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Multi-elemental composition of botanical preparations and probabilistic evaluation of toxic metals and metalloids intake upon dietary exposure

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ABSTRACT

The aim of this study was to evaluate the inorganic elemental composition (49 elements) of 29 botanical preparations obtained from fruits, leaves, peels, seeds, roots, fungi, and spirulina by using inductively coupled-mass spectrometry and a mercury analyzer. Simultaneously, the risk associated with the chronic dietary exposure to 12 toxic metals and metalloids among the European population was evaluated by using a probabilistic approach based on Monte Carlo simulations. The analysis revealed worrying intake levels of Al, As, and Ni, primarily stemming from the consumption of spirulina-, peel-, and leaf-based botanicals by younger age groups. The intake of As from all analyzed botanicals posed a significant risk for infants, yielding margins of exposure (MOEs) below 1, while those deriving from peel-based botanicals raised concerns across all age groups (MOEs = 0.04–2.3). The consumption of peel-based botanicals contributed substantially (13–130%) also to the tolerable daily intake of Ni for infants, toddlers, and children, while that of spirulina-based botanicals raised concerns related to Al intake also among adults, contributing to 11–176% of the tolerable weekly intake of this element. The findings achieved underscore the importance of implementing a monitoring framework to address chemical contamination of botanicals, thus ensuring their safety for regular consumers.

1. Introduction

The term "botanical preparations" in Europe refers to plant material, algae, fungi, and lichens (i.e., botanicals) that have been processed through techniques like fragmentation, pressing, extraction, drying, concentration, distillation, or fermentation (EFSA, 2009a).

In recent years the use and consumption of such products have been following the growing trend towards adopting healthy eating habits and the perception that they are safe and with no side effects owing to their "natural" origin (Filipiak-Szok et al., 2015). Indeed, recent studies highlighted that the consumption of these products is now well established within the Western society, with consumption rates rising not only among adults but also among children, who often consume multiple botanicals simultaneously (Egan et al., 2011; Vargas-Murga et al., 2011; Lieberman et al., 2015; Barnes et al., 2016; Binns et al., 2018).

Botanical preparations can find application in the food industry (being employed as food ingredients, flavorings, or blends for infusion), in the production of concentrated food supplements, and in the pharmaceutical industry to produce specific drugs and alternative medicines (Ichim and de Boer, 2021). When employed for the production of food supplements destined to the European market, they can be sold in different dose forms such as powders, tablets, capsules, pills, or liquids (Directive, 2002/46/EC). Because of these diverse potential applications, the classification and regulatory framework that govern botanicals and botanical preparations are determined by their intended use.

At present, there is an extensive array of botanical preparations available in the market, exhibiting substantial differences in chemical composition not only between various species, sub-species, and varieties but also within different parts of the same species (Smichowski and Londonio, 2018). Multiple factors such as agricultural practices and geo-climatic conditions, as well as different manufacturing processes can also significantly influence the final chemical composition of botanical preparations. This extensive variability can result in substantial uncertainty not only concerning the overall nutritional composition

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and health benefits associated with their consumption, but also regarding their overall risk profile, due to the possible presence of chemical contaminants originating from raw materials. These contaminants may include polycyclic aromatic hydrocarbons, alkaloids, mycotoxins, pesticide residues, dioxins, and toxic metals (Tumir et al., 2010; Filipiak-Szok et al., 2015).

The occurrence of potentially toxic metals and metalloids in botanical preparations has been well documented in the literature (Dolan et al., 2003; Raman et al., 2004; Garcìa-Rico et al., 2007; Rzymski et al., 2019; Augustsson et al., 2021) and it has also been frequently notified on the Rapid Alert System for Food and Feed (RASFF, 2023). Notably, most notifications have involved significant contamination levels of Pb, Hg, As, Zn, and Ni in products whose raw materials originated from a wide range of countries, particularly from heavily industrialized regions (Alagić et al., 2015; Papazoglou and Fernando, 2017; Żukowska et al., 2021; RASFF, 2023).

The presence of toxic metals and metalloids in botanical preparations may not necessarily indicate an immediate threat to human health upon consumption. Indeed, certain metals play crucial roles in various physiological processes, offering potential health benefits when consumed in appropriate amounts. For instance, Fe is essential for hemoglobin synthesis, while Zn contributes to immune function and wound healing, and Cu aids in the formation of connective tissues and Fe absorption (Fraga, 2005). However, prolonged human exposure to these elements through dietary intake can lead to toxicity (Moghaddam et al., 2020), with Fe overload resulting in organ damage and high levels of Zn and Cu impairing metabolic processes. High levels of Ni intake can cause immunological disorders and respiratory issues, while Al overexposure has been associated with neurotoxicity and bone disorders (Briffa et al., 2020; Abd Elnabi et al., 2023). Although Sn and U toxicity are less common, excessive exposure can still lead to adverse health effects, particularly on the kidneys and the urinary system (Abd Elnabi et al., 2023). Conversely, medium to long-term exposure to even small amounts of the well-known toxic elements As, Cd, Cr, Pb, and Hg can pose severe health risks, including kidney and liver diseases, nervous system disorders, reproductive toxicity, and carcinogenicity (Avula et al., 2010; Tripathy et al., 2015; Deswal et al., 2022).

Hence, in a period marked by the rising popularity and diversity of botanical preparations, ensuring their safety through comprehensive compositional analysis and meticulous monitoring becomes imperative for the safeguarding of public health. This assessment should extend beyond the examination of their nutritional content and should encompass a thorough investigation into the presence of potentially harmful metals and metalloids, with the final goal to proactively mitigate the risks associated with dietary consumption (Gupta et al., 2010; Korfali et al., 2013; Kong et al., 2020).

In this context, previous studies have investigated the presence of toxic metals and metalloids across diverse botanical formulations, including medicinal plants and dietary supplements, aiming to estimate the associated risks for consumers upon ingestion (Ćwielag-Drabek et al., 2020; Kong et al., 2020; Moghaddam et al., 2020; Augustsson et al., 2021; Karami et al., 2021; Rubio et al., 2021; Deswal et al., 2022; Torović et al., 2023). However, the majority of these studies often focused solely on a specific product type derived from individual botanical varieties, leading to limited insights. Furthermore, these investigations commonly measured only a restricted set of toxic elements, potentially neglecting and underestimating the overall risk associated with less common inorganic contaminants. Additionally, the risk assessment often relied on deterministic methodologies rather than probabilistic approaches and did not consider variations in susceptibility across different age groups within the population, thereby introducing a wide range of uncertainty into the evaluation.

Given these limitations, the present study aims to provide a comprehensive overview of the elemental composition of a diverse set of botanical preparations and potential health risks associated with their consumption, contributing valuable insights for informed decision-making in public health and regulatory contexts. For this purpose, inductively coupled plasma-mass spectrometry (ICP-MS) and a mercury analyzer were employed to quantify 49 elements, including macro, micro, trace, and ultra-trace elements (Al, As, B, Ba, Be, Bi, Ca, Cd, Ce, Co, Cr, Cs, Cu, Dy, Er, Eu, Fe, Gd, Hf, Hg, Ho, K, La, Li, Lu, Mg, Mn, Mo, Na, Nd, Ni, P, Pb, Pr, Rb, Sb, Se, Sm, Sn, Sr, Tb, Th, Tl, U, V, W, Y, Yb, and Zn), across various botanical preparations. Additionally, the concentrations of 12 selected potentially toxic metals and metalloids (Al, As, Cd, Cr, Cu, Fe, Hg, Ni, Pb, Sn, U, and Zn) were evaluated for their contribution to the individual body burden of different age groups of the European population consuming botanicals. This was accomplished by conducting an exposure assessment and a risk characterization using a probabilistic method based on Monte Carlo simulations.

2. Materials and methods

2.1. Sample collection

The present study included n = 29 botanical preparations sourced from various origins, all of which were available in the EU market and sold in homogeneous powder form. These samples were categorized into seven distinct groups based on the botanical group and plant part used to produce the final product: fruits (n = 7); fungi (n = 4); leaves (n = 6); peels (n = 3); roots (n = 6); seeds (n = 2); and spirulina (n = 1). Table 1 provides a comprehensive list of the botanical name, commercial product name, plant part used, and the country of origin for each of the analyzed samples.

