrolysis (600 °C) and measurement by atomic fluorescence spectroscopy (CVAFS) – the MDL of MeHg at around 50 μg kg^{-1} dw (Rieder et al., 2011). In the most recent study. Ruiz-De-Cenzano et al. (2016) presented method of inorganic Hg (I-Hg) and organic Hg (O-Hg) determination in 0.2 g dried mushroom samples. Their method based on extraction of THg with diluted hydrochloric acid followed by generation and measurement of Hg vapour by CVAFS to determine I-Hg, and degradation by oxidation of O-Hg with $KBr/KBrO_3$ mixture at 50 °C in a water bath and generation and measurement of Hg vapour by CVAFS to determine THg (O-Hg concentration is calculated from the difference between THg and I-Hg). The MDL values were 3.2 μg kg^{-1} dw for THg and 0.6 μg kg^{-1} dw for I-Hg, and the MQL values were 10.5 μg kg^{-1} dw for THg and 1.9 μg kg^{-1} dw for I-Hg (Ruiz-De-Cenzano et al., 2016).

One of the achievements of the presented method are values of the MDL and MQL for MeHg of 1.0 μg kg^{-1} and 3.1 μg kg^{-1} in dehydrated fungal matrices, respectively (Table 2).

These values are equivalent to 0.1 and 0.31 μg kg^{-1} fresh (wet or whole weight) fungal matrices (assuming moisture content at 90%). Thus, this methodology could be further tested as to its possible suitability for the MeHg control, in addition to THg, in dried vegetables as well as in dairy products, which contain more fat than mushrooms or vegetables, and in which there is generally little of this element. In summary, an efficient, robust, fully validated and relatively fast method to determine MeHg in parallel to THg in fungal materials has been presented. Based on the obtained results it can be concluded that:

- ✓ the recovery of MeHg from CRMs, achieved using the proposed analytical procedure, is at a satisfactory level. The acceptable recovery for this type of analysis should be in the range of 80% to 120%;
- ✓ the CRMs were used to establish trueness of the method and all results obtained for both MeHg and THg concentrations were within the certified range, confirming the suitability of the method. Therefore, the trueness of the method was confirmed;
- ✓ the method is characterized by both high precision and repeatability (CV < 5%);
- ✓ for an individual operator, it is possible to perform a MeHg extraction for 8 samples daily (per 8 h of worktime), followed by THg and MeHg determination using the CV-AAS technique;
- ✓ indicative concentrations of MeHg have been presented for four fungal con-trol materials that have known official (indicative) values for THg. As the choice of a suitable reference material is primarily driven by the users' desire for its compatibility with the tested material due to the chemical composition and physical form of the matrix and due to the content of the substance to be determined, the analysed materials may constitute an appropriate response to the current demand for reference materials and control materials for fungi with a designated MeHg content;

- ✓ the applicability of the method has been demonstrated by the analysis of several species of mushrooms and sclerotia of the fungus *Pachyma hoelen*. These fungal materials of various type and geographical origin showed a range of concentrations of THg (0.011 to 10 mg kg^{-1} dw) and MeHg (0.0062 to 0.17 mg kg^{-1} dw) as well as a range of percentage contributions of MeHg in THg concentration, from 0.43 to 85.2%.

Table 3. Assigned values of THg and determined values of THg and MeHg in the fungal control materials and certified reference materials of other type available commercially

