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PRELIMINARY STUDY OF TRACE ELEMENTS IN WILD MACROFUNGI FROM ALTOS DE CANTILLANA, CENTRAL CHILE

Abstract: Wild edible mushrooms are a popular food considering their nutritional value. However, some mushroom species can harm human health by accumulating some elements excessively. To evaluate the pollution level of toxic elements in wild edible and non-edible mushrooms from two private natural areas in the Altos de Cantillana mountain range in Central Chile (Altos de Cantillana Natural Reserve and Cerro Poqui Nature Sanctuary) present in them were quantified. All mushrooms contained Pb, Zn, Fe, Cu, and Ni. Mushrooms obtained in Los Altos de Cantillana have higher amounts of metals. In mushrooms of the type *Bovista brunnea* (sample 27) there are maximum amounts of Pb (566.8 μg/g), Zn (1152.3 μg/g), and Cu (568.6 μg/g) while those of the type *Lycoperdon* sp. (sample 14) have maximum amounts of Fe (17806.9 μg/g) and Ni (27.6 μg/g). On the other hand, only the species *Stereum hirsutum* (samples 1 and 4) has very low amounts of As (3.9 μg/g and 6.5 μg/g) and only this one and *Phaeoclavulina flaccida* contain low amounts of Cd (0.02 μg/g and 0.04 μg/g). On the other hand, Sb and Au were not found in any sample; all values were < LOQ (Limit of quantification). Although intraspecies differences were observed, not all were significant. It is important to highlight the analysis of wild mushroom species that people can consume, such as the genus *Cyttaria*, which should be evaluated for trace element content.

Keywords: wild edible mushrooms, wild mushrooms, metals content, trace elements, Altos de Cantillana

Introduction

Edible mushrooms are a favourite food of many people especially in some Asian and European countries due to their delicious taste and nutritional value [1-3]. Edible mushrooms are highly nutritious and can be compared with eggs, milk and meat in terms of nutrient profile. Fruiting bodies of mushrooms are appreciated for their properties, including low in calories, high in proteins, vitamins, and minerals [4]. *Agaricus bisporus*, the most cultivated mushroom in the world, exhibits a high proportion of fatty acids. Literature survey shows that palmitic, stearic, oleic and linoleic acids are the most abundant fatty acids in *Agaricus* species [5-7]. Mushrooms have also been used for preventing

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diseases such as hypertension, hypercholesterolemia [8, 9]. They are the fleshy and edible fruit bodies of several species of macrofungi and they can appear either below ground (hypogeous) or above ground (epigeous). Macroscopic mushrooms are distinguished into two significant phyla, Basidiomycota and Ascomycetes. Some of the edible mushrooms have been artificially cultivated, and need a variety of nutrients from the surrounding environment for growth and reproduction, but most of them are wild grown. Because mushroom consumption increasing worldwide, it is important to know about the concentration of different toxic metals due to a large variety of different mushroom species is considered edible. Edible species, such as *Agaricus bisporus*, *Hericium erinaceus*, *Flammulina velutipes*, *Lentinu sedodes*, *Pleurotus eryngii*, and *Pleurotus ostreatus* have significant antioxidant and free radical scavenging activities [10]. Such properties have also been reported for medicinal mushrooms, including *Agaricus blazei*, *Sparassis crispa*, *Phellinus linteus*, *Ganoderma lucidum*, and *Inonotus obliquus* [11]. Artificially planted mushrooms are generally less susceptible to chemical contamination due to the controllable sources of raw materials. Others mushrooms accumulate metallic elements by absorbing them from the soil substrate via mycelia and some species by rhizomorphs (mycelial cords), which they form underground [12-15]. Mushrooms in contact with soil, air, and rain may be used as biological monitors for trace elements. Specifically mushrooms (macromycetes, macrofungi) *Amanita strobiliformis* hyperaccumulates Ag [16], *Cyanoboletus pulverulentus* and *Sarcosphaera coronaria* hyperaccumulate As [17, 18], and *Cystoderma carcharias* is a facultative hyperaccumulator of Cd [19, 20]. Interestingly, mushrooms can accumulate extremely high concentrations of elements even when they grow above soils with low contents of these elements [21]. Some of the mushroom species are capable of accumulating certain heavy metals like arsenic, mercury, lead, copper, cadmium, and selenium. More than 50 species of mushrooms were reported with high concentration of arsenic into their fruit bodies [22-25].