2.2. Reagents and standards

The Milli-Q® water purification system (Millipore, Bedford, USA) was utilized to obtain ultrapure water with a conductivity of 0.055 μS cm^{-1}, which was employed for the preparation of all solutions. Sub-boiled nitric acid, derived from nitric acid (65%, w/w) of Selectipur quality (Lach-Ner, Neratovice, Czech Republic) was obtained using the BSB-939-IR distillation apparatus (Berghof, Eningen, Germany). Hydrogen peroxide (Trace Select, ≥30%, w/w) was obtained from Fluka Chemie AG (Buchs, Switzerland). The internal standard solution (ISTD) was obtained from a stock solution of Rh (1 g L^{-1}) acquired from SCP Science (Montreal, Canada). Carbon reference solutions were obtained from a stock solution (10 g L^{-1} of C) prepared from urea of TraceSelect quality (Fluka Chemie AG, Buchs, Switzerland). Stock solutions used for calibration included: solution "A", with concentrations of 10 mg L^{-1} for Al, As, B, Ba, Be, Bi, Cd, Co, Cr, Cs, Fe, Hf, Li, Mo, Ni, Pb, Rb, Sb, Se, Sn, Sr, Th, Tl, V, and W (prepared from the Supelco ICP multi-element standard solution IV, Merck, Darmstadt, Germany and single-element standards with a concentration of 1 ± 0.002 g L^{-1} obtained Analytika Ltd., Prague, Czech Republic or SCP Science, Montreal, Canada); solution "B", with concentrations of 1 mg L^{-1} of Ce, La, Nd, Pr, and U (solution “B1”), and 0.20 mg L^{-1} of Dy, Er, Eu, Gd, Ho, Lu, Sm, Tb, Y, and Yb (solution “B2”) (prepared from the Astasol mix “M008”, Analytika Ltd., Prague, Czech Republic); solution "C", with concentrations of 50 mg L^{-1} of Ca, Cu, K, Mg, Mn, Na, P, and Zn (prepared from single-element standards of 1 g L^{-1} obtained from Analytika Ltd., Prague, Czech Republic).

2.3. Quantification of elements via ICP-MS

2.3.1. Sample preparation and mineralization

Both samples and certified reference materials (CRMs) were readily available in dried, powdered, and homogeneous form, allowing them to be directly subjected to mineralization without the need for any preliminary sample preparation steps before ICP-MS analyses. Specifically, botanical preparations and CRMs underwent digestion in a closed microwave system Speedwave XPERT (Berghof, Eningen, Germany) featuring dual magnetrons with a combined maximum power of 2 kW.

Table 1
List of botanical samples analyzed in this study.

Botanical name	Commercial product name	Plant part	Country of origin
Gardenia jasminoides E.	Gardenia extract	Fruit	Unknown[a]
Garcinia mangostana L.	Garcinia Mangostana fruit rind extract	Fruit	China
Hippophae rhamnoides	Sea Buckthorn extract	Fruit	Unknown[a]
Crategus monogyna	Hawthorn extract	Fruit	China
Capsicum frutescens L.	Cayenne extract	Fruit	China
Ligustrum lucidum Ait.	Lingustrin lucidum extract	Fruit	China
Alliumsativum L.	Black garlic extract	Fruit	China
Orthosiphon aristatus	Java tea extract	Leaves	China
Gymnema Sylvestre	Gymnema Sylvestre extract (75%)	Leaves	Unknown[a]
Rosmarinus Officinalis L.	Rosemary extract	Leaves	China
Ginkgo biloba L.	Ginkgo Biloba extract	Leaves	China
Camellia sinensis L.	Green Tea Extract	Leaves	China
Taraxacum officinale	Dandelion extract	Leaves	China
Juglans reggia	Walnut extract	Peel	China
Vitis vininfera L.	Grape Skin extract	Peel	China
Punica granatum.L	Pomegranate extract	Peel	China
Cordyceps sinensis	Cordyceps sinensis extract (4:1)	Mycelium	Unknown[a]
Ganoderma lucidum	Reishi mushroom extract	Mycelium	China
Cordyceps sinensis	Cordyceps sinensis extract	Mycelium	China
Cordyceps militaris	Cordyceps synensis extract (beta glucan)	Mycelium	China
Spirulina platensis	Spirulina powder	Alga	China
Beta vulgaris L.	Beetroot powder E2.6	Root	China
Beta vulgaris L.	Red beet powder 3BET	Root	Poland
Smilax china L.	Sarsaparilla extract	Root	China
Beta vulgaris L.	Beetroot Red extract E50	Root	China
Scutellaria baicalensis	Skullcap extract	Root	Unknown[a]
Urtica dioica L.	Nettle Root extract	Root	China
Sapindaceae Paullinia cupana K.	Guarana extract (10% caffeine)	Seed	Unknown[a]
Paullinia cupana	Red yeast rice extract	Seed	China

[a] Samples of unknown origin were manufactured either in the Czech Republic or Spain.

The process involved high-pressure resistant TFM™-PTFE vessels DAC100, capable of withstanding up to 100 bars. Sample mineralization was accomplished by digesting 100 mg of pulverized sample (or CRM) with a mixture of 2 mL of 30% and 6 mL of 16% HNO_3. This digestion process was carried out following a specific temperature program with three steps: i) a 5-min ramp-up time and 5 min of holding time at 170 °C; ii) a ramp to 230 °C in 5 min and a 35-min hold; iii) a final 5-min ramp and 5-min hold time at 100 °C.

Following digestion, samples were diluted to 25 mL with ultrapure water (0.05 μS cm^{-1} resistivity) and subjected to elemental analysis by ICP-MS, with each sample prepared in triplicate. Blanks, consisting of deionized water and reagents, underwent a similar preparation procedure.

2.3.2. ICP-MS instrument parameters

The Agilent 7900 ICP-MS, equipped with standard nickel cones, a glass concentric nebulizer MicroMist (400 μL min^{-1}), a Peltier-cooled (2 °C) quartz spray chamber, and a 2.5-mm internal diameter quartz torch was utilized for the multi-element quantification. A low-pulsation, 10-roller peristaltic pump with three separate channels facilitated precise delivery of samples and internal standard (ISTD). The instrument featured an octopole-based collision cell to effectively remove multiple polyatomic interferences using kinetic energy discrimination (KED) in either standard helium ("He") or high-energy helium ("HE He") mode. The ICP-MS MassHunter software automatically tuned the instrument during each startup to optimize sensitivity for elements with low, middle, and high mass-to-charge ratios. Working parameters of the collision cell for helium ("He") and high-energy helium ("HE He") modes were manually adjusted, with consistent plasma and ion lens tuning parameters for all cell modes (refer to Supplementary Table S1 for technical details).

Analyzing analyte concentrations involved creating multi-element calibration curves. These curves were generated by examining calibration solutions at five different concentrations of standards. The concentration ranges for the standards were 0–100 μg L^{-1} for elements like Al, As, B, Ba, Be, Bi, Cd, Co, Cr, Cs, Fe, Hf, Li, Mo, Ni, Pb, Rb, Sb, Se, Sn, Sr, Th, Tl, V, and W; 0–10 μg L^{-1} for Ce, La, Nd, Pr, and U; 0–2 μg L^{-1} for Dy, Er, Eu, Gd, Ho, Lu, Sm, Tb, Y, and Yb; and 0–10 mg L^{-1} for Ca, Cu, K, Mg, Mn, Na, P, and Zn (see Varrà et al., 2021 for more details). Linear calibrations with a coefficient of determination >0.999 were obtained for all elements. To compensate for possible instrumental drift and matrix effects, a 200 μg L^{-1} Rh ISTD was simultaneously aspirated and mixed with samples.

2.4. Quantification of mercury via direct mercury analyzer

Analysis of botanical samples and CRMs for total mercury content was conducted using the specialized atomic absorption spectrometer, the AMA-254 (Altec Ltd., Prague, Czech Republic). The analytical procedure involved in situ dry-ashing followed by gold amalgamation. The samples were weighed in a nickel boat and subjected to the following conditions: initial drying at 120 °C for 60 s, subsequent combustion in an oxygen atmosphere at approximately 750 °C for 150 s. The amalgamator was then heated to 900 °C, facilitating the quantitative release of trapped mercury from the gold amalgamator to the measuring cuvette detection system within 45 s at 900 °C. Monitoring was carried out by measuring the absorbance of the peak area at 253.7 nm. The carrier gas, oxygen (99.5%), was maintained at a flow rate of 170 mL min^{-1}.

2.5. Quality assurance/quality control

The accuracy of quantifying analytes was assessed using CRMs, namely GBW 10052 (Green Tea) and GBW07603 (Bush Leaves) obtained from the National Institute of Metrology and Institute of Geophysical and Geochemical Exploration in Beijing, China, CRM NCS ZC73015 Milk Powder from the National Research Centre for Certified Reference Materials (NRCRM) in Beijing, China, P–WBF CRM 12–2-04 Essential and Toxic Elements in Wheat Bread Flour from pb-anal in Kosice, Slovakia, and CRM12-2–03 P-Alfalfa Essential and Toxic Elements in Lucerne from pb-anal in Kosice, Slovakia. The precision of the method was evaluated through intra-day and inter-day analyses, involving the examination of individual CRMs three times within the same day and over

three days within a month, respectively. The results of element quantification demonstrating a high level of agreement between the target and found values, underscoring the trueness of the obtained data (refer to Supplementary Material, Table S2). The method exhibited also satisfactory precision, as indicated by the percent relative standard deviations (RSD%) for intra-day and inter-day precision, with values mostly below 10% (see Supplementary Material, Table S2).

The detection limits of the method (MLODs) were computed as concentrations corresponding to three times the standard deviation (SD) obtained from measuring 10 replicates of a blank sample, accounting for the sample dilution factor. Notably, the MLODs in all cases were found to be well below the requirements for this analysis, enabling the determination of selected elements at background levels. Table S3 in the Supplementary Material provides a summary of MLODs and the relative sensitivities of ICP-MS for individual elements, using Rh ISTD.

