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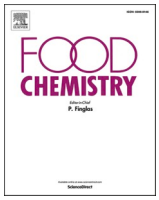

Review

Decoding trace element speciation in mushrooms: Analytical techniques, comprehensive data review, and health implications

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ABSTRACT

This review focuses on trace element speciation in edible mushrooms, providing information on analytical methods, available literature data, and health risk assessment. All steps of analytical procedures were presented, including extraction, separation and quantification. It compiles fragmented literature data on trace element speciation, focusing on arsenic, chromium, selenium, mercury, and antimony. Key findings include non-bioaccumulative chromium, the prevalence of Sb(V), mercury accumulation in contaminated sites, diverse arsenic and selenium speciation. Safe intake limits by agencies like USEPA indicate low risk for Cr(VI) and Sb but significant hazards from mercury and methylmercury, especially in contaminated areas: about 10 % of samples exceed safe limits for inorganic arsenic, and selenium enrichment often surpasses safety thresholds. The review underscores the need for standardized methods, speciation analyses of all toxicologically relevant species, and research on cooking impacts to improve health risk evaluations: establishing safe conditions for mushroom consumption remains a far-fetched goal.

1. Introduction

Mushrooms have played a fundamental role in the gastronomy of many cultures, being considered valuable foods due to their attractive sensory and culinary characteristics (Azizur Rahman et al., 2022; Kolniak-Ostek et al., 2022). Actually, mushrooms became a food commodity, with an estimated worldwide production of 44 million tons in 2021 (Food and Agriculture Organization of the United Nations, 2023) and a value of $42 billion (More, 2024). Recently, the number of studies related to their nutritional profile has increased, showing their high content in vitamins, proteins, fibers, essential amino acids and carbohydrates (Berthélémy, 2008; Ouzouni et al., 2009). In addition, mushrooms have been shown to contain several biologically and physiologically active compounds that are responsible for their antioxidant, anti-tumor, anti-inflammatory or anti-diabetic effects (Jayakumar et al., 2011; Kalač, 2009; Lakhnarayan Kumar Bhagarathi et al., 2023). They are considered functional foods due to their beneficial effects on human health accordingly, as their introduction into the diet favors the regulation of various biological processes (Mleczek et al., 2021; Sarikurkcu et al., 2015). The latter benefits, along with the population interest in adopting healthier eating habits has boosted mushroom consumption worldwide, with mushroom production increasing by 50 % in ten years (2012−2022). Mushroom consumption increased also in several European countries such as Spain, Denmark and Sweden which used to have a large mycophobic gastronomy (Ostos et al., 2015; Peintner et al., 2013).

However, mushroom consumption may also present health risks. Excluding the inadvertent ingestion of toxic wild species, the foremost concern pertains to the bioaccumulative capability exhibited by the fruiting bodies of fungi. The latter is beneficial when essential minerals are involved but may pose a risk when toxic elements are accumulated from the substrate (Braeuer et al., 2022; Chen et al., 2023; de Oliveira et al., 2023; Demirbas, 2001; Ostos et al., 2015; Sarvan et al., 2021). Research focused on the assessment of element accumulation because organic contaminants in the fruiting bodies are seldom detected and their contents do not pose significant health risk for consumption: non-

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polar species may hardly accumulate in the mostly polar constituents of fungi tissues (Gałgowska & Pietrzak-Fiećko, 2021; Kamal et al., 2009). Striking examples of element bioaccumulation include a 1000-fold enrichment of arsenic (As) by *Sacrosphaera coronaria* (7 $mg{\cdot}Kg^{-1}$ in soil and 8900 $mg{\cdot}Kg^{-1}$ in the fruiting body (Braeuer et al., 2020)), and up to 15-fold for mercury (*B. edulis* and *A. rubescens*) (Senila et al., 2024). Bioaccumulation is influenced by a range of extrinsic factors, including soil type, pH, moisture, organic matter content, atmospheric pollution, climatic conditions, anthropogenic activities, and geological processes. Additionally, intrinsic factors such as the specific element studied, mushroom species, morphology, size, tissue type, developmental stage, and age of the mycelium also play a significant role, further contributing to the complexity of understanding bioaccumulation (Campos et al., 2009; Đurđić et al., 2021; Kalač, 2010; Kavčič et al., 2019; Kokkoris et al., 2019).

Elemental contents exceeding the safe limits for food consumption have already been reported in the literature. Examples include edible wild species such as *Boletus edulis, Morchella angusticeps* and *Tricholoma matsutake* exceeding As, lead (Pb) and cadmium (Cd) safe limits in the Yunnan Province in China (B. Liu et al., 2015), an average chromium (Cr) content (0.74 $mg{\cdot}Kg^{-1}$) in 12 species of mushrooms from markets in China higher than the Chinese limit for total Cr in vegetables (0.5 $mg{\cdot}Kg^{-1}$), in addition to several samples of *Boletus* spp. exceeding the World Health Organization (WHO) limits for food by up to five times (Falandysz et al., 2019). Accordingly, possible risks associated with mushroom consumption were evidenced in a recent review (Dowlati et al., 2021).

Nevertheless, the total content of elements is not a good indicator for risk assessment since diverse elemental species exhibit varying toxicological traits ("element species" are defined as element forms differing in oxidation states, isotopic composition, covalently bound or complexed organic species; "speciation analysis" is the analytical activities of identifying and/or measuring the quantities of one or more individual chemical species in a sample; "speciation of an element" is its distribution among defined chemical species in a system, (Nordberg et al., 2004)). Several toxicologically relevant species have been pinpointed and their risk for human health assessed (Apostoli et al., 2006; Hughes et al., 1995): archetypal examples include the redox speciation of chromium, with Cr(VI) being a carcinogenic species (WHO, 1988) and Cr(III) an essential element (Wise et al., 2022), and the organic speciation of mercury (Hg), mainly methylmercury (MeHg), presenting bioaccumulation and biomagnification risks (Costet et al., 2024; Lozano et al., 2021; Stern & Smith, 2003). Arsenic also shows a rich speciation, both in terms of oxidation state (inorganic As (III) and As(V) are considered toxic (Feldmann & Krupp, 2011; Taylor et al., 2017), and organic species, the most abundant being usually arsenobetaine (AB), which is considered of no toxicological relevance (Nearing et al., 2014; Straif et al., 2009). Similar to arsenic, selenium (Se), which is an essential element, may also be found in different oxidation states (IV and VI) and organic forms (e.g. selenomethionine, methylselenocysteine, selenocysteine and selenoethionine), each species, or species group, showing different chemical properties (Dong et al., 2021; Maseko et al., 2013; Milovanovic et al., 2019; Niedzielski et al., 2015; Prange et al., 2019; Shi et al., 2023). The unique chemical characteristics exhibited by elemental species not only govern their toxicity but also typically influence their interactions within the biosphere: the availability for uptake by mushrooms can be species specific, thus further complicating the picture (see e.g. the different bioavailability of Cr (III) and Cr (VI) (Figueiredo et al., 2007)).

At present, our knowledge and comprehension of the potential risks associated with exposure to chemical species through the consumption of mushrooms remains notably limited. Data related to chemical species in mushroom tissues are themselves limited and have not undergone thorough evaluation, except for one review specifically focused on arsenic speciation (Zou et al., 2019). This paucity of observations appears to stem from challenges in delineating and implementing analytical protocols tailored to the intricate tissue matrix of mushrooms. The extraction, separation, and detection of the numerous chemical species present a formidable challenge to current analytical techniques: the development of standardized, highly efficient, automated, reliable, and high-throughput methods remains undefined.

