Journal of Food Composition and Analysis 108 (2022) 104419

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Original Research Article

Toxic elements and trace elements in *Macrolepiota procera* mushrooms from southern Spain and northern Morocco

Marta Barea-Sepúlveda [a], Estrella Espada-Bellido [a,*], Marta Ferreiro-González [a], Hassan Bouziane [b], José Gerardo López-Castillo [c], Miguel Palma [a], Gerardo F. Barbero [a]

[a] *Department of Analytical Chemistry, Faculty of Sciences, University of Cadiz, Agrifood Campus of International Excellence (ceiA3), IVAGRO, 11510, Puerto Real, Cadiz, Spain*

[b] *Laboratory of Applied Botany, Department of Biology, Faculty of Sciences, University Abdelmalek Essaâdi, Mhannech II, 2121, Tetouan, Morocco*

[c] *Unidad de Protección de la Salud, Distrito Sanitario Granada-Metropolitano, Consejería de Salud y Familias, Junta de Andalucía, 18150, Gójar, Granada, Spain*

ARTICLE INFO

Keywords:
Parasol mushroom
Wild edible mushrooms
Foods
Toxic elements
Trace elements
Spain
Morocco
Health risk assessment

ABSTRACT

Anthropogenic activities, such as mining and fossil fuel combustion, produce large amounts of pollutants that affect environmental homeostasis. Wild edible mushrooms fructify exposed to environmental conditions, proving to be efficient accumulators of trace elements and toxic and potentially toxic elements. Due to the increasing consumption of mushrooms worldwide, this is of public health concern. In this work, the total content of chromium (Cr), arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb), copper (Cu), zinc (Zn), and selenium (Se) was determined by ICP-MS in the caps and stipes of the high valued wild edible mushroom *Macrolepiota procera* collected in several locations of the South of Spain and the North of Morocco. The results obtained have indicated that the cap of *M. procera* contains a broad spectrum of both toxic elements and trace elements, occurring in higher contents in this part of the fruiting body with respect to the stipe. Moreover, Cu was the predominant element found in the samples studied, followed by Zn in most of the cases. The one-way ANOVA/Kruskal-Wallis test indicated that there were no significant differences in metal and metalloid content between the geographical areas studied. In addition, the results obtained through Hierarchical Cluster Analysis (HCA) and Principal Component Analysis (PCA) support the conclusions drawn through univariate statistical studies, indicating that there is no obvious clustering trend for the *M. procera* cap samples based on the sampling area. The health risk assessment for *M. procera* caps showed a cause for concern related to Cr, Cd, As, and Hg due to frequent consumption of around 300 g of fresh caps per day during the mushrooming season.

1. Introduction

The up-level content of metals and metalloids in the soil, air and water due to anthropogenic activities is a high-priority issue because of its adverse effects on living organisms and the environment (Kiran et al., 2021). Elements such as mercury (Hg), lead (Pb), cadmium (Cd), chromium (Cr), and arsenic (As) are considered toxic for organisms, even at low contents, because they can interact either with non-specific biomolecules or cause oxidative damage due to their catalytic ability in redox reactions (Jan et al., 2015). Nevertheless, almost all biological processes, as well as health care and prevention of mineral micronutrient-related diseases, require a proper intake of some trace elements such as copper (Cu), zinc (Zn), and selenium (Se) (Alloway, 2013). Notwithstanding, exceeding the maximum allowable levels of some of these essential micronutrients can affect the normal metabolism of the organism, compromising its well-being (Kfle et al., 2020; Rebello et al., 2021).

Currently, it is well documented the ability of various wild foods to accumulate toxic elements and trace elements. Specifically, wild-growing mushroom fruiting bodies are likely to be efficient accumulators of toxic elements and trace elements, being considerably higher than those of crops, plants, cultivated mushrooms, and even soil (Liu et al., 2015a; Zhu et al., 2011a). Cd and Hg can be an example of toxic elements that are found at higher levels in fruiting bodies of some wild-edible mushrooms than in the underlying soil and plants (Aloupi et al., 2012; Demirbaş, 2001; Gucia et al., 2012). On the other hand, a

---

* Corresponding author at: Department of Analytical Chemistry, Faculty of Sciences, University of Cadiz, Agrifood Campus of International Excellence (ceiA3), IVAGRO, 11510, Puerto Real, Cadiz, Spain.

E-mail address: estrella.espada@uca.es (E. Espada-Bellido).

https://doi.org/10.1016/j.jfca.2022.104419
Received 19 October 2021; Received in revised form 14 January 2022; Accepted 20 January 2022
Available online 29 January 2022

great content of trace elements such as Cu and Zn in several mushroom species have also been reported (Mirończuk-Chodakowska et al., 2019; Zhu et al., 2011b). The uptake of toxic elements and trace elements by fungi depends on various environmental factors (climate, pH, organic matter content, soil adsorption capacity, type of substrate, etc.) and on the fungus itself (species, distribution and age of the mycelium, decomposer activity and nutrition, biochemical composition, anatomical part, etc.) (Gadd, 2007; Kalač, 2010; Kokkoris et al., 2019; Nikkarinen and Mertanen, 2004; Nnorom et al., 2020; Stefanović et al., 2016; Wang et al., 2017). The former determines the mobility and availability of the elements, and the latter defines the greater accumulation capacity of fungi concerning plants and the different uptake aptitudes shown by the diverse fungi species. It is a well-known fact that mushrooms have been considered a delicacy in the human diet over the years (Lalotra et al., 2016; Valverde et al., 2015), nonetheless, more comprehensive knowledge about their nutritional properties has increased mushroom consumption, especially during the mushrooming season. Due to this fact, in 2020 the global mushroom market size was valued at $46.1 Bn, and it is estimated to expand at a compound annual growth rate (CAGR) of 9.5 % from 2021 to 2028 (Global Mushroom Market, 2020).

In the last decades the accumulation of toxic elements and trace elements in mushrooms has gained a greater scientific relevance since mushrooms have become an interesting product for the consumers. Several articles on this topic have been published, focusing mainly on wild mushrooms of culinary interest, such as *Macrolepiota procera* and *Lactarius deliciosus* among others, mainly from the regions of China, Turkey, and Poland (Aloupi et al., 2012; Falandysz et al., 2017b, 2017a; Gucia et al., 2012; Kojta and Falandysz, 2016; Liu et al., 2015a; Malinowska et al., 2004; Mleczek et al., 2018; Sarikurkcu et al., 2020, 2011; Türkmen and Budur, 2018; Zhang et al., 2008). Spain possesses a wide variety of mushrooms and truffles, making this region a true paradise for fungal lovers. The 2020 annual household consumption panel data issued by the Ministry of Agriculture, Fisheries and Food from Spain indicated that the quantities consumed nationwide totaled 70,221 Tm, representing an estimated expenditure of 293,354 € by the Spanish consumers in this sector (Ministerio De Agricultura, 2022). Of all the mushrooms consumed in this country, *Agaricus bisporus* occupies a prominent place with approximately 70 % of the total consumption, followed by the *Pleurotus ostreatus* and other seasonal mushrooms, such as *L. deliciosus* or *M. procera* (Haro et al., 2020; Valverde et al., 2015). Notwithstanding the increase in consumption nationwide, the studies carried out on the content of toxic elements and trace elements in mushrooms species from Spain are scarce, especially in the southern region of the country. For example, Ostos et al. (2015) studied the Hg content in 10 species of mushrooms collected in different areas of the South of Spain, including the culinary valued *M. procera*, *L. deliciosus* and *Amanita caesarea*. On the other hand, Melgar et al. (2014) evaluated As contents and the associated health risk in six species of wild mushrooms from northern Spain, including *Boletus aereus* and *M. procera*. In turn, Melgar et al. (2016) determined the Cd content in 28 edible mushroom species, such as the high-valued species *M. procera*, *Boletus edulis*, *P. ostreatus*, and *L. deliciosus*. Likewise, Alonso et al. (2003) determined the Cu and Zn content in 28 wild mushroom species from northwestern Spain, which included species of the families *Russulaceae*, *Agaricaceae* and *Boletaceae*. On the other hand, Haro et al. (2020) determined the mineral composition of 18 mushroom species collected in southern Spain, including *M. procera*. In addition, previous studies carried out by our research group have focused on the determination of both toxic elements and trace elements in species of the genus *Lactarius* in regions of southern Spain and northern Morocco (Barea-Sepúlveda et al., 2021). Thus, given the relevant role that mushrooms are acquiring in the Spanish diet, providing information on the composition of trace elements and potentially toxic elements, as well as their intake rates through this wild food, can be of great importance for both consumers and food safety agencies.

Table 1

A detailed list of sampling design of analyzed *M. procera* mushrooms from the studied areas along with their corresponding sample ID, specimens' number (*n*), collection location, sampling year, geographical coordinates, and habitat description.

Sample ID	Number of specimens	Location/ sampling year	Latitude	Longitude	Habitat description
(1)	*n* = 12	Mtahen – Parc Naturel of Bouhachem (Chaouen, Morocco) 2017	35° 15’ 62" N	5° 24’ 9.8" W	Close to the highway / Cork oak
(2)	*n* = 13	Taza – Parc Naturel of Bouhachem (Chaouen, Morocco) 2017	35° 16’ 0.84" N	5° 26’ 3.5" W	Far from the urban nucleus / Pine
(3)	*n* = 18	Parc Naturel of Bouhachem (Chaouen, Morocco) 2017	35° 09’ 52" N	5° 11’ 10.2" W	Far from the urban nucleus / Pine
(4)	*n* = 15	Parc National of Talassemtane (Chaouen, Morocco) 2017	35° 09’ 53.9" N	5° 11’ 9.2" W	Far from the urban nucleus / Cork oak
(5)	*n* = 11	Derdara (Chaouen, Morocco) 2017	35° 05’ 48" N	5° 15’ 48.6" W	Close to the highway / Cork oak
(6)	*n* = 17	Sendero Valdeinfierno (Cadiz, Spain) 2017	36° 13’ 38.3" N	5° 35’ 9.8" W	Far from the urban nucleus / Cork oak
(7)	*n* = 14	Sendero Valdeinfierno (Cadiz, Spain) 2017	36° 13’ 36.9" N	5° 35’ 9.6" W	Far from the urban nucleus / Cork oak
(8)	*n* = 18	Pinar del Rey (Cadiz, Spain) 2017	36° 14’ 5.1" N	5° 23’ 54.4" W	Close to the urban nucleus / Pine
(9)	*n* = 14	Pinar del Rey (Cadiz, Spain) 2017	36° 14’ 19’’ N	5° 23’ 39.8" W	Close to the urban nucleus / Pine
(10)	*n* = 19	Sendero El Palancar (Cadiz, Spain) 2017	36° 14’ 55’’ N	5° 33’ 32.9" W	Far from the urban nucleus / Pine
(11)	*n* = 16	Sendero Valdeinfierno (Cadiz, Spain) 2018	36° 13’ 36.7" N	5° 35’ 9.5" W	Far from the urban nucleus / Cork oak
(12)	*n* = 11	Puerto de Galiz (Cadiz, Spain) 2018	36° 33’ 28.7’’ N	5° 36’ 13.4’’ W	Far from the urban nucleus / Cork oak

M. procera, commonly recognized as Parasol mushroom, is a basidiomycete and saprophytic mushroom of the *Agaricaceae* family, which cap is popularly consumed during the mushrooming season in both the North and the South of Spain. This culinary mushroom species uses to

