Toxic trace elements in dried mushrooms: effects of cooking and gastrointestinal digestion on food safety

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Abstract

Mushrooms can accumulate toxic trace elements. The objectives of the present study are to evaluate levels of mercury, cadmium, lead, and arsenic in dried mushrooms, to determine the effect of cooking on the contents of these elements, and to evaluate their bioaccessibility in the mushrooms ready for consumption. The results showed that Hg levels in *Amanita ponderosa*, *Boletus edulis*, *Marasmius oreades*, and *Tricholoma georgii*, as well as Cd levels in some samples of *Amanita caesarea* and *T. georgii*, exceeded the legislated limits. Cooking significantly reduced the levels of As (26–72%), whereas the reduction in levels of Hg, Cd, and Pb was much lower. However, the bioaccessibility of As (63–81%) was higher than the values obtained for the metals (<40%). Taking the effects of cooking and gastrointestinal digestion into account gives a more realistic estimate of the risk associated with the consumption of mushrooms.

Keywords: mushrooms, arsenic, cadmium, mercury, lead, cooking, bioaccessibility

1. Introduction

Mushrooms are considered a healthy food, because the energy they provide is relatively low, around 360–419 kcal/kg, they are a source of proteins with a higher nutritional value than other plants, and they are important sources of minerals (Kalač, 2013). They also contain compounds of high biological value, such as polyunsaturated fatty acids, phenolic compounds, tocopherols, ascorbic acid, and carotenoids (Reis, Barros, Martins & Ferreira, 2012). These food matrices are also considered important sources of dietary fiber, consisting mainly of insoluble fiber (chitin and β -glucans).

Some species of mushrooms, however, can accumulate high concentrations of toxic metals such as mercury (Hg), cadmium (Cd), and lead (Pb), and metalloids such as arsenic (As). The concentrations of these elements can still be high even when the mushrooms have been collected in uncontaminated areas (Falandysz & Borovička, 2013). Among these elements, Hg and Cd have been investigated the most, with results showing that mushrooms act as bio-accumulators of these metals. The bioconcentration factors (mushrooms/soil) can reach values of 210 for Hg (Falandysz, 2014) and 1400 for Cd (Melgar, Alonso & García, 2016). The edible mushrooms of the genera *Boletus*, *Macrolepiota*, *Agaricus*, and *Lepista* are those with the highest concentrations of Hg (Kalač & Svoboda, 2000), with inorganic Hg being the predominant chemical form (Ruiz de Cenzano, López-Salazar, Cervera & De la Guardia, 2016). The genus *Agaricus* also has the highest concentrations of Cd (Svoboda, Zimmermannová & Kalač, 2000; Tuzen, Sesli & Soylak, 2007).

Although Pb studies are scarcer than studies of Hg and Cd, they have shown a direct relationship between Pb concentrations in mushrooms and soil contamination due to traffic pollution (Jorhem & Sundström, 1995). *Lycoperdon perlatum*, *Macrolepiota rhacodes*, and *Lepista nuda* have been identified as large accumulators of this metal (Kalač & Svoboda, 2000; Sesli, Tuzen, & Soylak, 2008). With regard to As, high concentrations have been

reported in the genus *Laccaria* (1.8–163 mg/kg dry weight) (Slekovec & Irgolic, 1996; Vetter, 2004; Zhang, Li, Yang, Liu & Wang, 2015), although the bioconcentration factors are much lower than those reported for Hg and Cd (Slekovec & Irgolic, 1996). Speciation studies show that mushrooms can present a wide variety of chemical forms of As, and even that the inorganic form, the most toxic species existing in food, can be the predominant form (Llorente-Mirandes, Barbero, Rubio & López-Sánchez, 2014; Nearing, Koch & Reimer, 2014).

Most research to date has been conducted on fresh mushrooms, whereas there is much less information on contents in dried mushrooms, commercialization and consumption of which have increased in recent years (CBI, 2016). According to previous studies, the drying process applied to mushrooms to obtain the dried forms does not produce significant losses of their nutritional value. This process significantly concentrates the amounts of phenolic compounds, carbohydrates, and proteins, owing to the loss of moisture (Reid, Munyanyi & Mduluza, 2016). It is expected that the dehydration process has the same effect for metals and metalloids.