Therefore, the aim of the present review paper is to report on available information about trace element speciation and speciation analysis in edible mushroom tissues and to provide a first species specific risk assessment for the ingestion of mushrooms. In details, the paper will present the following topics: a) summary of the most common analytical protocols employed for the speciation analysis of trace elements in mushrooms; b) compilation of the available information on the speciation of toxic (As, Cr, Hg and antimony (Sb)) and essential (Se) elements in mushroom edible species; c) tentative species-specific risk assessment associated to mushroom ingestion; d) information gaps and possible paths towards knowledge and understanding advancement.

2. Analytical methods

2.1. Mushrooms sampling, drying and grinding

In general, sample collection and preparation before analysis follow a common procedure in all the collected papers. Wild mushrooms were collected with ceramic or plastic knives, cleaned from debris, kept in polymeric bags, oven-dried or lyophilized and finally pulverized to obtain a homogeneous powder that can be easily digested (Falandysz et al., 2022). The use of non-metal tools minimized the risk of contamination. If the researchers were interested in the potential difference between caps and stipes of the mushrooms, the latter were carefully separated after collection. Samples of commercial mushrooms were collected dry or fresh in local markets, supermarkets or shops and treated as mentioned above (see e.g. Chen & Liu, 2023; Kolniak-Ostek et al., 2022 for the common sampling and sample treatment procedure). The effect of cooking in the presence of vegetable or animal fat was also assessed in two cases only (Falandysz et al., 2022; Y. Liu et al., 2023).

2.2. Extraction and analysis

2.2.1. As

Arsenic speciation was treated extensively in the critical review by Braeuer and Goessler (Braeuer & Goessler, 2019). It provides information on the arsenic species commonly found in mushrooms and details the analytical techniques used for their selective determination: more recent research works (dated 5 years ago) not evaluated by this review will be covered here. Fractionation and bioaccessibility studies will be presented first.

An anion-exchange chromatography-inductively coupled plasma-mass spectrometry (IC-ICP-MS) method was tested for the selective determination of AB, As(III), As (V), dimethylarsinate (DMA) and methylarsonate (MA) (Komorowicz et al., 2019). In this study UAE (Ultrasound Assisted Extraction), MAE (Microwave Assisted Extraction) and EAE (Enzymatic assisted extraction) were performed and thoroughly compared. For the first two procedures, mushroom powders were extracted in ultrapure water while the enzymatic procedure was carried out with the Unified BARGE (Bioaccessibility Research Group of Europe) method consisting of an extraction with simulated digestive fluids such as saliva, gastric juice, gastrointestinal juice, and bile. The fluids were tested both singularly and sequentially, adding the simulated biological fluid to the mushroom's powder under a thermosttated stirring. The samples were then centrifuged, filtered, and analyzed as in the reference cited above (Komorowicz et al., 2019). The arsenic species composition in the interesting *Cordyceps sinensis*, a parasitic mushroom widely used in the traditional Chinese medicine to treat lungs and kidney diseases, was covered in detail (Li, Liu, et al., 2019). In this case, the powdered mushroom was extracted at 37 °C for 12 h with simulated gastric juice: pepsin and nitric acid in different proportions. The

extracted solutions were analyzed by anion-exchange chromatography coupled with ICP-MS (AE-HPLC-ICP-MS) and AB, As (III), DMA, arsenocholine (AC), MA, and As(V) were selectively determined. To investigate the different distribution of arsenic species, different proteins (water-soluble protein, salt-soluble protein, alkali-soluble protein, and alcohol-soluble proteins), crude water polysaccharide and lipid were first separated and then the arsenic species composition determined. The procedure for the protein extraction starts with ultrasound assisted treatment after 12 h at room temperature in a solution of NaCl (5 %), NaOH (0.08 mol L^{-1}) and EtOH (70 %) respectively. The samples are then centrifuged, and the supernatant is collected and mixed with acetone. The proteins are then left to precipitate and centrifuged a second time before eliminating the acetone with nitrogen. The pure proteins obtained were dissolved in a buffer solution (pH 7.5), filtered and analyzed. Water-soluble polysaccharides were extracted by utilizing a water extraction method followed by ethanol precipitation. Subsequently, lipids were extracted from the sample powder through three microwave extractions at room temperature in n-hexane, with careful separation of the supernatant obtained post-centrifugation.

Speciation studies were recently performed using a simple extraction in water. The analysis of the bracket fungus *Fomitopsis betulina* from contaminated (former mine in UK) and pristine site (woodland in Quebec) allows us to evaluate the effect of contamination over the arsenic species composition (Button et al., 2020). Mushroom powder was extracted with ultrapure water and shaken for 3 h at 60 rpm. The solutions obtained were centrifuged and the supernatant was collected, filtered, and analyzed using HPLC-ICP-MS. XANES (X-ray absorption near-edge structure) spectroscopy measurements were also carried out. A highly extensive work has been carried out by (Zou, Zhou, Li, Yang, Wen, Song, et al., 2020) on 266 samples of Chinese mushrooms collected between 2017 and 2018, comprising nine different species. An aliquot of powdered sample was extracted in water in an ultrasound bath at 60° for 35 min and centrifuged for 15 min. The supernatant collected was filtered and then analyzed using HPLC-ICP-MS with an ion exchange column. The optimization of the extraction method and extractants followed this procedure. *Tricholoma matsutake* samples were accurately weighed and extracted with ultrapure water, methanol-water (1:1, *v*/v), 1 % (*v*/v) HNO_3, and 1 % (v/v) H_3PO_4, respectively. Ultrasound assisted extraction was performed at 60 °C for 30 min, water bath extraction at 90 °C for 2.5 h and shaking extraction at room temperature for 2.5 h. A similar extraction procedure was used in (Braeuer, Borovička, et al., 2021; Braeuer, Walenta, et al., 2021), in which freeze-dried mushroom powder was extracted in water using an ultrasound bath for 20 min at room temperature. In this case hydrogen peroxide was added to an aliquot of each solution to convert all the labile arsenic species to their pentavalent oxygenated equivalent. The analysis was carried out as usual with HPLC- ICP-MS, exploiting both a cation- and anion-exchange column (with pyridine and an ammonium phosphate as mobile phase, respectively) to successfully determine As(V), MA, AC, tetramethylarsoniun ion (TETRA), trimethylarsoniopropionate (TMAP), DMA, AB, dimethylarsinoyl-acetate (DMAA) and homoarsenocholine and trimethylarsine oxide (TMAO). In the same year, a total diet study was conducted in Japan in order to gain a better understanding of the arsenic intake of the population (Suzuki et al., 2022). The total diet study samples were extracted with 0.3 M nitric acid repeatedly, centrifuged and the supernatant collected was mixed with a 5 % ammonia solution (to reach a pH of 2.7) and analyzed post filtering with HPLC- ICP-MS. The most recent studies all use a simple ultrasound assisted extraction in ultrapure water with slight changes in the extraction time (Chen et al., 2023; Chen & Liu, 2023; Y. Liu et al., 2023). However, another group of authors (Li, Wang, et al., 2019) has carried out an ultrasound assisted extraction employing a methanol/water (1:1 v/v) solution. The solution was centrifuged, the supernatant decanted and the extraction was repeated twice. The selective determination of both organic and inorganic arsenic was carried out with HPLC-ICP-MS equipped with an ion-exchange column using a solution of $(NH_4)H_2PO_4$ and $(NH_4)_2CO_3$ as the mobile phase. In general, water is largely employed as a solvent in arsenic extractions, while HPLC coupled with ICP-MS seems to be the ideal combination for the analysis.

As a final remark, special care should be taken when analyzing inorganic arsenic species As(III) and As(V), as they may easily convert into each other if reducing or oxidizing chemicals are added during the extraction or analytical phase. Quality control procedures are reported in the cited papers to tackle this issue: some of the authors studied the species interconversion before the analysis and did not detect any, whereas others used a species specific spike and recovery strategy to investigate possible interconversion obtaining satisfactory recoveries (see e.g. Li, Liu, et al., 2019; Li, Wang, et al., 2019).