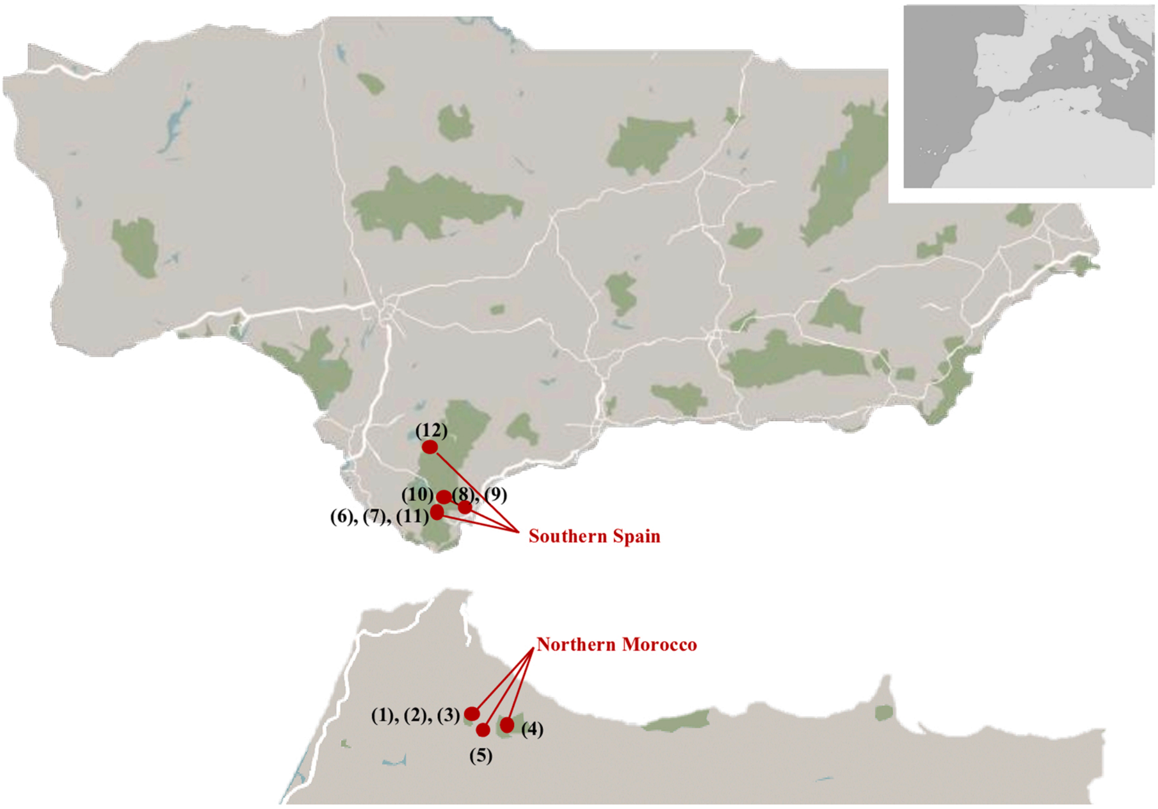

Fig. 1. Geographical location of the sampling areas of the *M. procera* samples analyzed in the present study.

grow alone or distributed in pastures, roadsides, and forest clearings during the autumn period, and is characterized by a soft brown cap of around 30 cm in diameter with a very fibrous stipe, which makes it inedible. Nevertheless, several authors reported evidence of this mushroom species' ability to accumulate toxic elements and trace elements, especially in its edible part (Alonso et al., 2003; Gucia et al., 2012; Kojta et al., 2016; Širić et al., 2016). Therefore, in order to evaluate the exposure to toxic elements and trace elements through the consumption of wild edible mushrooms, this article aimed to determine the content of five toxic elements (Cr, As, Cd, Hg, and Pb) and three trace elements (Zn, Cu, and Se) in the caps and stipes of the high culinary valued *M. procera* mushrooms collected in a total of twelve locations in different regions of northern Morocco and southern Spain. Cr, As, Cd, Hg and Pb have been selected for this study to assess the risks of the consumption of *M. procera* due to their classification as possible and potential carcinogens for human health by the International Agency for Research on Cancer (IARC) and, therefore, because of the adverse effects that they can cause in the body due to prolonged exposure to them. For their part, Cu, Zn and Se were chosen to evaluate the possible health benefits that may occur through the consumption of *M. procera* due to their classification as trace elements and their implication in the regulation of various metabolic processes. Morocco was also selected as a geographical sampling area due to its closeness to the South of Spain and the growing mycological activity during the mushrooming season, involving the consumption of wild-edible species from this region. The Translocation Factor (TF) was calculated to understand the accumulation potential of *M. procera* mushroom. Furthermore, consumers' exposure and health implications were evaluated by using two well-recognized safety criteria: the Estimated Daily Intake of Metals (EDIM) and the Health Risk Index (HRI) for the eight studied metals and metalloids.

2. Material and methods

2.1. Sample collection

In this study, the complete fruiting bodies of *M. procera* mushroom (Table 1) were collected from twelve different geographical locations in northern Morocco and southern Spain during the sampling season, resulting in a total of twelve samples divided into caps and stipes ($n = 24$). A map showing the geographic locations sampled in both regions is shown in Fig. 1. The studied specimens were identified as parasol mushroom according to their distinctive morphological characteristics, which are specific and unmistakable within the *Agaricaceae* family and other mushrooms species and families. The sampling area in southern Spain covered a total of seven points of interest in the province of Cadiz, while in northern Morocco five areas were studied in the province of Chaouen. Furthermore, the habitat description at each geographical location examined was established considering the type of vegetation and the proximity of the area to urban nuclei and/or highways. On the other hand, the *M. procera* samples consisted of at least ten complete specimens collected *in situ* in each of the areas to form a representative pool of sample for each geographical location studied. The samples were properly prepared for their analysis upon their arrival at the laboratory. For this purpose, all fruiting bodies collected were washed with deionized water and divided into caps and stipes. Subsequently, all anatomical parts from each of the geographic locations sampled were dried in an oven at 50 °C for 48 h until constant weight. The dried caps and stipes samples of *M. procera* were finally homogenized with the help of an agate mortar and stored in polyethylene (PE) bottles perfectly labeled according to the anatomical part and sampling area.

2.2. Chemicals and reagents

All the reagents used for the sample acid digestions were from high-analytical grade and 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. Block acid digestion procedure

The acid digestions were performed by means of a DigiPREP Jr block digestion system from SCP Science (Montreal, Quebec, Canada) capable of operating in a temperature range up to 180 °C and with a graphite heating block with 24 positions for 50 mL polypropylene (PE) digestion tubes (DigiTubes; SCP Science; Montreal, Quebec, Canada). Firstly, the subsamples of dried and pulverized (0.25 g) both caps and stipes were placed in the digestion tubes with 5 mL of HNO_3, 2 mL of HCl, and 2 mL of nanopure water. Then, they were digested by applying a procedure of gradual temperature increase for 20 min to 65 °C and maintaining this temperature for a total of 30 min. After a cooling step a second digestion was carried out by adding 3 mL of H_2O_2, gradually rising the temperature for 30 min up until reaching 110 °C and then maintaining it for a total of 60 min. Prior to analysis, the digested samples were filtered using a –600 mbar vacuum port through a 0.45 μm filter (DigiFILTER; SCP Science; Montreal, Quebec, Canada) and then transferred to a clean 50 mL volumetric DigiTube, which was completed to 50 mL with nanopure water. All samples were prepared by triplicate.

2.4. Elemental analysis and quality assurance

The contents of Cr, As, Cd, Hg, Pb, Cu, Zn, and Se in the caps and stipes of *M. procera* samples were determined by an Inductively Coupled Plasma-Mass Spectrometer (Thermo X Series II ICP-MS, Waltham, MA, USA) equipped with a concentric nebulizer, cyclonic spray chamber, quadrupole mass analyzer, and collision/reaction cell. Throughout the analyses, the Xt interface, kinetic energy discrimination (KED), and CCT H_2 (7 %)/He were applied. The ICP-MS instrumental conditions were: CCT H_2(7 %)/He: 4.5 mL min^{-1}; pole bias voltage: –17.0 V; hexapole bias voltage: –20.0 V; 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}; sampling depth: 80.0 mm; and RF power: 1380 W. An internal standard of 103Rh, 72Ge, 191Ir, 209Bi, and 45Sc prepared from individuals' solutions (SCP Science; Montreal, Quebec, Canada) of 1000 μg mL^{-1} was used to correct the temporal variations of signal intensity during the analyses. Furthermore, the analytical methodology used for the determination of the elemental contents was validated and controlled through triplicates, blanks, and a Certified Reference Material (CRM), namely *B. edulis* powder Control Material CS-M-3 (Institute of Nuclear Technology and Chemistry; Warsaw, Poland). No relevant interferences for the quantified elements were found in the blank samples. The recovery levels in the reference material (CS-M-3) were found to be in an acceptable range of 70 – 130 % for the determined elements. Limits of Detection (LOD) for Cr, As, Cd, Hg, Pb, Cu, Zn, and Se were between 0.002 and 0.1 mg $kg^{-1}$of dry weight (DW).

2.5. Translocation factor (TF)

To estimate the transfer of the eight studied toxic elements and trace elements between the anatomical parts (caps and stipes) of *M. procera* samples, the translocation factor (TF) was calculated (Dimitrijevic et al., 2021; Stefanović et al., 2016). The TF is defined as the ratio between the metal and metalloid content in the cap to stipe and is calculated according to the following expression (1):

$$\mathrm{TF} = \frac{C_C}{C_S} \tag{1}$$

where C_C is the metal or metalloid content in the cap and C_S is the content of the metal or metalloid in the stipe.

2.6. Estimated daily intake of metals (EDIM)

The Estimated Daily Intake of Metals (EDIM) was calculated for the edible part of *M. procera* fruiting body using the content obtained for the cap expressed in mg kg^{-1} of dry weight (DW), as shown in the following Eq. (2):

$$EDIM = \frac{C_c \cdot D_{food\ intake}}{BW} \tag{2}$$

where C_c is the metal or metalloid content (mg kg^{-1}) in the cap, $D_{food\ intake}$ refers to the mushroom daily intake, and *BW* is the average person body weight in kg. A food ration of 300 g of fresh mushrooms (30 g of dried mushrooms per day) (Falandysz et al., 2017a; Gucia et al., 2012; Kalacï, 2000; Kojta et al., 2016; Liu et al., 2015a; Sarikurkcu et al., 2020) and a regular consumer of 70 kg body weight (Kalacï, 2000; Sarikurkcu et al., 2020; Záhorcová et al., 2016) were considered.

2.7. Health risk index

The Health Risk Index (HRI) was calculated according to Eq. (3) to evaluate the potentially human health risk due to the exposure to the studied toxic elements and trace elements in the edible anatomical part of *M. procera* samples:

$$\mathrm{HRI} = \frac{EDIM}{R_f D} \tag{3}$$

where EDIM is the daily intake of metals and metalloids through the consumption of the edible cap of *M. procera* in our study and R_fD is the maximum acceptable daily oral dose of a toxic substance. Based on the data proposed by Sarikurkcu et al. (2020) and the data in the U.S. EPA. Integrated Risk Information System (Integrated Risk Information System, 2022), the reference doses for Cr, As, Cd, Hg, Pb, Cu, Zn, and Se are 3, 0.3, 1, 0.3, 3.5, 40, 300, and 0.5 μg kg^{-1} body weight per day, repectively.