As with many other foods, assessment of the risk associated with consumption is usually based on the contaminant contents in the food as it is marketed (fresh or dried), and it does not take into account two aspects that may change the risk substantially: cooking and bioaccessibility (fraction of the element solubilized during digestion and available for intestinal absorption). The few studies on the effect of cooking indicate a decrease in the contents of Hg, Pb, Cd, and As after applying boiling or frying methods to fresh mushrooms (Cheyns, Waegeneers, Van de Wiele & Ruttens, 2017; Cibulka, Čurdová, Miholová & Stěhulová, 1999; Svoboda, Kalač, Špička & Janoušková, 2002). With regard to bioaccessibility, it is necessary to bear in mind that carbohydrates are the main nutrients in mushrooms (35–70% dry weight) and that they are mostly resistant to human digestive

enzymes (Cheung, 2013), which could make the solubilization of contaminants during digestion difficult. The only study on the bioaccessibility of these contaminants in mushrooms is the one carried out by Sun, Liu, Yang & Zhuang (2012), which showed that only 50% of the Cd present in cooked mushrooms was available for absorption.

The objectives of the present study are to determine the contents of Hg, Cd, Pb, and As in dried mushrooms, to evaluate the effect of cooking on these contents, and to study the bioaccessibility of these elements and the factors that condition it. By evaluating these factors, the study covers a gap in knowledge about the food safety of these products and helps to improve the assessment of the risk associated with their consumption.

2. Materials and methods

2.1. Samples

Eleven species of dried mushrooms, acquired from establishments in Spain, were analyzed (Table 1). For each species, at least two samples from different batches were evaluated, each of which was divided into two portions. The portion of raw product destined for analysis was homogenized and stored at 4 °C. In the portion destined to be cooked, the culinary treatment indicated by the manufacturer was applied without the addition of any other ingredients; afterwards, the sample was drained, homogenized, and stored at –20 °C until analysis. Only the species that presented the highest contents of the toxic trace elements under study were selected for evaluation of the effect of cooking and bioaccessibility.

2.2. *In vitro* gastrointestinal digestion model

The method used for the evaluation of bioaccessibility was a static model previously described by Jadán-Piedra, Sánchez, Vélez & Devesa (2016) that simulates the gastric and

intestinal stages of the human digestion process. The samples were weighed in Erlenmeyer flasks (2 g of cooked sample), and 90 mL of deionized water was added. The mixture was homogenized by mechanical stirring. The pH was adjusted to 2 with 6 mol/L HCl (Merck, Spain), and the weight of the mixture was brought to 100 g with deionized water. Subsequently, porcine pepsin (enzymatic activity 944 U/mg protein; Sigma, Spain) (2 mg pepsin/100 g of solution) was added and the Erlenmeyer flasks were covered with Parafilm and incubated at 37 °C for 2 h with agitation (120 rpm).

Once the gastric phase had finished, the pH was adjusted to 6.5 by the addition of NH₃ (Scharlau, Spain). Porcine pancreatin (activity equivalent to 4 × US Pharmacopoeia specifications/mg pancreatin; Sigma, Spain) and porcine bile extract (glycine and taurine conjugated with deoxycholic acid and other bile salts; Sigma, Spain) (0.25 mg of pancreatin and 1.5 mg of bile extract per 100 g of solution) were added. The mixture was incubated again under agitation (120 rpm, 2 h, 37 °C). After the intestinal stage, the volume in the flask was transferred to polypropylene tubes and the soluble or bioaccessible fraction was separated by centrifugation (10000 rpm, 20 min, 4 °C).

From the bioaccessible fraction, Hg, Cd, As, and Pb were quantified by applying the methodologies described in sections 2.3 to 2.5. The bioaccessibility was calculated using the following expression:

$$\text{Bioaccessibility (\%)} = [S/C] \times 100$$

where S is the content of the element solubilized during digestion; C is the content of the element in the sample.

Applying this method *in vitro*, the effect of certain parameters of the gastrointestinal digestion on the bioaccessibility of these elements was evaluated. At the gastric level, the influence of the gastric pH (2, 3, and 4) and the pepsin concentration (0, 1, 2, 4, 8, and 13 mg/g of sample) was studied. From the intestinal digestion parameters, the effects of pH (4.5,

6, and 7) and the bile salts concentration (3, 10, and 300 mg/g sample) were analyzed. Additionally, the effect of the major insoluble fibers in mushrooms – chitin (Santa Cruz Biotechnology, Germany) and β -glucan (Sigma, Spain) – on bioaccessibility was determined. These compounds were added at a concentration of 100 mg/L to standard solutions of the toxic trace elements (1 mg/L, final volume 20 mL), and the whole solution was subjected to the gastrointestinal digestion process described above.