Control and certified reference materials*	Kind of material	THg (mg kg^{-1} dw)	THg (mg kg^{-1} dw)	Recovery (%)	MeHg (mg kg^{-1} dw)**	MeHg (mg kg^{-1} dw)**	Recovery (%)
		Assigned or certified value	Determined value		Assigned or certified value	Determined value	
Control material CS-M-5	*S. bovinus*	0.185 ± 0.028 (0.157 - 0.213)	0.1924 ± 0.0011	104.01 ± 0.60	NA	0.01195 ± 0.00045	NA
Control material CS-M-4	*L. scabrum*	0.463 ± 0.024 (0.439 - 0.487)	0.4683 ± 0.0093	101.1 ± 2.0	NA	0.01520 ± 0.00068	NA
Control material CS-M-3	*B. edulis*	2.85 ± 0.10 (2.745 - 2.953)	3.035 ± 0.019	100.55 ± 0.67	NA	0.01929 ± 0.00025	NA
Control material CS-M-2	*A. campestris*	0.1641 ± 0.0040	0.1747 ± 0.0041	101.5 ± 2.7	NA	0.00654 ± 0.00030	NA
CRM BCR-463	Tuna fish	2.85 ± 0.16	2.90 ± 0.14	101.9 ± 1.2	3.04 ± 0.16	2.645 ± 0.072	87.0 ± 2.3
CRM DOLT-4	Dogfish liver	2.58 ± 0.22	2.39 ± 0.27	92.70 ± 0.41	1.33 ± 0.12	1.322 ± 0.019	99.4 ± 1.3
CRM DORM-2	Fish protein	Not applicable	Not applicable	NA	4.472 ± 0.030	4.311 ± 0.036	96.45 ± 0.73

Notes: *(six determinations per each compound and material), **(as Hg); CRM (Certified reference material).

Table 4. The examples of total Hg and MeHg content in mushrooms randomly sampled from Europe, North America and Asia and examined using developed methodology

Fungal materials data		THg[#]	U (k=2)	MeHg[#]	U (k=2)	% MeHg[~]
Latin name of mushroom	Common name	(mg/kg^{-1} dw)				
Boletus edulis (Poland, Pomerania, Wdzydze Landscape Park, 1999-2001; whole, n = 45)[*]	King Bolete	2.215	0.043	0.0331	0.0014	1.5
Boletus edulis (Poland, Wielkopolska, Porażyn , 2008; whole, n = 13)	King Bolete	1.876	0.026	0.07684	0.00076	4.1
Boletus edulis (Poland, Pomerania, Pomlewo, 2015; caps, n = 31)	King Bolete	1.893	0.043	0.09280	0.00015	4.9
Boletus edulis (Poland, Pomerania, Tuchola Pinewoods, Lubiatowo, 2016; whole, n = 23)	King Bolete	0.352	0.015	0.0137	0.0014	3.9
Boletus edulis (Poland, Pomerania, Kościerzyna, 2001; caps, n = 15)	King Bolete	3.137	0.087	0.0405	0.0014	1.3
Boletus edulis (Poland, Pomerania, Kościerzyna, 2001; stipes, n = 15)	King Bolete	2.152	0.042	0.0106	0.0032	0.50
Calvatia gigantea (Poland, Sobowidz, 2019; whole, n=1)	Giant Puffball	5.507	0.010	0.09842	0.00087	1.8
Cortinarius caperatus (Poland, Tuchola Pinewood, 2016; caps, n = 15)	Gipsy	1.539	0.021	0.0689	0.0047	4.5
Cortinarius caperatus (Poland, Tuchola Pinewood, 2016; stipes, n = 15)	Gipsy	0.595	0.011	0.00909	0.00033	1.5
Lactarius vellereus (Poland, 2003; caps)	Fleecy Milkcap	3.16	0.014	0.07907	0.00094	2.5
Lactarius vellereus (Poland, 2003; stipes)	Fleecy Milkcap	1.848	0.015	0.04314	0.00053	2.3
Lactarius vellereus (Poland, Tricity Landscape Park, Oliwa, 2003; whole, n = 10)	Fleecy Milkcap	1.5073	0.0092	0.0764	0.0011	5.1
Lactarius controversus (Poland, Tricity Landscape Park, Osowa, 2006; whole, n = 10)	Fenugreek Milkcap	1.3754	0.0097	0.1593	0.0034	11.6
Macrolepiota procera (Poland, Pomerania, Dębnica Kaszubska, 2003, caps, n = 30)	Field Parasol	3.337	0.048	0.09991	0.00031	3.0
Macrolepiota procera (Poland, Pomerania, Kościerzyna, 2001, caps, n = 10)	Field Parasol	2.701	0.015	0.08274	0.00038	3.1
Chlorophyllum rhacodes (Poland, Pomerania, Pomlewo, 2016; caps, n = 16)	Shaggy Parasol	0.8841	0.057	0.04205	0.00025	4.8
Tricholoma equestre (Poland, Ontoga, 2003; caps, n = 13)	Yellow Knight	0.869	0.043	0.0368	0.0010	4.2
Tricholoma equestre (Poland, Ontoga, 2003; stipes, n = 13)	Yellow Knight	0.409	0.026	0.0258	0.0022	6.3
Tricholoma equestre (Poland, Kępice, 2003; caps, n = 8)	Yellow Knight	0.836	0.017	0.01934	0.00030	2.3
Tricholoma equestre (Poland, Kępice, 2003; stipes, n = 8)	Yellow Knight	0.601	0.010	0.00908	0.00067	1.5
Tricholoma equestre (Poland, Pomerania, Sierakowice, 2007; caps, n = 7)	Yellow Knight	0.879	0.011	0.0572	0.0072	6.5
Xerocomus subtomentosus (Poland, Sobowidz, 2018; whole, n=1)	Suede Bolete	1.439	0.012	0.0547	0.0013	3.8
Xerocomus subtomentosus (Poland, Sobowidz, 2018; stipe, n=1)	Suede Bolete	3.576	0.016	0.0708	0.0011	2.0
Xerocomus subtomentosus (Poland, Sobowidz, 2018; cap, n=1)	Suede Bolete	6.214	0.014	0.0987	0.0018	1.6
Albatrellopsis ellisii (USA, whole, n = 1)	Sheep Polypore	0.0267	0.0010	0.00620	0.00024	23.2