2.8. Statistical analysis and software

Results are presented as means of triplicate determinations and standard deviation (*s.d.*). All the statistical analyses were performed using RStudio software (R version 4.0.5, Boston, MA, USA). The differences between the elemental content in the caps of *M. procera* according to the sampling area (northern Morocco and southern Spain) were assessed using both univariate and multivariate statistics, such as the analysis of variance (ANOVA), Hierarchical Cluster Analysis (HCA), and Principal Component Analysis (PCA). For the univariate statistics analysis, we previously tested whether the quantitative variables were normally distributed by Shapiro-Wilk test using the *shapiro.test* function from the *stats* package. The homogeneity of variance was evaluated with the Levene's test using the *leveneTest* from *car* package. A One-Way ANOVA using the *aov* function from the *stats* package was performed in those cases where a normal distribution of the quantitative variables was found. When no normal distribution of the data was observed the non-parametric ANOVA, namely Kruskal-Wallis test, was carried out using the *kruskal.test* function from the *stats* package. Regarding the multivariate analysis, HCA was performed using the *hclust* function from the *stast* package. Selection of the Linkage method for the HCA was established by calculating and comparing the agglomerative coefficient obtained from different Linkage methods (Average, Single, Complete and Ward) using the *agnes* function of the *cluster* package. The HCA

Table 2
Concentrations of toxic elements and trace elements for the caps and stipes of *M. procera* mushrooms samples (mg kg^{-1} dry weight). Values are presented as mean ± standard deviation ($n = 3$).

Element	Anatomic part	Sample ID											
		#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12
Cr	Cap	0.550 ± 0.02	0.495 ± 0.02	0.866 ± 0.03	14.9 ± 0.04	1.83 ± 0.02	2.34 ± 0.21	3.23 ± 0.2	8.21 ± 0.07	0.600 ± 0.03	2.37 ± 0.05	1.97 ± 0.1	4.72 ± 0.2
	Stipe	1.56 ± 0.03	0.263 ± 0.02	1.77 ± 0.03	13.4 ± 0.4	3.56 ± 0.1	0.728 ± 0.02	2.95 ± 0.02	17.8 ± 0.3	1.63 ± 0.02	1.49 ± 0.01	1.16 ± 0.2	3.23 ± 0.2
As	Cap	0.813 ± 0.06	0.355 ± 0.03	< 0.06	1.27 ± 0.08	0.633 ± 0.07	0.262 ± 0.04	0.459 ± 0.06	0.880 ± 0.005	0.969 ± 0.03	2.67 ± 0.1	1.71 ± 0.05	0.732 ± 0.01
	Stipe	0.534 ± 0.07	0.254 ± 0.06	0.363 ± 0.03	1.18 ± 0.07	0.441 ± 0.04	0.331 ± 0.02	0.314 ± 0.02	0.500 ± 0.04	0.510 ± 0.03	1.61 ± 0.05	1.23 ± 0.03	0.459 ± 0.06
Cd	Cap	1.50 ± 0.03	0.408 ± 0.02	0.181 ± 0.01	2.812 ± 0.1	0.369 ± 0.02	0.474 ± 0.01	0.593 ± 0.05	0.275 ± 0.02	0.862 ± 0.03	4.71 ± 0.04	1.55 ± 0.03	0.443 ± 0.01
	Stipe	0.827 ± 0.04	0.241 ± 0.02	0.149 ± 0.02	1.67 ± 0.09	0.165 ± 0.01	0.162 ± 0.01	0.176 ± 0.02	0.0730 ± 0.01	0.206 ± 0.01	1.10 ± 0.04	0.675 ± 0.02	0.593 ± 0.05
Hg	Cap	0.759 ± 0.04	1.15 ± 0.02	1.60 ± 0.03	0.496 ± 0.02	1.65 ± 0.04	2.47 ± 0.04	3.13 ± 0.2	1.80 ± 0.001	2.12 ± 0.09	1.08 ± 0.03	0.902 ± 0.01	5.25 ± 0.01
	Stipe	0.509 ± 0.02	0.873 ± 0.03	2.00 ± 0.01	0.287 ± 0.02	0.739 ± 0.04	1.32 ± 0.06	1.59 ± 0.02	0.957 ± 0.01	1.07 ± 0.03	0.638 ± 0.003	0.648 ± 0.04	3.13 ± 0.2
Pb	Cap	0.307 ± 0.002	0.325 ± 0.01	0.915 ± 0.02	1.95 ± 0.09	0.638 ± 0.004	1.15 ± 0.03	1.43 ± 0.06	1.52 ± 0.03	1.44 ± 0.03	0.408 ± 0.008	0.312 ± 0.003	1.52 ± 0.02
	Stipe	0.223 ± 0.006	0.170 ± 0.01	1.76 ± 0.02	2.09 ± 0.1	0.324 ± 0.012	0.372 ± 0.01	0.474 ± 0.01	0.798 ± 0.01	0.351 ± 0.01	0.345 ± 0.005	0.138 ± 0.001	1.428 ± 0.05
Cu	Cap	125 ± 1	61.1 ± 1	96.1 ± 2	63.8 ± 3	195 ± 2	93.8 ± 2	108 ± 5	107 ± 2	131 ± 3	134 ± 3	113 ± 1	152 ± 2
	Stipe	88.7 ± 2	59.6 ± 1	176 ± 3	53.8 ± 3	113 ± 2	65.2 ± 3	77.8 ± 1	60.8 ± 1	93.1 ± 1	122 ± 1	98 ± 4	108 ± 5
Zn	Cap	65.8 ± 1	81.1 ± 1	92.9 ± 3	85.1 ± 4	93.4 ± 2	99.7 ± 2	129 ± 7	88.9 ± 0.3	98.6 ± 4	89.8 ± 1	86.7 ± 1	161 ± 1
	Stipe	43.4 ± 1	54.3 ± 1	70.2 ± 1	57.4 ± 3	45.1 ± 1	58.3 ± 2	106 ± 3	52.2 ± 0.7	50.6 ± 1	64.4 ± 1	59.8 ± 3	129 ± 7
Se	Cap	1.71 ± 0.03	1.77 ± 0.1	3.32 ± 0.1	1.49 ± 0.1	2.53 ± 0.2	2.84 ± 0.2	3.16 ± 0.1	2.30 ± 0.1	3.69 ± 0.2	2.30 ± 0.1	1.36 ± 0.04	4.03 ± 0.1
	Stipe	1.33 ± 0.2	1.50 ± 0.06	3.01 ± 0.3	1.12 ± 0.2	1.34 ± 0.01	2.07 ± 0.2	2.09 ± 0.01	1.34 ± 0.03	2.34 ± 0.1	1.65 ± 0.09	1.11 ± 0.04	3.16 ± 0.1

Table 3
The translocation factor (TF) of *M. procera* samples for the studied toxic elements and trace elements.

Elements	Sample ID											
	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12
Cr	0.353	1.88	0.489	1.11	0.514	3.21	1.09	0.461	0.368	1.59	1.69	1.46
As	1.52	1.40	–	1.08	1.43	0.793	1.46	1.76	1.90	1.66	1.40	1.60
Cd	1.81	1.69	1.21	1.69	2.234	2.93	3.38	3.77	4.18	4.28	2.30	0.747
Hg	1.49	1.32	0.799	1.72	2.24	1.88	1.96	1.88	1.99	1.69	1.39	1.68
Pb	1.38	1.92	0.520	0.937	1.97	3.09	3.02	1.91	4.10	1.18	2.27	1.07
Cu	1.41	1.02	0.545	1.19	1.23	1.44	1.39	1.76	1.41	1.01	1.16	1.41
Zn	1.52	1.49	1.32	1.48	2.07	1.71	1.22	1.70	1.95	1.39	1.45	1.24
Se	1.29	1.14	1.10	1.25	1.89	1.38	1.51	1.72	1.58	1.40	1.23	1.28

results were plotted in a dendrogram using the *fviz_dend* function from the *factoextra* package. PCA was performed using the *prcomp* function from the stats package. The *fviz_eig* function from the *factoextra* package was used to extract and visualize the output of this multivariate data analysis. The scores and loadings obtained from the PCA were graphically displayed using the *ggplot* function from the *ggplot2* package.

3. Results

3.1. Toxic elements and trace elements content

The contents of trace elements and toxic elements are shown in Table 2 for the different anatomic parts in the analyzed samples, expressed as mean of triplicates in mg kg^{-1} DW. All the studied toxic elements and trace elements included in our study were detected in all the samples except for As in the cap for sample #3 collected in Parc Naturel of Bouhachem (Chaouen, Morocco). Overall, the minimum and maximum levels in the present study were (Table 2): 0.263 ± 0.02 and 17.8 ± 0.3 mg kg^{-1} for Cr, 0.254 ± 0.06 and 2.67 ± 0.1 mg kg^{-1} for As, 0.0730 ± 0.01 and 4.71 ± 0.04 mg kg^{-1} for Cd, 0.287 ± 0.02 and 5.25 ± 0.01 mg kg^{-1} for Hg, 0.138 ± 0.001 and 2.09 ± 0.1 mg kg^{-1} for Pb, 53.8 ± 3 and 195 ± 2 mg kg^{-1} for Cu, 43.4 ± 1 and 161 ± 1 mg kg^{-1} for Zn, and 1.11 ± 0.04 and 4.03 ± 0.1 mg kg^{-1} for Se, respectively.

3.2. Translocation factor

Translocation factor (TF) was calculated to evaluate the accumulation trend of the determined toxic elements and trace elements in the different anatomical parts of the *M. procera* samples. As a criterion, a TF greater than 1 indicates that the content of the specific metal or metalloid is higher in the cap than in the stipe (Dimitrijevic et al., 2021; Stefanović et al., 2016). The TF values obtained for each metal and metalloid in all the samples are shown in Table 3.

3.3. Statistical analysis

Differences between the elemental content of the edible part (cap) of *M. procera* samples were analyzed according to the sampling area using univariate statistics. The results obtained regarding the normality (Shapiro-Wilk test) and homoscedasticity (Levene's test) of the

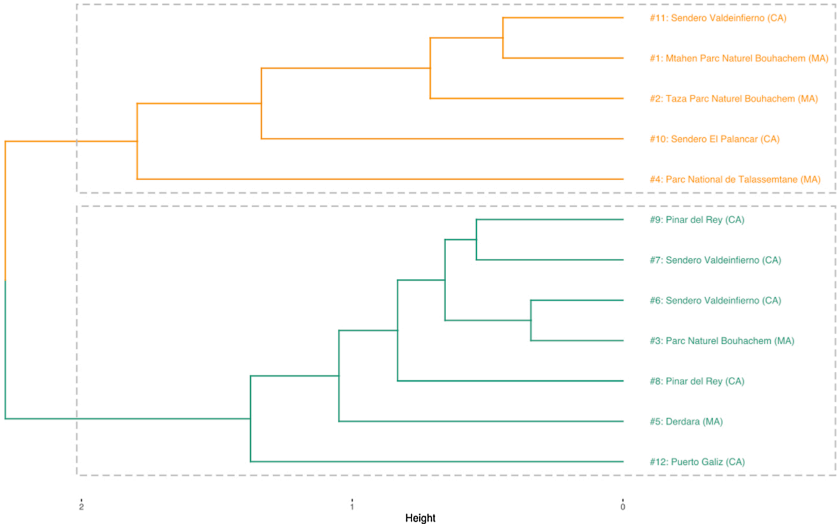

Fig. 2. Dendrogram of HCA analysis combined with Ward's linkage and Euclidean distance. Cap samples ($n = 12$) are colored according to the cluster ("green" and "orange") to which they belong.

quantitative variables are shown in Table A1 as an appendix, along with the statistical comparison between the means of the elemental content based on the sampling areas using the One-Way ANOVA/Kruskal-Wallis tests. Furthermore, HCA and PCA were performed as unsupervised multivariate pattern recognition techniques to search for grouping trends based on the geographic area sampled in order to contrast the results obtained in the univariate statistical study. The HCA and PCA results are presented in Figs. 2 and 3 , respectively.