2.3. Determination of mercury

Mushroom samples (0.25 g raw, 0.5 g cooked) or the bioaccessible fractions (4 mL) were placed in Teflon reactors, 4 mL of 14 mol/L HNO₃ (Merck, Spain) and 1 mL H₂O₂ (30% v/v, Panreac, Spain) were added, and they were irradiated (180 °C, 15 min) in the microwave system (model MARS, CEM, Vertex, Spain). The digest was allowed to rest for 12 h to eliminate nitrous vapor, after which it was made up to 10 mL with 0.6 mol/L HCl, and then Hg was quantified by cold vapor generation coupled to atomic fluorescence spectrometry (CV-AFS, model 10.025 Millennium Merlin, PS Analytical, UK). The analytical conditions were: reducing agent, 2% (m/v) SnCl₂ (Scharlau, Spain) in 1.8 mol/L HCl, 4.5 mL/min; reagent blank, 0.6 mol/L HCl, 9 mL/min; carrier gas, Ar, 0.3 L/min; drying gas, air, 2.5 L/min; delay time, 15 s; analysis time, 40 s; memory wash time, 60 s. Quality control for quantification by CV-AFS was performed by analyzing a water reference material (RTC QC1014, LGC Standards, Spain), with a certified Hg concentration of 40.8 ± 1.19 μ g/L.

2.4. Determination of cadmium and lead

Mushroom samples were digested by microwave oven under the conditions described above for Hg analysis (section 2.3). Subsequent quantification was carried out using a graphite furnace atomic absorption spectrometer (GF-AAS, model AAnalyst 600, Perkin-Elmer,

Germany) with longitudinal Zeeman effect correction, pyrolytic tubes with transverse heating, and integrated L'vov platform equipped with an autosampler (model AS-800). Discharge lamps without electrodes and wavelengths of 228.8 nm for Cd and 283.3 nm for Pb were used. As a matrix modifier, a solution of 0.067 mg $\text{H}_2\text{PO}_4\text{NH}_4$ (Merck, Spain) and 0.003 mg $\text{Mg}(\text{NO}_3)_2$ (Merck, Spain) in 10 μL 1% HNO_3 (v/v) was used for both elements. The temperature program used in the graphite furnace is shown in the supplementary material (S1). Quality control of the quantification was carried out by analysis of an EnvironMAT drinking water reference sample (EP-H-2; SPC Science, Canada) certified for Cd (0.079 mg/L) and Pb (0.26 mg/L).

2.5. Determination of arsenic

Samples (1 g of raw mushrooms, 2 g of cooked mushrooms, or 10 mL of bioaccessible fraction) were treated with 1 mL of ashing aid suspension [20% m/v MgNO_3 (Merck) and 2% m/v MgO (Merck)] and 5 mL of 7 mol/L HNO_3 . The mixture was evaporated on a sand bath (Raypa, Spain) until total dryness and subsequently mineralized in a muffle furnace (Heraeus, Spain), applying a temperature-time cycle in which, after a gradual rise in temperature (50 $^{\circ}\text{C}/\text{h}$), the samples were maintained at 450 $^{\circ}\text{C}$ for 12 h. The white ash obtained after mineralization was dissolved in 6 mol/L HCl and a reducing solution [5% m/v KI (Panreac, Spain) and 5% m/v ascorbic acid (Merck, Spain)]. The ratio of reducing solution to the final volume of the mixture was 1:5. Subsequently, the solution was filtered through Whatman No. 1 paper and brought to a final volume (10 mL) with 6 mol/L HCl . Arsenic contents were quantified by hydride generation coupled to atomic fluorescence spectrometry (HG-AFS, model 10.044 Excalibur, PS Analytical, UK). The instrumental conditions for the quantification were as follows: sample flow, 9 mL/min; reducing solution, 1.2% NaBH_4 (m/v) (Scharlau, Spain) in 0.1 mol/L NaOH (m/v) (Panreac, Spain), 4 mL/min; acid solution, 4.5

mol/L HCl, 9 mL/min; carrier gas, Ar, 300 mL/min; drying gas, air, 2.5 L/min; primary current, 27.5 mA. The quality control was carried out by analysis of an EnvironMAT drinking water reference sample (EP-H-2) with a certified As content of 0.159 mg/L.