2.6. Statistical analysis

Prior to conducting any statistical analyses, a series of preliminary checks were performed on the data matrix including the element concentrations. These checks included an assessment for normal distribution using the Shapiro-Wilk's test and an examination for homoscedasticity using the Box's M test (at a significance level of 95%). In cases where the elemental data deviated from a normal distribution pattern and homoscedasticity, the Box-Cox transformation was applied. The transformed elemental data underwent the one-way Analysis of Variance (ANOVA) followed by the Tukey's post hoc test to determine if statistically significant differences ($p \leq 0.05$) existed among the distinct botanical groups for each element. Specifically, the comparison was conducted among fruits, leaves, peels, seeds, roots, and fungi sample groups. The statistical evaluation excluded the spirulina sample from this analysis, as it was a unique case within the category of botanicals, consisting of only one sample.

To provide a concise summary of the data, the results were finally presented as means, medians, and 95% upper and lower confidence intervals (CIs) on the original scale after reversing the Box-Cox transformation. Statistical tests were all performed using the OriginPro 2023 software package (v. 10.0.0.154, Origin Lab Corporation, USA).

2.7. Dietary exposure assessment to toxic metals and metalloids and risk characterization

2.7.1. Food consumption data

The chronic dietary exposure to toxic metals and metalloids deriving from the consumption of botanical preparations was estimated by selecting EU consumers of "Herbal formulations and plant extracts" as the target population, since the samples analyzed in the present work were meant for the European market. Chronic mean dietary consumption data of all the European countries were retrieved from specific surveys conducted at national level and available on the EFSA Comprehensive European Food Consumption Database (chronic food consumption grams per kilogram of body weight per day (g kg $bw^{-1}day^{-1}$)) - consumers only) (EFSA, 2023a). Consumption data of the following population age groups were considered: infants (0–12 months); toddlers (13–36 months); other children (37 months–9 years); adolescents (10–17 years); adults (18–65 years); elderly (over 65 years) (EFSA, 2023a). Given that consumption data of each country were not available for all age groups, the assessment was carried out considering a total of 17 out of 27 European countries (i.e., those states for which consumption data of at least one age group was available), namely: Austria, Belgium, Bosnia-Herzegovina, Croatia, Cyprus, Estonia, Finland, Hungary, Ireland, Italy, Lithuania, Netherlands, Portugal, Macedonia, Serbia, Slovenia, and UK. Average consumption rates of botanicals at European level (g kg $bw^{-1}day^{-1}$ ± SD) were finally calculated for each age group by combining multiple data from different countries as shown in Table 2.

Table 2
Chronic food consumption of "Herbal formulations and plant extracts" retrieved from the EFSA Comprehensive European Food Consumption Database (EFSA, 2023b) and employed to calculate the mean (±SDs) chronic food consumption grams per kilogram of body weight per day (g kg $bw^{-1}day^{-1}$) used for the exposure assessment.

Age group	N. of countries	Mean (g kg $bw^{-1}day^{-1}$)	SD
Infants	2	1.2	0.071
Toddlers	10	0.3	0.26
Children	11	0.1	0.11
Adolescent	8	0.04	0.035
Adult	15	0.08	0.095
Elderly	10	0.03	0.025

2.7.2. Probabilistic exposure and health risk assessment

The exposure assessment and risk characterization were carried out in order to determine i) the amount of potentially toxic metals and metalloids to which chronic consumers of "Herbal formulations and plant extracts" are exposed to through the consumptions of these products, ii) and the contribution of metal and metalloid intake to the thresholds of concern established by EFSA and the Joint FAO/WHO Expert Committee on Food Additives. Among all the elements quantified, the following ones were selected based on their potential health risks and the availability of specific thresholds of concern as toxicological reference values (health-based guidance values, HBGVs) or reference points (benchmark dose lower limits, BMDLs): Al, As, Cd, Cr, Cu, Fe, Hg, Ni, Pb, Sn, U, and Zn. The thresholds of concern were the following: tolerable daily intake (TDI) for Cr (III) (0.3 mg kg $bw^{-1}day^{-1}$), Ni (13 μg kg $bw^{-1}day^{-1}$) and U (0.6 μg kg $bw^{-1}day^{-1}$); provisional maximum tolerable daily intake (PMTDI) for Cu (0.5 mg kg $bw^{-1}day^{-1}$), Fe (0.8 mg kg $bw^{-1}day^{-1}$) and Zn (0.3 mg kg $bw^{-1}day^{-1}$); tolerable weekly intake (TWI) for Al (1 mg kg $bw^{-1}week^{-1}$), Cd (2.5 μg kg $bw^{-1}week^{-1}$) and inorganic Hg (4 μg kg $bw^{-1}week^{-1}$); provisional tolerable weekly intake (PTWI) for Sn (14 mg kg $bw^{-1}week^{-1}$); benchmark dose lower confidence limits associated with a 1% change in the incidence of an adverse effect (BMDL01) for inorganic As (*i*As) (0.3–8 μg kg $bw^{-1}day^{-1}$); BMDL01 of neurotoxicity for Pb (0.5 μg kg $bw^{-1}day^{-1}$); BMDL01 of cardiovascular diseases for Pb (1.5 μg kg $bw^{-1}day^{-1}$); benchmark dose lower confidence limits associated with a 10% change in the incidence of nephrotoxicity (BMDL10) for Pb (0.63 μg kg $bw^{-1}day^{-1}$) (EFSA, 2023b; FAO/WHO, 2023).

Exposure assessment and risk characterization were performed by using a probabilistic approach based on Monte Carlo simulations. This approach was chosen to minimize the uncertainties inherent in deterministic methods that do not account for the distribution of available data (Guo et al., 2019). Specifically, the distribution frequencies of both chronic food consumption data (lognormally distributed) (Table 2) and elemental concentrations (lognormally distributed), were firstly used to simulate the estimated daily intake (EDI) or the estimated weekly intake (EWI) of each one of the 12 elements under investigation, for each age group and for each of the seven botanical groups the samples were divided into. For this purpose, the following formulas were employed:

$$\text{EDI} = \text{element concentration } (\mu g\ kg^{-1}) / 1000 \times \text{food consumption rate } (\mu g\ kg\ bw^{-1}day^{-1})$$

$$\text{EWI} = \text{element concentration } (\mu g\ kg^{-1}) / 1000 \times \text{food consumption rate } (\mu g\ kg\ bw^{-1}day^{-1}) \times 7$$

After these steps, the forecasted EDIs or EWIs were compared with HBGV values (for Al, Cd, Cr, Cu, Fe, Hg, Ni, Sn, U, and Zn) or BMDL values (for As and Pb). This comparison was done to determine the probability distributions of the contribution percentages to the TDI or TWI and the Margin of Exposure (MOE), respectively. For these purposes, the following formulas were employed:

Table 3
Mean[b], median[c], and 95% confidence interval (CI, lower–upper)[d] of elemental concentrations (μg kg^{-1}) of the botanical preparation samples.