The largest producer, consumer, and exporter of edible fungi is China globally; the output exceeded 41 million tonnes (tonne = 10^3 kg = 10^6 g = Mg) in 2021 and keeps a high growth rate of about 5 % per year [26, 27]. Large-scale cultivation of medicinal mushrooms is mainly carried out in Asian country, where the mushroom-based pharmaceutical and nutraceutical industry is highly developed [28].

Mushrooms are collected for consumption as a good source of digestible proteins, minerals, carbohydrates, amino acids (e.g., leucine, lysine, methionine, tryptophan), carbohydrates, as well as fibres (mainly in the form of chitin), vitamins (B_1, B_2, B_{12}, C, D, niacin, folic acid), phenolics, organic acids, sterols, alkaloids, terpenoids polysaccharides, polyphenols, etc. These components give mushrooms various properties, including antitumor, anti-inflammatory, antioxidant, antiviral, antibacterial, antiparasitic, antidiabetic, and cytotoxic properties, among others [29, 30]. The first and foremost compound in mushrooms are polysaccharides, responsible, for example, for their health properties, including improving the immune system. Bioactive polysaccharides found in mushrooms include α and β glucan, galactans, chitin, xylans, hemicelluloses, and mannans [31, 32]. Shiitake mushrooms are also rich in polysaccharides and protein, containing almost all essential amino acids the human requires [33]. They have been utilised to extract polysaccharides [34], proteins [35], phenolic compounds [36], terpenoids [37], minerals, and vitamins [38].

According to the reported background, it can be concluded that mushrooms present notable duality: they are nutritional and possess medicinal properties, yet their extraordinary capacity to absorb metals from the soil, sometimes their consumption is not appropriate, especially those that grow in some polluted places. However, they can serve as biomarkers for the area under study and could potentially be used for the remediation of contaminated waters. Trustworthy and traceable measurement of inorganic elements such as Cd, Pb and Hg is important for the safety evaluation of mushrooms as a food product. Metals are directly and/or indirectly involved in all aspects of mushrooms growth, metabolism and differentiation. While many metals are essential, e.g. K, Na, Mg, Ca, Mn, Fe, Cu, Zn, Co and Ni, many others have no apparent essential function, e.g. Rb, Cs, Al, Cd, Ag, Au, Hg and Pb. Both essential and nonessential heavy metals are toxic for fungi, when present in excess. The mushroom is able to sequester essential trace metal ions from various sources, where the metals can be present in concentrations ranging from trace to toxic levels [39, 40]. With this objective, we decided to conduct this study, starting with detecting toxic and some essential metals in mushrooms that grow in different places of Altos de Cantillana mountain range (central Chile). The metals chosen were: Zn, Fe, Cu, Pb, Ni, Cd, Au and nonmetals as Sb and As, because many mushrooms have been reported to have high tolerance levels, allowing them to bioaccumulate rapidly. These analytes were chosen due to their toxicity and high presence in environmental and food media. Some of them, even at very low concentrations, are cytotoxic, carcinogenic, mutagenic and sometimes even have higher concentrations in the mushrooms than in the surrounding water and soil. It has been determined that the presence of these metals and nonmetals has increased over time, due to industrial activity, mining, and the use of fertilisers. This allows mushrooms growing near mining areas to absorb some of these metals. The ability of fungi to uptake metals depends on their concentration, chemical form, interactions with other metals, and soil characteristics, among other factors.