3.4. *Health risk assessment*

3.4.1. *Estimated daily intake of metals*

To assess the possible risks to human health associated with the consumption of the edible part of the *M. procera* samples, the Estimated Daily Intake of Metals (EDIM) was calculated for the five toxic elements (Cr, As, Cd, Hg, and Pb) as well as for the three trace elements (Cu, Zn, and Se). Firstly, the results obtained for metal and metalloid content in the edible part (caps) were used, and a serving portion of 30 g of dried *M. procera* caps and an average consumer of 70 kg was assumed. The EDIM results are shown in Table 4.

3.4.2. *Health risk index*

The evaluation of the potential health risk was performed by calculating the Health Risk Index (HRI) as the ratio between the EDIM from consuming the caps of the studied samples and the R_fD (Reference Dose) for each metal and metalloid. The results have been included in Table 4. HRI's values equals or below 1 for a given metal would indicate that the consumption of caps of this species collected in a particular geographical location is considered safe to the consumer (Liu et al., 2015a; Sarikurkcu et al., 2020).

4. Discussions

4.1. *Toxic elements and trace elements content*

In general, Cu was the predominant element found in the samples studied, followed by Zn in most of the cases. The rest of the elements were found in relatively lower contents. The results obtained for Cu in the present study were compared with those previously reported by our research group for samples of the genus *Lactarius* collected in northern Morocco and southern Spain (Barea-Sepúlveda et al., 2021). We observed that *M. procera* accumulates a higher Cu content than these ectomycorrhizal species, where Zn was the major element. In turn, this is consistent with the results presented by Alonso et al. (2003), which indicated that this saprophytic species tends to accumulate higher contents of this trace element than other ectomycorrhizal species. On the other hand, in a comparison of the content of macronutrients, trace elements and toxic elements among several edible mushroom species, Mleczek et al. (2021) reported that *M. procera* was a rich source of Cu. Furthermore, previous studies by other authors have reported similar results suggesting a good and active regulation of Cu uptake and sequestration in the fruiting bodies of *M. procera*, making it a good source of this trace element (Falandysz et al., 2008; Gucia et al., 2012). This phenomenon could be explained by the higher decomposition and catalase activities of *M. procera*, resulting in a more effective metal uptake mechanism for Cu (Alonso et al., 2003, 2000).

4.1.1. *Toxic and potentially toxic elements*

Chromium: The content of Cr (Table 2) in the caps ranged from 0.495 ± 0.02 mg kg^{-1} to 14.9 ± 0.04 mg kg^{-1} DW, being the lowest content for sample #2 collected in Taza in the Parc Naturel of Bouhachem (Chaouen, Morocco) and the highest for sample #4 collected in the Parc National of Talassemtane (Chaouen, Morocco). Meanwhile, Cr contents in the stipes ranged from 0.263 ± 0.02 – 17.8 ± 0.3 mg kg^{-1} DW, with the highest content observed for sample #8 collected in Pinar del Rey (Cadiz, Spain) and the lowest for sample #2 collected in Taza in the Parc Naturel of Bouhachem (Chaouen, Morocco). Information on Cr contents in the caps, stipes, and fruiting bodies of *M. procera* mushroom has been reported by several authors: Kojta et al. (2016) reported contents of 0.26 – 1.3 mg kg^{-1} in the caps and 0.58 – 0.98 mg kg^{-1} in the stipes. On the other hand, Gucia et al. (2012) obtained contents in the range of 0.10 – 0.80 mg kg^{-1} in the caps and 0.11 – 1.2 mg kg^{-1} in the stipes. In the study by Širić et al. (2016) contents of 2.24 mg kg^{-1} in stipes and 3.62 mg kg^{-1} in caps were found. Árvay et al. (2014) conducted a study on the entire fruiting body indicating contents of 0.7 mg kg^{-1}. In turn, Mleczek et al. (2021) determined the Cr content in the fruiting body of *M. procera*, obtaining a range of content in this toxic element of 0.025 – 3.43 mg kg^{-1}. Thus, according to the literature, it was observed that the Cr contents in our study were generally above those reported by other authors.

Arsenic: In the caps, the highest content of As (Table 2) was 2.67 ± 0.1 mg kg^{-1} DW corresponding to sample #10 collected at Sendero El Palancar (Cadiz, Spain), whereas the lowest content was observed in sample #6 collected at Sendero de Valdeinfierno (Cadiz, Spain) with a value of 0.262 ± 0.04 mg kg^{-1} DW of As. The content of this metalloid in the stipe of the *M. procera* samples ranged from 0.254 ± 0.06 – 1.61 ±

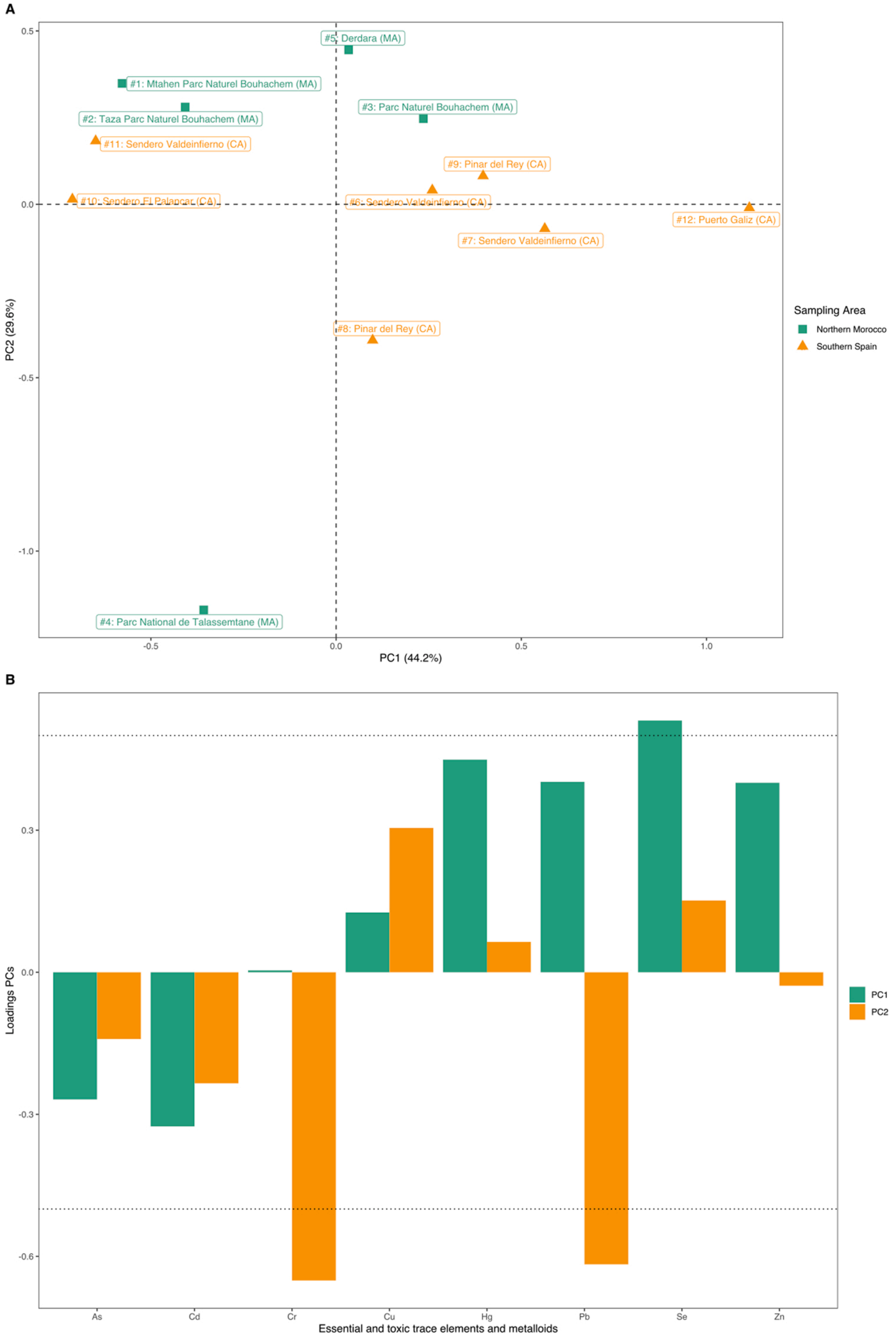

Fig. 3. **(A)** Score obtained for PC1 and PC2 for all the samples (*n* = 12); **(B)** Loadings obtained in PC1 and PC2.

0.05 mg kg^{-1} DW, being the highest for sample #10 collected in the Sendero El Palancar (Cadiz, Spain) and the lowest content for sample #2 collected in Taza in the Parc Naturel of Bouhachem (Chaouen, Morocco). The As contents in *M. procera* reported in the literature ranged from 0.49 – 1.8 mg kg^{-1} in the caps (Stefanović et al., 2016), 0.54–1.3 mg kg^{-1} in the stipes (Stefanović et al., 2016), and 0.29 – 2.8 mg kg^{-1} for the entire fruiting bodies (Falandysz et al., 2017b). Furthermore, Mleczek et al. (2021) determined the As content in the fruiting body of *M. procera*, ranging from 0.094 to 1.51 mg kg^{-1}. In general, the results obtained for As levels in our study were below the contents previously reported in other investigations.

Cadmium: The lowest/highest contents of Cd (Table 2) in the caps were observed in sample #3 (0.181 ± 0.01 mg kg^{-1} DW) from Parc Naturel of Bouhachem (Chaouen, Morocco) and #10 (4.71 ± 0.04 mg kg^{-1} DW) from Sendero El Palancar (Cadiz, Spain), respectively. Regarding the stipes, the highest amount of Cd was found in sample #4 (1.67 ± 0.09 mg kg^{-1} DW) from Parc National of Talassemtane (Chaouen, Morocco), while the lowest one was detected for sample #8 (0.0730 ± 0.01 mg kg^{-1} DW) collected in Pinar del Rey (Cadiz, Spain). Several researchers reported that Cd contents in *M. procera* mushroom were found at levels of 0.54–1.33 mg kg^{-1} for fruiting bodies (Cocchi et al., 2006; Kosanić et al., 2016; Melgar et al., 2016). In turn, Mleczek

Table 4
Estimated Daily Intake of Metals (EDIM) and Health Risk Index (HRI) in the caps of *M. procera* samples.