2.6. Statistical analysis

The statistical analysis was performed by applying Student's *t*-test or analysis of variance of one factor or two factors (ANOVA) with multiple post hoc comparisons using the Fisher LSD test (SigmaPlot, version 13). Differences were considered significant when $p < 0.05$.

3. Results

3.1. Contents in samples of raw dried mushrooms

Table 2 shows the Hg, Cd, As, and Pb contents in the dried mushrooms. The elements with higher concentrations were Hg (0.003–10.3 mg/kg, median: 0.53 mg/kg) and Cd (0.049–4.53 mg/kg, median: 0.47 mg/kg). The highest levels of Hg were found in *Amanita ponderosa* (7.20–10.3 mg/kg), *Tricholoma georgii* (3.45–4.26 mg/kg), and *Boletus edulis* (0.95–1.84 mg/kg). These last two species of mushroom were also the ones that presented the highest Cd contents [*T. georgii* (0.855–2.82 mg/kg), *B. edulis* (0.756–1.31 mg/kg)], together with *Amanita caesarea* (0.85–4.53 mg/kg).

The As contents ranged between 0.025 and 1.01 mg/kg (median: 0.25 mg/kg), and the Pb contents between 0.034 and 1.94 mg/kg (median: 0.18 mg/kg). The contents found in some samples can be considered high; 7 of the 32 samples evaluated exceeded 0.5 mg As/kg and 2 of them presented values higher than 1 mg Pb/kg.

3.2. Contents in cooked samples and the effect of cooking

To evaluate the effect of cooking on the levels of these metals/metalloids, the mushroom species with the highest concentrations of these elements were selected (*A. caesarea*, *A. ponderosa*, *B. edulis*, *Marasmius oreades*, *T. georgii*). The comparison between raw and cooked mushroom contents was made on a dry weight basis (Figure 1).

The culinary treatment produced a reduction in the levels of all the toxic elements, although the magnitude of the reduction varied according to the element. Arsenic was reduced in all the samples (range: 26–72%, median: 52%). In 60% of all the mushrooms there was a decrease in Cd (range: 31–54%, median: 48%) and Pb (range: 23–34%, median: 25%), and in 20% of the samples a reduction of Hg (range: 10–35%, median: 21%) was observed. The reduction of the element levels occurred partly as a result of solubilization in the water used for cooking. The percentages of elements in the water with respect to the levels in the sample were, in ascending order: Hg (0.1–5%) < Pb (4–12%) < Cd (11–38%) < As (28–52%).

3.3. Bioaccessibility of Hg, Cd, As, and Pb

3.3.1. Bioaccessible contents

Table 3 shows the bioaccessible contents (mg/kg) and the bioaccessibility (%) of the toxic elements in the cooked mushrooms. For all the samples, bioaccessible levels of Pb were lower than the limit of detection of the methodology, therefore no results are provided for this element. For Hg and Cd, the bioaccessibilities did not exceed 40%. These results indicate that gastrointestinal digestion caused a substantial reduction in the risk associated with the intake of Pb, Cd, and Hg. In contrast, the bioaccessibility of As was high (63–81%%), and therefore, for this metalloid, the risk reduction after gastrointestinal digestion was much lower.

3.3.2. Influence of the various parameters of the digestion and the composition of the sample on bioaccessibility

The low bioaccessibility observed for Hg, Cd, and Pb could be a consequence of a) incomplete digestion of the matrix, hindering release of these metals, and/or b) the presence in the matrix of compounds that formed insoluble complexes with the metals. To evaluate the effect of these two factors on the bioaccessibility the following approaches were adopted:

A) Modification of the digestion conditions. The digestion of the sample, and therefore the release of the elements, could be affected by the conditions used in the static digestion model. During the digestive process, pH, and saline and enzymatic concentrations vary in a range that is governed to some extent by the type of food ingested. In order to determine if the change in these parameters could influence the bioaccessibility of the elements studied, they were modified within the usual ranges experienced in human digestion.

Of all the parameters analyzed, only the gastric and intestinal pH significantly affected the solubilization of Hg and Cd during the digestion of the mushrooms (Figure 2). An increase in the gastric pH led to a reduction in the bioaccessibility of Hg (reduction: 36–80%) and, to a lesser extent, of Cd (reduction: 15–21%) in the two species of mushroom tested. The intestinal pH affected the bioaccessibility of each element differently. An increase in the pH reduced the bioaccessibility of Cd (20–40%) and increased that of Hg (45–107%). The effect of the pH could be a consequence of changes in the ionization states of the metals or of compounds that can bind these metals, favoring the formation of complexes with lower solubility.