Experimental

Sampling site, sampling, and species identification: The mushroom samples were collected in two private natural areas, Altos de Cantillana Natural Reserve (Metropolitan Region-Chile, between 373 m - 438 m altitude) and Cerro Poqui Nature Sanctuary (O'Higgins Region-Chile, between 341 m - 698 m altitude), on September 28th and October 5th, 2023, respectively, marking the beginning of the dry season. Both sample sites are in Altos de Cantillana's mountain range, representing the Mediterranean central Chile climate and forests. The studied area comprises various vegetation communities and is currently a priority site for conservation due to its high endemism and, at the same time, high anthropogenic pressure and vulnerability to climate change [41]. Table S1 summarises the geographic coordinates (using an Etrex 10 GPS, Garmin, USA) and the classification of the diverse macrofungal species assigned based on the macroscopic characteristics of the samples compared with the local species recognition guide [42]. Figure 1 shows representative photographs of the sampling sites and some specimens collected and subsequently analysed.

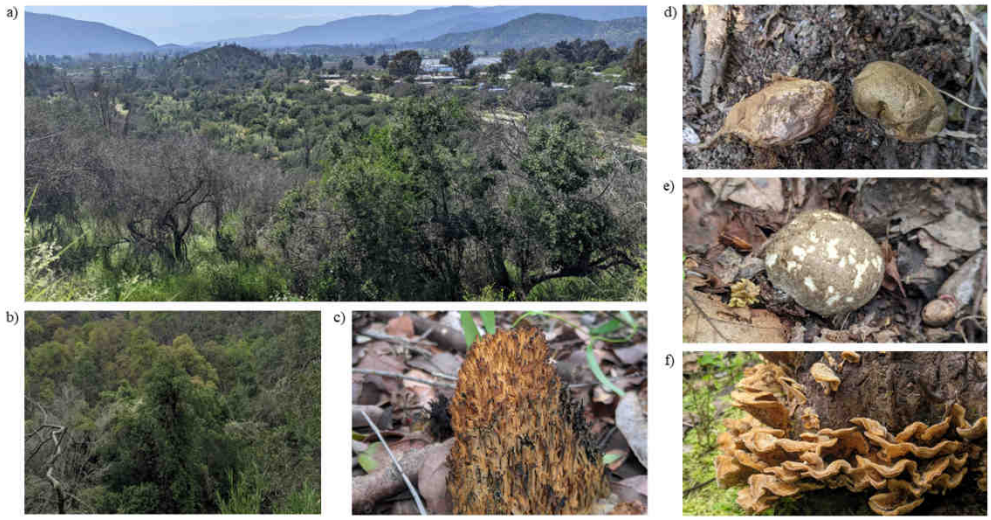

Fig. 1. Representative images of the sampling sites and some collected specimens: a) Altos de Cantillana Natural Reserve, b) Cerro Poqui Nature Sanctuary, c) *P. flaccida*, d) *Lycoperdon* sp., e) *B. brunnea*, f) S. *hirsutum*

Pretreatment and analysis of total metals: The samples were collected and stored in the dark and at room temperature, until analysis. A digestion of 0.5 g - 1.0 g of fungi biomass samples was mineralised using the wet method in Teflon bombs with 10 mL of nitric acid (Instra Grade, J.T. Baker, USA) for 2 h at 150 °C in a homemade ceramic oven with an internal temperature sensor and external control. After cooling, the digested samples were filtered through 0.45 µm (Advantec-MFS, Japan) and made up to 50.0 mL with deionised water. For arsenic analysis, 10 mL of the previous solution is taken, 0.5 mL of sulfuric and 0.25 mL perchloric acid (Suprapur Grade, Merck) are added. The mixture was then maintained at 200 °C in a reflux system for one hour. Finally, it was filtered through 0.45 µm and adjusted to a final volume of 25 mL with 0.1 M HCl [40, 41]. Metal ions were quantified using GFAAS (Thermo Scientific iCE3000 Series AA Spectrometer, USA) and a conventional FAAS (novAA 350, Analytik Jena, Germany) with the manufacturer's optimal conditions and an external calibration curve. The analytical merits figures are in Table 1, which includes information on the linear range, LOD (Limit of detection), LOQ (Limit of quantification), regression linear coefficient, and internal quality control with synthetic spiked solutions information during the calibration (validation). Standard quality was performed using AAS (from Merck), and the salts and acids used were according to the manufacturer's recommendations by Suprapur Quality (from Merck). Milli-Q water of 18,2 MΩ cm quality was used.