	Fruits (n = 7)	*Leaves (n = 6)*	*Peels (n = 3)*	*Seeds (n = 2)*	*Roots (n = 6)*	*Fungi (n = 4)*	*Spirulina (n = 1)*
Al[a]	11[a]	29[a]	16[a]	8.8[a]	10[a]	12[a]	209
	13	39	16	17	14	12	
	7.50–17.4	16.4–50.7	11.2–21.8	2.21–38.6	5.22–20.4	6.18–24.9	
As	87[abc]	134[ab]	272[a]	88[abc]	80[bc]	52[c]	599
	88	140	353	139	91	51	
	67.3–118	79.9–273	103–2291	43.3–282	60–114	43.0–65.4	
B[a]	5.4[ab]	8.4[a]	13[a]	1.6[b]	2.6[b]	1.9[b]	2.6
	12	12	9.2	1.7	2.4	3.0	
	2.71–11.3	4.59–15.6	8.55–21.5	1.18–2.28	1.62–4.39	1.07–3.63	
Ba[a]	0.9[ab]	2.2[a]	1.3[ab]	0.3[b]	0.9[ab]	0.7[ab]	5.7
	0.7	3.1	1.2	0.8	1.3	0.7	
	0.55–1.48	0.99–4.69	0.59–2.92	0.03–1.89	0.55–1.49	0.32–1.72	
Be	0.9[b]	2.1[a]	1.3[ab]	1.2[ab]	0.8[b]	0.7[b]	18
	0.8	2.3	1.0	1.2	0.7	1.1	
	0.61–1.46	1.39–3.38	0.82–2.21	0.99–1.44	0.58–1.28	0.44–1.27	
Bi	0.8[bc]	2.9[a]	1.7[ab]	0.5[bc]	0.5[c]	0.5[c]	36
	0.7	4.5	1.8	0.9	0.6	0.4	
	0.52–1.13	1.80–4.79	0.77–4.23	0.19–1.78	0.29–0.99	0.35–0.61	
Ca[a]	331[ab]	746[a]	865[a]	116[b]	379[ab]	624[ab]	1844
	220	960	1582	166	243	578	
	234–477	315–2005	424–1914	48.2–320	224–670	312–1352	
Cd	3.8[b]	7.8[ab]	20[a]	12[ab]	10[ab]	24[a]	51
	4.2	6.8	23	77	9.1	24	
	2.46–5.70	3.84–14.8	11.2–33.5	0.09–208	4.52–20.5	20.0–28.7	
Ce	15[a]	23[a]	14[a]	7.6[a]	8[a]	9.2[a]	362
	16	20	13	20	15	6.8	
	9.59–22.4	13.7–41.0	9.77–20.5	1.54–48.7	3.92–18.58	5.28–16.5	
Co	41[bc]	109[ab]	272[a]	27[bc]	30[c]	23[c]	384
	73	72	964	38	47	24	
	25.3–69.0	56.5–226	64.8–1707	11.7–72.3	15.6–61.4	14.0–39.9	
Cr[a]	1.3[a]	0.9[ab]	0.8[ab]	0.2[b]	0.6[ab]	0.3[b]	0.9
	1.9	0.9	1.4	0.4	0.8	0.2	
	0.77–2.17	0.46–1.50	0.39–1.54	0.01–1.07	0.37–1.05	0.15–0.54	
Cs	7.8[a]	21[a]	40[a]	24[a]	12[a]	19[a]	51
	9.0	24	39	57	11	20	
	3.54–18.4	7.46–67.3	30.7–53.9	5.52–137	8.90–16.0	14.5–25.1	
Cu[a]	0.3[b]	2.1[a]	3.8[a]	0.9[ab]	1.2[a]	1.8[a]	0.9
	0.3	2.4	3.8	1.7	1.1	2.2	
	0.17–0.56	0.93–4.86	1.15–12.5	0.21–3.72	0.73–1.96	1.01–3.1	
Dy	0.7[a]	1.7[a]	1.5[a]	0.4[a]	0.6[a]	1.0[a]	35
	1.0	1.0	1.0	0.9	0.9	1.0	
	0.48–1.15	1.00–3.13	0.95–2.51	0.13–1.99	0.31–1.37	0.66–1.41	
Er	0.4[a]	1.0[a]	0.7[a]	0.3[a]	0.4[a]	0.6[a]	26
	0.6	0.7	0.6	0.5	0.5	0.6	
	0.30–0.65	0.60–1.85	0.50–1.11	0.09–1.22	0.22–0.84	0.40–0.84	
Eu	0.4[ab]	1.0[a]	1.0[ab]	0.2[b]	0.4[ab]	0.5[ab]	7.6
	0.4	1.0	1.3	0.4	0.6	0.6	
	0.28–0.68	0.54–1.98	0.65–1.42	0.06–0.99	0.25–0.84	0.27–0.87	
Fe[a]	34[ab]	47[a]	60[a]	7.6[b]	23[ab]	39[ab]	613
	49	60	60	21	21	50	
	20.6–54.9	25.2–82.6	54.2–66.4	0.49–50.7	13.2–39.1	21.7–65.3	
Gd	1.0[a]	1.7[a]	1.5[a]	0.8[a]	0.6[a]	0.9[a]	29
	0.9	1.0	1.1	1.1	0.9	0.8	
	0.63–1.61	0.95–3.00	0.88–2.81	0.31–2.08	0.29–1.38	0.62–1.34	
Hf	0.8[a]	1.1[a]	0.6[a]	0.7[a]	1.0[a]	0.7[a]	12
	0.6	2.4	0.6	0.7	1.2	0.8	
	0.49–1.49	0.59–2.65	0.45–0.85	0.48–0.95	0.75–1.39	0.52–0.97	
Hg[e]	1.0[a]	1.8[a]	1.0[ab]	1.3[a]	1.0[a]	0.50[b]	6.0
	1.2	2.0	0.6	2.1	1.0	0.6	
	0.78–1.41	1.35–2.43	0.51–2.92	0.59–4.33	0.78–1.42	0.45–0.62	
Ho	0.2[a]	0.4[a]	0.3[a]	0.1[a]	0.1[a]	0.2[a]	7.9
	0.2	0.2	0.2	0.2	0.2	0.2	
	0.10–0.24	0.21–0.66	0.18–0.46	0.03–0.38	0.07–0.28	0.15–0.29	
K[a]	1557[c]	12078[ab]	21836[a]	1745[bc]	5497[bc]	10147[ab]	15407
	1159	21966	14438	2743	8388	9532	
	672–3314	4898–27044	5683–68415	425–5734	2494–11241	8856–11598	
La	8.4[a]	12[a]	7.7[a]	4.1[a]	4.3[a]	4.6[a]	205
	9.0	9.4	7.5	11	7.5	3.6	
	5.90–12.2	7.12–21.5	6.28–9.40	0.80–26.8	2.14–9.16	2.52–8.48	
Li	82[a]	91[a]	208[a]	28[b]	112[a]	98[a]	146
	83	95	228	30	117	86	
	56.7–125	52.1–174	127–366	18.5–44.3	88.8–143	57.9–180	
Lu	0.1[ab]	0.1[a]	0.1[ab]	0.03[b]	0.1[ab]	0.1[ab]	4.9
	0.1	0.1	0.1	0.1	0.1	0.1	
	0.04–0.09	0.08–0.26	0.07–0.13	0.01–0.16	0.04–0.15	0.05–0.12	

(*continued on next page*)

Table 3 (*continued*)

	Fruits (n = 7)	*Leaves (n = 6)*	*Peels (n = 3)*	*Seeds (n = 2)*	*Roots (n = 6)*	*Fungi (n = 4)*	*Spirulina (n = 1)*
Mg[a]	191[b]	539[ab]	621[ab]	316[ab]	476[ab]	991[a]	2348
	85	527	459	908	630	888	
	106–332	210–1268	390–965	25.9–2236	314–708	665–1453	
Mn[a]	1.9[c]	16[a]	8.7[abc]	5.4[abc]	4.3[bc]	10[ab]	30
	3.2	22	7.5	21	3.8	5.0	
	0.98–3.53	8.01–31.4	5.62–13.0	0.19–54.1	2.12–8.12	4.17–20.1	
Mo	55[b]	195[a]	235[a]	102[ab]	54[b]	137[ab]	100
	40	250	268	254	44	166	
	28.6–94.1	145–255	199–266	0.95–605	34.2–81.2	80.9–214	
Na[a]	362[bc]	1090[ab]	760[ab]	90[c]	1293[a]	513[ab]	7467
	349	920	652	98	1222	523	
	191–716	559–2221	568–1025	58.0–143	681–2557	269–1022	
Nd	3.4[a]	6.7[a]	5.5[a]	1.9[a]	2.4[a]	3.1[a]	118
	3.5	4.7	4.2	4.8	4.4	2.2	
	2.15–5.49	3.89–11.8	3.33–9.20	0.38–11.9	1.12–5.53	1.95–5.08	
Ni[a]	0.6[ab]	0.7[ab]	1.8[a]	0.4[ab]	0.4[b]	0.5[ab]	1.0
	0.9	0.8	1.4	0.7	0.6	0.5	
	0.38–0.94	0.45–1.14	0.52–9.80	0.18–1.41	0.25–0.62	0.34–0.65	
P[a]	224[c]	1579[b]	372[c]	933[bc]	357[c]	4398[a]	8768
	309	1659	719	2445	489	4688	
	112–419	877–2697	132–898	63.1–5893	204–594	3606–5330	
Pb	29[c]	98[ab]	196[a]	14[c]	45[bc]	36[bc]	301
	31	122	186	54	53	43	
	21.2–40.7	53.2–181.2	65.1–581	1.31–144	25.5–79.4	26.5–48.8	
Pr	1.3[a]	2.4[a]	1.8[a]	0.7[a]	0.9[a]	1.0[a]	44
	1.4	1.9	1.5	2.0	1.7	0.8	
	0.81–2.04	1.40–4.24	1.23–2.80	0.15–4.85	0.39–1.97	0.62–1.79	
Rb[a]	1.9[b]	9.2[a]	10[ab]	5.0[ab]	3.1[ab]	8.3[ab]	2.3
	2.4	7.7	8.2	12	4.3	8.6	
	0.64–4.79	3.50–22.1	4.53–22.3	0.57–28.5	1.63–5.50	7.21–9.59	
Sb	3.5[b]	31[a]	23[a]	1.9[b]	5.3[b]	4.2[b]	23
	2.3	44	17	3.5	7.7	5.0	
	2.18–5.57	17.7–56.4	12.7–42.9	0.46–7.88	2.68–10.5	3.13–5.61	
Se	27[ab]	44[a]	47[a]	11[b]	14[b]	21[ab]	223
	56	50	25	24	13	22	
	15.7–45.7	34.4–56.3	19.1–113	1.97–57.0	9.72–19.5	14.8–30.6	
Sm	1.1[a]	2.0[a]	1.9[a]	0.6[a]	0.7[a]	1.0[a]	32
	1.1	1.3	1.4	1.3	1.2	0.8	
	0.75–1.62	1.12–3.56	1.13–3.42	0.13–3.04	0.33–1.70	0.67–1.57	
Sn	9.0[bc]	18[ab]	39[a]	3.6[c]	7.8[bc]	8.1[bc]	81
	5.0	19	69	4.7	8.8	8.4	
	5.09–16.4	12.1–27.5	19.6–80.1	1.62–8.38	4.27–14.6	5.85–11.4	
Sr[a]	2.2[a]	4.9[a]	4.9[a]	0.3[b]	2.5[a]	3.3[a]	16
	1.7	6.8	5.9	0.5	2.1	4.8	
	1.29–3.88	1.64–15.1	2.16–11.2	0.09–0.97	1.50–4.28	1.50–7.47	
Tb	0.1[a]	0.3[a]	0.3[a]	0.1[a]	0.1[a]	0.2[a]	5.5
	0.2	0.2	0.2	0.2	0.2	0.2	
	0.08–0.20	0.17–0.55	0.16–0.49	0.02–0.39	0.05–0.25	0.12–0.25	
Th	1.8[a]	2.6[a]	2.4[a]	1.0[a]	1.5[a]	1.3[a]	138
	2.7	4.4	2.1	2.9	2.0	1.2	
	1.17–2.90	1.19–6.40	1.46–4.12	0.20–7.56	0.73–3.31	0.79–2.36	
Tl	0.9[b]	6.6[a]	7.7[a]	1.4[ab]	6.4[a]	0.6[b]	33
	1.7	5.5	8.7	3.2	8.9	0.7	
	0.39–1.78	2.62–15.7	3.98–14.4	0.20–7.59	3.76–10.7	0.40–0.89	
U	9.2[a]	12[a]	22[a]	0.8[b]	5.1[ab]	4.2[ab]	67
	7.8	6.5	15	1.0	14	4.2	
	5.26–15.4	6.09–22.7	12.5–37.2	0.32–1.91	1.68–13.7	1.66–9.77	
V	38[bc]	111[a]	115[ab]	15[c]	44[abc]	51[abc]	378
	66	89	150	21	64	52	
	24.8–59.3	58.2–213	79–168	5.33–42.0	27.5–70.9	22.5–114	
W	11[a]	17[a]	15[a]	3.8[a]	13[a]	6.7[a]	63
	10	18	19	4.6	20	4.6	
	6.85–15.8	11.7–23.8	10.3–20.4	0.57–8.26	5.70–25.1	1.74–20.1	
Y	6.1[a]	11[a]	7.4[a]	3.1[a]	4.4[a]	6.1[a]	258
	7.3	6.8	5.8	5.2	6.6	6.3	
	3.83–9.88	6.32–19.5	4.86–11.6	1.03–11.2	2.32–8.93	4.26–8.77	
Yb	0.4[a]	0.9[a]	0.6[a]	0.3[a]	0.5[a]	0.5[a]	30
	0.6	0.6	0.4	0.4	0.7	0.5	
	0.28–0.59	0.53–1.58	0.42–0.80	0.10–0.95	0.25–0.91	0.40–0.77	
Zn[a]	2.5[d]	8.8[b]	6.6[bc]	4.5[bcd]	3.4[cd]	23[a]	10
	2.2	9.0	6.1	11	4.5	23	
	1.75–3.47	6.11–12.3	5.23–8.35	0.31–26.1	2.18–4.99	20.1–26.7	