B) Influence of food components on bioaccessibility. Insoluble fibers were added to the digestion in accordance with the concentrations of these compounds in mushrooms. Sari, Prange, Lelley & Hambitzer (2017) evaluated a wide variety of mushrooms and reported β -glucan contents that ranged between 10 and 40 g/100 g dry weight. The reported chitin contents are slightly lower (0.4–10 g/100 g of dry weight of mushroom) (Nitschke, Altenbach, Malolepszy & Möllenken, 2011).

Figure 3 shows the contents of the bioaccessible fractions obtained in the presence and absence of chitin or β -glucan for the aqueous solutions of Hg and Cd (1 mg/L). The presence of these fibers produced a reduction in the bioaccessibility of both elements (Cd: 46–51%, Hg: 21–25%), and therefore they affected the quantity of toxic element available for absorption.

4. Discussion

Prolonged exposure to the trace elements evaluated in the present study produces adverse effects, in many cases with the development of some types of cancer (IARC, 1993, 2004, 2006; ATSDR, 2012). Because of this toxicity their presence in food is not desirable, and there are various regulations that limit their contents in many food matrices, although sometimes the scope of the legislation is less wide-ranging than might be expected on the basis of existing toxicological data. European legislation only limits the maximum contents of Pb and Cd in three species of mushroom: *Agaricus bisporus* (button mushroom), *Pleurotus ostreatus* (oyster mushroom), and *Lentinula edodes* (shiitake mushroom). The maximum permitted levels are set at 0.3 mg/kg for Pb (Commission Regulation (EU) 2015/1005) and 0.2 mg/kg for Cd (Commission Regulation (EU) No 488/2014). These regulations refer to fresh mushrooms but say that, when the maximum levels specified are applied to dried foodstuffs, changes of the concentration of the contaminant caused by drying processes shall be taken into account (Commission Regulation (EC) No 1881/2006). Given the loss of water that occurs during the mushroom drying process, the fresh to dehydrated transformation factor could be estimated at 10. Of the species covered by the regulations, only shiitake mushrooms were analyzed in the present study, and the transformation of their levels of Pb and Cd did not exceed the limit allowed by the European Union.

Other countries are more restrictive in the evaluation of mushroom food safety and set maximum levels for all species. In China (GB 2762-2012) the maximum regulated levels are: Pb, 1 mg/kg (10 mg/kg for dried); Hg, 0.1 mg/kg (1 mg/kg for dried); As, 0.5 mg/kg (5 mg/kg for dried mushrooms). In the case of Cd, China establishes a maximum content level of 0.2 mg/kg (2 mg/kg for dried), or 0.5 mg/kg for shiitake mushrooms (GB 2762-2012). Some of the samples analyzed in the present study exceeded the Chinese limits for Cd and Hg. The Cd limit was exceeded in a sample of *Tricholoma georgii* and two samples of *Amanita caesarea*. Non-compliances for Hg were detected in samples of *Amanita ponderosa* (7.20–10.3 mg/kg), *Boletus edulis* (1.42 and 1.84 mg/kg), *Hydnum repandum* (1.16 mg/kg), *Marasmius oreades* (1.66 and 1.71 mg/kg), and *Tricholoma georgii* (3.45–4.24 mg/kg). According to the Chinese regulations, there could be a food safety problem with these mushroom species, and therefore the European Union should extend the monitoring and control of contaminants to a greater number of species than those currently contemplated in its regulations.

It has been shown that for certain food/metal(loid) combinations there is a modification of the risk associated with intake as a consequence of pre-consumption processing. Solubilization of the toxic elements in the cooking or canned liquid and concentration of them as a result of loss of water and other soluble compounds during cooking are some of the causes of the modification of their contents in the cooked or processed products (Devesa, Vélez & Montoro, 2008; Hajeb, Sloth, Shakibazadeh, Mahyudin & Afsah-Hejri, 2014). In the present study, when comparing the contents in dry weight (Figure 1), it is evident that in all the samples there were significant losses in As after cooking (28–52%). The reductions of the other elements after cooking were not significant, except in the case of a sample of *A. caesarea*, where a reduction of 38% for Cd was observed. The different behavior, during cooking, for As compared to other trace elements can be explained by the fact that its

interaction with the food matrix is different; Hg, Cd, and Pb are metal cations, whereas As is an oxyanion.