Statistical analysis: According to the different requirements for presenting the results, graphs were created using GraphPad Prism 8.0, OriginPro 2024 (with Google Map Import App), and Statgraphics Centurion software. The one-way ANOVA test with multiple comparisons ANOVA test was used to compare the results.

Table 1

Analytical performance by FASS and GFAAS for the metal analysis in real samples

Metal	Linear range	LOD	loq	R^2	Spiked concentration	Founded concentration	Relative error [%]
As*	LOQ-45 µg/L	2.80 µg/L	9.30 µg/L	0.9950	10.00 µg/L	10.02 µg/L	0.2
Au	LOQ-18 mg/L	0.89 mg/L	2.96 mg/L	0.9986	3.00 mg/L	3.10 mg/L	3.3
Cd	LOQ-1.5 mg/L	0.06 mg/L	0.18 mg/L	0.9993	0.50 mg/L	0.54 mg/L	8.0
Cu	LOQ-5 mg/L	0.41 mg/L	0.71 mg/L	0.9940	2.00 mg/L	1.95 mg/L	–2.5
Fe	LOQ-7 mg/L	0.25 mg/L	0.95 mg/L	0.9979	1.00 mg/L	1.08 mg/L	8.0
Ni*	LOQ-12 µg/L	0.80 µg/L	2.60 µg/L	0.9976	5.00 µg/L	503 µg/L	0.6
Pb	LOQ-2.5 mg/L	0.11 mg/L	0.37 mg/L	0.9966	0.50 mg/L	0.52 mg/L	4.0
Sb*	LOQ-30 µg/L	4.50 µg/L	14.80 µg/L	0.9931	15.00 µg/L	14.98 µg/L	–0.1
Zn	LOQ-2.5 mg/L	0.09 mg/L	0.33 mg/L	0.9991	0.50 mg/L	0.51 mg/L	2.0

*Metals analysed with GF-AAS

Results and discussion

The initial identification of the species collected in the field was conducted in collaboration with the park rangers of each enclosure. Once in the laboratory, species names were assigned based on the local guide consulted and the macro characteristics of the specimens [43]. Table S1 shows the identified species from many collected specimens; 32 were chosen before analysis, comparisons, and statistical tests. The GPS location of each specimen is also reported, along with their division and order. The samples were identified based on the substrate where the organism was found and the macroscopic characteristics of its fruiting bodies. The different species were classified into two phylogenetic divisions: *Basidiomycota* and *Ascomycota*. The following species are presented in decreasing order the numbers of collected specimens: *S. hirsutum*, *Lycoperdon* sp., *P. papilionaceus*, *T. versicolor*, *C. critina*, *C. atramentaria*, *B. brunnea*, *Cyttaria* sp., and *P. flaccida* (Table S1) founded in the sampling area.

The data from the analysis of each individual sample can be found in Table 2, along with the analytical parameters of the GFFAAS and FAAS technique listed in Table 1. All the correlation coefficients of the standard calibration were greater than 0.99 ($R^2 > 0.99$). Similar figures of analytical merit such as linear range, precision and accuracy have been obtained with similar instruments in the study of trace elements in mushrooms as biomonitors in forest areas [44]. At the same time, the relative errors were less than or equal to 8 % concerning the control synthetic spiked solutions during the analysis of the samples. This method was used because certified reference material for the matrix under study was not available. For the presentation of the data in Table 2, the guidelines from Hites [45] were followed, used whereby reporting, depending on the number of samples below the limit of detection (< LOD). In the case of the Cu and Pb metals, where samples below LOD constituted less than 50 % of the total population, they were reported as LOD/2. It is important to note that Sb and Au were all samples below the detection limit. For As and Cd, very few samples showed detectable signals; two individuals of the same species (*S. hirsutum*) were notable for As, with values in the same order of concentration in ppb. A similar situation occurred for Cd, with 3 individuals falling within the same concentration range. The Sb, Au, As, and Cd data were not considered for subsequent analyses.