[a] Data expressed in mg kg^{-1}.

[b] Mean values are reported in the first row of each element and were calculated by reversing the Box-Cox transformed data. Values in the same row followed by different superscript letters are different at $p \leq 0.05$ according to the ANOVA results.

[c] Median values are reported in the second row of each element.

[d] 95% lower and upper CI values are reported in the third row of each element and were calculated by reversing the Box-Cox transformed data.

[e] Hg was determined by means of AMA-254 mercury analyzer.

%TDI or %TWI = EDI or EWI / HGBV × 100

MOE = BMDL / EDI

Regarding the risk characterization for Pb, MOE values that fell below 10 were taken into consideration, even though EFSA guidelines indicate that exposure risk is significant at MOE values below 1 (EFSA, 2010). This approach was adopted for a more cautious assessment.

All the Monte Carlo simulations were run through 50,000 iterations using the Crystal Ball software (ver. 11.1.3.0.0, Oracle, Inc., Tx, USA).

3. Results and discussion

3.1. Elemental profile of the botanical preparation samples

The concentrations of elements measured in this work are summarized and presented as mean values (back-transformed data), median values, and 95% CI (lower–upper) in Table 3.

A great variability of concentration values of all the 49 analyzed elements was observed across the 7 different botanical groups, as well as within the different samples of each botanical group. This scenario can be attributed to the wide range of species used to obtain botanical preparations, the diversity of plant parts utilized, and their geographical origins. Indeed, biological, geochemical, and climatic factors influence the process of mobility and availability of elements, impacting the final chemical composition of plant biomass (Kabata-Pendias, 2004). Moreover, the inorganic composition of botanical preparations is affected by multiple agronomic factors (e.g., fertilization, growth conditions, purity of the harvest, etc.) (Alagić et al., 2015; Rempelos et al., 2023) and, for certain plant species, also by seasonal changes (Mirdehghan and Rahemi, 2007). In addition, the transfer of elements to the end-product may occur in the subsequent steps of post-harvest handling, including processing, transportation, and storage (Costa et al., 2019).

3.1.1. Macro- and micro-elements

In all 29 analyzed botanical preparations, the most abundant elements resulted to be K, Ca, Na, P and Mg. Among these, K showed the highest concentration values, exceeding 1% of weight (>1000 mg kg^{-1}) across all botanical groups (Table 3). High concentrations of K, Ca, Na, P and Mg, with values close to the results found in the present work, were already reported in several studies, confirming that that these types of products are generally a good source of essential macro- and micro-elements (Carr et al., 2003; Chen et al., 2009; Mahlangeni et al., 2017; Mleczek et al., 2018; Rzymski et al., 2019; Santos et al., 2019; Augustsson et al., 2021; Farias et al., 2022). As observed in Table 3, the peels group displayed the highest mean value of K (21,836 mg kg^{-1}), while the fruits group had the lowest mean concentration (1557 mg kg^{-1}). Concentrations of K spanning from 44,507–3929 mg kg^{-1} were previously reported in walnut green peels collected in Iran and in garlic cloves coming from Russia and China, respectively (Polyakov et al., 2020; ZamaniBahramabadi et al., 2022), which correspond to the same type of botanical preparations included in the peels and fruit groups analyzed in the present work (see Table 1, *Materials and Methods* section). Ca and Mg concentrations exceeded 1% of the weight of the samples exclusively in the spirulina sample, showing values of 1844 and 2348 mg kg^{-1}, respectively. In the other botanical groups, the mean concentration values ranged from 1 to 0.1 % of weight (1000–100 mg kg^{-1}), with the lowest Ca levels of 116 mg kg^{-1} found in the seeds group, and the lowest Mg levels of 191 mg kg^{-1} found in the fruits group (Table 3). The highest concentrations of P were recorded in spirulina, fungi, and leaves groups, with mean values of 8768, 4398 and 1579 mg kg^{-1} respectively. The lowest mean value of P was observed in the fruits group (224 mg kg^{-1}). This observation indicated that P was the element with the widest variability in concentrations across the 7 botanical groups (Table 3). Similarly, Na concentrations showed a great variability among the botanical groups, ranging from a minimum of 90 mg kg^{-1} in seeds to a maximum of 7467 mg kg^{-1} in spirulina.

Fe and Al also showed a relevant variability, being higher that 0.1% of weight (>100 mg kg^{-1}) only in the spirulina sample (613 and 209 mg kg^{-1}, respectively). No statistically significant differences were found among samples of the other botanical groups according to Al average concentrations ($p > 0.05$). Al is naturally present in the environment; however, its concentration and carryover into food and feed may increase as a result of human activities, such as mining and industrial production of compounds containing this element (EFSA, 2008). In general, unprocessed foods have been reported to contain Al levels below 5 mg kg^{-1}, whereas 5–10 mg kg^{-1} have been usually found in processed foods (EFSA, 2008). Al concentrations found in this work were above this range in 5 out of 7 botanical groups, with samples of the leaves group, along with the spirulina sample, showing the highest concentrations (Table 3). This result is consistent with the literature, which indicates that leafy foods such as tea leaves and herbs often exhibit very high levels of Al (EFSA, 2008). High concentrations of Al in spirulina have been previously documented as well, and these elevated levels are often associated with factors such as water acidification, soil erosion, and wastewater discharges (Rubio et al., 2021).

3.1.2. Trace and ultra-trace elements

Zn, Mn, Rb, and B were present at trace level (between 100 and 1 mg kg^{-1}) in all botanical groups, while other elements such as Sr, Ba, Cu, Cr, were found in trace amounts in some groups (e.g., in the peels group) and in ultra-trace amounts (<1 mg kg^{-1}) in other groups (e.g. in the seeds group). Among all the botanicals, the seeds group exhibited the lowest average concentrations of these elements.

Essential metals like Fe, Cu, Zn, and Mn play crucial biological roles but become toxic at high concentrations (Tuzen, 2003). Metal concentrations similar to those measured in the fruits group were previously observed in Sea buckthorn, while concentrations similar to those found in peels and roots group closely aligned to those reported in walnut and *Vitis vinifera* peels and in red beets, respectively (Sadhu et al., 2015; Uraku, 2015; Mahlangeni et al., 2017; Żukowska et al., 2021; Zamani-Bahramabadi et al., 2022).

