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Elemental and radiological characterization of commercial *Cetraria islandica* (L.) Acharius pharmaceutical and food supplementation products

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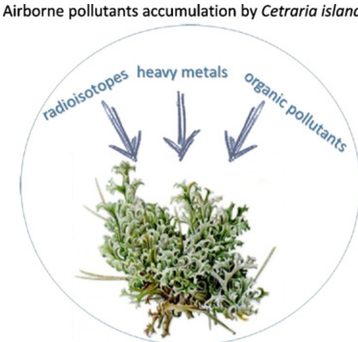
HIGHLIGHTS

- Essential and nonessential elements were determined by EDPXRF.
- Natural radionuclides were determined by alpha spectrometry.
- ^{210}Po was much higher than those reported by UNSCEAR for leafy vegetables in the world.
- Contribution to toxic element intake does not appear to pose a threat.
- Toxic elements level should be monitored to protect consumers against potential adverse health effects.

GRAPHICAL ABSTRACT

Airborne pollutants accumulation by *Cetraria islandica*



Airborne pollutants accumulation by *Cetraria islandica*.

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ABSTRACT

An elemental and radiological characterization was performed on *Cetraria islandica* (L.) Ach. pharmaceutical and food supplementation products purchased in local specialty shops in Italy. Essential elements (K, Ca, P, S, Cl, Mn, Fe, Cu, Zn, Ni, Br, I) and nonessential or toxic elements (Al, Ti, Si, Rb, Sr, As, Cd, Sn, and Pb) were determined by Energy Dispersive Polarized X-Ray Fluorescence Spectrometry; natural radionuclides (^{238}U, ^{234}U, ^{230}Th, ^{210}Po, ^{232}Th, and ^{228}Th) were determined by alpha spectrometry. The results show that *C. islandica*, whose nutritional value was assessed referring to recommended nutrient intakes, could serve as an important source of essential elements. Moreover, as expected, lichens concentrate airborne ^{210}Po, whose activity ranged from 132 to 489 Bq kg^{-1}_{dw}. This value was much higher than those reported by UNSCEAR for leafy vegetables in the world. In addition, total As and Cd were <1 mg kg^{-1}_{dw} and Pb mean concentration was 9.25 mg kg^{-1}_{dw}. Health risks associated with the toxic elements contained in *C. islandica* (L.) products were calculated using risk estimators. Their contribution to total elemental intake does not appear to pose a threat, but the concentrations of these elements should be continuously monitored to protect consumers against potential adverse health effects.

1. Introduction

The term Island moss or *Lichen islandicus* denotes whole or cut dried thallus of *Cetraria islandica* (L.) Ach. s.l. from the Parmaliaceae family (EMA, 2014). *C. islandica* is a lichen that grows in damp places, usually

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on rocks or the bark of trees, especially conifers. It grows in North America and all over Europe, especially in the Arctic and Subarctic regions. Long-living and very slow growing, lichens are composite organisms consisting of a symbiotic association between fungi and algae. Lichens obtain most of their nutrients directly from the air (Mattsson, 1975). Indeed, due to their lack of roots and large surface area, their uptake from the substrate is minimal compared to that which they obtain from wet and dry deposition (Shukla et al., 2014). As a consequence, lichens accumulate atmospheric pollutants (organic compounds, radioisotopes and heavy metals) more efficiently than other forms of vegetation. In fact, lichens have frequently been used to monitor pollutant deposition over wide areas since the pollution concentrations found in their thalli can be directly correlated with those in the environment (Bosh-Roig et al., 2013). Stable elements are removed through biological elimination processes, while radioactive elements are removed, via radioactive decay. Lichens are among the most radiation resistant living plants (Mattsson, 1975). However, their fixation of radionuclides is a passive phenomenon and does not occur through a physiological process. Given their ability to accumulate airborne pollutants, lichens can pose serious health risks to consumers. In fact, via ingestion, the heavy metals and radionuclides that have accumulated in these species can enter, the human body and may disturb the normal functioning of its systems (Mousavi et al., 2014).