Previous studies show greater losses of toxic trace elements during the cooking of mushrooms, although there are no cases for dried samples. Falandysz and Drewnowska (2017) reported a reduction of up to 56% in Hg content after boiling *Amanita fluva* mushrooms, although the raw samples were blanched prior to boiling. Cibulka et al. (1999) also observed a loss of Hg ($\approx 30\%$) during heat treatment of fresh mushrooms at high temperatures that simulated a frying process. The differences in this latter study may also have been caused by the use of more drastic cooking treatments than cooking in water.

Most of the reduction of the levels of these elements was due to their loss from the mushrooms to the water used for cooking. In these specific cases there may be a risk reduction, as long as the cooking liquid is discarded before consumption. The CBI reported (2016) that one of the main uses of these dried mushrooms is to make soups, in which case there would not be a decrease in the associated risk.

After ingestion, food undergoes a series of processes that could also affect the associated risk. The release of the toxic compound from the food matrix during digestion and its subsequent intestinal absorption are essential steps for it to reach the systemic circulation and exert its effects. *In vitro* bioaccessibility studies are used to assess the amount of the compound that remains available for absorption after digestion. It has been shown that in many cases *in vitro* studies accurately reflect the data obtained in animals and even in humans (Versantvoort, van de Kamp & Rompelberg, 2004). The bioaccessibility tests carried out in the present study show that the amounts of Hg, Cd, and Pb released from the matrix after digestion were low. This suggests that a considerable part of these metals was not absorbed, and therefore they would not accumulate in the target organs and would not cause adverse effects. However, the bioaccessibility of As was high, with values that were close to 100% in

several samples. As in the case of cooking, the behavior of this metalloid was different from that of the three metals.

The low bioaccessibility of the metals observed in the mushrooms could be due to several causes, as previously indicated: a) low digestibility of the sample; b) the elements were released from the matrix in the form of complexes with low solubility; c) after their release from the matrix they bound to other elements or compounds also released from the matrix during digestion, forming complexes of low solubility. Some studies indicate that mushrooms are difficult matrices to digest. The digestive enzymes secreted by the pancreas and the brush border cannot hydrolyze the glycosidic bond of β -glucans (Aida, Shuhaimi, Yazid & Maaruf, 2009). In fact, the increase in the concentrations of the enzymes and bile salts used in the simulated digestion did not lead to changes in the bioaccessibility of the metals, indicating that these enzymes do not play an important part in digestion.

The results obtained in this study also showed that the reduced bioaccessibility of the toxic elements was influenced by the insoluble fibers present in the mushrooms (Figure 3), which probably formed complexes of low solubility with the elements. The binding of heavy metals by chitin has been previously documented, especially for Pb and Cd. Xiong (2010) showed Cd chelation in aqueous solution, with a maximum at pH 5.4. The author demonstrated by infrared analysis that Cd binds to the hydroxyl and acetylamino groups of chitins. The binding of Hg is even more efficient, with percentages close to 90%, and with an optimum pH around 7 (Barriada, Herrero, Prada-Rodríguez & Sastre de Vicente, 2008). This latter study showed that, once the chitin–Hg complex is formed, metal desorption is difficult. In our study, higher uptake of Cd by chitin was observed, possibly because the pH range used during the digestion was lower than the optimum pH for Hg binding.

5. Conclusion

The *in vitro* data show that both cooking (As) and gastrointestinal digestion (Hg, Cd, and Pb) reduce the available amounts of elements that would be expected to reach the systemic circulation after ingestion if only the contents of the raw food are considered. The influence of these factors should be confirmed *in vivo*, to see whether, although some of these mushrooms have Hg and Cd values above legislated limits, the quantity of these elements that would reach the systemic circulation after processing and digestion would not be such as to represent a risk, from a toxicological point of view.

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Table 1. Mushroom samples analyzed.

Scientific name	Common name
<i>Amanita caesarea</i>	Caesar's Amanita
<i>Amanita ponderosa</i>	Gurumelo
<i>Boletus edulis</i>	King Bolete
<i>Cantharellus lutescens</i>	Yellow Foot
<i>Cantharellus tubaeformis</i>	Autumn Chanterelle
<i>Hydnum repandum</i>	Wood Hedgehog
<i>Lactarius deliciosus</i>	Saffron Milkcap
<i>Lentinula edodes</i>	Shiitake
<i>Lepiota procera</i>	Parasol
<i>Marasmius oreades</i>	Fairy Ring
<i>Tricholoma georgii</i>	Saint George's mushroom

Table 2. Hg, Cd, As, and Pb contents in raw dried mushrooms. Values expressed as mg/kg (mean $\pm$ SD, n = 3).

