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Original Research Article

Metal concentrations in *Lactarius* mushroom species collected from Southern Spain and Northern Morocco: Evaluation of health risks and benefits

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ABSTRACT

In this work, the concentration of five non-essential (Cr, As, Cd, Pb and Hg) and three essential elements (Cu, Zn and Se) in five different species from the genus *Lactarius* collected from Southern Spain and Northern Morocco were determined by ICP-MS after a block acid digestion. Zn was found to be the major element in all the studied samples, followed by Cu and Cr in almost all the cases. The data obtained from the Health Risk Index (HRI) assessment suggested that As content in *L. semisanguifluus* and *L. deliciosus* collected from different locations in Granada and Cadiz provinces (Spain) may pose a risk to human health. The comparison between the Daily Intake of Metals (DIM) and the Recommended Dietary Allowance (RDA) indicated that the consumption of the studied mushrooms only covers a small percentage of the daily requirements of Cu, Zn and Se.

1. Introduction

The consumption of edible either cultivated or wild mushrooms in Spain, has increased considerably in recent years according to the Spanish Agency of Food and Nutrition (AESAN). This could be due to their beneficial properties, and particularly to their antioxidant and other nutritional characteristics, since they are an important source of phenolic compounds, vitamins, proteins, amino acids, and fiber (Lalotra et al., 2016; Ouzouni et al., 2009). Although mushrooms are considered as one of the main health-promoting foods, literature reveals that their fruiting bodies can assimilate and accumulate a number of heavy metals and metalloids, which are amongst the most hazardous and harmful pollutants for humans because of their persistence and ability to bioaccumulate throughout the food chain (Kalač, 2010; Malinowska et al., 2004; Mendil et al., 2005; Proskura et al., 2017).

In the context of nutrition, a healthy diet includes different amounts of dietary minerals and trace elements that are required for certain biochemical reactions and cellular functions. Unlike dietary minerals, some trace elements such as copper, selenium and zinc are needed in small doses for proper body functioning. However, a deficit or excess of specific dietary or trace elements may lead to acute or chronic disorders or diseases. On the other hand, heavy metals and metalloids such as chromium, mercury, cadmium or arsenic can also be supplied to the body through food. These non-essential elements have no biological function and can therefore be toxic even in low concentrations (Tchounwou et al., 2012).

Within this framework, the scientific community has made a great effort to inform on the content of heavy metals in different fungi species from different geographical locations. In fact, most of the articles published on this field of research have focused on samples from China, Poland and Turkey (Falandysz et al., 2017a,b; Gucia et al., 2012; Kojta and Falandysz, 2016; Liu et al., 2015; Mendil et al., 2005; Sarikurkcu et al., 2011; Türkmen and Budur, 2018; Zhang et al., 2008; Zhu et al., 2011). To mention some of them, Liu et al. (2015) studied the accumulation of eight heavy metals (Cu, Zn, Fe, Mn, Cd, Cr, Ni and Pb) in 14 species of wild mushrooms from China, including some of the highly valued ones, like *Pleurotus ostreatus* and *Agaricus bisporus.* On the other hand, Falandysz et al. (2002) studied the total mercury content in 13 species of wild mushrooms collected from Borecka Forest in Poland. *Lactarius deliciosus*, which is a culinary important fungus, is among the

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species studied.

Although Spain boasts a wide diversity of mushrooms and a growing consumption trend, the number of studies on this subject is very limited and most of them have focused on samples from the northern regions in the country despite the fact that mycological activities are steadily growing in its southern regions (Alonso et al., 2003; Campos and Tejera, 2011; Campos et al., 2009; Melgar et al., 2009, 2016). To date, there are few studies on the accumulation of heavy metals to cover southern Spain (Andalusia). For example, Ostos et al. (2015) studied the mercury content in 10 wild edible mushrooms from the South of Spain: namely, *Lactarius deliciosus, Macrolepiota procera, Morchella* spp., *Pleurotus eryngii, Terfezia arenaria, Agrocybe aegerita, Boletus aereus, Boletus aereus, Amanita caesarea,* and *Cantharellus cibarius*. On the other hand, Haro et al. (2020) analyzed the mineral composition (Ca, Mg, K, Na, Fe, Cu, and Zn) in 18 species of wild-growing, including *Lactarius deliciosus*, *Lactarius sanguifluus*, *Macrolepiota procera*, and *Pleurotus ostreatus*, species which are highly appreciated for culinary and commercial reasons. With much-acclaimed attention being paid to mushrooms in this area, there is a current need to study mineral contents in the most frequently consumed species among those that grow in southern Spain.

The mushrooms of the genus *Lactarius* are ectomycorrhizal fungi well known to mushroom lovers. Together with those of the genus *Russula*, the species of the genus *Lactarius* are part of the family Russulaceae. They are characterized by the presence of a milky fluid (latex) that is exuded when cut and because their flesh has a brittle consistency. In addition to these two characteristics, *Lactarius* species are flattened-convex cap mushrooms and have a variable coloring from white to red, including orange and ocher tones (Lee et al., 2019). Although it is true that within the genus *Lactarius* there are some species with a bad reputation because of their toxicity, such as *Lactarius torminosus,* there are some edible species which are highly valued in culinary markets. In Spain, *Lactarius deliciosus* is the most frequently collected and consumed wild edible mushroom. Nevertheless, there are other highly appreciated edible species such as *Lactarius vinosus, Lactarius rugatus, Lactarius sanguifluus* and *Lactarius semisanguifluus* that are increasingly consumed nationwide.

Therefore, the present study has focused on determining the content of eight heavy metals, five toxic elements (Hg, Pb, Cr, Cd, and As) and three essential elements (Zn, Cu, and Se) in five *Lactarius* mushroom species: *Lactarius deliciosus, Lactarius vinosus, Lactarius semisanguifluus, Lactarius sanguifluus,* and *Lactarius rugatus* collected from different locations in Southern Spain and Northern Morocco. This latter country has been selected because of its proximity to the Andalusian region and the growing Andalusian mycological tourism to different Moroccan regions. Furthermore, the different risks and benefits of the studied mushrooms were also evaluated to gain some knowledge and to value their quality as well as their toxicological aspects. In addition, metal accumulations in *Lactarius vinosus* and *Lactarius rugatus* have been studied.