Figures S1 and S2 show the distributions of the different species at the sampling points at Altos de Cantillana and Cerro Poqui sites, respectively. The bubble graphs indicate the

spatially distributed concentration of each metal in proportion to the metal. In the Altos de Cantillana Nature Reserve (Fig. S1), random distributions can be observed with no apparent relationship to the cyclical section of the sampling. A similarity can be seen between the proportions of Zn-Cu-Pb and Fe-Ni. Meanwhile, in the Cerro Poqui Nature Sanctuary (Fig. S2), the linear layout of the sampling can be better seen, with noticeable similarities between Zn-Pb. It should be noted that in this last site, lower values were found at a lower altitude and close to the beginning of the trail and the entrance to the park, not correlated with the height of the rest of the specimens.

Table 2

Content of total Zn, Fe, Cu, Pb, Ni, As, Cd, Sb[*], and Au[*] in samples [µg/g d.m.]

Sample	Zn	Fe	Cu	Pb	Ni	As	Cd
1	176 ±8	2962 ±16	65 ±2	30[***]	2.8 ±0.3	3.9 ±0.6	0.018 ±0.004
2	105 ±7	1885 ±14	25 ±0	40 ±6	2.4 ±0.3	< LOQ[**]	< LOQ
3	183 ±13	206 ±3	9 ±1	64[***]	2.2 ±0.3	< LOQ	< LOQ
4	102 ±5	2121 ±11	56 ±0	17[***]	3.0 ±0.3	6.5 ±1.0	< LOQ
5	197 ±14	240 ±2	78[***]	67[***]	6.9 ±0.5	< LOQ	< LOQ
6	173 ±10	325 ±2	69 ±3	51[***]	1.4 ±0.2	< LOQ	< LOQ
7	185 ±9	545 ±5	49 ±0	35[***]	2.3 ±0.2	< LOQ	< LOQ
8	125 ±7	355 ±3	34 ±0	37[***]	1.0 ±0.1	< LOQ	< LOQ
9	165 ±10	3301 ±18	84 ±1	60 ±9	3.3 ±0.4	< LOQ	< LOQ
10	428 ±32	1847 ±15	232 ±4	171 ±22	4.8 ±0.3	< LOQ	< LOQ
11	365 ±24	6560 ±31	221 ±6	139 ±25	7.9 ±0.6	< LOQ	< LOQ
12	379 ±25	11372 ±10	206 ±3	177 ±21	18.0 ±0.9	< LOQ	< LOQ
13	177 ±11	157 ±2	128 ±2	19[***]	1.8 ±0.2	< LOQ	< LOQ
14	1076 ±82	17807 ±215	103 ±2	528 ±76	27.6 ±0.2	< LOQ	< LOQ
15	230 ±12	5400 ±22	299 ±2	76 ±8	7.3 ±0.5	< LOQ	< LOQ
16	608 ±47	424 ±7	259[***]	309 ±14	4.6 ±0.3	< LOQ	< LOQ
17	790 ±66	428 ±12	331 ±8	427 ±87	12.1 ±0.8	< LOQ	< LOQ
18	285 ±14	1021 ±7	80[***]	27[***]	2.9 ±0.3	< LOQ	< LOQ
19	186 ±11	147 ±1	36 ±1	42[***]	1.4 ±0.2	< LOQ	< LOQ
20	310 ±18	487 ±13	58 ±8	33[***]	2.5 ±0.2	< LOQ	< LOQ
21	173 ±12	122 ±5	28 ±1	67 ±12	3.0 ±0.2	< LOQ	< LOQ
22	113 ±8	81 ±1	45[***]	54 ±5	0.8 ±0.1	< LOQ	< LOQ
23	821 ±62	2558 ±23	9 ±1	355 ±67	7.0 ±0.5	< LOQ	< LOQ
24	458 ±38	2470 ±6	20 ±2	196[***]	6.8 ±0.5	< LOQ	< LOQ
25	234 ±16	608 ±8	79 ±2	67[***]	3.8 ±0.2	< LOQ	< LOQ
26	201 ±11	1178 ±5	170 ±2	18[***]	2.2 ±0.1	< LOQ	< LOQ
27	1152 ±99	22 ±6	569[***]	567[***]	6.7 ±0.5	< LOQ	< LOQ
28	356 ±21	82 ±5	272 ±1	126 ±17	8.3 ±0.6	< LOQ	< LOQ
29	104 ±7	55 ±5	21 ±1	43 ±4	2.4 ±0.2	< LOQ	< LOQ
30	313 ±24	86 ±9	7 ±0	99[***]	12.8 ±0.9	< LOQ	< LOQ
31	208 ±11	572 ±3	589 ±7	50 ±10	1.9 ±0.2	< LOQ	0.036 ±0.005
32	156 ±9	752 ±2	394 ±4	47 ±6	1.6 ±0.2	< LOQ	0.039 ±0.006