Mushroom	Hg	Cd	As	Pb
<i>Amanita caesarea</i>	0.327 $\pm$ 0.040-	0.850 $\pm$ 0.099	0.478 $\pm$ 0.074	0.185 $\pm$ 0.006
	0.164 $\pm$ 0.027	4.53 $\pm$ 0.062	0.706 $\pm$ 0.074	1.30 $\pm$ 0.221
	0.924 $\pm$ 0.022	3.70 $\pm$ 0.0002	0.275 $\pm$ 0.016	0.307 $\pm$ 0.022
<i>Amanita ponderosa</i>	10.3 $\pm$ 0.856	0.256 $\pm$ 0.010	0.040 $\pm$ 0.004	0.064 $\pm$ 0.0004
	8.19 $\pm$ 0.583	0.283 $\pm$ 0.023	0.294 $\pm$ 0.011	0.083 $\pm$ 0.004
	7.20 $\pm$ 0.646	0.476 $\pm$ 0.017	0.411 $\pm$ 0.003	0.044 $\pm$ 0.003
<i>Boletus edulis</i>	0.953 $\pm$ 0.014	1.31 $\pm$ 0.052	0.094 $\pm$ 0.008	0.094 $\pm$ 0.001
	1.84 $\pm$ 0.027	0.756 $\pm$ 0.029	0.219 $\pm$ 0.003	0.114 $\pm$ 0.016
	1.42 $\pm$ 0.140	1.18 $\pm$ 0.305	0.141 $\pm$ 0.009	1.94 $\pm$ 0.049
<i>Cantharellus lutescens</i>	0.021 $\pm$ 0.002	0.240 $\pm$ 0.009	0.089 $\pm$ 0.008	0.383 $\pm$ 0.032
	0.036 $\pm$ 0.003	0.605 $\pm$ 0.022	0.176 $\pm$ 0.013	0.354 $\pm$ 0.028
	0.003 $\pm$ 0.0002	0.622 $\pm$ 0.017	0.214 $\pm$ 0.004	0.645 $\pm$ 0.035
<i>Cantharellus tubaeformis</i>	0.170 $\pm$ 0.002	0.194 $\pm$ 0.009	0.134 $\pm$ 0.017	0.604 $\pm$ 0.088
	0.164 $\pm$ 0.012	0.499 $\pm$ 0.014	0.070 $\pm$ 0.001	0.269 $\pm$ 0.015
	0.160 $\pm$ 0.008	0.554 $\pm$ 0.016	0.025 $\pm$ 0.002	0.219 $\pm$ 0.029
<i>Hydnum repandum</i>	1.16 $\pm$ 0.101	0.182 $\pm$ 0.015	0.542 $\pm$ 0.058	0.149 $\pm$ 0.017
	0.523 $\pm$ 0.042	0.243 $\pm$ 0.008	1.01 $\pm$ 0.019	0.176 $\pm$ 0.008
	0.522 $\pm$ 0.014	0.131 $\pm$ 0.005	0.320 $\pm$ 0.009	0.194 $\pm$ 0.020
<i>Lactarius deliciosus</i>	0.273 $\pm$ 0.006	0.132 $\pm$ 0.007	0.509 $\pm$ 0.035	0.139 $\pm$ 0.008
	0.095 $\pm$ 0.043	0.463 $\pm$ 0.007	0.488 $\pm$ 0.012	0.077 $\pm$ 0.005
<i>Lentinula edodes</i>	0.019 $\pm$ 0.002	0.392 $\pm$ 0.030	0.260 $\pm$ 0.021	0.082 $\pm$ 0.017
	0.008 $\pm$ 0.001	0.326 $\pm$ 0.004	0.078 $\pm$ 0.006	0.034 $\pm$ 0.007
	0.011 $\pm$ 0.004	0.419 $\pm$ 0.001	0.117 $\pm$ 0.011	0.067 $\pm$ 0.018
<i>Lepiota procera</i>	0.100 $\pm$ 0.011	0.049 $\pm$ 0.001	0.333 $\pm$ 0.037	0.062 $\pm$ 0.010
	0.575 $\pm$ 0.005	0.485 $\pm$ 0.041	0.303 $\pm$ 0.003	0.169 $\pm$ 0.030
	0.544 $\pm$ 0.019	1.39 $\pm$ 0.054	0.201 $\pm$ 0.019	0.290 $\pm$ 0.030
<i>Marasmius oreades</i>	0.775 $\pm$ 0.077	0.296 $\pm$ 0.016	0.602 $\pm$ 0.025	0.237 $\pm$ 0.017
	1.71 $\pm$ 0.125	0.343 $\pm$ 0.030	0.153 $\pm$ 0.012	0.517 $\pm$ 0.007
	1.66 $\pm$ 0.025	0.286 $\pm$ 0.008	0.563 $\pm$ 0.004	0.520 $\pm$ 0.004
<i>Tricholoma georgii</i>	4.26 $\pm$ 0.145	1.65 $\pm$ 0.082	0.245 $\pm$ 0.014	0.106 $\pm$ 0.010
	3.45 $\pm$ 0.102	0.855 $\pm$ 0.011	0.233 $\pm$ 0.016	0.065 $\pm$ 0.003
	3.51 $\pm$ 0.203	2.82 $\pm$ 0.290	0.557 $\pm$ 0.003	0.556 $\pm$ 0.006