2. Material and methods

2.1. Sampling

A total of eighteen samples of *Lactarius* mushrooms (Table 1) were collected in late fall and early winter from different locations in Southern Spain and Northern Morocco. The five *Lactarius* species studied have been identified by their morphological characteristics, which are specific and unmistakable for the varieties studied. The sampling area in Southern Spain included eleven spots of interest in the provinces of Cadiz, Granada and Malaga, whereas in Northern Morocco two areas located in the provinces of Tetouan and Chaouen were studied. The description of these sampling areas have been established depending on whether they are close or not to urban nucleus as indicated in Table 1. The mushrooms sampling consisted on collecting at least 10 specimens of each mushroom species from each location. This was considered as a representative pool of samples for each area. The samples were properly

Table 1
List of all mushroom samples from the studied areas along with their corresponding code, specie, specimens' number (n), collection location, sampling year, geographical coordinates, location and habitat description.

ID	Specie	Location/ sampling year	Latitude	Longitude	Location and habitat description
#1	*L. deliciosus* (*n* = 26)	Dchar Akjiouene (Chaouen, Morocco) 2017	35° 04′ 36.9″N	5° 13′ 49.5′ ’W	Close to the urban nucleus / Pines
#2	*L. deliciosus* (*n* = 20)	Alimadene (Tetouan, Morocco) 2017	35° 16′ 8.2″ N	5° 26′ 7.4″ W	Far from the urban nucleus / Pine forest
#3	*L. deliciosus* (*n* = 16)	Pinar de la Dehesa de las Yeguas (Cadiz, Spain) 2017	36° 33′ 7.0″ N	6° 7′ 50.9″ W	Close to the urban nucleus / Pines
#4	*L. deliciosus* (*n* = 18)	Pinar del Rey (Cadiz, Spain) 2017	36° 14′ 6.3″ N	5° 23′ 55.9″ W	Close to the urban nucleus / Pines
#5	*L. deliciosus* (*n* = 19)	Sendero El Palancar (Cadiz, Spain) / 2017	36° 14′ 50.5″ N	5° 33′ 39.9″ W	Far from the urban nucleus / Pine forest
#6	*L. deliciosus* (*n* = 14)	Jimena de la Frontera (Cadiz, Spain) 2017	36° 26′ 23.2″ N	5° 27′ 31.0″ W	Far from the urban nucleus / Pine forest
#7	*L. deliciosus* (*n* = 20)	Cortes de la Frontera (Malaga, Spain) 2018	36° 34′ 13.3″ N	5° 24′ 3.7″ W	Far from the urban nucleus / Pine forest
#8	*L. deliciosus* (*n* = 22)	Fuente del Espino (Granada, Spain) 2018	37º 4′ 9.4″ N	3° 5′ 26.6″ W	Far from the urban nucleus / Pine forest
#9	*L. deliciosus* (*n* = 12)	Puerto Real (Cadiz, Spain) 2018	36° 31′ 21.7″ N	6° 7′ 40.7″ W	Close to the urban nucleus / Pines
#10	*L. vinosus* (*n* = 13)	Pinar El Colorado (Cadiz, Spain) 2017	36° 20′ 13.9″ N	6° 5′ 45.9″ W	Close to the urban nucleus / Pines
#11	*L. vinosus* (*n* = 19)	Sendero El Palancar (Cadiz, Spain) 2017	36° 14′ 51.5″ N	5° 33′ 37.9″ W	Far from the urban nucleus / Pine forest
#12	*L. vinosus* (*n* = 12)	Puerto Real (Cadiz, Spain) 2018	36° 31′ 21.7″ N	6° 7′ 40.7″ W	Close to the urban nucleus / Pines
#13	*L. vinosus* (*n* = 11)	Pinar de la Dehesa de las Yeguas (Cadiz, Spain) 2017	36° 33′ 22.4″ N	6° 7′ 59.5″ W	Close to the urban nucleus / Pines
#14	*L. semisanguifluus* (*n* = 16)	Puerto de la Mora (Granada, Spain) 2018	37° 16′ 58.9″ N	3° 27′ 36.6″ W	Far from the urban nucleus / Pine forest
#15	*L. semisanguifluus* (*n* = 19)	Sierra de Huetor (Granada, Spain) 2018	37° 16′ 45.6″ N	3° 26′ 21.9″ W	Far from the urban nucleus / Pine forest
#16	*L. rugatus* (*n* = 12)	Cortes de la Frontera (Malaga, Spain) 2018	36° 33′ 58″ N	5° 24′ 28.5″ W	Far from the urban nucleus / Pine forest
#17	*L. sanguifluus* (*n* = 21)	Sierra de Huetor	37° 17′ 19.2″ N	3° 27′ 24.9″ W	Far from the urban

(*continued on next page*)

Table 1 (*continued*)

ID	Specie	Location/ sampling year	Latitude	Longitude	Location and habitat description
		(Granada, Spain) 2018			nucleus / Pine forest
#18	*L. sanguifluus* (*n* = 16)	Cortes de la Frontera (Malaga, Spain) 2018	36° 34′ 12.5″ N	5° 24′ 4.4″ W	Far from the urban nucleus / Pine forest

prepared for their analyses. The specimens were washed with tap-water and then with deionized water to remove any soil traces. Then, they were dried at 50 °C for 48 h. Finally, the dried mushrooms were homogenized and powdered by means of an agate mortar and stored in polyethylene (PE) bottles.

2.2. Chemicals and reagents

The highest mineral, acids and oxidants standards were purchased from SCP Science (Montreal, Quebec, Canada): HCl PlasmaPURE (34–37 %), HNO_3 PlasmaPURE (67–69 %) and from Sigma-Aldrich (St. Louis, MO, USA): H_2O_2 ($\geq$ 30 %). All the solutions were prepared using nanopure water obtained by passing twice-distilled water through a Milli-Q system (18 MΩ/cm, Millipore, Bedford, MA, USA).

2.3. Digestion procedure

The subsamples of dried and powdered mushrooms (0.25 g) were placed in the digestion tubes (DigiTUBES, SCP Science) with 2 mL nanopure water, 5 mL HNO_3, and 2 mL HCl. Then, they were digested employing a DigiPREP Jr block digestion system from SCP Science with 24 positions (Montreal, Quebec, Canada). A gradually rising temperature procedure was applied to reach a final temperature of 110 °C (140 min run). After a cooling step, 3 mL of H_2O_2 was added, followed by a second digestion process under the same heating conditions. Prior to their analysis, the mushroom digested samples were filtered through a 0.45 μm filter and then transferred into a 50 mL volumetric DigiTube, which was made up to 50 mL with nanopure water. All the samples were prepared in triplicate.