2.2.2. Cr

The studies addressing the redox speciation of chromium in the different morphological parts of the mushroom are indeed limited. The interesting work of (Figueiredo et al., 2007) employed a two-step, alkaline procedure to selectively extract Cr(VI): the quantification of Cr (VI) is performed by electrothermal atomization atomic absorption spectrometry. The validation of the method was carried out with both a lichen and soil certified reference material. This procedure of extraction and analysis was also used to selectively determine Cr(VI) in mushrooms collected in unpolluted sites in the Czech Republic (Šíma et al., 2019).

2.2.3. Se

A great variety of selenium species were discovered in biota, differing in oxidation state, Se (VI) and (IV), or covalently bound species (like the amino acids selenomethionine or selenocysteine among others). Accordingly, there is no generalized universal method for the extraction and quantification of all of these species simultaneously. Organic species were usually extracted employing enzymatic methods: different enzymes (pepsin, trypsin, proteinase K or pronase) were used, either individually or sequentially, to obtain the different organic species (Dong et al., 2021; Gergely et al., 2006; Huerta et al., 2006; Maseko et al., 2013; Milovanovic et al., 2019; Shi et al., 2023; Stefánka et al., 2001; Wilburn et al., 2004). Generally, these enzymatic methods use temperatures between 37 °C and 50 °C, under agitation and in some cases incubating the samples in the dark for 3 to 24 h (Maseko et al., 2013; Milovanovic et al., 2019; Stefánka et al., 2001). However, some authors (Huerta et al., 2005; Tadayon & Mehrandoost, 2014) performed different extractions using water, a mixture of organic compounds such as DAN (2,3-diaminonaphthalene) (0.003 M) or Trixton X-114 (2 %) or cloud point extraction (CPE) (Tadayon & Mehrandoost, 2014), the latter employing a temperature range between RT and 85 °C for 30–40 min, in some cases using an ultrasonic bath.

In the case of the inorganic selenium (Se (IV) and Se (VI)) the same methods used for the extraction of organic compounds can be used, although other authors also use phosphoric acid as an extractant (Niedzielski et al., 2015), with both methods giving similar results.

The diverse chemical forms of selenium also present challenges in the determination step, making the selection of a single analytical method unfeasible. HPLC-ICP-MS was mostly employed, but different techniques, namely size-exclusion, anion-exchange or reversed-phase, were applied to achieve an efficient separation of the different species (Dong et al., 2021; Gergely et al., 2006; Huerta et al., 2005, 2006; Milovanovic et al., 2019; Wilburn et al., 2004). However, other techniques were also employed for Se speciation, such as LC-UV-HG-AFS (Liquid Chromatography coupled with Hydride Generation-Atomic Fluorescence Spectrometry) (Shi et al., 2023), XANES (X-ray absorption near-edge structure) spectroscopy (Prange et al., 2019), HPLC-HG-AAS (High-Performance Liquid Chromatography coupled with Hydride Generation-Atomic Absorption Spectrometry) (Niedzielski et al., 2015) or using UV–Vis, after cloud point extraction (Tadayon & Mehrandoost, 2014). Interestingly, HPLC coupled to mass spectrometry detection seems not to have been applied yet in mushrooms, although it may be expected to provide useful elemental (oxidation state after chromatographic

separation) and molecular information.

2.2.4. Hg

Mercury and its organic species are notable for their toxicity. There is a wide variety of organic mercury species, including methylmercury, ethylmercury and phenylmercury, but research has mainly focused on inorganic mercury and methylmercury so far, due to their widespread presence in the environment. Other mercury species are extremely rare: ethylmercury and phenylmercury were detected in mushrooms in two samples only in a recent study (Zou, Zhou, Li, Yang, Wen, Li, et al., 2020).

The first studies focused on the quantification of mercury and methylmercury in mushrooms grown in both contaminated and not-contaminated soils using neutron activation (Stegnar et al., 1973) or reduction-volatilization (Minagawa et al., 1980) for total mercury; gas chromatography was employed for methylmercury determination. Another study was conducted over a mining polluted area (Bargagli & Baldi, 1984). MeHg was extracted by a benzene-cysteine solution, then treated with HNO_3 to digest the organic phase and quantified using cold vapor atomic absorption spectrophotometry. A different organic solvent, namely a methanolic, 25 % (*w/v*) KOH solution was subsequently used by other authors (Fischer et al., 1995) followed by the same analytical technique to quantify methylmercury.

However, more recently, water based extractants were mostly employed. They usually contain halides (chloride or bromide) and sulfur-based ligands (e.g. cysteine) that complex inorganic and organic mercury facilitating their extraction from mushroom. In addition, authors have focused on developing more sensitive methodologies that are capable of extracting and quantifying methylmercury in biological samples at the ultratrace level, such as in mushrooms. Some of the most recent studies performed a sample digestion based on sequential oxidation with HCl assisted by manual agitation, using alternative techniques other than the more common chromatographic procedures, such as cold vapor atomic absorption spectrophotometry (Ruiz-de-Cenzano et al., 2016), obtaining a recovery rate of 98.5 %. The extracted solution was treated with a mixture of $KBr/KBrO_3$ and hydroxylamine hydrochloride to selectively determine inorganic Hg (i-Hg); organic mercury (o-Hg) was estimated by difference after determination of the total Hg content.

Furthermore, regarding extraction, the authors have focused on studying its efficiency using different reagents (HCl, toluene, L-cysteine, HBr or water), as well as different techniques, such as ultrasound-assisted, microwave-assisted or conventional extraction (Chen et al., 2023; Falandysz et al., 2022; Pilz et al., 2011; Rutkowska et al., 2022). Specifically, Pilz et al. concluded, after performing several extractions under different conditions and with different reagents, that the best recovery percentages (higher than 92 %) were obtained by extracting with L-cysteine (1 % (m/v)) and using ultrasound, being the same methodology employed by Chen et al. Regarding separation, a reversed-phase, C18 column was employed, whereas quantification was performed by CVG-ICP-MS (Chemical Vapor Generation and Inductively Coupled Plasma Mass Spectrometry) or simply by ICP-MS.

An interesting work of comparison between species distribution in both cooked and raw mushrooms was published in 2022 (Falandysz et al., 2022). In this work, the digestion procedure was carried out following the method by (Maggi et al., 2009) in which an acid treatment, employing HBr, is followed by one extraction and one back-extraction using toluene and L-cysteine. Finally, quantification by atomic absorption spectroscopy after thermal decomposition treatment was performed.

An optimized extraction procedure is presented in the most recent work regarding mercury speciation in mushrooms (Rutkowska et al., 2022). The procedure involves a hydrolysis step with 4 mL of 48 % HBr with a vertical shaker, followed by a double extraction with toluene. A back extraction employing L-cysteine (1 % *v*/v) is finally performed. The extraction steps were optimized and good recoveries (87 % - 99.4 %) were achieved. The determination of both total and methylmercury was performed by oxygen combustion-gold amalgamation using a Direct Mercury Analyser (DMA-80).

Generally, several extraction and quantification strategies have been adopted (Bergin et al., 2021; Rieder et al., 2011; Sarvan et al., 2021). Sarvan et al., added 10 mL of 6 M HCl to 1 g of sample and carried out a nitrogen-assisted distillation for 70 min. Then, MeHg was extracted as MeHgCl and collected in HCl 6 M, filled up to 15 mL with distilled water and BrCl (0.2 M) was added to convert MeHg into Hg^{2+}; the solution was kept in the dark for 1 h and mercury was measured by ICP-MS.