Among ultra-trace elements, concentrations of rare earth elements (REEs) including La, Ce, Eu, Gd, Nd, Pr, Sm, Dy, Er, Ho, Lu, Tb, Y, and Yb, were found to be very similar across all sample groups, except for the spirulina sample whose concentrations were evidently higher ($p \leq 0.05$, Table 3). Rzymski and colleagues analyzed the presence of REEs in 13 spirulina-based food supplements and found an average cumulative concentration of 2140 μg kg^{-1} (Rzymski et al., 2019). However, their study also included Sc and Tm, making the concentration slightly higher than the 1283 μg kg^{-1} calculated in the present study. REEs, due to increased human emission in recent decades, can now be considered as an emerging category of pollutants with a possible correlation to cytotoxic effects and other health concerns (Pagano et al., 2015; Zhuang et al., 2017; Gwenzi et al., 2018). The concentrations of REEs found in the present work can be considered relatively low, even though currently, there is no regulation in the EU concerning this series of elements. The only threshold identified is in China, where a limit of 7.0 mg kg^{-1} (dry weight) has been established (SAC, 2012; Rzymski et al., 2019).

Toxic heavy metals and metalloids As, Cd, Hg, and Pb were all found as ultra-trace elements (<1 mg kg^{-1}). As concentrations resulted to be the highest (52–599 μg kg^{-1}), followed by those of Pb (14–301 μg kg^{-1}), Cd (3.8–51 μg kg^{-1}), and Hg (0.50–6.0 μg kg^{-1}) (Table 3). The European Commission has not set any maximum levels (MLs) for toxic metals and metalloids in botanical preparations. However, MLs are in place for food supplements, including Pb (3.0 mg kg^{-1}), Cd (1.0 mg kg^{-1} and 3.0 mg kg^{-1} for algae-based supplements), and Hg (0.10 mg kg^{-1}), and these limits can also apply to botanical preparations as ingredients (Commission Regulation (EU) No. 2023/915). In the present work, none of the 29 analyzed samples exceeded these limits, although the levels of Pb and Cd in some botanical groups can be considered relatively high and close to the MLs (Table 3). This is especially true for spirulina, which was

the most contaminated sample when compared to the mean values of the other botanical groups. This result is generally consistent with findings reported by other authors who have investigated spirulina-based food supplements in various dosage forms such as powders, capsules, and tablets (Al-Dhabi, 2013; Rzymski et al., 2019; Ćwielag-Dbarek et al., 2020; Rubio et al., 2021). As previously discussed for Al, contamination of spirulina by toxic metals may be related to the characteristics of the marine environment in which the alga grows. Additionally, MLs of 200 μg kg^{-1} and 50 μg kg^{-1} of Pb in small fruits/berries and garlic, respectively (intended for consumption as food), have been established at European level, but all the samples belonging to the fruits group and analyzed in the present study resulted compliant to these limits as well (Commission Regulation (EU) No. 2023/915).

The contamination levels in the peels botanical group, especially for As, were very high. Although the group mean for As was lower than that of spirulina, the upper value of the 95% CI was higher (2291 μg kg^{-1}, Table 3). To the best of the authors' knowledge, no other studies on heavy metal contamination in peel extracts have been conducted and, therefore, further investigation is necessary. However, the ability of walnut green peel to absorb heavy metals has already been demonstrated, suggesting its usefulness for soil bioremediation purposes (Yu et al., 2021).

In the leaves botanical group, high contamination levels were observed for As, Cd, and Pb. Similar levels were reported in a gingko leaf extract sample and in samples of green tea leaves by other authors (Dolan et al., 2003; Chen et al., 2009; Augustsson et al., 2021). Indeed, previous studies have noted that leaves are highly sensitive to atmospheric pollution, and contamination by these toxic elements is not uncommon in specific areas (Tomašević et al., 2004; Alagić et al., 2015).

Another important aspect emerging from the results achieved is the relatively low concentration of heavy metals and metalloids observed in the sample belonging to the fungi botanical group. These findings are somewhat surprising, as various fungal species commonly used for dietary or medicinal purposes, such as *Ganoderma lucidum*, *Cordyceps militaris*, and *Cordyceps sinensis*, have often been described in the literature as natural high accumulators of heavy metals (Mleczek et al., 2018). Similarly, the concentrations of As, Cd, Hg, and Pb in samples from the fruits and roots groups were generally lower, up to one order of magnitude, compared to those reported in other studies (Sadhu et al., 2015; Huang et al., 2023). Finally, it is worth noting that the two samples of guarana seed extract (included in the seeds group) had higher concentrations of Hg and Pb but similar Cd concentrations to those reported in similar botanical products by Caldas and colleague (Caldas and Machado, 2004).

3.2. Probabilistic health and dietary risk assessment

The results of the probabilistic risk assessment, obtained through Monte Carlo simulations, are presented in Table 4. For brevity, only the contribution percentages of calculated EIs of toxic metals and metalloids exceeding 10% of TDI/TWI and MOE values below 10,000 (for As) and 10 (for Pb) were included. This choice was made to emphasize the dietary exposure scenarios related to the consumption of "Herbal formulations and plant extracts" which may pose the highest risk, aligning with EFSA guidelines for these contaminants (EFSA, 2005; EFSA, 2009b; EFSA, 2010; EFSA, 2017). Therefore, among the twelve investigated elements, only the results regarding Al, As, Cd, Fe, Ni, Pb and U are presented and discussed.

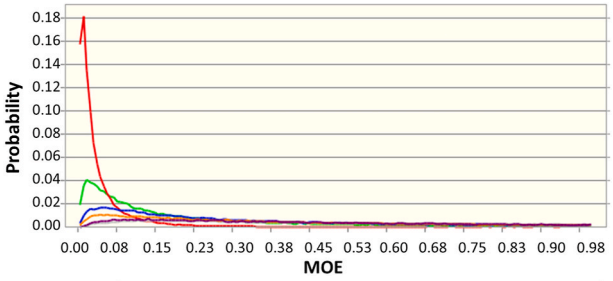
3.2.1. Arsenic

Table 4 shows that one contaminant of concern for consumers of all age groups in this study is As, irrespective of whether the lowest (l.c.l.) or highest (h.c.l.) confidence limits of the BMDL01 values were used in calculating the MOEs. The peels botanical group was found to pose the major threat. Indeed, the consumption of this botanical category resulted in the lowest MOE values across all age groups (MOEs: 0.04 to 61),

Table 4
Mean contribution percentages (%) ± standard deviation values to PMTDI/TDI (Fe, Ni, U) or TWI (Al, Cd) and mean MOE ± standard deviation values (As and Pb).

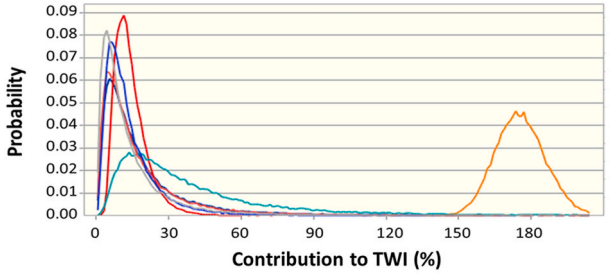
Population	Elements	Spirulina (n = 1)	Leaves (n = 6)	Peel (n = 3)	Seed (n = 2)	Roots (n = 6)	Fungi (n = 4)	Fruit (n = 7)
Infants	Al	176 ± 10.4	41 ± 39.3	14 ± 6.75	14 ± 17.8	19 ± 21.3	18 ± 21.4	14 ± 12.1
	As (l.c.l.)[a]	0.4 ± 0.02	0.8 ± 0.73	0.04 ± 0.071	2.6 ± 1.33	2.9 ± 1.16	4.8 ± 1.09	2.8 ± 1.25
	As (h.c.l.)[b]	11 ± 0.66	22 ± 19.6	1.1 ± 1.83	70 ± 35.4	76 ± 31.0	127 ± 29.2	74 ± 33.4
	Cd	17 ± 1.01	/	/	26 ± 36.2	/	/	/
	Fe	92 ± 5.42	11 ± 10.5	/	/	/	/	/
	Ni	/	11 ± 9.27	130 ± 212	/	/	/	/
	Pb (neuro)[c]	1.4 ± 0.08	4.9 ± 4.85	2.7 ± 3.64	23 ± 42.3	12 ± 14.7	/	/
	Pb (kidney)[d]	1.8 ± 0.10	6.2 ± 6.10	3.4 ± 4.35	29 ± 58.8	16 ± 20.1	/	/
	Pb (s.b.p.)[e]	4.2 ± 0.24	15 ± 14.4	8.0 ± 10.6	/	/	/	/
	U	13 ± 0.79	/	/	/	/	/	/
Toddlers	Al	41 ± 38.0	/	/	/	/	/	/
	As (l.c.l.)[a]	3.4 ± 3.18	6.5 ± 9.90	0.4 ± 0.81	21 ± 24.7	23 ± 25.1	39 ± 37.8	23 ± 25.1
	As (h.c.l.)[b]	91 ± 85.3	175 ± 276	9.1 ± 22.4	567 ± 647	620 ± 680	1028 ± 1021	600 ± 670
	Fe	21 ± 20.2	/	/	/	/	/	/
	Ni	/	/	30 ± 69.1	/	/	/	/
	Pb (neuro)[c]	11 ± 10.8	/	22 ± 51.2	/	/	/	/
	Pb (kidney)[d]	14 ± 13.5	/	27 ± 51.6	/	/	/	/
Children	Al	17 ± 16	/	/	/	/	/	/
	As (l.c.l.)[a]	8.4 ± 8.14	16 ± 26.3	0.9 ± 2.08	53 ± 62.7	57 ± 64.3	95 ± 96.5	56 ± 63.5
	As (h.c.l.)[b]	221 ± 213	428 ± 687	22 ± 57.5	1389 ± 1607	1514 ± 1703	2515 ± 2553	1469 ± 1704
	Ni	/	/	13 ± 36.0	/	/	/	/
Adolescents	As (l.c.l.)[a]	20 ± 16.8	36 ± 55	2.0 ± 4.60	126 ± 133	137 ± 135	228 ± 202	133 ± 136
	As (h.c.l.)[b]	537 ± 446	1030 ± 1460	54 ± 119	3366 ± 3518	3366 ± 3571	6083 ± 5356	3548 ± 3599
Adults	Al	11 ± 13.6	/	/	/	/	/	/
	As (l.c.l.)[a]	17 ± 21.0	33 ± 61.4	1.7 ± 4.48	106 ± 153	116 ± 164	192 ± 248	112 ± 162
	As (h.c.l.)	444 ± 546	846 ± 1576	44 ± 120	2811 ± 4241	3032 ± 4178	5038 ± 6427	2940 ± 4137
Elderly	As (l.c.l.)[a]	23 ± 16.3	43 ± 56.5	2.3 ± 4.59	141 ± 133	154 ± 135	255 ± 197	150 ± 136
	As (h.c.l.)[b]	602 ± 430	1163 ± 1548	61 ± 134	3776 ± 3553	4113 ± 3574	6826 ± 5226	3983 ± 3589