Jahnoporus hirtus (USA, Oregon, 1997; whole, n = 1)	Bitter Iodine Polypore	0.0907	0.0055	0.00867	0.00020	9.6
Boletus aereus (China, Yunnan, 2012; caps, n = 10)	Bronze (Queen) Bolete	10.27	0.13	0.0877	0.0013	0.85
Boletus aereus (China, Yunnan, 2012; caps, n = 10)	Bronze (Queen) Bolete	3.514	0.025	0.01529	0.00054	0.43
Boletus bainiugan (China, Yunnan, 2015; caps, n = 17)	Bái niú gān	6.941	0.054	0.173	0. 014	2.5
Boletus bainiugan (China, Yunnan, 2015; stipes, n = 17)	Bái niú gān	2.377	0.013	0.0401	0.0038	1.7
Retiboletus griseus (China, Yunnan, 2015; caps, n = 14)	Gray Bolete	0.561	0.017	0.02732	0.00075	4.9
Retiboletus griseus (China, Yunnan, 2015; stipes, n = 14)	Gray Bolete	0.3623	0.0042	0.01051	0.00035	2.9
Agaricus arvensis (China, Inner Mongolia, 2015; caps, n = ~ 60)	Horse Mushroom	1.212	0.031	0.0728	0.0016	6.0
Agaricus arvensis (China, Inner Mongolia, 2015; stipes, n = ~ 60)	Horse Mushroom	0.716	0.013	0.02797	0.00062	3.9
Pachyma hoelen (China, Yunnan, 2012 , n = 1)	Hoelen, Poria	0.01342	0.00029	0.01144	0.00097	85.2
Pachyma hoelen (China, Yunnan, 2012 , n = 1)	Hoelen, Poria	0.0108	0.0013	0.00916	0.00095	84.8

Notes: *Number of specimens in composite sample); #Mean content after analysis of six aliquots; ~Calculated before rounding off a result.

Acknowledgements

Donation of the polypore samples (*Albatrellopsis ellisii* and *Jahnoporus hirtus*) by Dr. Tjakko Stijve is acknowledged.

Contributions

MR: Conceptualization, contributed regents, performed the experiments, analyzed and interpreted data, drawn figures, wrote, reviewed and edited the paper. JF: Conceptualization, contributed materials, analyzed and interpreted data, wrote, reviewed and edited the paper, supervision. MS: Performed the experiments, contributed materials and reagents, help with quaerenda. PS: Help with quaerenda; reviewed the paper. M M-S: Performed the experiments, help with quaerenda. PK: Contributed reagents and analysis tools, reviewed the paper, supervision.

Dedication

This manuscript is dedicated to the memory of Dr. Tjakko Stijve (1937 - 2020).