On the other hand, Rieder et al., employed a mixture of sulfuric acid (5 %), KBr (18 %) and 1 mL of 1 M $CuSO_4$. After suspending the sample in this solution, methylmercury was extracted in CH_2Cl_2, MeHg was ethylated with 1 % $NaBEt_4$, and preconcentrated by purge and trap. Methyl-Hg was then thermally desorbed, separated on a GC column and, after converting it to Hg^0, measured using a cold vapor atomic fluorescence detector.

Finally, Bergin et al., carried out the extraction of MeHg using 5 mL of 4 M HNO_3, acetate buffer and $NaBEt_4$, and the quantification was done employing an automated methyl mercury analysis system (Tekran 2700).

2.2.5. Sb

Antimony redox speciation was determined by (Sousa Ferreira et al., 2009) after extracting 1 g of sample in 10 mL of H_2SO_4 0.5 M, as the latter solution showed the best recovery during the optimization phase (99 %). Then, extracts were centrifuged, and the solids washed with 10 mL of 0.1 % (*w/v*) EDTA, centrifuged and the supernatant mixed with the first extract. After acidification to 3.5 M HCl, one aliquot was analyzed with HG-AFS, to obtain information about Sb(III), as it was observed that Sb(V) reacts with $NaBH_4$ with a slower reaction kinetic than Sb(III). So, its signal depends on the hydride generation conditions. Thus, the concentration of Sb(V) is determined by difference from total antimony as determined on a second extracted sample aliquot, where Sb (III) is reduced to total Sb using a 1 % KI and 0.2 % ascorbic acid mixture.

On the other hand, (Koch et al., 2000) carried out the speciation of Sb performing the extraction of 0.5 g of dried samples using 10–15 mL of $MeOH/H_2O$ (1:1) sonicating for 20 min. After that, the liquid was decanted and centrifugated 5 times and the extracts were dried using a rotavapor until a volume of 1–2 mL and they were diluted using deionized water. The analysis of the samples was carried out by hydride generation-gas chromatography-atomic absorption spectrometry (HG-GC-AAS) where samples react with $NaBH_4$ (2 % *w*/w) and the gases generated were separated and detected by AAS.

2.3. Speciation of other elements

The determination of isotopic ratios provides useful information regarding natural and man-induced processes and, although usually not included in “classical” speciation studies, it falls in the IUPAC definition of speciation (Nordberg et al., 2004) and will therefore be covered in this review as well.

In the intricate field of trace element speciation in mushrooms, cadmium (Cd) (Borovička et al., 2023), lead (Pb) (Borovička et al., 2014; Đurđić et al., 2021; Komárek et al., 2007), magnesium (Mg) (Andronikov et al., 2022; Fahad et al., 2016), copper (Cu) (Andronikov et al., 2022), vanadium (V) (Malinovsky & Kashulin, 2016), and zinc (Zn) (Andronikov et al., 2022) have also been scrutinized to understand heavy metal accumulation mechanisms.

Notably, *Thelephora pencillata* from the Czech Republic has been singled out as a significant Cd hyperaccumulator (Borovička et al., 2023), surpassing accumulation rates in species like *Amanita muscaria*, *Boletus edulis*, and *Russula cyanoxantha* by over 300 times. This study utilized thermal ionization mass spectrometry (TIMS) to assess Cd isotopic composition in mushroom fruiting bodies, revealing variable $\delta^{114/}$

^{110}Cd values within the Karlina Pila mushroom community. These findings suggest species-specific Cd isotopic fractionation at the soil/fruiting body interface. Furthermore, Cd hyperaccumulation in *T. penicillata* aligned perfectly with mycoavailable soil Cd fractions, unlike other species such as *Neoboletus erythropus*.

While only one study focused on Cd isotopes, multiple works in Eastern Europe explored lead isotopes (Borovička et al., 2014; Đurđić et al., 2021; Komárek et al., 2007) with lead accumulation up to hundreds of mg/kg in polluted areas: polysaccharides and pigments in mushroom mycelium plays a key role in this process. Studies on *Macrolepiota procera* in unpolluted areas in Serbia, disclosed mushrooms' predisposition to exchangeable/acid-extractable fractions of topsoil, influenced mainly by traffic emission pollution (Đurđić et al., 2021). Similar studies on saprotrophic mushroom species in the Czech Republic suggested Pb transport from depths of approximately 13–17 cm or deeper (Borovička et al., 2014). Evidence from Komárek et al. (2007) highlighted age-dependent Pb uptake, indicating mixing from exchangeable and reducible soil fractions. Despite three works only reported on the topic, it is reasonable that the Pb-isotopic composition in mushrooms is determined by the isotopic pattern of the bioavailable Pb fraction in soils, challenging mushrooms' suitability as pollution bioindicators.

Studies on other elements, like Mg, Cu, and Zn in *Xerocomus subtomentosus* (Andronikov et al., 2022), revealed insights into their biogeochemical cycling. Additionally, Mg speciation studies (Fahad et al., 2016) indicated ectomycorrhizal fungi's efficiency in mobilizing, transporting, and storing Mg. Vanadium isotopic studies (Malinovsky & Kashulin, 2016) in *Amanita muscaria* showed promise in discerning mushrooms' geographical origins. The main vanadium species was identified in this mushroom as amavadin complex (Braeuer, Borovička, et al., 2021; Braeuer, Walenta, et al., 2021).

Finally, size-exclusion chromatography has aided in understanding molecular weight distribution patterns across mushroom species (Wuilloud et al., 2004). Variation in element fractionation patterns (silver, arsenic, cadmium, mercury, lead, and tin) depending on extraction mediums and mushroom species emphasizes the importance of extraction steps in elemental speciation analyses. Studies have also revealed Cd species predominantly residing in cellular compartments exceeding 1 kDa in *T. pencillata*, suggesting effective mechanisms to mitigate Cd intracellular toxicity.

In summary, the intricate interplay among isotopes, complexes, and molecular weight distributions, as investigated by diverse researchers, contributes to a more profound comprehension of fungal bioaccumulation. However, the current scarcity in the number of these studies impedes the development of a comprehensive interpretation, highlighting the need for more extensive research to facilitate the formulation of robust models in this complex domain. Exploring in vitro studies with cultivable mushroom species could further elucidate isotope fractionation mechanisms at the soil/mycelia interface and potential species-specific variations.

3. Data presentation and discussion

3.1. Survey of literature data

This section collects all the available data on trace elements speciation in edible mushroom samples: a synopsis is reported in Table 1, whereas the entire set of data is reported as Table S1 in the Additional Info (data for Cr, Sb, Hg, As, Se are reported in different worksheets in Table S1). Only speciation data regarding edible mushrooms were collected as the final aim of this review is the health risk assessment of mushroom consumption: moreover, edible species are by far the most studied compared to non-edible ones. Generally, data are sparse with the typical approach involving the speciation analysis of only one element in different fungal anatomical parts. A limited number of papers investigated the effect of possible controlling factors, including differences in mushroom tissues (stalk and cap), cultivation or collection in the wilderness, cooking, and soil contamination. Investigations were accordingly not systematic, as may be easily expected because of the high dimensionality of the experimental space, involving approx 1000 edible mushroom species (Boa, 2004), with geographical range extents covering up to several thousand kilometers ((Bazzicalupo et al., 2019), see also the Final remarks section for further discussion). Arsenic and mercury speciation were mainly studied, with reports on 110 and 93 mushroom species, respectively (20 species only are reported in the present review with regards to As speciation: see Braeuer & Goessler, 2019 for pre-2019 data). Ten to twenty mushroom species were investigated for Cr and Se, and five only for antimony. In addition to the limited extension of the dataset, the employment of various analytical techniques, particularly diverse extraction solutions, adds complexity to the situation, thereby constraining the comparability of data across different authors' reports. (see Section 2.2; see below the Final remarks section for comments). Establishing any overarching trends is therefore challenging in the following discussion, therefore, we highlighted the most interesting findings, emphasized trends, offered hypotheses for data interpretation, and extracted knowledge whenever possible. Data are presented according to the investigated element.