	Estimated Daily Intake of Metals (EDIM, µg kg body weight^{-1} per day)								Health Risk Index (HRI)							
Sample ID	Cr	As	Cd	Hg	Pb	Cu	Zn	Se	Cr	As	Cd	Hg	Pb	Cu	Zn	Se
#1	0.236	0.348	0.643	0.325	0.132	53.6	28.2	0.733	0.079	1.16	0.643	1.08	0.0380	1.34	0.0940	1.47
#2	0.212	0.152	0.175	0.493	0.139	26.2	34.8	0.759	0.071	0.507	0.175	1.64	0.0400	0.655	0.116	1.52
#3	0.371	–	0.078	0.686	0.392	41.2	39.8	1.42	0.124	–	0.078	2.29	0.112	1.03	0.133	2.85
#4	6.39	0.544	1.21	0.212	0.837	27.4	36.5	0.638	2.13	1.82	1.21	0.707	0.239	0.684	0.122	1.28
#5	0.784	0.271	0.158	0.709	0.273	83.7	40.0	1.09	0.261	0.904	0.158	2.36	0.0780	2.09	0.133	2.17
#6	1.00	0.112	0.203	1.06	0.493	40.2	42.7	1.22	0.334	0.374	0.203	3.53	0.141	1.01	0.142	2.44
#7	1.38	0.197	0.254	1.34	0.613	46.2	55.5	1.35	0.461	0.656	0.254	4.47	0.175	1.16	0.185	2.71
#8	3.52	0.377	0.118	0.771	0.651	45.9	38.1	0.986	1.17	1.26	0.118	2.57	0.186	1.15	0.127	1.97
#9	0.257	0.415	0.369	0.909	0.617	56.2	42.3	1.58	0.0860	1.38	0.369	3.03	0.176	1.40	0.141	3.16
#10	1.016	1.14	2.02	0.462	0.175	57.2	38.5	0.987	0.339	3.81	2.02	1.54	0.0500	1.43	0.128	1.97
#11	0.843	0.734	0.666	0.386	0.134	48.8	37.1	0.583	0.281	2.45	0.666	1.29	0.0380	1.22	0.124	1.17
#12	2.02	0.314	0.190	2.25	0.652	65.1	68.8	1.73	0.675	1.05	0.190	7.49	0.186	1.63	0.229	3.45
R$_f$D (µg kg body weight^{-1} per day) [a]	3 [d]	0.3 [d]	1[d]	0.3 [d]	3.5 [e]	40 [e]	300 [d]	0.5 [d]								
PTDI (µg kg body weight^{-1} per day) [b]	–	2.14 [f]	0.82 [f]	0.57 [f]	–	–	–	–								
PTMDI (µg kg body weight^{-1} per day) [c]	–	–	–	–	–	5000[f]	300-1000 [f]	–								

[a] R$_f$D – Reference dose.
[b] PTDI – Provisional tolerable daily intake.
[c] PTMDI – Provisional tolerable maximum daily intake.
[d] USEPA – U.S. Environmental Protection Agency.
[e] Sarikurkcu, C. et al. (2020).
[f] JECEFA - The Joint FAO/WHO Expert Committee on Food Additives.

et al. (2021) determined the Cd content in the fruiting body of *M. procera*, obtaining a range of content in this toxic element of 0.044 – 0.637 mg kg^{-1}. For caps and stipes, Širić et al. (2016) reported contents of 2.54 mg kg^{-1} (caps) and 2.07 mg kg^{-1} (stipes), while Gucia et al. (2012) obtained levels of 1.1 – 3.2 mg kg^{-1} in caps and 0.14 – 1.7 mg kg^{-1} in stipes. In general, the results obtained through our analyses indicated that the Cd contents of these samples were in line with the literature.

Mercury: The highest content of Hg (Table 2) in the caps was 5.25 ± 0.01 mg kg^{-1} DW, corresponding to sample #12 collected in Puerto de Galiz (Cadiz, Spain). On the other hand, the lowest content was observed in sample #4 from Parc National of Talassemtane (Chaouen, Morocco) with a value of 0.496 ± 0.02 mg kg^{-1} DW. About the stipes, Hg contents were observed between 0.287 ± 0.02 and 3.13 ± 0.2 mg kg^{-1} DW, with the lowest content for sample #4 collected in the Parc National of Talassemtane (Chaouen, Morocco) and the highest for #12 from Puerto de Galiz (Cadiz, Spain). The Hg contents found in other studies were in the range of 0.616 – 2.80 mg kg^{-1} for fruiting bodies (Cocchi et al., 2006; Mleczek et al., 2021; Širić et al., 2016), 1.58 – 3.00 mg kg^{-1} for caps (Alonso et al., 2000; Ostos et al., 2015), and 0.91 – 1.87 mg kg^{-1} for stipes (Alonso et al., 2000; Ostos et al., 2015). Comparing the results obtained with those previously reported by these authors, it has been observed that, in general, the Hg contents here are consistent with the literature.

Lead: In caps and stipes, Pb (Table 2) occurred in the ranges of 0.307 ± 0.002 and 1.95 ± 0.09 mg kg^{-1} DW and 0.138 ± 0.001 and 2.09 ± 0.1 mg kg^{-1} DW, respectively. Regarding the caps, the highest Pb contents were observed in sample #4 collected in the Parc National of Talassemtane (Chaouen, Morocco), while the lowest were found in sample #1 collected in Mtahen in the Parc Naturel of Bouhachem (Chaouen, Morocco). For the stipes, the lowest/highest contents were found in sample #11 from the Sendero de Valdeinfierno (Cadiz, Spain) and sample #4 from the Parc Natural of Talassemtane (Chaouen, Morocco), respectively. The Pb contents previously reported by other authors were found to range from 0.183 mg kg^{-1} to 2.79 mg kg^{-1} on the whole fruiting body (Mleczek et al., 2021; Yamaç et al., 2007), 0.097 – 3.62 mg kg^{-1} in the caps (Lalotra et al., 2016; Širić et al., 2016), and 0.043 – 2.24 mg kg^{-1} in the stipes (Lalotra et al., 2016; Širić et al., 2016). In general, the Pb levels found in the samples studied were in accordance with the contents reported in the literature.

4.1.2. Trace elements

Copper: The amount of Cu (Table 2) in the stipes ranged from 53.8 ± 3 – 176 ± 3 mg kg^{-1} DW, with the highest content observed in sample #3 collected in Parc Naturel of Bouhachem (Chaouen, Morocco) and the lowest in sample #4 from Parc National of Talassemtane (Chaouen, Morocco). Meanwhile, for the caps, the highest contents of this trace element were observed in sample #5 (195 ± 2 mg kg^{-1} DW) collected in Derdara (Chaouen, Morocco) and the lowest for sample #2 (61.1 ± 1 mg kg^{-1} DW) collected in Parc Naturel of Bouhachem (Chaouen, Morocco). Regarding Cu contents with respect to *M. procera* mushroom in the literature, levels of this trace metal were found in the range of 200 – 368 mg kg^{-1} in fruiting bodies (Alonso et al., 2003; Tuzen et al., 2007). In turn, Mleczek et al. (2021) determined the Cu content in the fruiting body of *M. procera*, obtaining a range of content in this trace element of 4.98 – 138 mg kg^{-1}. With respect to caps and stipes, Kojta et al. (2016) indicated contents of 94 – 220 mg kg^{-1} in caps and 63 – 120 mg kg^{-1} in stipes, while Gucia et al. (2012) reported contents of 100 – 200 mg kg^{-1} in caps and 61 – 210 mg kg^{-1} in stipes. Thus, the results obtained through our analysis agree with those reported in previous studies.

Zinc: The highest/lowest contents of Zn (Table 2) in the caps were found in sample #12 (161 ± 1 mg kg^{-1} DW) from Puerto de Galiz (Cadiz, Spain) and sample #1 (65.8 ± 1 mg kg^{-1}) collected in Mtahen in the Parc Naturel of Bouhachem (Chaouen, Morocco), respectively. In turn, the lowest content in the stipes was observed in sample #1 (43.4 ± 1 mg kg^{-1}) from Mtahen in the Parc Naturel of Bouhachem (Chaouen, Morocco), whereas the highest content was found in sample #12 (129 ± 7 mg kg^{-1}) collected in Puerto de Galiz (Cadiz, Spain). The Zn contents reported in other studies ranged from 34.5 – 157 mg kg^{-1} for the fruiting body (Alonso et al., 2003; Mleczek et al., 2021; Tuzen et al., 2007), and between 74 –190/ 46 – 110 mg kg^{-1} for caps and stipes (Gucia et al., 2012), respectively. The results obtained in the present study are in line with data observed in the literature.

Selenium: In caps and stipes, Se (Table 2) was observed in the ranges of 1.36 ± 0.04 and 4.03 ± 0.1 mg kg^{-1} DW and 1.11 ± 0.04 and 3.16 ± 0.1 mg kg^{-1} DW, respectively. For both, caps and stipes, the highest Se contents were observed in sample #12 collected in Puerto de Galiz (Cadiz, Spain), while the lowest were found in sample #11 collected in Sendero Valdeinfierno (Cadiz, Spain). With regard to Se contents available for this species in the literature, Cocchi et al. (2006) reported levels of 3.19 mg kg^{-1} in the fruiting bodies. On the other hand, Tuzen et al. (2007) indicated levels of this metalloid of 66.9 mg kg^{-1} also in the fruiting body. Regarding stipes and caps, Stefanović et al. (2016) obtained Se contents in the range of 0.40 – 1.6 mg kg^{-1} for caps and 0.38 – 1.6 mg kg^{-1} for stipes, while Falandysz (2008) reported contents of 39 mg kg^{-1} in caps and 25 mg kg^{-1} in stipes. In relation to literature, the Se contents in the present study are generally in agreement with the results presented by Cocchi et al. (2006) and Stefanović et al. (2016), and below the levels reported by Tuzen et al. (2007) and Falandysz (2008).

4.2. Translocation factor

According to Table 3, it can be observed that the TF for Cr was in the range of 0.353 – 3.21, accumulating in general higher Cr contents in the caps (TF > 1) except for samples #1 Mtahen in the Parc Naturel of Bouhachem (Chaouen, Morocco), #3 from Parc Naturel of Bouhachem (Chaouen, Morocco), #5 Derdara (Chaouen, Morocco), and #8 and #9 from Pinar del Rey (Cadiz, Spain), which means that in these five samples the content is higher in the stipe. The TF values for As were found to range from 0.793 to 1.90, with most of them being greater than 1. This fact indicates that the contents of this metalloid are higher in the caps. Only sample #6 collected at Sendero de Valdeinfierno (Cadiz, Spain) showed a higher As content in the stipe (TF < 1). Regarding Cd, the TF was found in values ranging from 0.747 to 4.28. This non-essential and potentially toxic element was mostly found in higher contents in the cap, except for sample #12 from Puerto de Galiz (Cadiz, Spain). It is noteworthy that, among the eight elements studied, the highest TFs were found in this metal, corresponding to samples #9 (TF = 4.18) from Pinar del Rey (Cadiz, Spain) and #10 (TF = 4.28) from Sendero El Palancar (Cadiz, Spain). For its part, the TFs values for Hg were found in the ranges of 0.799 – 2.24, with most of them presenting values greater than 1, except for sample #3 collected in the Parc Naturel of Bouhachem (Chaouen, Morocco), which TF indicates that Hg accumulates more in the stipe for this sample. The TF values for Pb were found to range from 0.520 to 4.10. It is remarkable that Pb is the second metal in this study with the highest TFs, especially for sample #9 (TF = 4.10) from Pinar del Rey (Cadiz, Spain). In general, this toxic element was observed to be found in higher contents in the caps. A higher accumulation of Pb in the stipes (TF < 1) was only observed in samples #3 from Parc Naturel of Bouhachem (Chaouen, Morocco) and #4 from Parc National of Talassemtane (Chaouen, Morocco). In the case of Cu, it was found that contents are generally higher in the caps, with TF in the range of 0.545 – 1.76. However, the content of this essential metal is higher in the stipe (TF < 1) for sample #3 from the Parc Naturel of Bouhachem (Chaouen, Morocco). For Zn and Se, all samples had a higher content of these two elements in the caps, with the TF values for the former being between 1.22 and 2.07, and 1.10 – 1.89 for the latter.

In short, the elements studied were generally found in higher contents in the caps. According to the literature, the distribution of toxic elements and trace elements along the fruiting body has been described as uneven, with higher concentrations in the spore-forming part (although not in the spore), a lower content in the rest of the cap and the lowest level in the stipe (Thomet et al., 1999; Elekes et al., 2010). This fact may be due to the different nature and concentration of proteins shown by the various structures of the carpophore, with a more complex electrophoretic spectrum in the cap than in the stipe (Chang and Chan, 1973; Gadd, 1993). Given this context, the results observed in the present study agreed with those reported for other mushroom species. Furthermore, these results are in line with those reported by several studies on the metal content in *M. procera*, where it was indicated that this species tends to accumulate higher contents of some metals in the cap (Gucia et al., 2012; Kojta et al., 2016).