Funding

This study had part financial support by the National Science Centre under grant no. UMO-2016/23/N/NZ9/02746.

References

Antunović Z, Mioč B, Klir Ž, Širić I, Držaić V, Lončarić Z, Bukvić G, Novoselec J (2020) Concentrations of mercury and other elements in ewes' milk: Effect of lactation stage. Chemosphere 261:128128

Árvay J, Tomáš J, Hauptvogl M, Kopernická M, Kováčik A, Bajčan D, Massányi P (2014) Contamination of wild-grown edible mushrooms by heavy metals in a former mercury-mining area. J Environ Sci Health Part B 49**:**815–827

Árvay J, Záhorcová Z, Tomáš J, Hauptvog M, Stanovič R, Harangozo L (2015) Mercury in edible wild-grown mushrooms from historical mining area – Slovakia: Bioaccumulation and risk assessment. J Microbiol Biotech Food Sci 4 (special issue 3):1-4

Árvay J, Demková L, Hauptvogl M, Michalko M, Bajčan D, Stanovič R, Tomáš J, Hrstková M, Trebichalský P (2017) Assessment of environmental and health risks in former polymetallic ore mining and smelting area, Slovakia: Spatial distribution and accumulation of mercury in four different ecosystems. Ecotoxicol Environ Saf 144:236–244

Árvay J, Hauptvogl M, Demková L, Harangozo L, Šnirc M, Bobuľská L, Štefániková J, Kováčik A, Silvia Jakabová S, Jančo I, Kunca V, Relić D (2022) Mercury in *Scarletina bolete* mushroom (*Neoboletus luridiformis*): Intake, spatial distribution in the fruiting body, accumulation ability and health risk assessment. Ecotoxicol Environ Saf 232:113235

Bargagli R, Baldi F (1984) Mercury and methyl mercury in higher fungi and their relation with the substrata in a cinnabar mining area. Chemosphere 13:1059–1071

Bergin R, Koch I, Rutter A, Shirley J, Zeeb B (2021) Evaluating mercury concentrations in edible plant and fungi species in the Canadian Arctic environment. J Environ Qua. 1−12: https://doi.org/10.1002/jeq2.20253

Donadt C, Cooke CA, Graydon JA, Poesch MS (2021) Mercury bioaccumulation in stream fish from an agriculturally-dominated watershed. Chemosphere 262:128059

Drewnowska M, Sąpór A, Jarzyńska G, Nnorom IC, Sajwan KS, Falandysz J (2012) Mercury in *Russula* mushrooms: Bioconcentration by Yellow-ocher Brittle Gills *Russula ochroleuca*. J Environ Sci Health Part A 47:1577–1591

EU (2006) COMMISSION REGULATION (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union L 364/5

Falandysz J, Lipka K, Mazur A (2007) Mercury and its bioconcentration factors in fly agaric (*Amanita muscaria*) from spatially distant sites in Poland. J Environ Sci Health Part A 42:625–1630

Falandysz J, Gucia M (2008) Bioconcentration factors of mercury by Parasol Mushroom (*Macrolepiota procera*). Environ Geochem Health 30:121–125

Falandysz J (2014) Distribution of mercury in Gypsy *Cortinarius caperatus* mushrooms from several populations: An efficient accumulator species and estimated intake of element. Ecotoxicol Environ Saf 110:68–72

Falandysz J, Zhang J, Wang YZ, Saba M, Krasińska G, Wiejak A, Li T (2015a) Evaluation of mercury contamination in fungi boletus species from latosols, lateritic red earths, and red and yellow earths in the circum-pacific mercuriferous belt of southwestern China. PLoS ONE 10(11):1–19

Falandysz J, Zhang J, Wang Y, Krasińska G, Kojta A, Saba M, Shen T, Li T, Liu H (2015b) Evaluation of the mercury contamination in mushrooms of genus *Leccinum* from two different regions of the world: accumulation, distribution and probable dietary intake. Sci Total Environ 537:470–478

Falandysz J (2016) Mercury bio-extraction by fungus *Coprinus comatus*: a possible bioindicator and mycoremediator of polluted soils? Environ Sci Pollut Res 23:7444–7451