Results expressed of media ±Standard deviation (n = 2) in µg/g d.m.
[*]Sb and Au were not found in any sample; all values were < LOD
[**]< LOQ: value less than the LOQ reported
[***]Value calculated with LOD/2 in the case of values with < LOD if that value was < 50 % of the total population, like a correction for censored environmental measurements [42]

Figure 2 shows the data for the metals considered for each species' analyses (Zn, Fe, Cu, Pb, Ni). In general, quite different ranges of values are obtained for the different metals. Still, when using one-way ANOVA and multiple comparison analysis with

ANOVA, for some species, the Fe content is the only one different from all the other metals analysed, specifically in *S. hirsutum*, *Lycoperdon* sp., *C. citrina*, and *C. atramemntaria*. The species *B. brunnea* and *Cyttaria* sp. did not show differences in the contents of any metal.

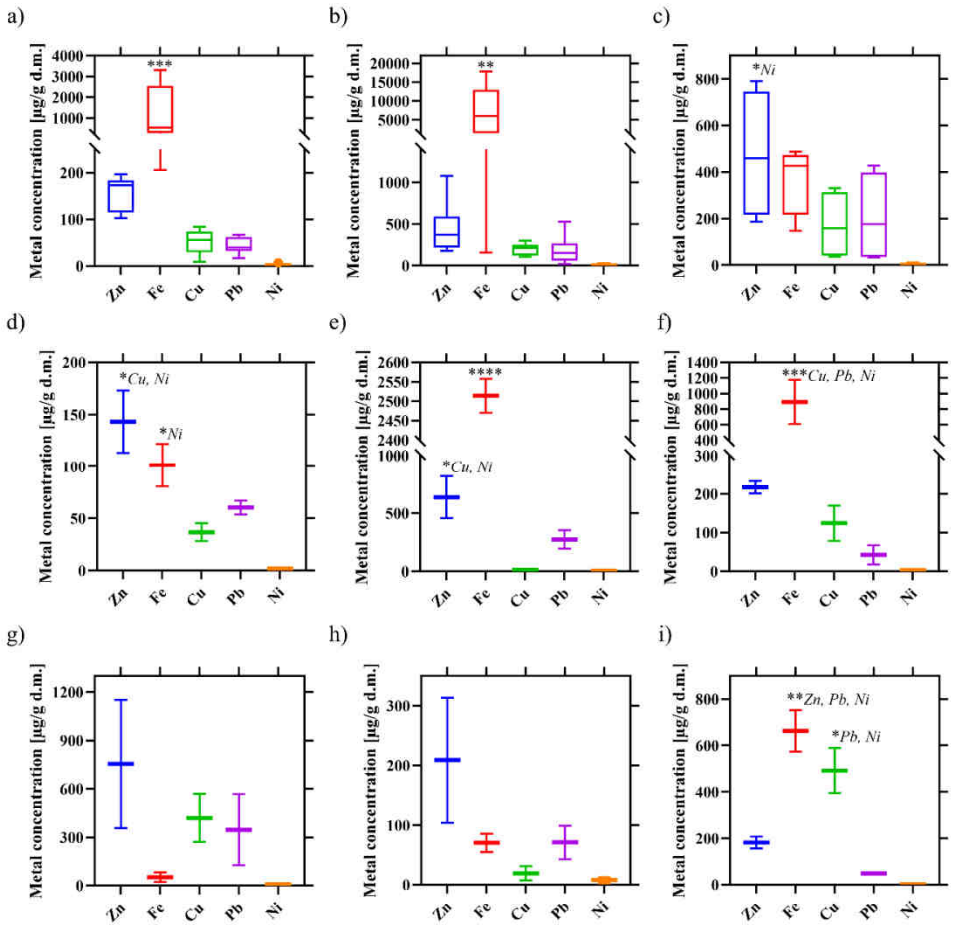

Fig. 2. Tukey box-plot of the content of total metals [µg/g d.m.] in the different species samples: a) *S. hirsutum*, b) *Lycoperdon* sp., c) *P. papilionaceus*, d) *T. versicolor*, e) *C. citrina*, f) *C. atramentaria*, g) *B. brunnea*, h) *Cyttaria* sp., i) *P. flaccida*

The differences among the various species can be observed for the same type of graphs, but separated by the metals analysed (data not shown). No significant differences were found between the contents of Zn, Pb, and Ni. A notable difference in Fe content was observed only in the species *S. hirsutum* and *Lycoperdon* sp. Conversely, for Cu *P. flaccida* differed from all the other species. Additionally, species *B. Brunner* and *Cyttaria* sp. differed from other specified species. In species recollected near mining areas in Slovakia, like *Lycoperdon perlatum*, researchers found high levels of mercury and cadmium as well as extremely elevated concentrations of Pb and Cu, reaching hundreds of milligrams per

kilogram of dry matter [47]. This contrasts with our results, where the analysed metals were found in the *Lycoperdon* sp., with an exception for Cd. Differences have also been reported based on the type of substrate on which the fungi grow, with the fungi grow, with fungi growing on wood generally accumulating fewer metals (as observed in *S. hirsutum* and *T. versicolor*) [48]. In this study, no significant differences were evident among the various species, except for Cu, which does not conform to the substrate pattern