2.4. Elemental analysis

The heavy metals content in the mushrooms was determined by means of an Inductively Coupled Plasma-Mass Spectrometer (Thermo X Series II ICP-MS, Waltham, MA, USA) equipped with cyclonic spry chamber, concentric nebulizer, collision/reaction cell and quadruple mass analyzer. During the analyses, Xt interface, CCT H_2 (7%)/He and Kinetic Energy Discrimination (KED) were applied. The ICP-MS instrumental parameters set for the elemental determinations were: RF power: 1400 W; sampling depth: 80.0 mm; auxiliary Ar flow rate: 1.0 L min^{-1}; nebulizer Ar flow rate: 1.0 L min^{-1}; plasma Ar flow rate: 14.0 L·min^{-1}; CCT H_2 (7%)/He): 4.5 mL min^{-1}; hexapole bias voltage: –20.0 V; pole bias voltage: –17.0 V. An internal standard of ^{45}Sc, ^{72}Ge, ^{103}Rh, ^{191}Ir, and ^{209}Bi prepared from individual solutions containing 1000 μg mL^{-1} (SCP Science, Montreal, Quebec, Canada) were used to correct the temporary variations of the signal intensity.

2.5. Quality control

The measurements of the heavy metals content were validated and controlled by calibrating the ICP-MS with standard solutions. The accuracy of the analytical method was evaluated based on the analysis of triplicates, blanks and a Certified Reference Material (CRM): *Boletus edulis* powder Control Material CS-M-3, Institute of Nuclear Technology and Chemistry in Warsaw, ICHTJ, Poland. Recovery levels at 70–130 % of the certified reference material (CS-M-3) was considered as acceptable for most of the elements to be determined. The Limit of Detection (LOD) values for the elements of interest expressed as mg kg^{-1} (dw) were as follows: 0.416 for Cr, 0.076 for As, 0.041 for Cd, 0.132 for Hg, 0.020 for Pb, 0.412 for Cu, 2.020 for Zn, and 0.378 for Se.

2.6. Data analysis and software

RStudio software (R version 4.0.0, Boston, MA, USA) was used for the statistical evaluation and multivariate analysis of the data. The heatmap.2 function from Ggplots package was used for performing the non-supervised chemometric tool, Hierarchical Cluster Analysis (HCA). For the statistical analysis, the normal distribution of the quantitative variables was verified by means of Shapiro-Wilk tests. Levene's test was used to determine the homogeneity of the variance of the groups. The data obtained were normally distributed ($p > 0.05$) for Cr, Zn, and Se according to mushrooms species and, in all the cases, the variances were equal ($p > 0.05$). Some relevant variations between mushrooms element concentrations were detected by One-way ANOVA using Tukey's test at 5% significance level. Kruskal–Wallis tests followed by Tukey's range test at 5% significance level were used to compare the content of the elements that were not normally distributed. Statistically significant differences ($p < 0.05$) were only found between Se contents in *L. rugatus* and *L. semisanguifluus*.

2.7. Daily Intake of Metals (DIM)

The Daily Intake of Metals (DIM) was calculated according to the following expression (Igbiri et al., 2018):

$$DIM = \frac{C_{metal} \cdot D_{food\ intake}}{BW} \quad (1)$$

where C_{metal} is the metal concentration in a mushroom sample expressed in mg kg^{-1}, $D_{food\ intake}$ is the mushroom daily intake in kg and BW is the average $person^{-1}$ body weight also in kg. For our study, a serving of 300 g of fresh mushrooms (30 g of dried mushrooms per day) and a regular 70 kg body weight consumer were considered in accordance with the proposal by Liu et al. (2015) as well as by Sarikurkcu et al. (2020).

2.8. Health risk index (HRI)

In order to assess the risk to human health as a consequence of exposure to heavy metals through the intake of wild edible mushrooms, the Health Risk Index (HRI) was calculated according to the following equation (Sarikurkcu et al., 2020):

$$HRI = DIM \Big/ R_fD \quad (2)$$

where DIM is the daily intake of metals through the consumption of the wild edible mushrooms in our study and R_fD is the maximum acceptable daily oral dose of a toxic substance. According to the data from Integrated Risk Information System (IRIS) and the data proposed by Sarikurkcu et al. (2020), the reference doses for Cr, As, Cd, Pb, Hg, Cu, Zn, and Se would be 3, 0.3, 1, 3.5, 0.3, 40, 300, and 0.5 μg/kg body weight/day, respectively.

3. Results and discussion

The results on elemental contents are shown in Table 2 expressed in mg kg^{-1} dry weight (dw). All the studied metals and metalloids included in our study were detected in all the mushroom samples except for As and Hg, which were below the limit of detection (LOD) in 6 and 7 of the samples, respectively. Overall, the mushrooms collected from the different sampling locations in Granada (Spain) were those with the highest content in toxic metals. The major element found in the five studied species was Zn, followed by Cu and Cr in most of the cases. The

Table 2
Non-essential and essential trace minerals concentration of *Lactarius* mushrooms samples (mg·kg^{-1} dry weight). Values are presented as mean ± standard deviation (*n* = 3).