4.3. Statistical analysis

A univariate statistical study was carried out to evaluate whether there are significant differences between the means of the toxic elements and trace elements content depending on the sampling zones. As it can be seen in Table A1, data were normally distributed ($p > 0.05$) just for As, Pb, Cu, and Se, and, in all the cases, homoscedasticity ($p > 0.05$) was found. Therefore, the One-Way ANOVA was applied for those cases where the data followed a normal distribution (As, Pb, Cu, and Se) to establish the significant differences among samples of different geographical origin. By contrast, for those quantitative variables where a normal distribution of the data was not observed (Cr, Cd, Hg, and Zn), the non-parametric Kruskal Wallis test was used. Both analyses were not statistically significant ($p > 0.05$), indicating similar mean levels for the sampling areas studied. Therefore, based on the eight toxic elements and trace elements determined in the present study, non-statistically significant evidence allows us to establish that *M. procera* samples can be differentiated according to their geographical origin. As it is known, mushrooms accumulate toxic elements and trace elements through the hyphae network located in the upper soil horizon. Consequently, the soil where the fruiting bodies grew, and therefore the geographical location, is a factor that may influence the uptake of both toxic elements and trace elements by mushrooms. The results obtained here may indicate that the sampled areas in the Chaouen (northern Morocco) and Cadiz (southern Spain) provinces could present similar soil geochemical characteristics (pH, organic matter content, texture, toxic elements and trace elements contents, etc.). Notwithstanding, more information on the soils where they fructified would be needed to be able to contrast these results. To corroborate the results obtained through this univariate statistical study, two unsupervised multivariate analysis techniques, HCA and PCA, were applied. For this purpose, the mean contents in mg kg^{-1} of the toxic elements and trace elements determined in the *M. procera* caps were used as variables, thus obtaining a data matrix D_{nxm} where n is the number of samples ($n = 12$) and m the number of toxic elements and trace elements determined ($m = 8$). Prior to the application of the multivariate analysis the data matrix was normalized using Min-Max normalization, which consists on linearly transforming the original data so that each variable is assigned a value of 0 for its minimum value and 1 for its maximum value, with the rest of the values in a range between 0 and 1. First, HCA was performed. For this analysis, the Euclidean distance was selected for inter-individual similarity matrix calculation and Ward's method as the inter-group measure. The choice of Ward's method was established by calculating and comparing the agglomerative coefficient obtained with different linkage methods (Average, Complete, Single, and Ward). This coefficient allows finding the linkage method which a stronger clustering structure is identified, being an agglomerative coefficient equal to 1 the highest value. Here, Ward's method was the linkage method which presented the most closely to 1 agglomerative coefficient, being 0.64. The results obtained through HCA are shown in the dendrogram in Fig. 2. As can be seen, the *M. procera* cap samples are grouped into two main clusters: green cluster and orange cluster. The green cluster included a total of seven samples, five of them belonging to caps of this edible species collected in southern Spain and the remaining two to samples collected in northern Morocco. The orange cluster is composed of a total of five samples, three of them from northern Morocco and the two others from southern Spain. Nonetheless, this dendrogram is not completely consistent since there is no clear tendency to group the collected caps according to the geographical area in which they were obtained. These results are consistent with those obtained in the univariate statistical study. PCA was also carried out to corroborate the HCA clustering trend. Fig. 3A shows the plot of the scores obtained for the first two principal components (PC1 and PC2) for all the caps samples ($n = 12$), and Fig. 3B shows the plot of the loadings obtained for each principal component (PCs). PCs 1 and 2 explained 44.2 % and 29.6 % of the variance of the data, respectively, which implies a total accumulated variance of 73.8 %. In this case, PC1 (Fig. 3A) was mainly responsible for the separation of the samples. Nevertheless, as in the HCA, they were not clearly differentiated according to the sampling area. For their part, Cr, Pb, and Se had a greater weight on this PC (Fig. 3B). The results obtained from PCA were thus in agreement with those obtained by HCA and the univariate statistical study.

4.4. Health risk assessment

4.4.1. Estimated daily intake of metals

The EDIM results were compared with the values established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) for the Provisional Tolerable Maximum Daily Intake (PTMDI) and Provisional Tolerable Daily Intake (PTDI) of As, Cd, Hg, Cu, and Zn, and with the R_fD values established for Cr, Pb and Se. On average, the highest EDIM values obtained have been observed for Cu, and specifically for sample #5 collected in Derdara (Chaouen, Morocco; 83.7 μg kg body weight^{-1} per day). On the other hand, for As, Cu, and Zn the EDIMs of all samples were below the PTDI and PTMDI established for these metals (As: 2.14 μg kg body weight^{-1} per day, Cu: 5000 μg kg body weight^{-1} per day, and Zn: 300 – 1000 μg kg body weight^{-1} per day). Similar observations were found for Pb, which EDIM values for all the samples studied were lower than the established R_fD (3.50 μg kg body weight^{-1} per day). On the contrary, all EDIMs values obtained for Se were above the R_fD (0.50 μg kg body weight^{-1} per day) established for this metalloid. Regarding Cr, it was observed that in general the EDIM values were below the R_fD (3.00 μg kg body weight^{-1} per day), except for sample #4 from Parc National of Talassemtane (Chaouen, Morocco; 6.39 μg kg body weight^{-1} per day) and sample #8 collected in Pinar del Rey (Cadiz, Spain; 3.52 μg kg body weight^{-1} per day), which values exceeded the established reference value. The EDIM for Cd are mostly below the PTDI (0.82 μg kg body weight^{-1} per day). Above this reference value were only observed for samples #4 collected in Parc National of Talassemtane (Chaouen, Morocco; 1.21 μg kg body weight^{-1} per day) and #10 from Sendero El Palancar (Cadiz, Spain; 2.02 μg kg body weight^{-1} per day). Regarding Hg, values above the PTDI (0.57 μg kg body weight^{-1} per day) were mostly observed, specifically for samples #3 from Parc Naturel of Bouhachem (Chaouen, Morocco; 0.686 μg kg body weight^{-1} per day), #5 from Derdara (Chaouen, Morocco; 0.709 μg kg body weight^{-1} per day), #6 and #7 from Sendero de Valdeinfierno (Cadiz, Spain; 1.06 and 1.34 μg kg body weight^{-1} per day, respectively), #8 and #9 from Pinar del Rey (Cadiz, Spain; 0.771 and 0.909 μg kg body weight^{-1} per day, respectively) and #12 from Puerto de Galiz (Cadiz, Spain, 2.25 μg kg body weight^{-1} per day).

4.4.2. Health risk index

The studied samples showed HRI values below 1 just for Pb and Zn (Table 4), indicating that the consumption of caps of *M. procera* from these sampling sites is safe in terms of these two toxic and trace elements, and especially for Pb, which is a probable human carcinogen, Group 2A, according to the IARC (Rousseau et al., 2005). Meanwhile, the situation was similar for the HRIs obtained for Cr and Cd, where values of less than 1 were mainly observed in the samples studied. For Cr, exceptions to this trend were detected for sample #4 from Parc National of Talassemtane (Chaouen, Morocco) and #8 collected in Pinar del Rey (Cadiz, Spain), which HRI values were above 1 and, therefore, may represent a risk to the health of consumers during the mushrooming season. As it is well-known, trivalent (Cr^{3+}) and hexavalent (Cr^{6+}) are the most common and stable forms of Cr found in nature. The trivalent form is poorly soluble in water and an indispensable element for the proper functioning of the organism since it participates in the metabolism of glucose, cholesterol, and fatty acids. The hexavalent form is water-soluble and highly reactive, constituting a form of major toxicological concern due to its negative effects on the body, such as respiratory and digestive system disorders (Naz et al., 2016; Tseng et al., 2019). Consequently, Cr^{3+} has been classified by the International Agency for Research Cancer (IARC) in Group 3 as not classificable as to its carcinogenicity to humans, and Cr^{6+} in Group 1 as potentially carcinogenic to humans (Naz et al., 2016). In the present study, Cr was determined as total Cr. Thus, as no speciation was performed, there is no available information about the main form in which this metal was found in the samples. Nonetheless, owing to the toxicological concerns associated with one of the forms of this element, it is not possible to exclude health risks related to exposure to Cr through the consumption of *M. procera* mushrooms from these two geographical locations. Regarding Cd, samples #4 from Parc National of Talassemtane (Chaouen, Morocco) and #10 collected from Sendero El Palancar (Cadiz, Spain) have presented HRI values above 1, so the consumption of *M. procera* caps from these locations is not safe in terms of this element. On the other hand, Cd is a toxic element that is found principally in its bivalent state (Cd^{2+}) for most of the compounds it forms. This toxic element can accumulate in tissues and organs, mainly kidneys, and can lead to serious diseases such as cancer (Waalkes, 2003). Thus, Cd and its compounds have been classified as Group 1 carcinogenic for humans by the IARC (Kim et al., 2015). Due to the toxicological attention concerning Cd and based on the HRIs obtained, the consumption of *M. procera*'s caps from the two geographical locations mentioned could involve a risk to human health due to excessive exposure to this toxic element.

The HRI values observed for As were generally found to be above 1, with the exception (HRI < 1) of sample #2 collected in Taza in the Parc Naturel of Bouhachem (Chaouen, Morocco), #5 collected in Derdara (Chaouen, Morocco), and #6 and #7 collected on Sendero de Valdeinfierno (Cadiz, Spain). As is a metalloid element with adverse health effects due to its high toxicity in its inorganic form, specifically in its trivalent (As^{3+}; arsenite) and pentavalent states (As^{5+}; arsenate). The trivalent form of arsenic is reactive to thiol, causing inhibition of enzyme systems or protein disruption with such sulfur groups. On the other hand, the pentavalent form deactivates mitochondrial oxidative phosphorylation because of phosphate competition in the formation of adenosine triphosphate (Crinnion, 2017; Kuivenhoven and Mason, 2020). For this reason, inorganic As has been classified by the Agency for Toxic Substances and Disease Registry (ASTDR) as a Group 1 carcinogen for humans (ATSDR, 2007). In turn, the HRI values for Hg were found to be above 1, except for sample #4 (HRI < 1) from Parc National of Talassemtane (Chaouen, Morocco). Hg is a highly toxic metal that exist in many inorganic forms such as metallic mercury (Hg°) and mercurous (Hg_2^{++}) or mercuric (Hg^{++}) salts, and mercury-based organic compounds, including methylmercury. Toxicity in humans varies according to the form, dose, and rate of mercury exposure. The main target organs of mercurous and mercuric salts are the lining of the intestine and kidney, whereas methylmercury is widely distributed throughout the body (Bernhoft, 2012). Currently, metallic mercury and inorganic mercury compounds are classified by the IARC in Group 3 as not classificable as to its carcinogenicity to human, and methylmercury compounds as possibly carcinogenic to humans (IARC, 1993). Therefore, the consumption of the edible part of *M. procera* from the geographical locations studied, except for the samples that presented a HRI < 1, may represent a risk to human health in terms of As and Hg exposure.