[a] l.c.l. = Lower confidence level.
[b] h.c.l = Higher confidence level.
[c] Pb (neuro) = neurotoxic BMDL01 (0.5 μg kg bw^{-1} day^{-1}).
[d] Pb (kidney) = kidney cancer; BMDL10 (0.63 μg kg bw^{-1} day^{-1}).
[e] Pb (s.b.p.) = sistolic blood pressure; BMDL01 (1.5 μg kg bw^{-1} day^{-1}).



	MOE					
Percentile	Infants	Toddlers	Children	Adolescents	Adults	Elderly
5%	0.0031	0.01	0.03	0.08	0.04	0.10
50%	0.020	0.13	0.30	0.79	0.54	0.93
95%	0.15	1.30	3.22	7.61	6.41	8.45

Fig. 1. Probability distribution of the MOEs to As from Montecarlo simulations and associated with the consumption of the botanicals included in the peels group by different population age groups (MOE values at the 5th, 50th, and 95th percentiles are listed in the table below the chart).



	Contribution to TWI (%)						
Percentile	Fruits	Peels	Spirulina	Fungi	Roots	Leaves	Seeds
5%	3.14	6.15	159	2.59	2.82	7.76	1.94
50%	10.7	12.9	175	11.9	12.6	29	9.17
95%	36	27	193	55	57	110	43

Fig. 2. Probability distribution of the contribution to the TWI (%) of Al from Montecarlo simulations and associated with the consumption of different botanicals by the infants age group and the related percentiles (TWI % at the 5th, 50th, and 95th percentiles are listed in the table below the chart).

followed closely by spirulina (MOEs: 0.4–602), and the leaves group (MOEs: 0.8–1163) (Table 4). The graphic representation of the Monte Carlo simulation of the distribution of MOE values for As deriving from the consumption of botanicals of the peels group is shown in Fig. 1. As observed, the MOE values at the 5th and 50th percentiles were below the threshold value of 1 for all the population age groups considered, suggesting a potential health concern for a significant portion of the population consuming these botanical products. Globally, these low MOEs to As resulted from the very high daily dietary intake of As calculated from the consumption rate of the botanicals of the peels group, which ranged from 0.0019 to 28 μg kg $bw^{-1}day^{-1}$ depending on the age group.

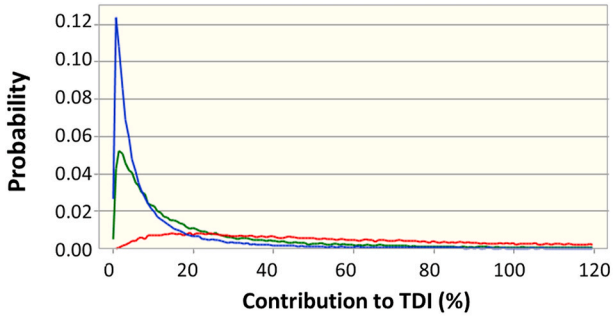
In 2021, EFSA reported mean dietary exposure levels to inorganic As (*i*As) across the European population. For the young population (infants, toddlers, and other children), exposure ranged between the minimum lower bound (LB) value of 0.07 and the maximum upper bound (UB) level value of 0.61 μg kg bw^{-1} day^{-1} (min LB-max UB). Lower levels were estimated in the adult population (adults, elderly, and very elderly), ranging between 0.03 and 0.15 μg kg bw^{-1} day^{-1}. The most important foods contributing to these levels of exposure to *i*As, within all age groups, were reported to be rice, rice-based products, grains, grain-based products, and drinking water (EFSA, 2021).

When compared to the established range of BMDL01 values of 0.3–8 μg kg bw^{-1} day^{-1}, LB mean dietary exposure estimates were found to be below this range across the whole population, while UB mean dietary intake levels were within this range only when concerning infants, toddlers, and other children (EFSA, 2009b, 2021). Within this context, it is important to underline that previous studies have indicated that some botanical and microalgal food supplements are primarily composed of organic forms of As, even though certain *i*As species were detected as well (Hedegaard et al., 2013; Rzymski et al., 2019). In the current study, the lack of speciation data for As, which would distinguish between organic and inorganic (toxic) species in the analyzed samples, adds uncertainty to the exposure assessment.

3.2.2. *Aluminum*

The results presented in Table 4 highlight that the overall intake of Al associated with the consumption of different botanicals can be a concern for several age groups. As a matter of fact, the EWI of Al were found to range from 0.0039 to 1.8 mg kg $bw^{-1}week^{-1}$, leading to a contribution to the TWI of over 10% in several cases. In particular, the consumption of spirulina and leaves groups of botanicals by infant was found to contribute to 176% and 41% of the Al TWI, respectively. When evaluating the other age groups, the 10% contribution to the TWI was solely exceeded through the consumption of spirulina by toddlers (41%), children (17%), and adults (11%) (Table 4). Hence, infants emerged as the most at-risk population group. This situation was confirmed by analyzing the distribution curves of the contribution to the Al TWI forecasted through Monte Carlo simulations (Fig. 2), from which it emerged that infants already exceeded 100% TWI at the 5th percentile.

Although the contribution to the Al TWI of most of the botanical groups analyzed was found to be lower than 10%, it is important to note that multiple routes of exposure and various foods in the overall diet may contain this element. Among these, baked goods, cereals, beverages, infant formulae, tea leaves, cocoa, and spices have been identified as the primary dietary sources of exposure to Al (EFSA, 2008). Considering the notable risk of exceeding the Al TWI as identified by EFSA, along with the documented neurotoxicity, embryotoxicity, and developmental toxicity associated with chronic dietary exposure to Al (EFSA, 2008), the results of the present study suggest that special attention should be given to the administration of these products to infants and toddlers. In particular, it may be advisable to limit or avoid their chronic consumption of spirulina- and leaves-based botanical preparations and derived products for these age groups.



	Contribution to TDI (%)		
Percentile	Infants	Toddlers	Children
5%	10.4	1.15	0.48
50%	69	11.6	4.76
95%	439	112	47

Fig. 3. Probability distribution of the contributions to the TDI (%) of Ni from Montecarlo simulations and associated with the consumption of botanicals included in the peels group by infants, toddlers, and children (TDI % at the 5th, 50th, and 95th percentiles are listed in the table below the chart).

3.2.3. Nickel

The EDI of Ni derived from the consumption of the botanical samples ranged between 0.018 and 17 μg kg $bw^{-1}day^{-1}$. In particular, intake levels of Ni related to consumption of botanicals of the peels group by infants, toddlers, and children contributed on average to 130%, 30%, and 13% to the TDI of Ni, respectively (Table 4). These mean contribution values were found to be higher than those found at the 50th percentile following probabilistic modelling (Fig. 3). However, at the 95th percentile, a highly concerning situation emerged, strongly suggesting that the toxicological threshold of concern for this element can be exceeded through the consumption of just one food product category, i.e., peels-based botanicals (Fig. 3). This implies the possibility of even more concerning exposure scenarios to this contaminant throughout the entire diet. Studies investigating the toxicity of Ni indicate that chronic oral exposure to this element is associated with adverse effects such as neurotoxicity, reproductive toxicity, and immunotoxicity (EFSA, 2020). The main contributors to the dietary exposure to Ni of the European general population were found to be grains and grain-based products, even though not significant health-related issues were reported (EFSA, 2020). Nonetheless, the results of this study emphasize the necessity of closely monitoring the consumption of peel-based botanical extracts among the younger population. Moreover, considering that developmental toxicity (post-implantation loss of embryos and/or fetuses) has been identified as one of the adverse effects of Ni (EFSA, 2020), the findings of the present work also underscore importance of paying special attention to the potential harmful effects on women of childbearing age consuming these products.