Falandysz J (2017) Mercury accumulation of three *Lactarius* mushroom species. Food Chem 214:96–101

Falandysz J, Dryżałowska A, Zhang J, Wang Y (2019a) Mercury in raw mushrooms and in stir-fried in deep oil mushroom meals. J Food Comp Anal 82:103239

Falandysz J, Zhang J, Mędyk M, Zhang X (2019b) Mercury in stir-fried and raw mushrooms from the *Boletaceae* family from the geochemically anomalous region in the Midu county, China. Food Control 102:17–21

Falandysz J, Mędyk M, Saba M, Zhang J, Wang Y, Li T (2020a) Mercury in traditionally foraged species of fungi (macromycetes) from the karst area across Yunnan province in China. Appl Microbiol Biotechnol 104:9421–9432

Falandysz J, Saba M, Zhang J, Hanć A (2020b) Occurrence, distribution and estimated intake of mercury and selenium from sclerotia of the medicinal fungus *Wolfiporia cocos* from China. Chemosphere 247:125928

Falandysz J, Shi J, Monti C (2020c) Environmental cycling and fate of mercury: 2020. Chemosphere 261:127766

Falandysz J, Saba M, Rutkowska M, Konieczka P (2021) Total mercury and methylmercury (MeHg) in braised and crude *Boletus edulis* carpophores during various developmental stages. Environ Sci Pollut Res 29:3107–3115

Fernandes A, Falandysz J, Širič I (2020) The toxic reach of mercury and its compounds in human and animal food webs. Chemosphere 261:127765

Ferreira SLC, Lemos VA, Silva LOB, Queiroz AFS, Souza AS. da Silva EGP, dos Santos WNL, das Virgens CF (2015) Analytical strategies of sample preparation for the determination of mercury in food matrices — A review. Microchem J 121:227–236

Fischer RG., Rapsomanikis S, Andreae MO, Baldi F (1995) Bioaccumiilation of methylmercury and transformation of inorganic mercury by macrofungi. Environ Sci Technol 29:993–999

Gao Y, De Galan S, De Brauwere A, Baeyens W, Leermakers M (2010) Mercury speciation in hair by headspace injection-gas chromatography-atomic fluorescence spectrometry (methylmercury) and combustion-atomic absorption spectrometry (total Hg). Talanta 82:1919–1923

Górecki J, Díez S, Macherzynski M, Kalisińska E, Gołaś J (2013) Improvements and application of a modified gas chromatography atomic fluorescence spectroscopy method for routine determination of methylmercury in biota samples. Talanta 115:675–680

Hanć A, Fernandes AR, Falandysz J, Zhang J (2021) Mercury and selenium in developing and mature fruiting bodies of *Amanita muscaria*. Environ Sci Pollut Res 28:60145–60153

Houserová P, Matějíček D, Kubáň V, Pavlíčková J, Komárek J (2006) Liquid chromatographic – cold vapour atomic fluorescence spectrometric determination of mercury species. J Sep Sci 29:248–255

Jarzyńska G, Falandysz J (2011) The determination of mercury in mushrooms by CV-AAS and ICP-AES techniques. J Environ Sci Health Part A 46:569–573

Kavčič A, Mikuš K, Debeljak M, Teun van Elteren J, Arčon I, Kodre A, Vogel-Mikuš K (2019) Localization, ligand environment, bioavailability and toxicity of mercury in *Boletus* spp. and *Scutiger pes-caprae* mushrooms. Ecotoxicol Environ Saf 184:109623

Kojta AK, Falandysz J (2016) Soil-to-mushroom transfer and diversity in total mercury content in two edible *Laccaria* mushrooms. Environ Earth Sci 75:18

Konieczka P, Namieśnik J (2007) Walidacja procedur analitycznych. Ocena i kontrola jakości wyników pomiarów analitycznych. Wydawnictwo Naukowo-Techniczne, Warszawa.