Table 1
List of elements and chemical species analyzed, minimum and maximum concentration of each of them, expressed in mg element/kg dry weight and number of mushroom species studied.

Element	Chemical species	Minimum content (mg/kg dw)	Maximum content (mg/kg dw)	N° mushroom species
Cr	**Total Cr**	0.08	1.49	**19**
	Cr (VI)	<LOD (0.003)	0.58	
Sb	**Total Sb**	0.0194	120	**9**
	Sb (III)	0.0046	0.0114	
	Me_3Sb	<0.005	<0.01	
	Sb (V)	0.0147	5	
Hg	**Total Hg**	0.002	35.2	**82**
	MeHg	0.0001	0.63	
	Total Hg contaminated	0.12	144	**35**
	MeHg contaminated	0.01	7.9	
As	**Total As**	0.07	81.52	**22**
	Organic As	0.0424	81.5	
	As (III)	0.0134	2.76	
	As (V)	0.014	3.83	
	AB	0.00194	29.54	
Se	**Total Se**	0.5	770.7	**13**
	Se (IV)	0.01	156.3	
	Se (VI)	0.1	69.9	
	Selenomethionine	0.02	33.9	
	Selenoethionine	24.7	68	
	Selenocysteine	1.5	116.5	
	Methylselenocysteine	0.031	36.36	

3.1.1. As

Arsenic exhibits a diverse speciation, encompassing redox states (As (III) and As(V)), as well as inorganic and organic species, with organic arsenic usually considered of less toxicological relevance with respect to inorganic As (Braeuer & Goessler, 2019; Chen et al., 2023; Chen & Liu, 2023; Y. Liu et al., 2023). Inorganic arsenic in mushrooms include As (III) and As (V) oxoacids like H_3AsO_3 or H_3AsO_4 (and derived anions), and it accounts for 0.04 %–69 % of total As in mushroom tissues (iAs contents between 0.014 and 3.6 mg/Kg). As(V) is the most representative inorganic redox species (0.04 %–51.0 % of total arsenic), whereas As(III) was found in the content range 0.013–0.96 mg/Kg, from 0.1 % to 30 % of total arsenic.

Organic arsenic is on average prevailing with respect to inorganic As, representing a percentage between 8.8 % and 100 % of total arsenic

(median value 79.2 %). Methylarsonate (MA), dimethylarsinate (DMA), arsenobetaine (AB) and arsenocholine (AC), were determined in several studies, although rarely all of them were targeted. Available data show that arsenobetaine is the prevailing compound among organic species, accounting for 20.8 % (median value) of the total As content. The latter contrasts with AB constituting up to 90 % of total arsenic in e.g. seafood, which is the most important dietary contribution for arsenic to humans. Nevertheless, mushrooms are the only terrestrial organisms containing AB. (Luvonga et al., 2020).

It is interesting to note that the increase in total arsenic is not paralleled by an increase in inorganic arsenic species, see Fig. 3. Total arsenic content is accordingly not a good predictor for inorganic arsenic as the content of iAs does not show a clear trend with total As (As(III) vs total As: r − 0.0097, $n = 50$, $p = 0.47$; As(V) vs total As: $r = 0.00257$, $p = 0.49$, $n = 56$; both inorganic species vs total As: $r = -0.0058$, $p = 0.48$, $n = 106$). It is unclear at present whether this trend is due to iAs biotransformation by fungi, e.g. by methylation (Zhang et al., 2020), or to some kind of species selectivity in the uptake process as both processes, namely biotransformation and passive uptake (no biotransformation), are reported in the literature, which may be also dependent on mushroom species (Braeuer & Goessler, 2019). See also below for implications in risk assessment.

3.1.2. Cr

Chromium redox speciation was only reported for two case studies. The primary reason for the lack of studies may be the low content of this metal, and the even lower content of the Cr(VI) species, which limits the applicability of suitable analytical method. Thus, Figueiredo et al. analyzed a total of 15 species of mushroom collected along Northwest Portugal, determining total Cr and Cr(VI) in cap and stalk separately (Figueiredo et al., 2007). No statistically significant differences were found in total chromium contents (caps: 0.103 ± 0.045 mg/Kg (dry weight, dw), stalks: 0.143 ± 0.061 mg/Kg (dw)), and in Cr(VI) percentage (caps: 9.0 %; stalks: 12.9 %). The same authors also determined total Cr and Cr(VI) in the soils from which fruiting bodies were collected and determined the bioconcentration factors. The latter were below 1 for both total Cr and Cr(VI), pointing to a non bioaccumulative character for these mushroom species.

Sima et al. studied the content of total Cr and Cr(VI) in four different species of mushrooms (*C. rhacodes, S. grevillei, I. badia* and *X. chrysenteron*), collected in the Czech Republic, but the content of Cr (VI) resulted below the detection limit of their method (0.003 mg/Kg (dw); total chromium in the 0.08–0.17 mg/Kg (dw)) (Šíma et al., 2019). Here again, the bioconcentration factors for Cr transfer from soil to the fruiting body was calculated and points to no accumulation of Cr for these mushroom species (bioconcentration factors in the 0.006–0.013 range).

3.1.3. Se

Selenium is the only essential element whose speciation analysis is reported in the literature in some details. Similarly to arsenic, selenium also shows a rich speciation, including inorganic (selenite, selenate) and organic (selenomethionine, selenocysteine, methylselenocysteine, selenoethionine) species, with selenomethionine, selenite and selenate being the most common ones (see Table 1). This element is often provided in food supplements as it is deficitary in the diets of several populations worldwide (Kieliszek & Serrano Sandoval, 2023). The research interest accordingly focused on the understanding of selenium transfer from artificially enriched soils to the fruiting body and how Se speciation may shape or be affected during the uptake process (see Table 2 for Se species contents in enriched and natural conditions: please note that different experimental conditions, like employed amending species and concentration, soil structure and chemistry, and growing conditions, may all affect bioaccumulation: data are summarized in Table 2 to show the general effect of amendment only). Notwithstanding the wide range of experimental conditions, the content of selenium species in mushrooms is higher in amended soils than in non-enriched ones in almost all the investigated samples. For example, Niedzielski et al. determined total selenium, Se (IV) and Se (VI) in three species enriched with different concentrations of Se (VI) and Se (IV) salts (Niedzielski et al., 2015). The results obtained reveal that species such as *P. ostreatus* and *P. eryngii* convert this selenium supply into Se (VI) rather than Se (IV), achieving up to 7 times more than the former. It is unclear whether Se(VI) is preferentially uptaken or Se(IV) is biotransformed into Se(VI) after uptake. In addition, Dong et al. determined organic Se in *F. velutipes* mushrooms enriched with selenite salt ranged from 0 mg/Kg to 50 mg/Kg (Dong et al., 2021). It can be observed that organic selenium content increased due to the selenium supply, achieving the highest percentage of organic selenium with a 2.5 mg/Kg content of selenite salt.

Table 2
Minimum and maximum content, expressed as mg/kg dry weight, of each selenium species found in mushrooms grown in soils enriched and not enriched with selenium salts.