ID	Cr	As	Cd	Pb	Hg	Cu	Zn	Se
#1	7.70 ± 1.38	< 0.08	0.11 ± 0.004	0.20 ± 0.01	< 0.13	3.94 ± 0.04	61.7 ± 0.50	0.94 ± 0.01
#2	2.10 ± 0.05	0.37 ± 0.03	0.62 ± 0.02	0.57 ± 0.002	0.19 ± 0.01	6.44 ± 0.12	125.2 ± 1.00	0.92 ± 0.08
#3	3.40 ± 0.07	0.35 ± 0.02	0.51 ± 0.02	0.19 ± 0.002	< 0. 13	9.45 ± 0.19	101.2 ± 1.00	0.84 ± 0.07
#4	4.14 ± 0.17	< 0.08	0.20 ± 0.03	0.15 ± 0.003	0.23 ± 0.04	8.13 ± 0.37	101.7 ± 7.63	1.12 ± 0.03
#5	0.94 ± 0.03	1.20 ± 0.05	0.32 ± 0.03	0.07 ± 0.0001	0.24 ± 0.002	13.0 ± 0.24	132.1 ± 4.03	1.58 ± 0.18
#6	3.15 ± 0.57	< 0.08	0.32 ± 0.01	0.15 ± 0.04	< 0.13	9.09 ± 0.96	90.5 ± 0.93	1.08 ± 0.09
#7	2.15 ± 0.21	0.43 ± 0.02	0.23 ± 0.01	0.18 ± 0.0001	0.24 ± 0.01	9.36 ± 0.22	121.1 ± 3.30	1.20 ± 0.05
#8	9.30 ± 0.79	1.33 ± 0.12	0.32 ± 0.01	0.69 ± 0.05	0.44 ± 0.02	7.19 ± 0.39	111.2 ± 3.63	1.04 ± 0.08
#9	1.70 ± 0.04	0.37 ± 0.01	1.01 ± 0.03	0.37 ± 0.01	0.23 ± 0.01	10.7 ± 0.14	147.5 ± 4.75	0.68 ± 0.07
#10	6.16 ± 0.10	0.48 ± 0.04	0.40 ± 0.03	0.31 ± 0.01	< 0.13	5.07 ± 0.09	48.7 ± 0.19	2.01 ± 0.13
#11	1.60 ± 0.04	< 0.08	0.15 ± 0.01	0.11 ± 0.005	< 0.13	6.35 ± 0.04	57.8 ± 0.44	1.27 ± 0.19
#12	1.15 ± 0.11	0.85 ± 0.02	0.46 ± 0.002	0.11 ± 0.04	0.24 ± 0.003	8.98 ± 0.11	111.2 ± 0.75	1.37 ± 0.01
#13	2.44 ± 0.12	0.57 ± 0.07	0.28 ± 0.02	0.19 ± 0.01	< 0.13	8.12 ± 0.40	77.6 ± 5.17	1.04 ± 0.09
#14	7.32 ± 2.32	5.62 ± 0.05	1.24 ± 0.02	0.71 ± 0.01	0.42 ± 0.002	6.15 ± 0.36	58.4 ± 1.53	2.00 ± 0.02
#15	5.89 ± 0.48	3.58 ± 0.03	0.78 ± 0.01	0.90 ± 0.005	0.38 ± 0.01	5.78 ± 0.20	54.9 ± 0.34	2.01 ± 0.01
#16	3.00 ± 0.07	< 0.08	2.05 ± 0.06	0.12 ± 0.002	0.20 ± 0.003	43.7 ± 1.19	86.5 ± 2.12	0.46 ± 0.002
#17	5.50 ± 0.18	0.92 ± 0.05	1.27 ± 0.02	1.31 ± 0.06	0.42 ± 0.01	6.80 ± 0.13	64.1 ± 0.76	2.31 ± 0.07
#18	0.95 ± 0.02	< 0.08	0.20 ± 0.01	0.34 ± 0.01	< 0.13	3.87 ± 0.14	99.0 ± 3.61	0.74 ± 0.02

rest of the elements were detected in significantly lower concentrations. These results are in line with previous studies where *Lactarius* species and particularly *L. deliciosus* showed a trend to accumulate a high content of zinc, which is especially interesting from a nutritional point of view (Alonso et al., 2003; Aloupi et al., 2012; Kosanić et al., 2016). The greatest accumulation of zinc in these species of the genus *Lactarius* could be due to their ectomycorrhizal nature as well as to their genetic adaptation. Mycorrhizae is a symbiotic association between plant roots and fungal mycelium that plays an important role in nutrient absorption (Ruytinx et al., 2020). For instance, Adriaensen et al. (2004) noted that in the forests of the Northern Hemisphere the absorption of zinc by some trees, such as *Pinus sylvestris*, is largely modulated by the presence of ectomycorrhizal fungi.

3.1. Non-essential trace minerals

In this study, the highest concentration of chromium was measured in sample #8: *L. deliciosus* (9.30 ± 0.79 mg kg^{-1}) from Fuente del Espino (Granada, Spain), while the lowest concentrations were observed in #5: *L. deliciosus* (0.94 ± 0.03 mg kg^{-1}) from Sendero del Palancar (Cadiz, Spain). It has been found that the chromium concentrations reported by other authors were in the ranges 0.04–13.2 mg kg^{-1} in *L. deliciosus* (Aloupi et al., 2012; Campos and Tejera, 2011; Kosanić et al., 2016; Yamaç et al., 2007), 0.28–13.2 mg kg^{-1} in *L. sanguifluus* (Aloupi et al., 2012; Campos and Tejera, 2011) and in concentrations around 2.03 mg kg^{-1} in *L. semisanguifluus* (Aloupi et al., 2012). Therefore, the chromium content levels determined in our study for these species are in agreement with the above-mentioned studies.

The highest/lowest concentrations of arsenic were observed in sample #14: *L. semisanguifluus* (5.62 ± 0.05 mg kg^{-1}) from Puerto de la Mora (Granada, Spain) and #3: *L. deliciosus* (0.352 ± 0.0202 mg kg^{-1}) from Pinar de la Dehesa de las Yeguas (Cadiz, Spain), respectively. It was observed that arsenic contents for *L. deliciosus* in literature were in the range 0.09–2.99 mg kg^{-1} (Melgar et al., 2014; Pelkonen et al., 2006; Vetter, 2004; Xu et al., 2019). Thus, the arsenic contents detected in our study for *L. deliciosus* were similar to those reported previously. On the other hand, it is noteworthy that no information has been found on the accumulation of arsenic by *L. semisanguifluus* and *L. sanguifluus*. Therefore, we compared the results obtained for these two species with the literature concentrations of arsenic in *L. deliciosus*. In general, the arsenic concentrations for these species agree with the previously published research. With the exception of samples #14: *L. semisanguifluus* (5.62 ± 0.05 mg kg^{-1}) from Puerto de la Mora (Granada, Spain) and # *L. semisanguifluus* (3.58 ± 0.03 mg kg^{-1}) from Sierra de Huetor (Granada, Spain), whose arsenic concentrations are higher than those observed in the literature.

Regarding cadmium, the lowest concentrations of this metal were found in sample #1: *L. deliciosus* (0.11 ± 0.004 mg kg^{-1}) from Dchar Akjiouene (Chaouen, Morocco). By contrast, the highest levels were found in sample #16: *L. rugatus* (2.05 ± 0.06 mg kg^{-1}) from Cortes de la Frontera (Malaga, Spain). Cadmium concentrations reported in the literature were in the range 0.15–2.94 mg kg^{-1} for *L. deliciosus* (Aloupi et al., 2012; Kosanić et al., 2016; Melgar et al., 2016; Pelkonen et al., 2006; Yamaç et al., 2007) and at concentrations around 0.21 mg kg^{-1} for *L. sanguifluus* and 0.16 mg kg^{-1} for *L. semisanguifluus* (Aloupi et al., 2012). The cadmium content levels determined in our study were in agreement with those previously reported for *L. deliciosus,* while they were higher in the case of *L. sanguifluus* and *L. semisanguifluus.*

The highest and lowest concentrations of lead were respectively observed in sample #17: *L. sanguifluus* (1.31 ± 0.06 mg kg^{-1}) from Sierra de Huetor (Granada, Spain) and #5: *L. deliciosus* (0.073 ± 0.0001) from Sendero El Palancar (Cadiz, Spain). Lead contents reported by other studies were in the range 0.126–3.6 mg kg^{-1} for *L. deliciosus* (Aloupi et al., 2012; Campos and Tejera, 2011; Yamaç et al., 2007), and 0.08–4.20 mg kg^{-1} for *L. sanguifluus* (Aloupi et al., 2012; Campos and Tejera, 2011), while concentrations around 0.097 mg kg^{-1} were detected in *L. semisanguifluus* (Aloupi et al., 2012). When compared to the data reported by previously published studies, the lead concentrations registered in our work were lower in the case of *L. sanguifluus*, higher for *L. semisanguifluus* and similar to those previous studies when referred to *L. deliciosus*.