Le Croizier G, Lorrain A, Schaal G, Ketchum J, Hoyos-Padilla M, Besnard L, Munaron JM, Le Loc'h F, Point D (2020) Trophic resources and mercury exposure of two silvertip shark populations in the Northeast Pacific Ocean. Chemosphere 253:126645

Leopold K, Foulkes M, Worsfold P (2010) Methods for the determination and speciation of mercury in natural waters - A review. Anal Chim Acta 663:127–138

Maćkiewicz D, Falandysz J (2012) Total mercury in Yellow Knights (*Tricholoma equestre*) mushrooms and beneath soils. Bull Environ Contam Toxicol 89:755–758

Maggi C, Berducci MT, Bianchi J, Giani M, Campanella L (2009) Methylmercury determination in marine sediment and organisms by Direct Mercury Analyser. Anal Chim Acta 641:32–36

Manhães BMR, Santos-Neto EB, Tovar LR, Guari EB, Flach L. Kasper D, Galvão PMA, Malm, O, Gonçalves RA, Bisi TL, Azevedo AF, Lailson-Brito J (2021) Changes in mercury distribution and its body burden in delphinids affected by a morbillivirus infection: Evidences of methylmercury intoxication in Guiana dolphin. Chemosphere 263:128286

Médieu A, Point D, Receveur A, Gauthier O, Allain V, Pethybridge H, Menkes CHE, Gillikin DP, Revill AT, Somes ChJ, Collin J, Lorrain A (2021) Stable mercury concentrations of tropical tuna in the south western Pacific ocean: An 18-year monitoring study. Chemosphere. 263, 128024.

Melgar MJ, Alonso J, García MA (2009) Mercury in edible mushrooms and underlying soil: Bioconcentration factors and toxicological risk. Sci Total Environ. 407:5328–5334

Nawrocka A, Durkalec M, Szkoda J, Filipek A, Kmiecik M, Żmudzki J, Posyniak A (2020) Total mercury levels in the muscle and liver of livestock and game animals in Poland, 2009–2018. Chemosphere 258:127311

Ostos C, Pérez-Rodríguez F, Arroyo BM, Moreno-Rojas R (2015) Study of mercury content in wild edible mushrooms and its contribution to the Provisional Tolerable Weekly Intake in Spain. J Food Comp Ana. 37:136–142

Peregrino CP, Moreno MV, Miranda SV, Rubio AD, Leal LO (2011) Mercury levels in locally manufactured Mexican skin-lightening creams. Int J Environ Res Public Health 8:2516–2523

Ralston NVC, Raymond LJ (2010) Dietary selenium's protective effects against methylmercury toxicity. Toxicology 278:112–123

Ralston NVC, Raymond LJ (2018) Mercury's neurotoxicity is characterized by its disruption of selenium biochemistry. Biochim Biophys Acta (BBA) – Gen Subj 1862:2405-2416

Rieder SR, Brunner I, Horvat M, Jacobs A, Frey B (2011) Accumulation of mercury and methylmercury by mushrooms and earthworms from forest soils. Environ Pollut 159:2861–2869

Reyes LH, Rahman GMM, Kingston HMS (2009) Robust microwave-assisted extraction protocol for determination of total mercury and methylmercury in fish tissues. Anal Chim Acta 631:121–128

Ruiz-De-Cenzano M, López-Salazar O, Cervera ML, De La Guardia M (2016) Non-chromatographic speciation of mercury in mushrooms. Anal Methods 8:1774–1779

Rutkowska M, Bajger-Nowak G, Kowalewska D, Bzoma S, Kalisińska E, Namieśnik J, Konieczka P (2019) Methylmercury and total mercury content in soft tissues of two bird species wintering in the Baltic Sea near Gdańsk, Poland. Chemosphere 219:140–147

Širić I, Falandysz J (2020) Contamination, bioconcentration and distribution of mercury in *Tricholoma* spp. mushrooms from southern and northern regions of Europe. Chemosphere 251:126614

Stijve T, Roschnik R (1974) Mercury and methyl mercury content of different species of fungi. Trav Chim Aliment Hyg. 65:209–220

Sulimanec Grgec A, Kljaković-Gašpić Z, Orct T, Tičina V, Sekovanić A, Jurasović J, Piasek M (2020) Mercury and selenium in fish from the eastern part of the Adriatic Sea: A risk-benefit assessment in vulnerable population groups. Chemosphere 261:127742