Concerning Cu and Se, it was observed that the calculated HRI values exceed the established criterion, excepting samples #2 from Taza in the Parc Naturel of Bouhachem (Chaouen, Morocco) and #4 from Parc National of Talassemtane (Chaouen, Morocco), which HRI values were below 1 for Cu. Se is an essential metalloid included in trace elements classification necessary for the normal functioning of the body. In general, Se poisoning due to overdose is rare, especially if it comes from food sources. However, an intake of selenium above the recommended dose may contribute to the prevention of prostate cancer (Falandysz, 2008; Mirończuk-Chodakowska et al., 2019). Therefore, there is no evidence that consumption of *M. procera* mushrooms from all geographic areas studied can pose a health threat related to Se. Cu is an essential nutrient for humans found in many enzymes which are important in multiple systems, such as the immune, respiratory, and nervous systems. Nonetheless, it still can pose some risks to human health at elevated levels of exposure, mainly in the gastrointestinal tract (Taylor et al., 2019). Based on the results obtained in this research, it cannot be discarded that prolonged exposure to this trace element through the consumption of *M. procera* from the geographical locations mentioned above may have repercussions on the health of consumers. However, moderate consumption during the mushrooming season should not necessarily have negative health implications in terms of Cu.

5. Conclusions

This study evaluated the total content of toxic elements and trace elements in the caps and stipes of *M. procera* mushrooms collected from northern Morocco and southern Spain. Among the eight toxic elements and trace elements determined, Cu was found in the highest contents, followed by Zn. On the other hand, the TF calculation allowed us to establish that there is a greater tendency for metal accumulation in the caps. The one-way ANOVA/Kruskal-Wallis test indicated that there were no significant differences between the geographical areas studied in terms of the toxic elements and trace elements evaluated. Furthermore, the results obtained through HCA and PCA supported the conclusions reached through the univariate statistical study, suggesting that there was no clear trend of clustering of *M. procera* cap samples according to the sampling area. Finally, the health risk assessment showed that the daily consumption of caps during the mushrooming season, the edible part of this high-valued mushroom species, may compromise the health of the consumer in terms of Cr, Cd, As, and Hg, highlighting this latter toxic element where the highest HRI values were obtained.

Author contributions

Marta Barea-Sepúlveda: Conceptualization, Methodology, Software, Validation, Formal Analysis, Investigation, Data curation, Writing - Original Draft, Visualization, Revision.

Estrella Espada-Bellido: Conceptualization, Investigation, Data curation, Writing - Review & Editing, Supervision, Revision.

Marta Ferreiro-González: Software, Formal Analysis, Data curation.

Hassan Bouziane: Resources.

José Gerardo López-Castillo: Resources.

Miguel Palma: Resources, Funding acquisition.

Gerardo F. Barbero: Conceptualization, Investigation, Data curation, Writing - Review & Editing, Supervision, Revision.

Funding

This study was financially supported by the University of Cadiz and Aula Universitaria del Estrecho, projects 001ENE2019 and 002ENE2019.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Acknowledgments

The authors would like to thank Asociación Micológica del Estrecho (Mairei), Aula Universitaria del Estrecho (University of Cadiz), and Antonio Benítez Rodríguez from the Central Research Services for Science and Technology (University of Cadiz) for their help during the main stages of this research.

Appendix A

Table A1

Table A1

Results of Shapiro-Wilk test, Levene's test, and One-Way ANOVA/Kruskal Wallis test performed with a confidence level of 95 % for quantified each toxic element and trace element in the *M. procera* caps collected in northern Morocco and southern Spain.

Trace Element	Shapiro-Wilk test	Levene's test	One-Way ANOVA/Kruskal Wallis test
Cr	$p < 0.05$	$p = 0.546$	Chi-sq = 1.91 $p = 0.168$
As	$p = 0.126$	$p = 0.499$	$F = 1.35$ $p = 0.273$
Cd	$p < 0.05$	$p = 0.904$	Chi-sq = 534 $p = 0.465$
Hg	$p < 0.05$	$p = 0.232$	Chi-sq = 3.49 $p = 0.0618$
Pb	$p = 0.122$	$p = 0.801$	$F = 0.669$ $p = 0.433$
Cu	$p = 0.705$	$p = 0.138$	$F = 0.271$ $p = 0.641$
Zn	$p < 0.05$	$p = 0.361$	Chi-sq = 3.49 $p = 0.0618$
Se	$p = 0.612$	$p = 0.631$	$F = 1.73$ $p = 0.217$

References

Alloway, B.J., 2013. Heavy metals and metalloids as micronutrients for plants and animals. In: Alloway, B. (Ed.), Heavy Metals in Soils. Environmental Pollution, 22. Springer, Dordrecht, pp. 195–209. https://doi.org/10.1007/978-94-007-4470-7_7.

Alonso, J., Salgado, M.J., García, M.A., Melgar, M.J., 2000. Accumulation of mercury in edible macrofungi: influence of some factors. Arch. Environ. Contam. Toxicol. 38, 158–162. https://doi.org/10.1007/s002449910020.

Alonso, J., García, M.A., Pérez-López, M., Melgar, M.J., 2003. The concentrations and bioconcentration factors of copper and zinc in edible mushrooms. Arch. Environ. Contam. Toxicol. 42, 180–188. https://doi.org/10.1007/s00244-002-2051-0.

Aloupi, M., Koutrotsios, G., Koulousaris, M., Kalogeropoulos, N., 2012. Trace metal contents in wild edible mushrooms growing on serpentine and volcanic soils on the island of Lesvos, Greece. Ecotoxicol. Environ. Saf. 78, 184–194. https://doi.org/10.1016/j.ecoenv.2011.11.018.

Árvay, J., Tomáš, J., Hauptvogl, M., Kopernická, M., Kováčik, A., Bajčan, D., Massányi, P., 2014. Contamination of wild-grown edible mushrooms by heavy metals in a former mercury-mining area. J. Environ. Sci. Heal. - Part B Pestic. Food Contam. Agric. Wastes 49, 815–827. https://doi.org/10.1080/03601234.2014.938550.

ATSDR, 2007. Agency for Toxic Substances and Disease Registry. Toxicological Profile for Arsenic. Department of Health and Human Services, Public Health Service, United States.

Barea-Sepúlveda, M., Espada-Bellido, E., Ferreiro-González, M., Benítez-Rodríguez, A., López-Castillo, J.G., Palma, M., Barbero, G.F., 2021. Metal concentrations in Lactarius mushroom species collected from Southern Spain and Northern Morocco: evaluation of health risks and benefits. J. Food Anal. 99, 103859 https://doi.org/10.1016/J.JFCA.2021.103859.

Bernhoft, R.A., 2012. Mercury toxicity and treatment: a review of the literature. J. Environ. Public Health 2012, 460508. https://doi.org/10.1155/2012/460508.

Chang, S.-T., Chan, K.-Y., 1973. Quantitative and Qualitative Changes in Proteins During Morphogenesis of the Basidiocarp of Volvariella Volvacea. https://doi.org/. doi: 10.1080/00275514.1973.12019444, 65, pp. 355–364. https://doi.org/10.1080/00275514.1973.12019444.

Cocchi, L., Vescovi, L., Petrini, L.E., Petrini, O., 2006. Heavy metals in edible mushrooms in Italy. Food Chem. 98, 277–284. https://doi.org/10.1016/j.foodchem.2005.05.068.

Crinnion, W., 2017. Arsenic: the underrecognized common disease-inducing toxin. Integr. Med. A Clin. J. 16, 8–13. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6413640/.

Demirbaş, A., 2001. Heavy metal bioaccumulation by mushrooms from artificially fortified soils. Food Chem. 74, 293–301. https://doi.org/10.1016/S0308-8146(01)00155-8.

Dimitrijevic, M., Mitic, V., Djordjevic, D., Popovic, G., Krstic, N., Nikolic, J., Stankov-Jovanovic, V., 2021. Macroelements versus toxic elements in selected wild edible mushrooms of the Russulacea family from Serbia. J. Serbian Chem. Soc. 86 (10) https://doi.org/10.2298/JSC210410038D, 38–38.

Elekes, C.C., Busuioc, G., Ionita, G., 2010. View of the bioaccumulation of some heavy metals in the fruiting body of wild growing mushrooms. Not. Bot. Horti Agrobot. Cluj. 2, 38. https://doi.org/10.15835/nbha3824736.

Falandysz, J., 2008. Selenium in edible mushrooms. J. Environ. Sci. Heal. - Part C Environ. Carcinog. Ecotoxicol. Rev. 26 (3), 256–299. https://doi.org/10.1080/10590500802350086.

Falandysz, J., Kunito, T., Kubota, R., Gucia, M., Mazur, A., Falandysz, J.J., Tanabe, S., 2008. Some mineral constituents of Parasol Mushroom (Macrolepiota procera). J. Environ. Sci. Heal. - Part B Pestic. Food Contam. Agric. Wastes 43, 187–192. https://doi.org/10.1080/03601230701795247.

Falandysz, J., Chudzińska, M., Barałkiewicz, D., Drewnowska, M., Hanć, A., 2017a. Toxic elements and bio-metals in Cantharellus mushrooms from Poland and China. Environ. Sci. Pollut. Res. 24, 11472–11482. https://doi.org/10.1007/s11356-017-8554-z.

Falandysz, J., Sapkota, A., Dryżałowska, A., Mędyk, M., Feng, X., 2017b. Analysis of some metallic elements and metalloids composition and relationships in parasol mushroom Macrolepiota procera. Environ. Sci. Pollut. Res. 24 (18), 15528–15537. https://doi.org/10.1007/S11356-017-9136-9.

Gadd, G.M., 1993. Interactions of Fungi with toxic metals. New Phytol. 124, 25–60. https://www.jstor.org/stable/2558069.

Gadd, G.M., 2007. Geomycology: biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation. Mycol. Res. 111, 3–49. https://doi.org/10.1016/J.MYCRES.2006.12.001.

Global Mushroom Market Size, Market Share, Application Analysis, Regional Outlook, Growth Trends, Key Players, Competitive Strategies and Forecasts, 2021 to 2028: https://www.grandviewresearch.com/industry-analysis/mushroom-market.

Gucia, M., Jarzyńska, G., Rafał, E., Roszak, M., Kojta, A.K., Osiej, I., Falandysz, J., 2012. Multivariate analysis of mineral constituents of edible Parasol Mushroom (Macrolepiota procera) and soils beneath fruiting bodies collected from Northern Poland. Environ. Sci. Pollut. Res. Int. 19, 416–431. https://doi.org/10.1007/s11356-011-0574-5.

Haro, A., Trescastro, A., Lara, L., Fernández-Fígares, I., Nieto, R., Seiquer, I., 2020. Mineral elements content of wild growing edible mushrooms from the southeast of Spain. J. Food Anal. 91, 103504 https://doi.org/10.1016/j.jfca.2020.103504.

Integrated Risk Information System | US EPA [WWW Document], n.d. URL https://www.epa.gov/iris (Accessed 7.14.21).

Jan, A.T., Azam, M., Siddiqui, K., Ali, A., Choi, I., Haq, Q.M.R., 2015. Heavy metals and human health: mechanistic insight into toxicity and counter defense system of antioxidants. Int. J. Mol. Sci. 2015 (16), 29592–29630. https://doi.org/10.3390/IJMS161226183.

Kalač, P., 2010. Trace element contents in European species of wild growing edible mushrooms: a review for the period 2000-2009. Food Chem. 122, 2–15. https://doi.org/10.1016/j.foodchem.2010.02.045.

Kalacï, P., 2000. A review of trace element concentrations in edible mushrooms. Food Chem. 69, 273–281. https://doi.org/10.1016/S0308-8146(99)00264-2.

Kfle, G., Asgedom, G., Goje, T., Abbebe, F., Habtom, L., Hanes, H., 2020. The level of heavy metal contamination in selected vegetables and animal feed grasses grown in wastewater irrigated area, around Asmara, Eritrea. J. Chem. 296, 113231. https://doi.org/10.1155/2020/1359710.