described. In a study of global species such as *S. hirsutum*, Turkish researchers found similar values in the content of Fe and Pb in those analysed here since the values of these metals were 3027 mg/kg and 19.42 mg/kg, respectively [49]. On the other hand, Swislowski and Rajfur [44] studied the level of contamination (Mn, Fe, Ni, Cu, Zn, Cd, Pb) in soil and edible mushrooms from two forest areas of Poland (Staporkow and Kup). Obtaining that the soil collected from both study areas shows much higher concentrations of Mn, Fe and Pb, in comparison to mushrooms collected in these areas. For Ni, Cu, Zn and Cd, the content of analytes in soil was lower than the concentrations obtained in mushrooms. Compared with Polish standards of soil quality, the allowable concentrations of Ni, Cu, Zn, Cd and Pb were not exceeded. However accumulation of Cd, Cu and Zn in mushrooms was detected.

A multivariate principal component analysis (PCA) was performed to demonstrate group differences. The PCA analysis indicated that extracting two principal components can explain 85.5 % of the accumulated variance, which can also be verified graphically with the eigenvalue less than 1 (Fig. 3a). In Figure 3b, in a two-dimensional plane of the first and second principal components reveals a close relationship between Pb and Zn. When evaluating this relationship univariately, a high squared linear correlation coefficient of 0.9508 is observed (data not shown). Additionally, PCA analysis suggests that the metal contents of Fe and Ni could be grouped. At the same time, Cu does not fit into any group (Fig. 3b). In another polluted context, a quantitative evaluation of the relationship between element uptake by mushrooms from the substrate was conducted by calculating conducted the coefficient accumulation. A high accumulation of Zn was observed in mushrooms growing near the metal smelter [50]. However, these authors did not analyse the Pb content. They did not find the same classification observed under conditions without evident contamination or point source of pollution, as in this study. Although no tests were carried out in this work to calculate bioaccumulation concerning the substrate, in other articles that do address this issue [51], no correlations are made in the contents of the different metals that, according to the authors, this may indicate the presence or past existence of a potential source of natural or anthropogenic contamination.