Suvarapu LN, Baek SO (2017) Recent studies on the speciation and determination of mercury in different environmental matrices using various analytical techniques. Int J Anal Chem 2017:3624015

Vieira MA, Grinberg P, Bobeda CRR, Reyes MNM, Campos RC (2009) Non-chromatographic atomic spectrometric methods in speciation analysis : A review. Spectr Acta Part B At Spectrosc 64:459–476

Villar E, Cabrol L, Heimbürger-Boavida L (2020) Widespread microbial mercury methylation genes in the global ocean. Environ Microbiol Rep 12:77–287

Wang F, Xu S, Zhou Y, Wang P, Zhang X (2017) Trace element exposure of whooper swans (*Cygnus cygnus*) wintering in a marine lagoon (Swan Lake), northern China. Mar Pollut Bull 119:60–67

Wiejak A, Wang Y, Zhang J, Falandysz J (2016) Mercury in sclerotia of *Wolfiporia extensa* (Peck) Ginns fungus collected across of the Yunnan land. Spectr Spectr Anal 36:3083- 3086

Xu J, Buck M, Eklöf K, Ahmed OO, Schaefer JK, Bishop KU, Björn E, Bertilsson S, Bravo AG (2019) Mercury methylating microbial communities of boreal forest soils. Sci Rep 9:518.

Yokoyama H (2018) Mercury Pollution in Minamata. Springer briefs in environmental science. Springer, Singapore. https://doi.org/10.1007/978-981-10-7392-2

Zhang D, Yang S, Cheng H, Wang Y, Liu J (2019) Speciation of inorganic and organic species of mercury and arsenic in lotus root using high performance liquid chromatography with inductively coupled plasma mass spectrometric detection in one run. Talanta 199:620–627

Zhang J, Falandysz J, Hanć A, Lorenc W, Wang Y, Barałkiewicz D (2022) Occurrence, distribution, and associations of essential and non-essential elements in the medicinal and edible fungus “Fuling” from southern China. Sci Total Environ 831:155011

Zhu S, Chen B, He M, Huang T, Hu B (2017) Speciation of mercury in water and fish samples by HPLC-ICP-MS after magnetic solid phase extraction. Talanta 171:213–219

Supplementary Material

Practical guide

Applied Microbiology and Biotechnology

Methylmercury and total mercury in mushrooms from different regions of the world

Małgorzata Rutkowska[1*], Jerzy Falandysz[2#], Martyna Saba[3], Piotr Szefer[4], Małgorzata Misztal-Szkudlińska[4], Piotr Konieczka[1]

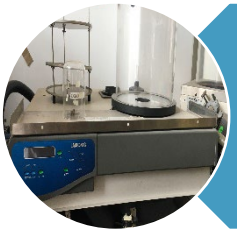
[1]Gdańsk University of Technology, Faculty of Chemistry, Department of Analytical Chemistry, 80-233 Gdańsk, 11/12 G. Narutowicza Street, Poland

[2]Medical University of Lodz, Faculty of Pharmacy, Department of Toxicology, 1 Muszyńskiego Street, 90-151 Łódź, Poland

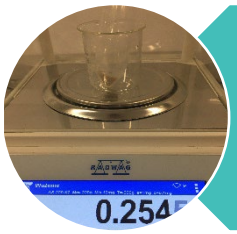
[3]University of Gdańsk, Faculty of Chemistry, Environmental Chemistry & Ecotoxicology, 80-308 Gdańsk, Poland

[4]Medical University of Gdańsk, Faculty of Pharmacy, Department of Food Sciences, Al. Gen. J. Hallera 107, 80-416 Gdańsk, Poland

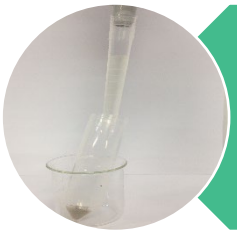
Samples of sporocarps (fruit bodies) and sclerotia of fungi