The highest amount of mercury was determined in sample #8: *L. deliciosus* (0.44 ± 0.02 mg kg^{-1}) from Fuente del Espino (Granada, Spain), while the lowest levels were found in sample #2: *L. deliciosus* (0.19 ± 0.01 mg kg^{-1}) from Alimadene (Tetouan, Morocco). With regard to *L. deliciosus,* the content varied from range 0.28 – 0.80 (stem) to 0.39–1.60 (cap) mg kg^{-1} (Alonso et al., 2000; Falandysz et al., 2002, 2003; Melgar et al., 2009; Ostos et al., 2015). However, our results concerning mercury content in *L. deliciosus* are in accordance with those reported in the literature. Remarkably, there is no information on the accumulation of mercury by *L. semisanguifluus* and *L. sanguifluus*. Therefore, we compared the results obtained for these two species with the literature concentrations of mercury in *L. deliciosus*. We observed that our mercury concentrations for these species agree with the previous studies.

The concentration of toxic elements in *L. rugatus* and *L. vinosus* were: 3.00 mg kg^{-1} Cr, < 0.08 mg kg^{-1} As, 2.05 mg kg^{-1} Cd, 0.12 mg kg^{-1} Pb, and 0.20 mg kg^{-1} Hg in the former, while the concentration ranges corresponding to the latter were: 1.15–6.16 mg kg^{-1} Cr, 0.48 – 0.85 mg kg^{-1} As, 0.15 – 0.46 mg kg^{-1}Cd, 0.11 – 0.31 mg kg^{-1} Pb, and 0.24 mg

kg^{-1} Hg. It should be highlighted that, to the date, there is no information regarding the content of these toxic elements in both *L. rugatus* and *L. vinosus*. Nonetheless, we have compared our non-essential trace minerals for these two species with the values reported in the literature for other edible species of the same genus (Aloupi et al., 2012; Campos and Tejera, 2011; Kosanić et al., 2016; Pelkonen et al., 2006; Vetter, 2004; Xu et al., 2019; Yamaç et al., 2007). Our results of toxic elements concentrations for *L. vinosus* and *L. rugatus* are in concordance with the literature.

3.2. Essential trace minerals

The lowest copper concentrations were found in sample #18: *L. sanguifluus* (3.90 ± 0.14 mg kg^{-1}) from Cortes de la Frontera (Malaga, Spain), whereas the highest concentrations were reported in sample #16: *L. rugatus* (43.7 ± 1.19 mg kg^{-1}) from Cortes de la Frontera (Malaga, Spain). The copper concentrations reported by previous studies were between 5.40 and 15.5 mg kg^{-1} in *L. deliciosus* (Alonso et al., 2003; Aloupi et al., 2012; Campos and Tejera, 2011; Kosanić et al., 2016; Yamaç et al., 2007), and between 4.20 and 7.00 mg kg^{-1} in *L. sanguifluus* (Aloupi et al., 2012; Campos and Tejera, 2011) with concentrations around 9.30 mg kg^{-1} in *L. semisanguifluus* (Aloupi et al., 2012). In general, the levels of copper concentrations determined by our analyses were similar to those indicated by previous studies with regard to *L. sanguifluus, L. semisanguifluus* and *L. deliciosus* copper contents.

The highest and lowest levels of zinc were measured in sample #9: *L. deliciosus* (147.5 ± 4.75 mg kg^{-1}) from Puerto Real (Cadiz, Spain) and #10: *L. vinosus* (48.7 ± 0.20 mg kg^{-1}) from Pinar El Colorado (Cadiz, Spain), respectively. Several researchers have reported that zinc concentrations were in the ranges 66.8–124 mg kg^{-1} in *L. deliciosus* (Aloupi et al., 2012; Campos and Tejera, 2011; Kosanić et al., 2016; Yamaç et al., 2007), 75.0–103 mg kg^{-1} in *L. sanguifluus* (Aloupi et al., 2012; Campos and Tejera, 2011) and at concentrations around 70.4 mg kg^{-1} in *L. semisanguifluus* (Aloupi et al., 2012). The results obtained from our analyses for zinc concentrations were higher in *L. deliciosus* and lower in *L. semisanguifluus* and *L. sanguifluus* in comparison with those reported by previous studies.

The highest selenium content was registered in sample #17: *L. sanguifluus* (2.31 ± 0.07 mg kg^{-1}) from Sierra de Huetor (Granada, Spain). On the contrary, the lowest levels were shown by sample #16: *L. rugatus* (0.46 ± 0.002 mg kg^{-1}) from Cortes de la Frontera (Malaga, Spain). Different studies have reported that selenium content in *L. deliciosus* was in the range 0.18–1.30 mg kg^{-1} (Falandysz, 2008; Stijve, 1977). The selenium concentrations determined in our study for *L. deliciosus* agreed with those previously reported. It is important to note that no studies have been found that determine the content of selenium in *L. semisanguifluus* and *L. sangifluus*. Therefore, our results regarding selenium for these two species have been compared with literature selenium concentrations for *L. deliciosus*. We have observed that selenium content found in #14: *L. semisanguifluus* (2.00 ± 0.02 mg kg^{-1}) from Puerto de la Mora (Granada, Spain), #15 *L. semisanguifluus* (2.01 ± 0.01 mg kg^{-1}) from Sierra de Huetor (Granada, Spain), and #17 *L. sanguifluus* (2.31 ± 0.07 mg kg^{-1}) from Sierra de Huetor (Granada, Spain), are higher than those observed in the literature.