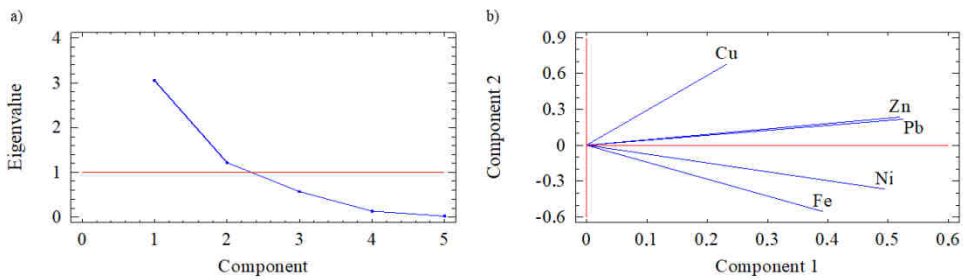

Fig. 3. Principal component analysis: a) Scree plot and b) plot of component weights

Interestingly, the current EU regulations regarding metal content in edible mushrooms are expressed on a wet-weight basis. The regulated limits metals for Pb and Cd, are set at 0.3 mg/kg and 0.2 mg/kg, respectively [48]. In the present study, the values were expressed based on dry weight. The only fungi edible species found and analysed, such as *Cyttaria* sp. [52], showed undetectable values for Cd, indicating compliance with regulations. However, the dry weight values for Pb were significantly higher than the wet weight regulations. Species of this genus, such as *C. berteroi* and *C. hariotii* are widely consumed in the central and southern areas of Chile and Argentina [53], hence the importance of monitoring toxic elements present in them. Therefore, future research should consider evaluating metal content based on the wet weight to assess the safety of consuming these mushrooms in the human diet. Other research has reported varying metal content in edible mushrooms growing in urban environments, with results differing based on species, habitat, and proximity to traffic [54]. The dry weight results suggest that, despite the samples being collected from nature conservation areas, it is necessary to monitor metal content, including those regulated by the EU, as significant levels can be present even without obvious contaminating sources. Future studies will seek to demonstrate the accumulation factors of these species, in addition to increasing the sample population and the sampling area near urban sites, to evaluate the effect of contamination by toxic elements in edible mushrooms.

Conclusion

In this work, the metal content (Zn, Fe, Cu, Pb, Ni, As, Cd, Sb, Au) of different macrofungal species collected in protected areas of the Altos de Cantillana mountain range (Central Chile) was analysed for the first time. The contents of the metals Sb and Au were all below the limit of detection. At the same time, very few samples presented detectable levels for Cd and As, which was insufficient to perform statistical studies. However, the content of the rest of the metals (Zn, Fe, Cu, Pb, Ni) in the different species collected with 32 individuals (*S. hirsutum*, *Lycoperdon* sp., *P. papilionaceus*, *T. versicolor*, *C. critina*, *C. atramentaria*, *B. brunnea*, *Cyttaria* sp., and *P. flaccida*) allowed observing differences in the contents of the same ones without following patterns according to the type of substrate of the fungi or according to species. Principal component analysis showed similar behaviours in Pb-Zn contents, obtaining a high correlation, not while in Cu. Although intraspecies differences were observed, not all were significant. This initial study also made it possible to highlight the importance of studying the species that can be consumed by people since, although the Cd contents were not detectable, the regulated Pb content in cultivated commercial mushrooms could be harmful to health in these wild mushrooms. More studies are needed to make these measurements simultaneous, with lower detection limits and a higher n, in addition to obtaining the bioconcentration factor concerning the substrate and considering non-pristine environments in which, for example, fungi of the genus *Cyttaria* may be available.

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