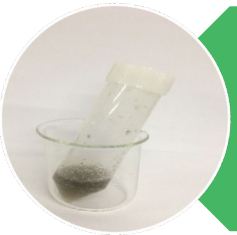
Freeze-drying and homogenization



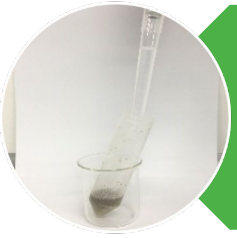
Weighing of approx. 250 mg of the sample



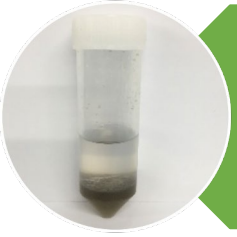
HBr hydrolisys (+4 mL)



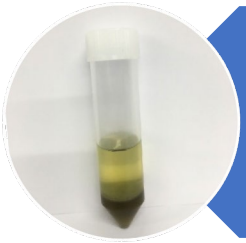
Shaking for 5 minutes



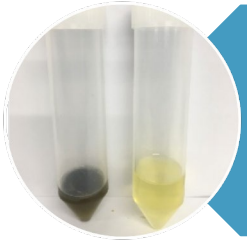
Adding 10 mL of toluene



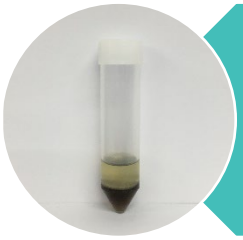
Mixing (20 min/300 rpm)



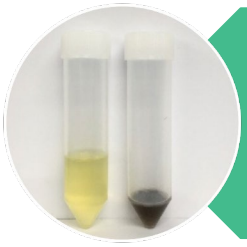
Centrifuging (10 min/4.8 rpm)



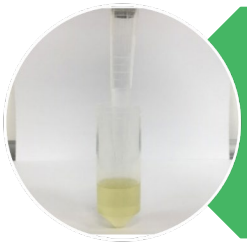
Collecting the supernatant in falcon tubes



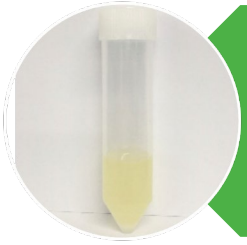
Repeat the whole procedure (+5 mL toluene; 20 min/300 rpm; 10 min/4.8 rpm)



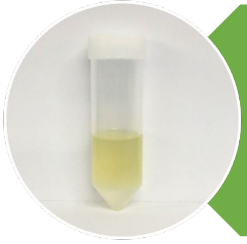
Collecting combined organic extracts



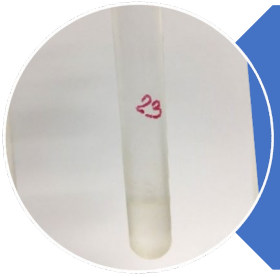
Back extraction with L-cysteine aqueous solution



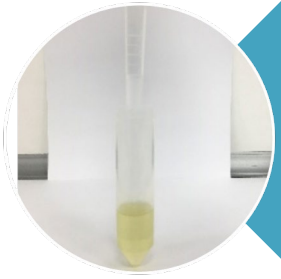
Shaking for 20 minutes/300 rpm



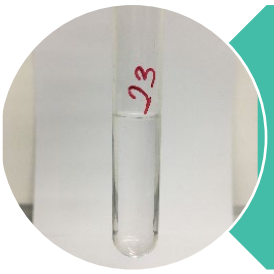
Centrifuging for 5 minutes/4.8 rpm



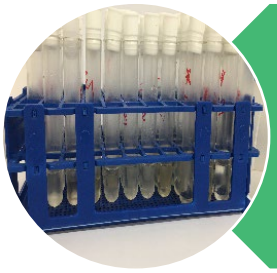
Collecting aliquot of L-cysteine extracts



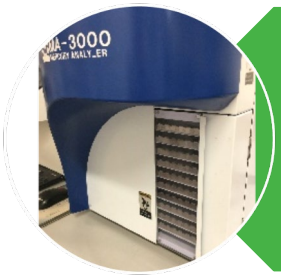
Repeat the whole procedure (+2 mL L-cysteine; 20 min/300 rpm; 5 min/4.8 rpm)



Collecting combined aliquot of L-cysteine extracts



Preparation of a set of samples



MeHg determination

Statistical analysis and uncertainty estimation of the obtained results