The measured concentrations of the essential elements in *L. rugatus* remained in 43.7 mg kg^{-1} Cu, 86.5 mg kg^{-1} Zn and 0.46 mg kg^{-1} Se. Furthermore, the concentration levels of the essential elements in *L. vinosus* were found to be in the ranges: 5.07–8.98 mg kg^{-1} Cu, 46.7–111.2 mg kg^{-1} Zn, and 1.04–2.01 mg kg^{-1} Se. As aforementioned, it should be highlighted that there is no information regarding the content of these essential trace elements in both *L. rugatus* and *L. vinosus*. Thus, we have compared our data for these two species with the values reported in the literature for other edible species of the same genus (Aloupi et al., 2012; Campos and Tejera, 2011; Falandysz, 2008; Kosanić et al., 2016; Stijve, 1977; Yamaç et al., 2007). Summarizing, our results of essential trace elements concentrations for *L. vinosus* and *L. rugatus* agree with those reported in the literature.

3.3. Chemometrics

A non-supervised method was applied to determine mushroom samples' grouping trends according to their Cr, As, Cd, Pb, Hg, Cu, Zn and Se content. Prior to the chemometric study, the data matrix was normalized to its maximum. A Hierarchical Cluster Analysis (HCA) was performed according to Ward's method using Euclidean distance measure. The results were graphically represented in Fig. 1 heatmap. The color key intends to represent concentration levels in an intuitive manner, where the darker tones of purple or blue indicate a lower content of a particular heavy metal in the mushroom sample and the gradual changes into green or yellow denote concentration increments.

The HCA revealed that the mushroom samples tended to group into 4 principal clusters (A, B, C and D) depending on the concentration of heavy metals and metalloids in their fruiting bodies. Cluster A included all the mushroom species (*L. semisanguifluus, L. sanguifluus* and *L. deliciosus*) collected from the different locations in Granada (Spain), which were clearly separated from the rest based on their highest content in Hg, Pb and Cr. Two subclusters could be distinguished within cluster A, the first one included *L. deliciosus* and the second one was formed by *L. sanguifluus* and *L. semisanguifluus* samples. Nevertheless, the latter two species seem to be more closely correlated in terms of heavy metal content. On one hand, cluster C was exclusively formed by *L. rugatus* from Malaga (Spain), which was distinguished from the other studied mushrooms for having the highest Cu and Cd contents. This was in agreement with our expectations, since *L. rugatus* differs from the other four studied species in respect of its macroscopic characteristics (cap, gills, stem, and latex). From a morphological point of view, *L. deliciosus, L. vinosus, L. sanguifluus,* and *L. semisanguifluus* are closely related species and, therefore, share a large number of features. On the other hand, Clusters B and D could be differentiated based on the high Zn and Hg content in D's samples. These two clusters included *L. semisanguifluus, L. sanguifluus, L.vinosus,* and *L. deliciosus* specimens collected from the different locations in Morocco, Cadiz (Spain) and Malaga (Spain). The results were completely in accordance with our expectations based on the similar soil composition at Cadiz, Morocco and Malaga sampled areas. The results of the cluster analysis have allowed us to observe that both, mushroom species and sampling area, are factors that seem to affect the accumulation of non-essential and essential trace elements. Nevertheless, this clustering was not completely consistent since there is not a perfect classification.

3.4. Health risks assessment

In order to evaluate the risks derived from consuming the studied mushrooms, the Estimated Daily Intake (DIM) was calculated for Cr, As, Cd, Hg, Pb, Cu, Zn, and Se. A serving of 30 g of dried mushrooms per day and a regular 70 kg body weight consumer (Table 3) were considered. The DIM results were compared with the Provisional Tolerable Daily Intake (PTDI) and the Provisional Maximum Tolerable Daily Intake (PMTDI) established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The results obtained in the present study show that the highest DIM corresponded to Zn and, specifically to sample #9: *L. deliciosus* sample from Puerto Real (Cadiz, Spain; 63.2 μg/kg body weight/ serving). On the other hand, the DIM calculated for Hg, Cu and Zn were below the PTDI and PMTDI in all the collected mushrooms. In contrast, the DIM for As in sample #14: *L. semisanguifluus* from Puerto de la Mora (Granada, Spain; 2.41 μg/kg body weight/ serving) was found to be above the established PTDI for As (2.14 μg/kg body weight/day). Similarly, a DIM above the established PTDI for Cd (0.82 μg/kg body weight/day) was observed in sample #16: *L. rugatus* from Cortes de la Frontera (Cadiz, Spain; 0.88 body weight/ serving). Regarding Cr, Pb, and Se, the JCEFA does not established a PTDI neither a PMTDI. Nevertheless, by comparing the DIM with the R_fD established by the for