Chemical species	Not enriched (mg/kg dw)	Enriched (mg/kg dw)
Total Se	0.5–21.5	5.1–771
Se (IV)	0.01–0.16	0.2–156
Se (VI)	0.1–0.24	0.3–69.9
SeMet	0.02–1.2	0.1–33.9
SeEt	–	24.7–68
$SeCys_2$	1.5–5.35	3.8–116
Met-Se-Cys	0.031–0.061	0.038–36.4

On the other hand, Maseko et al. determined the content of different species such as, selenomethionine, selenocysteine and methylselenocysteine in caps and stalks of *A. bisporus* after the enrichment with different concentrations of selenite (Maseko et al., 2013). The results reported show that there is no linear relationship between the concentration of selenite added to the substrate and the concentration of the different species determined, both in the caps and in the stalks. This fact suggests that selenium can be converted into different proteins via several metabolic pathways depending on the available concentration of selenium.

3.1.4. Hg

Mercury speciation analysis mainly aimed at distinguishing inorganic and organic mercury species, with methylmercury usually determined among organic species. The latter is actually the most abundant mercury organic species (de Oliveira et al., 2023) being also more toxic than inorganic Hg (Chen et al., 2023; Rutkowska et al., 2022).

A wide range of total Hg and methylmercury contents is reported in the literature, covering the 0.002–144 mg/kg range and the 0.0001–7.9 mg/kg range for total Hg and MeHg, respectively (complete data range, see Fig. 1). Most of the variability seems to be explained by samples being collected from both contaminated and pristine areas: around $^2/_3$ of the samples from the uncontaminated sites showed contents below 1 mg/kg and 0.04 mg/kg, for total Hg and MeHg, respectively (median values: 0.58 and 0.020 mg/kg). Much higher contents were instead reported for contaminated sites, with median values of 11 and 0.27 mg/kg for total Hg and MeHg, respectively. These data accordingly support mercury accumulation in the fruiting bodies of mushrooms: increased soil contents are expected to cause contamination of mushrooms by mercury and methylmercury. Nevertheless, literature studies do not report on mercury content and speciation in soils, reducing our ability to quantitatively describe the uptake process and any species-specific uptake. In this regard, inorganic mercury is reported as being mainly uptaken (Chiocchetti et al., 2020; Rutkowska et al., 2022), but its subsequent biotransformation, namely methylation, cannot be ruled out due to the large number of transformations known to be operated by mushrooms (Kavčič et al., 2019).

Finally, we investigated the correlation between the content of MeHg and total Hg (Fig. 2). Methylmercury is a minor fraction of total mercury

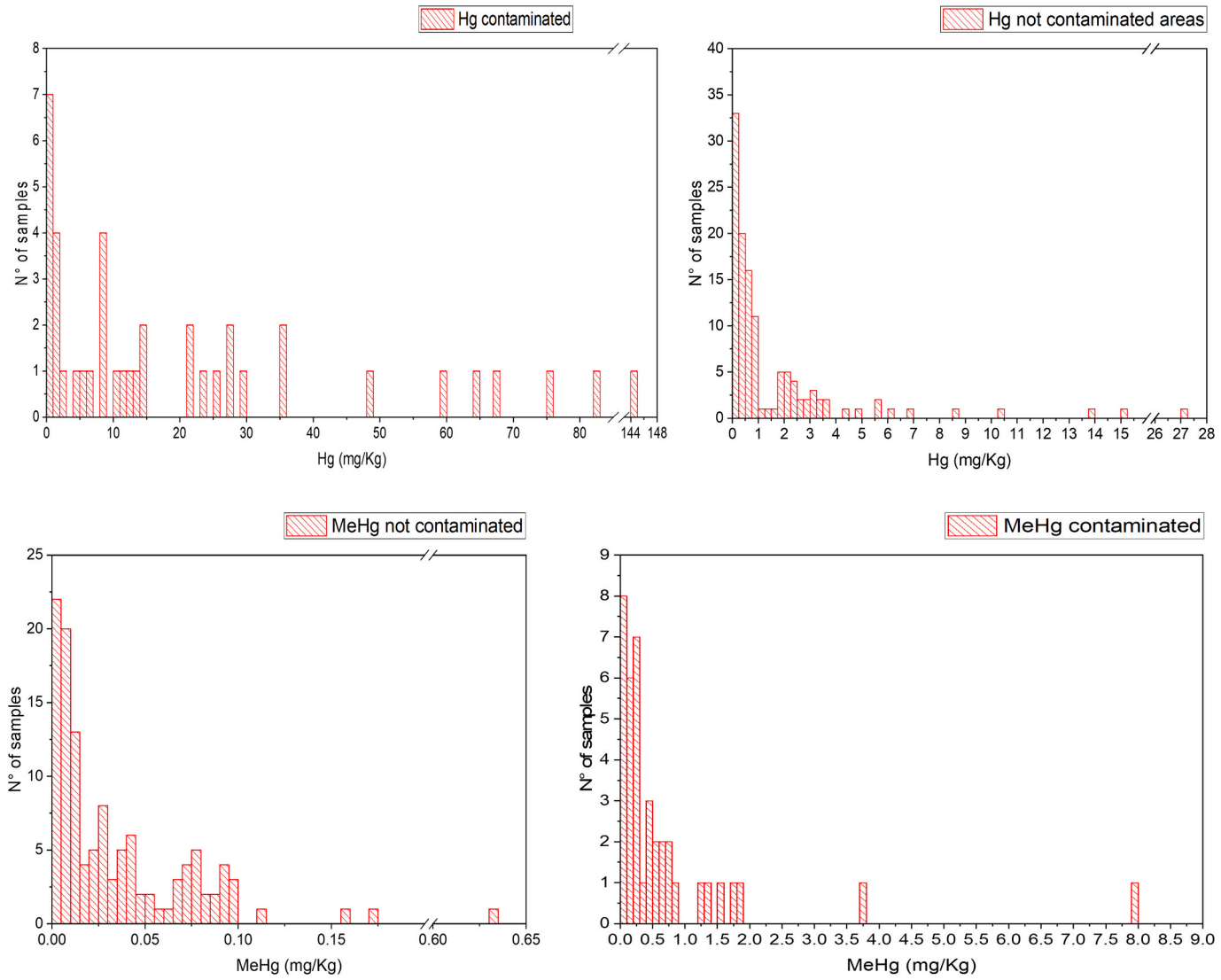

Fig. 1. Histogram of mercury and methylmercury contents found in mushrooms samples collected in not contaminated and contaminated areas.

in most of the samples, with a median value of 3.6 %. As a possible exception, the species *Pachyma hoelen* collected in China shows the highest percent of MeHg with respect to the total Hg, specifically 85.2 % (Rutkowska et al., 2022), although the total Hg content is very low (0.01 mg/kg). In Fig. 2, it can be observed that the increase of the total mercury content is correlated linearly with an increase of the methylmercury, especially in non-contaminated mushrooms (all data: $n = 160$, $r = 0.468$ p-value <0.01; non-contaminated areas: $n = 116$ (four high leverage data excluded), $r = 0.313$ p-value<0.01; contaminated areas: $n = 40$ (two high leverage data excluded), $r = 0.268$, p-value $= 0.047$).

3.1.5. Sb

As reported for chromium, there is a lack of studies related to the quantification of total antimony and its species. That also can be justified due to the low content of antimony in mushrooms and to the inherent difficulties in developing Sb speciation methods (Daus & Hansen, 2016). Therefore, Sousa-Ferreira et al. determined the total amount of Sb and carried out the speciation of Sb (III) and Sb (V) in five species of mushroom collected in Spain (Sousa Ferreira et al., 2009). The total content of Sb ranged between 0.020 and 0.031 mg/Kg (dry weight), whereas the content of Sb (III) and Sb (V) were in the range of 0.0046–0.011 mg/Kg (dw) and 0.0147–0.0212 mg/Kg (dw), respectively. Sb(V) is accordingly the most abundant species covering between 59 % and 76 % (Sb(III) in the 24 %–41 % range).