Table 3. Bioaccessibility of Hg, Cd, and As in cooked mushrooms. Values expressed as mg/kg fresh weight (mean $\pm$ standard deviation, n = 3) and percentages with respect to the total content in the mushroom.

Element	Mushroom	Bioaccessible fraction	
		mg/kg	%
Hg	<i>A. caesarea</i>	0.116 $\pm$ 0.017	40
	<i>A. ponderosa</i>	0.014 $\pm$ 0.004	25
	<i>B. edulis</i>	0.143 $\pm$ 0.006	27
	<i>M. oreades</i>	0.093 $\pm$ 0.013	28
	<i>T. georgii</i>	0.136 $\pm$ 0.008	21
Cd	<i>A. caesarea</i>	0.097 $\pm$ 0.012	18
	<i>A. ponderosa</i>	0.019 $\pm$ 0.005	12
	<i>B. edulis</i>	0.025 $\pm$ 0.002	27
	<i>M. oreades</i>	0.008 $\pm$ 0.002	25
	<i>T. georgii</i>	0.030 $\pm$ 0.003	12
As	<i>A. caesarea</i>	0.039 $\pm$ 0.006	63
	<i>A. ponderosa</i>	0.057 $\pm$ 0.010	66
	<i>B. edulis</i>	0.027 $\pm$ 0.003	70
	<i>M. oreades</i>	0.067 $\pm$ 0.006	81
	<i>T. georgii</i>	0.053 $\pm$ 0.005	69

Figure 1. Levels of Hg (A), Cd (B), As (C), and Pb (D) in raw and cooked mushrooms. Values expressed as mg/kg sample dry weight (dw) (mean $\pm$ standard deviation, n = 3). The asterisks indicate statistically significant differences between the contents in cooked samples with respect to the raw ones (p < 0.05).

Figure 2. Influence of gastric pH (S1A) and intestinal pH (S1B) on the bioaccessibility of Cd and Hg in mushrooms. Cd and Hg contents in the bioaccessible fraction expressed in mg/kg (mean $\pm$ standard deviation, n = 4). Different letters indicate statistically significant differences ($p < 0.05$) between the bioaccessible contents at different pH for the same sample.

Figure 3. Influence of insoluble fibers on the bioaccessibility of Cd and Hg in mushrooms. Cd and Hg contents in the bioaccessible fraction expressed in μg (mean $\pm$ standard deviation, $n = 4$). The asterisks indicate statistically significant differences ($p < 0.05$) of the bioaccessible contents in the digested standard solutions in the presence of chitin and β -glucan with respect to those digested without these fibers.

Supplementary material

Table S1. Temperature program of the graphite furnace used for the determination of Cd and Pb by means of GF-AAS.

Step	Temperature (°C)	Time (s)		Internal Ar (mL/min)
		Ramp	Hold	
Drying	110	1	30	250
Drying	130	15	30	250
Pyrolysis	500 (Cd), 850 (Pb)	10	30	250
Spraying	1400 (Cd); 1600 (Pb)	0	5	0
Cleaning	2450	1	3	250