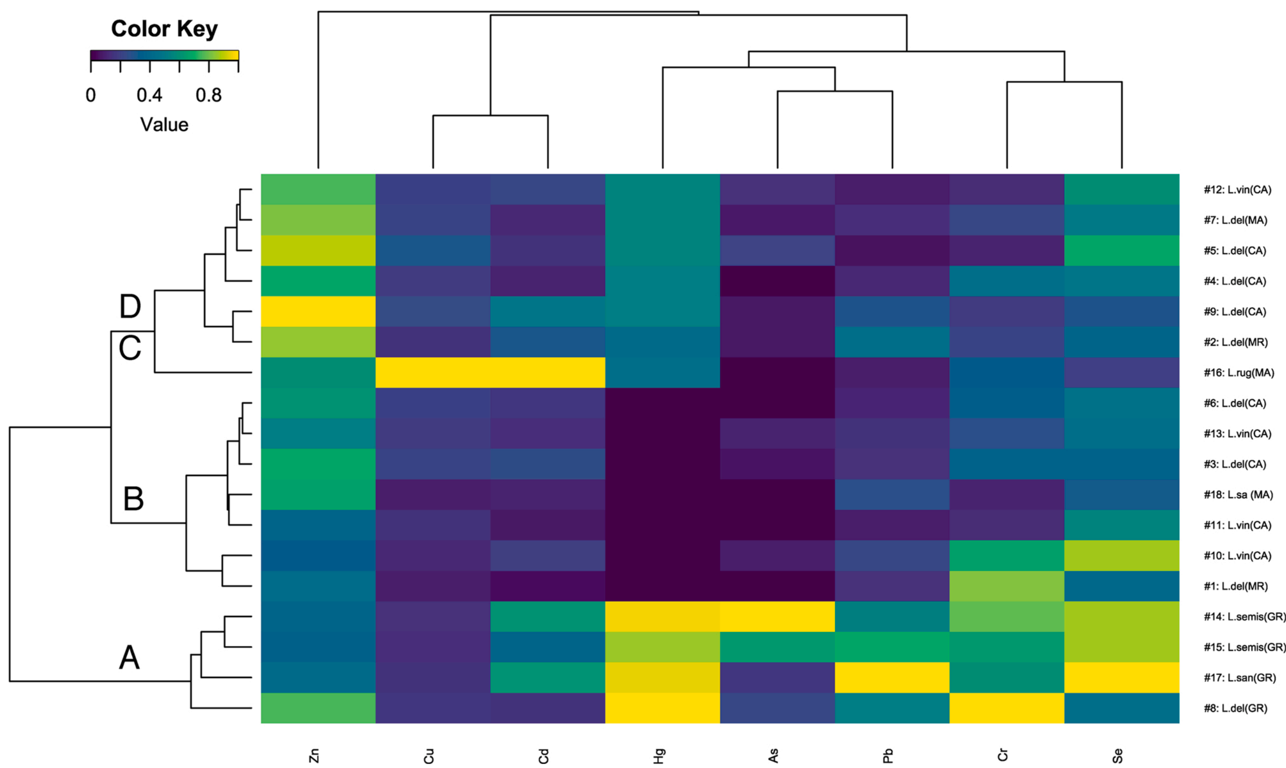

Fig. 1. Heatmap of the non-essential and essential trace elements concentrations (mean values) in the mushroom samples with presentation of a hierarchical tree plot.

these metals (Cr – 3.00 μg/kg body weight/day, Pb – 3.50 μg/kg body weight/day and Se – 0.50 μg/kg body weight/day) we found that Pb DIM for a 70 kg body weight consumer was lower than R_fD in all the collected mushrooms. On the other hand, the DIM for Cr in sample #1: *L. deliciosus* from Dchar Akjiouene (Chaouen, Morocco; 3.30 μg/kg body weight/ serving), #8: *L. deliciosus* from Fuente del Espino (Granada, Spain; 3.98 μg/kg body weight/ serving) and #14: *L. semisanguifluus* from Puerto de la Mora (Granada, Spain; 3.14 μg/kg body weight/ serving), exceeded the established R_fD. In the same way, the DIM for Se was observed to be above the R_fD (Table 3) in sample #5: *L. deliciosus* from Sendero El Palancar (Cadiz, Spain), #10: *L. vinosus* from Pinar El Colorado (Cadiz, Spain), #11: *L. vinosus* from Sendero del Palancar (Cadiz, Spain), #12: *L. vinosus* from Puerto Real (Cadiz, Spain), #14: *L. semisanguifluus* from Puerto de la Mora (Granada, Spain), #15: *L. semisanguifluus* from Sierra de Huetor (Granada, Spain), and #17: *L. sanguifluus* from Sierra de Huetor.

Moreover, the health risks evaluation was carried out by calculating the Health Risk Index (HRI) as the ratio between the Daily Intake of Metal (DIM) and the Reference Dose (R_fD) (Table 3). Mushrooms are considered to be safe for human consumption when their index is below or equal to 1 (Liu et al., 2015). According to the HRI results, which have been included in Table 3 for Cr, As, Cd, Pb, Hg, Cu, Zn, and Se, most of the species studied in this work do not pose a health risk to human consumers as a consequence of their heavy metal contents (HRI ≤ 1 or ~ 1). However, regarding As, the *L. semisanguifluus* specimens collected from Sierra de Huetor and Puerto de la Mora (Granada, Spain) registered 5.11 and 8.03 As HRI values, respectively. In the same way, the *L. deliciosus* samples collected from Sendero del Palancar (Cadiz, Spain) and Fuente del Espino (Granada, Spain) presented As HIR values above 1. Arsenic (As) is a common metalloid in nature, and it is one of the elements that raise toxicological concerns. Currently, it is classified as Group 1 of carcinogens (i.e., carcinogenic to humans) (ASTDR, 2007). The toxicity of As indeed depends on its chemical form and oxidation state (speciation). In general, inorganic arsenic is toxic in both its arsenite and arsenate form. Contrarily, organic arsenic compounds are less harmful. Even some highly methylated forms, such as arsenobetaine and arsenocholine, are considered not toxic. Notwithstanding, they cannot be expected fully safe for humans (Melgar et al., 2014; Zhang et al., 2020). Generally, most studies that determine arsenic in mushrooms are based on the total arsenic content due to the difficulty of distinguishing analytically between different forms of this metalloid (Cocchi et al., 2006; Falandysz, Drewnowska, et al. 2017; Melgar et al., 2014). Thus, the data presented in this study refer to the total arsenic content in the studied mushrooms. Regarding to the HRI obtained, the consumption of these particular mushroom species from the above-mentioned geographical locations could pose a risk to human health because of the exceeding exposure to As.

Se HRI for the *L. semisanguifluus* mushrooms collected from Sierra de Huetor and Puerto de la Mora (Granada, Spain) exceeded the reference value. Similarly, an HRI above 1 was also registered for *L. vinosus* mushrooms from Pinar del Colorado (Cadiz, Spain) as well as for the *L. sanguifluus* specimens from Sierra de Huetor (Granada, Spain). Selenium (Se) is an essential trace element, necessary for the proper functioning of the body. In general, Se toxicity due to overdose is rare, especially from dietary sources. Nevertheless, the consumption of higher than recommended Se doses could aid in prostate cancer prevention. Furthermore, Se can bind with toxic elements such as mercury and cadmium, preventing their toxicity (Falandysz, 2008; Mirończuk-Chodakowska et al., 2019). Therefore, there is no evidence that the consumption of the particular mushrooms from the above-mentioned geographical areas can pose a health risk in terms of selenium concentrations.

3.5. Health benefits assessment

In order to evaluate the benefits derived from the consumption of *Lactarius* mushrooms collected from southern Spain and northern Morocco, the DIM values obtained for Cu, Zn, and Se (Table 3) were compared with the Recommended Dietary Allowance (RDA) for adult men and women. The RDAs established for Cu, Zn, and Se are 900 μg/

Table 3
Daily intakes of metal (DIM) and health risk indexes (HRI) in *Lactarius* samples.