On the other hand, Koch et al. determined the concentration of three species of antimony, concretely Sb (III), Sb (V) and Me_3Sb and the total amount of Sb in mushrooms collected in Canada (Koch et al., 2000). The content of Sb (III) and Me_3Sb was below 0.01 mg/Kg (dry weight), while the content of Sb (V) ranged from 0.046 mg/kg (dry weight) to 5 mg/Kg (dry weight). Finally, the total content of Sb was ranged between 1.4 and 120 mg/Kg (dry weight).

3.2. Species specific risk assessment

International agencies established species specific safe limits when risk assessment indicated a relevant risk to be specifically associated to one element species or fraction. In particular, safe consumption limits in terms of daily intake of metal in milligram per kilogram of body weight were established following risk assessment procedures and expressed as element (or element species) mass (mg)/(body weight (kg)·day).

The USEPA (United States Environmental Protection Agency) established such limits, named reference dose (RfD) through its Integrated Risk Information System (IRIS) for total element and some of the element species reported in this review (United States Environmental Protection Agency) (data expressed as element (species or total) mass (mg)/(body weight (kg)·day)): Cr(VI): 0.003, total Sb: 0.0004, total Hg

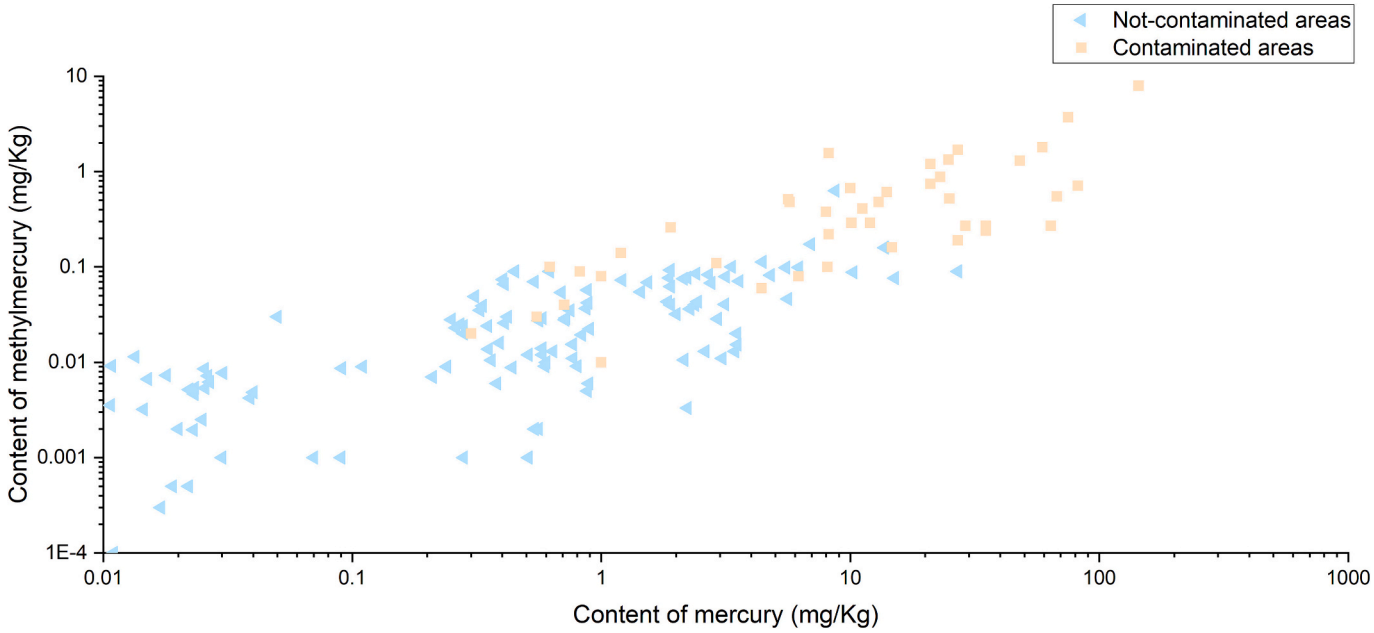

Fig. 2. Content of methylmercury versus content of total mercury, expressed in mg/kg in logarithmic scales, found in mushrooms in both contaminated and not-contaminated areas reported by the literature.

0.0003, MeHg 0.0001, inorganic As 0.0003 and total Se 0.005. USEPA states that "The RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups), that is likely to be without appreciable risk of deleterious noncancer effects during a lifetime. It is not a direct estimator of risk but rather a reference point to gauge the potential effects."(United States Environmental Protection Agency, 2024). These reference doses serve as valuable metrics for evaluating the safe intake levels of food, accommodating the acknowledged uncertainty. However, exceeding these doses does not necessarily precipitate the initiation of adverse health consequences.

In the present work we calculated daily intakes per kilogram of body weight assuming a daily consumption of 300 g of fresh mushrooms (equivalent to 30 g dry weight) and an adult body weight of 70 kg in accordance with literature studies (see e.g. Sarikurkcu et al., 2020) (Sarikurkcu et al., 2020): minimum and maximum values calculated using the data in Table S1 are reported in Table 3 along with the percentage of samples exceeding the Reference Dose values. An element-by-element discussion follows.

Observations indicate that the quantities of Cr(VI) and total Sb ingested through the consumption of 300 g of fresh mushrooms daily remain within prescribed thresholds, see Table 3. Current data imply that moderate mushroom consumption does not pose a notable health hazard regarding Sb and Cr(VI) exposure. However, insufficient measurements hinder definitive assessments.

Conversely, the consumption of mushrooms may pose a risk, when mercury and methylmercury are concerned. Ninety percent and 62 % of the mushroom samples from contaminated areas show contents above the RfD for total Hg and MeHg, respectively, whereas samples from uncontaminated areas showed much lower percentages, 44 % and < 1 % (total Hg and MeHg, respectively). While one might anticipate data regarding contaminated sites, the available dataset suggests that a possible risk may be also associated with mushrooms collected from pristine areas, calling for a careful evaluation of the risk associated with consumption. Regarding the species specific risk posed by MeHg, the determination of total mercury should be protective for MeHg too, notwithstanding the higher toxicity of the latter, standing the low fraction of MeHg measured in mushrooms (3.6 % median value, see Survey of literature data section and Fig. 1). This would lead to a strong simplification in the analytical procedure, avoiding species specific detection for risk assessment.

Regarding arsenic, only its inorganic species are considered of toxicological relevance so far: approximately 10 % of the samples resulted in an estimated ingestion that surpasses the Reference Dose (RfD) for inorganic arsenic (iAs). In contrast to methylmercury, relying solely on the detection of total arsenic may pose challenges in accurately assessing risks, as no definitive correlation between inorganic arsenic (iAs) and total arsenic has been identified (see data in Fig. 3). Accordingly, available data strongly suggest that the selective determination of iAs should be performed.

Selenium is the only essential element whose speciation was studied in mushroom anatomical parts, and it is well known to cause adverse

Table 3
Reference dose limit values for each chemical species, expressed as mg/day, supposing 70 kg as an average body weight, amount of element provided by the intake of 30 g of dry weight mushroom per day expressed in mg and percentage of samples exceeding the limit.