3.2.4. Lead

Pb is known for its chronic toxicity, with potential health risks spanning from developmental neurotoxicity, nephrotoxicity, cardiovascular issues, to possible carcinogenic effect (EFSA, 2011; IARC, 2023). The primary route of exposure to Pb is through food, particularly cereals, vegetables, and tap water (EFSA, 2010). Infants and children can face a higher risk of lead exposure, especially when consuming infant formulas. Pregnant women share a similar level of concern to infants and children due to the potential for neurodevelopmental disorders resulting from Pb exposure (EFSA, 2010). Conversely, the risk of significant adverse effects (mainly nephrotoxic and cardiovascular effects) in adults was reported to be low. Within the present study, the EDI of Pb ranged between 0.0013 and 0.50 μg kg $bw^{-1}day^{-1}$, and it was observed that infants consuming botanical preparations, whether spirulina-based or belonging to the leaves, peels, seeds, and roots groups, may be exposed to an increased risk of encountering neurotoxic effects. This is indicated by the mean MOE values for neurotoxic effects, which were found to be below 10 or above 10 but with high SD values, as shown in Table 4. Values of MOE related to neurotoxicity close to 10 were found also for toddlers consuming spirulina and peels botanicals, while for older age groups the resulting mean MOE values were such that the risk was considered lower (Table 4). These findings are consistent with the results reported by Torović and colleagues, who assessed the risk associated with the intake of Pb through the consumption of recommended doses of herbal food supplements. Indeed, in this study, infants were identified as the group with the highest exposure to Pb and the most susceptible to neurotoxic effects (MOE = 11), followed by toddlers (MOE = 13) (Torović et al., 2023).

3.2.5. Iron, cadmium, and uranium

The risk of Fe intoxication resulting from dietary intake is generally considered negligible in healthy individuals, but large acute intakes (>20 mg kg bw^{-1}) can lead to severe injuries, including fatal outcomes (EFSA, 2015). Conversely, chronic intoxication may occur in individuals who have other underlying conditions, such as hemolytic anemia (EFSA, 2015). Overall, there is no conclusive evidence indicating toxic effects resulting from dietary Fe intake and, for this reason, PMTDI rather than a TDI has been established for this element (FAO/WHO, 2023). Within the present assessment, the EDI of Fe from botanicals consumption ranged between 0.00071 and 0.74 mg kg $bw^{-1}day^{-1}$ and the contribution to the PMTDI of Fe was higher than 10% in three instances: infants consuming spirulina (92%) and botanicals from the leaves group (11%), and toddlers consuming spirulina (21%) (Table 4). Based on this, the potential intake of spirulina-based products in infants demands careful consideration, since the contribution to the PMTDI is nearly 100% and there is insufficient toxicological data available to evaluate the risk associated with such a high level of intake.

Cd risk assessment resulted in an EDI ranging from 0.0012 to 0.64 μg kg $bw^{-1}day^{-1}$ and highlighted a potential concern only for infants, where contributions of 26 % and 17% to the TWI due to the consumption of samples of the seeds group and spirulina were observed, respectively (Table 4). The main sources of dietary intake for this metal are vegetables, bread, cereals and offal, and its strong toxicity in humans could lead to respiratory diseases, renal dysfunction, endocrine disorders, and kidney and bone toxicity (Pan et al., 2010; EFSA, 2011). To date, the occurrence of toxic effects through dietary is unlikely, however some categories of botanical preparations may slightly increase this risk, especially for young age groups (EFSA, 2011).

Finally, the spirulina sample was found to be the sole botanicals whose U concentrations resulted in EDI for infants up to 0.081 μg kg $bw^{-1}day^{-1}$, which, in turn, contributed to 13% of the TDI of this element (Table 4). Even though U toxicity is contingent upon its solubility and oral bioavailability, acute exposure to substantial concentrations of U has been potentially linked to nephrotoxicity, reproductive/developmental disorders, and issues related to bone growth (EFSA, 2009c). However, to date, it has been observed that the risk related to the dietary consumption of U across all age groups in the European population can be regarded as minimal, as the TDI established for this element is not surpassed through the entire diet (EFSA, 2009c).

3.2.6. Comparative analysis with literature data

Comparing the results of the exposure assessment and risk characterization achieved in the present work with the literature is challenging due to variations in parameters used by different authors, including consumption data and risk assessment methodologies, even when studying the same food products. Nevertheless, some toxic metals investigated in this study yielded similar results to previous assessments of herbal and algal food supplements. For instance, in an exposure assessment using the recommended daily doses of different food supplements, Augustsson and colleagues also identified microalgal-based supplements as the primary source of Al, As, Cd, Ni, and Pb intake, with plant-based supplements ranking next (Augustsson et al., 2021). In this context, it is important to recognize that recommended consumption doses by manufacturers may exceed actual mean consumption data. Therefore, individuals who regularly follow these recommended doses may face a higher overall exposure risk. Furthermore, it is important to note multiple botanicals may be ingested daily for extended periods, potentially resulting in a total daily supplement intake of up to 3750 mg day^{-1} (Van den Berg et al., 2011). This can result in a chronic consumption pattern of multiple metals and metalloids (Torović et al., 2023), which has the potential to lead to what is referred to as "cocktail effects", wherein the toxic effects may be intensified compared to individual elements (Sani et al., 2023).

In conclusion, it is crucial to recognize that the input data used in the present work for probabilistic modeling (encompassing both consumption data and elemental concentrations) exhibited high SD values and non-normal distribution. As a consequence, a significant level of variability was observed in the risk assessment results. While this might be interpreted as an increase in uncertainty within the assessment, it is an inherent feature of probabilistic modeling. Unlike deterministic approaches that assume fixed values, probabilistic modeling considers the distribution of all data points, resulting in a more comprehensive and realistic representation of exposure, including extreme scenarios that deterministic models may overlook.

4. Conclusions

In the present study, a significant degree of contamination by toxic metals and metalloids was observed in various botanical preparations. Concentration levels were notably high for elements such as As, Al, and Ni, especially in products made from spirulina, plant peels or leaves.

The probabilistic risk assessment analysis revealed that infants, toddlers, and children are the population groups most susceptible to potential toxic effects resulting from chronic consumption of these products. Consequently, it is advisable for young individuals, as well as underweight subjects, to refrain from excessive or thoughtless consumption of these products which are mostly marketed and conceived as "natural" and "healthy". While the current study yielded valuable insights, it is imperative to acknowledge its inherent limitations. Variability in exposure patterns, influenced by diverse dietary habits, occupations, lifestyles, and geographical locations, may not have been fully accounted for. Furthermore, potential temporal and spatial variations in metal and metalloid concentrations within botanicals may have been insufficiently addressed. Additionally, variability in susceptibility among individuals, including pregnant or breastfeeding women and those with pre-existing health conditions, was not fully integrated into risk characterization due to the unavailability of robust food consumption data for these groups. Recognizing and mitigating these limitations is crucial for refining future research endeavors and advancing understanding of the intricate relationship between exposure to metals and metalloids and human health.

In summary, this study highlights the pressing need for producers of these food products to strengthen their quality control measures before releasing them into the market. This is imperative due to the substantial chemical variability and the wide array of potentially toxic contaminants these products can contain. Moreover, the study highlights the necessity for a more precise regulatory framework in the EU, based on a comprehensive assessment of available data and broad dietary exposure considerations. Such regulations are crucial to safeguard consumers who regularly incorporate, whether as formulated products or as ingredients in other foods, one or more botanicals within their diet. Another aspect to consider is the possibility of botanicals to be subjected to food fraud. In fact, adulteration and sophistication of botanicals can significantly worsen the risk profile associated with these products since different plant species have varying abilities to absorb and accumulate metals, and the presence of other botanical species or unknown ingredients in the samples can introduce bias, further complicating risk assessments.

In conclusion, it is recommended to continually monitor the composition of botanicals and botanical preparations available on the market, to ensure their safety regarding the potential presence of harmful levels of toxic metals and metalloids and guarantee high level of public health for regular consumers seeking an improvement or maintenance of their well-being and health.

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CRediT authorship contribution statement

Maria Olga Varrà: Writing – review & editing, Visualization, Validation, Software, Data curation. **Lenka Husáková:** Writing – review & editing, Validation, Methodology, Funding acquisition, Data curation. **Giovanni Tommaso Lanza:** Data curation, Software, Writing – original draft. **Martina Piroutková:** Formal analysis, Investigation. **Jan Patočka:** Formal analysis. **Sergio Ghidini:** Writing – review & editing, Funding acquisition. **Emanuela Zanardi:** Writing – review & editing, Supervision, Resources, Project administration.

Declaration of generative AI and AI-assisted technologies in the writing process

Not applicable.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2024.114664.

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