		Daily intakes of metals (DIM, μg/kg body weight/day)								Health risk index							
ID	Specie / Location	Cr	As	Cd	Pb	Hg	Cu	Zn	Se	Cr	As	Cd	Pb	Hg	Cu	Zn	Se
#1	*L. deliciosus* / Dchar Akjiouene	3.30	–	0.05	0.08	–	1.69	26.5	0.40	1.10	–	0.05	0.02	–	0.04	0.09	0.81
#2	*L. deliciosus* / Alimadene	0.89	0.16	0.27	0.25	0.08	2.76	53.7	0.40	0.30	0.53	0.27	0.07	0.27	0.07	0.18	0.79
#3	*L. delicious* / Pinar de la Dehesa de las Yeguas	1.45	0.15	0.22	0.08	–	4.05	43.4	0.36	0.48	0.50	0.22	0.02	–	0.10	0.14	0.72
#4	*L. delicious* / Pinar del Rey	1.77	–	0.08	0.06	0.10	3.48	43.6	0.48	0.59	–	0.08	0.02	0.33	0.09	0.15	0.96
#5	*L. delicious* / Sendero El Palancar	0.40	0.51	0.14	0.03	0.10	5.58	56.6	0.68	0.13	1.68	0.14	0.01	0.34	0.14	0.19	1.35
#6	*L. delicious* / Jimena de la Frontera	1.35	–	0.14	0.06	–	3.90	38.8	0.46	0.45	–	0.14	0.02	–	0.10	0.13	0.92
#7	*L. delicious* / Cortes de la Frontera	0.92	0.19	0.10	0.08	0.10	4.17	51.9	0.51	0.31	0.62	0.10	0.02	0.35	0.10	0.17	1.03
#8	*L. delicious* / Fuente del Espino	3.98	0.57	0.14	0.29	0.19	3.08	47.7	0.44	1.33	1.90	0.14	0.08	0.62	0.08	0.16	0.89
#9	*L. delicious* / Puerto Real	0.71	0.16	0.43	0.16	0.10	4.60	63.2	0.29	0.24	0.53	0.43	0.05	0.33	0.11	0.21	0.59
#10	*L. vinosus* / Pinar El Colorado	2.64	0.21	0.17	0.13	–	2.17	20.9	0.86	0.88	0.68	0.17	0.04	–	0.05	0.07	1.72
#11	*L. vinosus* / Sendero El Palancar	0.50	–	0.06	0.05	–	2.72	24.8	0.54	0.17	–	0.06	0.01	–	0.07	0.08	1.09
#12	*L. vinosus* / Puerto Real	0.49	0.37	0.20	0.05	0.10	3.85	47.7	0.59	0.16	1.22	0.20	0.01	0.34	0.10	0.16	1.18
#13	*L. vinosus* / Pinar de la Dehesa de las Yeguas	1.04	0.24	0.12	0.08	–	3.48	33.6	0.45	0.35	0.81	0.12	0.02	–	0.09	0.11	0.89
#14	*L. semisanguifluus* / Puerto de la Mora	3.14	2.41	0.53	0.30	0.18	2.64	25.0	0.86	1.05	8.03	0.53	0.09	0.60	0.07	0.08	1.72
#15	*L. semisanguifluus* / Sierra de Huetor	2.52	1.53	0.34	0.39	0.16	2.48	23.5	0.86	0.84	5.11	0.34	0.11	0.54	0.06	0.08	1.72
#16	*L. rugatus* / Cortes de la Frontera	1.29	–	0.88	0.05	0.08	18.7	37.1	0.20	0.43	–	0.88	0.01	0.28	0.47	0.12	0.39
#17	*L. sanguifluus* / Sierra de Huetor	2.36	0.39	0.54	0.56	0.18	2.91	27.5	0.99	0.79	1.31	0.54	0.16	0.59	0.07	0.09	1.98
#18	*L. sanguifluus* / Cortes de la Frontera	0.41	–	0.08	0.15	–	1.66	42.45	0.32	0.14	–	0.08	0.04	–	0.04	0.14	0.63
R_fD (μg/kg body weight/day) [a]		3[d]	0.3[d]	1[d]	3.5[e]	0.3[d]	40[e]	300[d]	0.5[d]								
PTDI (μg/kg body weight/day) [b]		–	2.14[f]	0.82[f]	–	0.57[f]	–	–	–								
PTMDI (μg/kg body weight/day) [c]		–	–	–	–	–	5000[f]	300–1000[f]	–								

[a] R_fD – Reference dose.
[b] PTDI – Provisional tolerable daily intake.
[c] PMTDI – Provisional maximum tolerable daily intake.
[d] USEPA – U.S. Environmental Protection Agency (USEPA, 2021).
[e] Sarikurkcu et al. (2020).
[f] JECFA - The Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2021).

day (both men and women), 8000−11000 μg/day (women and men, respectively) and 55 μg/day (both men and women) (Institute of Medicine (US) Panel on Dietary Antioxidants and Related Compounds, 2000; Institute of Medicine (US) Panel on Micronutrients, 2001). If we focus on those mushrooms whose consumption is safe for human health (HRI ≤ 1 or ~ 1) *L. deliciosus* collected from Alimadene (Tetouan, Morocco), Puerto Real (Cadiz, Spain), and Cortes de la Frontera (Malaga, Spain) are the ones that would most contribute to provide these three essential trace elements (Table 3). Nevertheless, the consumption of these mushrooms would only cover a minor percentage of our daily Cu, Zn and Se requirements. Therefore, daily diets including these mushrooms should be complemented with other foods that provide the necessary amounts of such essential trace elements.

4. Conclusions

Metals and metalloids contents in the fruiting bodies of 18 different wild edible *Lactarius* specimens collected from southern Spain and northern Morocco have been determined. The results from our analyses confirm that Zn is the main element to be found in all the mushroom species covered by our study, and particularly in *L. deliciosus*. The Hierarchical Cluster Analysis (HCA) has proven to be a useful tool to visualize the mushroom samples trend to be grouped together according to their metal content. In this regard, the closest similarity was observed between *L. sanguifluus* and *L. semisanguifluus*. Our study has also evaluated the potential risks to human health associated to the consumption of certain heavy metals and metalloids depending on their daily intake (DIM) and their health risk index (HRI). Based on these data, it has been confirmed that some particular mushroom samples exceeded the established permissible levels of As and Se. Nevertheless, Se levels in these particular mushrooms do not have to pose a risk for humans' health. Finally, the comparison of DIM against the recommended dietary allowance (RDA) suggested that the mushrooms in our study which are safe for consumption only cover a minor percentage of the daily requirements of Zn, Cu and Se. Therefore, for a healthy diet, not only a controlled consumption of these mushrooms would be recommended, but they should also be complemented by other foods that contribute to reach the daily consumption requirements for these elements.

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CRediT authorship contribution statement

Marta Barea-Sepúlveda: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization. **Estrella Espada-Bellido:** Conceptualization, Investigation, Data curation, Writing - review & editing, Supervision. **Marta Ferreiro-González:** Software, Formal analysis, Data curation. **Antonio Benítez-Rodríguez:** Methodology, Validation. **José Gerardo López-Castillo:** Resources. **Miguel Palma:** Resources, Funding acquisition. **Gerardo F. Barbero:** Conceptualization, Investigation, Data curation, Writing - review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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