Kim, H.S., Kim, Y.J., Seo, Y.R., 2015. An overview of carcinogenic heavy metal: molecular toxicity mechanism and prevention. J. Cancer Prev. 20, 232–240. https://doi.org/10.15430/JCP.2015.20.4.232.

Kiran, Bharti, R., Sharma, R., 2021. Effect of heavy metals: an overview. Mater. Today Proc. 2214–7853. https://doi.org/10.1016/J.MATPR.2021.06.278.

Kojta, A.K., Falandysz, J., 2016. Metallic elements (Ca, Hg, Fe, K, Mg, Mn, Na, Zn) in the fruiting bodies of boletus badius. Food Chem. 200, 206–214. https://doi.org/10.1016/j.foodchem.2016.01.006.

Kojta, A.K., Gucia, M., Krasińska, G., Saba, M., Nnorom, I.C., Falandysz, J., 2016. Mineral constituents of edible field parasol (Macrolepiota procera) mushrooms and the underlying substrate from upland regions of Poland: bioconcentration potential, intake benefits, and toxicological risk. Polish J. Environ. Stud. 25, 2445–2460. https://doi.org/10.15244/pjoes/62997.

Kokkoris, V., Massas, I., Polemis, E., Koutrotsios, G., Zervakis, G.I., 2019. Accumulation of heavy metals by wild edible mushrooms with respect to soil substrates in the Athens metropolitan area (Greece). Sci. Total Environ. 685, 280–296. https://doi.org/10.1016/j.scitotenv.2019.05.447.

Kosanić, M., Ranković, B., Rančić, A., Stanojković, T., 2016. Evaluation of metal concentration and antioxidant, antimicrobial, and anticancer potentials of two edible mushrooms Lactarius deliciosus and Macrolepiota procera. J. Food Drug Anal. 24, 477–484. https://doi.org/10.1016/j.jfda.2016.01.008.

Kuivenhoven, M., Mason, K., 2020. Arsenic Toxicity. StatPearls, Treasure Island (FL). https://www.ncbi.nlm.nih.gov/books/NBK541125/.

Lalotra, P., Gupta, D., Yangdol, R., Sharma, Y., Gupta, S., 2016. Bioaccumulation of heavy metals in the sporocarps of some wild mushrooms. Curr. Res. Environ. Appl. Mycol. 6, 159–165. https://doi.org/10.5943/cream/6/3/2.

Liu, B., Huang, Q., Cai, H., Guo, X., Wang, T., Gui, M., 2015a. Study of heavy metal concentrations in wild edible mushrooms in yunnan province, China. Food Chem. 188, 294–300. https://doi.org/10.1016/j.foodchem.2015.05.010.

Malinowska, E., Szefer, P., Falandysz, J., 2004. Metals bioaccumulation by bay bolete, Xerocomus badius, from selected sites in Poland. Food Chem. 84, 405–416. https://doi.org/10.1016/S0308-8146(03)00250-4.

Melgar, M.J., Alonso, J., García, M.A., 2014. Total contents of arsenic and associated health risks in edible mushrooms, mushroom supplements and growth substrates from Galicia (NW Spain). Food Chem. Toxicol. 73, 44–50. https://doi.org/10.1016/j.fct.2014.08.003.

Melgar, M.J., Alonso, J., García, M.A., 2016. Cadmium in edible mushrooms from NW Spain: bioconcentration factors and consumer health implications. Food Chem. Toxicol. 88, 13–20. https://doi.org/10.1016/j.fct.2015.12.002.

Mercury and Mercury Compounds (IARC Summary & Evaluation, Volume 58, 1993) [WWW Document], n.d. URL http://www.inchem.org/documents/iarc/vol58/mono58-3.html (Accessed 7.14.21).

Ministerio De Agricultura, Pesca Y Alimentación, Statistical Services from the Spanish Government: https://www.mapa.gob.es/es/alimentacion/temas/consumo-tendencias/panel-de-consumo-alimentario/series-anuales/default.aspx.

Mirończuk-Chodakowska, I., Socha, K., Zujko, M.E., Terlikowska, K.M., Borawska, M.H., Witkowska, A.M., 2019. Copper, manganese, selenium and zinc in wild-growing edible mushrooms from the eastern territory of "green lungs of Poland": nutritional and toxicological implications. Int. J. Environ. Res. Public Health 16, 1–22. https://doi.org/10.3390/ijerph16193614.

Mleczek, M., Rzymski, P., Budka, A., Siwulski, M., Jasińska, A., Kalač, P., Poniedziałek, B., Gąsecka, M., Niedzielski, P., 2018. Elemental characteristics of mushroom species cultivated in China and Poland. J. Food Anal. 66, 168–178. https://doi.org/10.1016/j.jfca.2017.12.018.

Mleczek, M., Siwulski, M., Budka, A., Mleczek, P., Budzyńska, S., Szostek, M., Kuczyńska-Kippen, N., Kalač, P., Niedzielski, P., Gąsecka, M., Goliński, P., Magdziak, Z., Rzymski, P., 2021. Toxicological risks and nutritional value of wild edible mushroom species -a half-century monitoring study. Chemosphere 263, 128095. https://doi.org/10.1016/j.chemosphere.2020.128095.

Naz, A., Mishra, B.K., Gupta, S.K., 2016. Human health risk assessment of chromium in drinking water: a case study of Sukinda chromite mine, Odisha, India. Expo. Heal. 82 (8), 253–264. https://doi.org/10.1007/S12403-016-0199-5.

Nikkarinen, M., Mertanen, E., 2004. Impact of geological origin on trace element composition of edible mushrooms. J. Food Anal. 17, 301–310. https://doi.org/10.1016/J.JFCA.2004.03.013.

Nnorom, I.C., Eze, S.O., Ukaogo, P.O., 2020. Mineral contents of three wild-grown edible mushrooms collected from forests of south eastern Nigeria: an evaluation of bioaccumulation potentials and dietary intake risks. Sci. African 8, e00163. https://doi.org/10.1016/J.SCIAF.2019.E00163.

Ostos, C., Pérez-Rodríguez, F., Arroyo, B.M., Moreno-Rojas, R., 2015. Study of mercury content in wild edible mushrooms and its contribution to the Provisional Tolerable Weekly Intake in Spain. J. Food Anal. 37, 136–142. https://doi.org/10.1016/j.jfca.2014.04.014.

Rebello, S., Sivaprasad, M.S., Anoopkumar, A.N., Jayakrishnan, L., Aneesh, E.M., Narisetty, V., Sindhu, R., Binod, P., Pugazhendhi, A., Pandey, A., 2021. Cleaner technologies to combat heavy metal toxicity. J. Environ. Manage. 296, 113231 https://doi.org/10.1016/J.JENVMAN.2021.113231.

Rousseau, M.-C., Straif, K., Siemiatycki, J., 2005. IARC carcinogen update. Environ. Health Perspect. 113, A580. https://doi.org/10.1289/EHP.113-1280416.

Sarikurkcu, C., Copur, M., Yildiz, D., Akata, I., 2011. Metal concentration of wild edible mushrooms in Soguksu National Park in Turkey. Food Chem. 128, 731–734. https://doi.org/10.1016/j.foodchem.2011.03.097.

Sarikurkcu, C., Popović-Djordjević, J., Solak, M.H., 2020. Wild edible mushrooms from Mediterranean region: metal concentrations and health risk assessment. Ecotoxicol. Environ. Saf. 190, 110058. https://doi.org/10.1016/j.ecoenv.2019.110058.

Širić, I., Humar, M., Kasap, A., Kos, I., Mioč, B., Pohleven, F., 2016. Heavy metal bioaccumulation by wild edible saprophytic and ectomycorrhizal mushrooms. Environ. Sci. Pollut. Res. 23, 18239–18252. https://doi.org/10.1007/s11356-016-7027-0.

Stefanović, V., Trifković, J., Djurdjić, S., Vukojević, V., Tešić, Ž., Mutić, J., 2016. Study of silver, selenium and arsenic concentration in wild edible mushroom Macrolepiota procera, health benefit and risk. Environ. Sci. Pollut. Res. 2321 (23), 22084–22098. https://doi.org/10.1007/S11356-016-7450-2.

Taylor, A.A., Tsuji, J.S., Garry, M.R., McArdle, M.E., Goodfellow, W.L., Adams, W.J., Menzie, C.A., 2019. Critical review of exposure and effects: implications for setting regulatory health criteria for ingested copper. Environ. Manag. 651 (65), 131–159. https://doi.org/10.1007/S00267-019-01234-Y.

Thomet, U., Vogel, E., Krähenbühl, U., 1999. The uptake of cadmium and zinc by mycelia and their accumulation in mycelia and fruiting bodies of edible mushrooms. Eur. Food Res. Technol. 209, 317–324. https://doi.org/10.1007/s002170050502.

Tseng, C.H., Lee, I.H., Chen, Y.C., 2019. Evaluation of hexavalent chromium concentration in water and its health risk with a system dynamics model. Sci. Total Environ. 669, 103–111. https://doi.org/10.1016/J.SCITOTENV.2019.03.103.

Türkmen, M., Budur, D., 2018. Heavy metal contaminations in edible wild mushroom species from Turkey's Black Sea region. Food Chem. 254, 256–259. https://doi.org/10.1016/j.foodchem.2018.02.010.

Tuzen, M., Sesli, E., Soylak, M., 2007. Trace element levels of mushroom species from East Black Sea region of Turkey. Food Control 18, 806–810. https://doi.org/10.1016/j.foodcont.2006.04.003.

Valverde, M.E., Hernández-Pérez, T., Paredes-López, O., 2015. Edible mushrooms: improving human health and promoting quality life. Int. J. Microbiol. 2015, 376387 https://doi.org/10.1155/2015/376387.

Waalkes, M.P., 2003. Cadmium carcinogenesis. Mutat. Res. Mol. Mech. Mutagen. 533, 107–120. https://doi.org/10.1016/J.MRFMMM.2003.07.011.

Wang, X., Liu, H., Zhang, J., Li, T., Wang, Y., 2017. Evaluation of heavy metal concentrations of edible wild-grown mushrooms from China. J. Environ. Sci. Heal. - Part B Pestic. Food Contam. Agric. Wastes 52, 178–183. https://doi.org/10.1080/03601234.2017.1261545.

Yamaç, M., Yildiz, D., Sarikürkcü, C., Çelikkollu, M., Solak, M.H., 2007. Heavy metals in some edible mushrooms from the Central Anatolia, Turkey. Food Chem. 103, 263–267. https://doi.org/10.1016/j.foodchem.2006.07.041.

Záhorcová, Z., Árvay, J., Hauptvogl, M., Tomáš, J., Harangozo, L., 2016. Heavy metals determination in edible wild mushrooms growing in former mining area - Slovakia: health risk assessment. Potravinarstvo 10, 37–46. https://doi.org/10.5219/528.

Zhang, D., Gao, T., Ma, P., Luo, Y., Su, P., 2008. Bioaccumulation of heavy metal in wild growing mushrooms from Liangshan Yi nationality autonomous prefecture, China. Wuhan Univ. J. Nat. Sci. 13, 267–272. https://doi.org/10.1007/s11859-008-0302-2.

Zhu, F., Qu, L., Fan, W., Qiao, M., Hao, H., Wang, X., 2011a. Assessment of heavy metals in some wild edible mushrooms collected from Yunnan Province, China. Environ. Monit. Assess. 179, 191–199. https://doi.org/10.1007/s10661-010-1728-5.

Zhu, F., Qu, L., Fan, W., Qiao, M., Hao, H., Wang, X., 2011b. Assessment of heavy metals in some wild edible mushrooms collected from Yunnan Province, China. Environ. Monit. Assess. 179, 191–199. https://doi.org/10.1007/s10661-010-1728-5.