Element	Chemical species	RfD limit values (mg/day)	Amount of element provided by the intake (mg/day)	Samples exceeding the limit (%)
Cr	Cr (VI)	0.21	0.00309–0.0174	–
Sb	Total Sb	0.028	0.000582–0.000921	–
Hg	Total Hg not contaminated	0.021	0.0006–0.81	44
	MeHg not contaminated	0.007	0.000003–0.0189	0.8
	Total Hg contaminated	0.021	0.0036–4.32	88
	MeHg contaminated	0.007	0.0003–0.237	62
As	As (III) + As (V)	0.021	0.00042–0.109	11
Se	Total Se	0.35	0.015–23.121	68

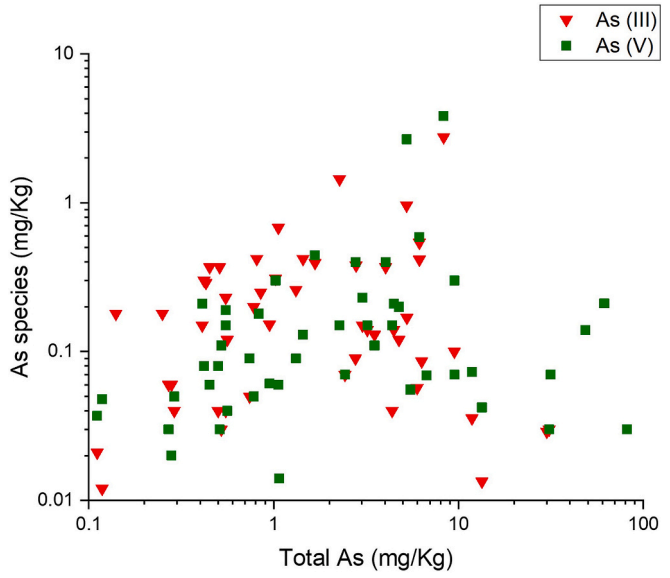

Fig. 3. Content of arsenic species (As (III) represented in red and As (V) represented in green) versus content of total arsenic, expressed in mg/kg in logarithmic scales, found in mushrooms in both contaminated and not-contaminated areas reported by the literature. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

effects in case of excessive oral exposure (see e.g. (Hadrup & Ravn-Haren, 2023) and references therein). Accordingly, USEPA set an RfD of 0.005 mg/(kg bw) (EPA. & Integrated Risk Information System (IRIS), 1991) and the European Food Safety Authority (EFSA) a tolerable upper intake level of 0.255 mg/day for adult men and women (Turck et al., 2023) for the safe oral intake of selenium. Data reported in Table 3 show that the intake of 68 % of the samples would provide an amount of selenium higher than the recommended safe level, with six samples exceeding the limit by ten times. These results are well in line with around ¾ of the samples being grown in Se enriched media: this practice should be carefully evaluated as the available data suggest that enriching the substrate with contents higher than approx. 1 mg/kg leads to a Se uptake in the fruiting bodies that would cause exceeding the limit set by USEPA and EFSA for oral consumption. The latter is clearly just an indication as assessing possible effects would require an extensive evaluation in addition to daily average mushroom consumption being much lower than the assumed portion of 300 g.

As an additional info on selenium soil amendment, its presence is reported to reduce the bioavailability of Hg and MeHg in plants, mushrooms and mammals, due to the formation of insoluble complexes (Braeuer et al., 2022; de Oliveira et al., 2023; Kavčič et al., 2019). Accordingly, selenium amendment may induce a protective action against mercury in addition to increasing the dietary source of selenium (refer to the preceding discussion regarding excessive enrichment).

4. Final remarks

The assessment of the literature reported in this review highlighted a strong limitation in data availability and research needs if the speciation of heavy metals in mushrooms is to be assessed in the framework of the health effects of their consumption. We propose a point by point list envisaging the main knowledge gaps, possible mitigation strategies, and future outlooks.

Knowledge gaps

1) The large number of edible mushroom species hinders any attempt to reach a decent level of knowledge on more than a few species.
2) Almost all studies beside three (Chen et al., 2023; Šíma et al., 2019; Wuilloud et al., 2004) focused on the speciation analysis of a single element. Health risk assessment is clearly hindered by this approach and indexes including more than one species, like e.g. the Hazard Index, cannot be calculated, strongly limiting our evaluation ability. The determination of all toxicologically relevant species are urgently needed to correctly assess the risk profile. At present, the health risk seems negligible for Cr(VI) exposure (although very limited data are available), limited for iAs (with a few sample showing high iAs content), non-negligible for total Hg, and connected to soil amendment for selenium.
3) We are far from defining safe conditions for mushroom consumption. Besides a clear indication for Hg, i.e. avoiding collection from contaminated sites, we do not know whether mushroom fruiting bodies may selectively accumulate one or more element species or transform and interconvert element species into each other. We do not know either how different mushroom species behave in this regard. Pioneering studies have attempted to clarify this phenomenon with respect to As and Se content ((Milovanovic et al., 2019; Nearing et al., 2015, 2016; Soeroes et al., 2005), but we are far from reaching sound knowledge in this field.
4) The effect of cooking is unknown, as only two papers studied its effect (Falandysz et al., 2022; Y. Liu et al., 2023). Most of the gastronomic preparations of mushrooms involve cooking, but we ignore if this process may cause species interconversion driven by e.g. temperature or pH, the latter due to the dressing type. In addition, it would be useful to know what fraction of the metal content is absorbed by the organism (bioavailability), for example, by performing extractions that simulate gastric juices.

Path to knowledge advancements (mitigation strategies and future outlook)

1) Studies should address the worldwide most consumed wild and commercial species, such as species belonging to the genus *Agaricus, Pleurotus, Lentinula, Boletus, Lactarius or Macrolepiota*. Cultivated mushrooms dominate the market, facilitating the implementation of this task. At present, data coverage is reasonable for iAs, although samples are mainly from Europe (see e.g. Walenta et al., 2023), limited for MeHg (Rutkowska et al., 2022) and selenium species, and scarce, if any, for Cr(VI), see Table 1.
2) Simplification and standardization of the procedures for speciation analysis would strongly increase sample throughput and address the work needed to implement the tasks reported in point 1. Research should focus on developing methods for the selective determination of the toxicologically relevant species (Cr(VI), MeHg, inorganic arsenic), which may be achieved by a simplified and high throughput procedure in case the species or fractions show markedly different chemical properties, as recently demonstrated by some of us (Spanu et al., 2019; Spanu et al., 2021; Spanu et al., 2022). Demonstrating their applicability to mushroom samples is ongoing. Alternatively, the development of simultaneous, multi-elemental, speciation analysis procedures seem at present difficult, mainly due to the different extraction and analytical conditions used in existing speciation methods.
3) Mushroom cultivation under controlled conditions will help in defining safe conditions for mushroom consumption. Species specific bioaccumulation factors may be determined by spiking the growing substrate with different element species and measuring their transfer to the fruiting bodies. The use of isotopically marked element species may also further advance our knowledge by understanding if the mushrooms simply accumulate an element species or operate biotransformation/conversion of the elements. Assessing the active processes for wild mushrooms is by far more challenging: determining element speciation in the soils where the mycetes growths may give some clues about active processes (e.g. bioaccumulation),

but it would mostly help in defining soil conditions leading to possible health risk through consumption.

4) The effect of cooking on element species and their conversion should be investigated. Here again, species specific isotope spiking may help in pinpointing species interconversion and the possible formation of toxic species.

In conclusion, our knowledge on element speciation in mushroom anatomical parts is very limited, hindering a proper health risk evaluation of mushroom consumption. We reckon that two major advancements, namely developing high throughput analytical speciation methods and understanding the extent of element species transfer to fruiting bodies would support a giant leap forward in our ability to provide consumers with indications for safe mushroom consumption.

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CRediT authorship contribution statement

Alejandro R. López: Writing – original draft, Data curation. **Elena Ortega-Caneda:** Investigation. **Estrella Espada-Bellido:** Writing – review & editing. **Davide Spanu:** Writing – review & editing, Visualization. **Martina Zava:** Writing – review & editing, Methodology. **Damiano Monticelli:** Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data are reported in the Supplementary Material

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Appendix A. Supplementary data

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