C. islandica has been used in folk medicines through the ages, and its use has been documented continuously in many pharmacopeoeias, pharmacognostical texts and handbooks (EMA, 2014). Today, many people in developed countries have begun turning to alternative or complementary therapies, including medicinal herbs. The part of the *C. islandica* containing active substances (drug) is the thallus, which is composed of 50% polysaccharides (lichenin and isolichenin), bitter lichen acids (usnic and cetraric acid), folic acid and other B vitamins. As reported in the WHO Monograph (WHO, 2009), its medicinal uses can be divided into the following categories: a) uses supported by clinical data, b) uses described in pharmacopoeias and other reliable documents and c) uses described in traditional medicine (Crawford, 2015). Lichen islandicus is used for treating irritation of the mouth and throat, loss of appetite, the common cold, dry cough, bronchitis, indigestion, fevers, lung disease, kidney and bladder complaints, and susceptibility to infection. *C. islandica* preparations, isolated polysaccharides and lichen acids have been investigated in pharmacological in vitro and in vivo experimental demonstrating several effects: antimicrobial effects (Turk et al., 2003), immunological effects (Ingólfsdóttir, 2000), anti-inflammatory effects (Freysdottir et al., 2008), anti-viral activity (Stubler and Buchenauer, 1996); antioxidative effects (Kotan et al., 2011), anti-proliferative effects (Haralsdottir et al., 2004). Some people also apply Iceland moss directly to poorly healing wounds. In Italy, thallus is included in the list of herbal substances and herbal preparations allowed in food supplements, and it is valued for its emollient and demulcent action, its effect on voice tone, as well as its ability to aid digestion and boost the body's natural defenses. Moreover, as it is very nutritious and rich in mineral elements, Iceland moss is used as an emergency food both in famine and non-famine years for bread (Airasiksinen et al., 1986). When bread is available, it can be mixed with wheat; alternatively, it can be used as a dessert jelly preparation. Finally, Iceland moss is also used as a flavoring in alcoholic beverages.

As a substance widely used as either a registered medicinal product, food supplement, it should contain only very small amounts of impurities. Indeed, WHO has emphasized the need for quality assurance of herbal products (WHO, 2007, 2009). Therefore, in view of the growing use of alternative medicines worldwide, the evaluation of the toxic elements, both stable and radioactive, contained in *C. islandica* products is of upmost importance to ensure the quality of these products and avoid any potential adverse human health risks they may pose.

The largest part of the radioactive dose received through ingestion of *C. islandica* products derives from primordial radionuclides such as ^{40}K and radionuclides of the ^{238}U and ^{232}Th series. These terrestrial radioisotopes are present more homogeneously in the environment than are artificial radionuclides.

Among the alpha emitters, ^{210}Po is estimated to contribute about 7% of the effective dose equivalent to humans from ingested natural radiation (UNSCEAR, 1988). However, ^{210}Po poses a relatively high radiation risk, even at minimal levels, due to its high linear energy transfer (LET). Its radiation dose per unit intake (Sv Bq^{-1}) is higher than that of other alpha emitters in the U and Th series, and its toxicity is about 5 times greater than that of ^{226}Ra (NRC, 1988).

The radionuclide ^{210}Po and its grandfather ^{210}Pb belong to the ^{238}U series. Their presence in the terrestrial environment arises from ^{222}Rn, which, once produced, may remain in soil interstitial air spaces, decay into ^{210}Pb and ^{210}Po within the mineral matrix of soil or be released into the atmosphere. ^{210}Pb and ^{210}Po return to the Earth's surface via both wet and dry deposition. The atmospheric fallout of these decay products results in the contamination of plants and the top layer of soil. Most of the natural radioactivity content in wild leafy plants derives from ^{210}Po as the result of the direct deposition of ^{222}Rn daughters from atmospheric precipitation; hence, their presence in all terrestrial plants is inevitable (Brown et al., 2011; Persson and Holm, 2011). Natural levels of ^{210}Pb and ^{210}Po in the environment can be increased locally by anthropogenic activities such as phosphate ore processing, coal-fired power stations, coal mining, metal smelting, etc., which produce higher than average levels of ^{210}Pb and ^{210}Po.

Regarding stable elements, contamination of the ecosystem by elements such as arsenicum (As), cadmium (Cd), mercury (Hg), lead (Pb), and antimony (Sb), which are ranked among hazardous substances (ATSDR, 2005), is a serious problem, threatening the habitat and the health of both wild animals and humans (Nacano et al., 2014; De Almeida Lopes et al., 2015). These elements are natural components of the Earth's crust and biological systems; however, their concentrations have increased in the ecosystem during the last few decades due to industrial and other anthropogenic activities (Meli et al., 2015; Desideri et al., 2016a).

The impact of toxic elements such as Pb, Cd and As on human health is of great, concern, as these elements may have adverse effects even at low levels when ingested over a long period of time. Due to its ability to concentrate heavy metals, *C. islandica* has been listed by the European Food Safety Authority (EFSA) in the new version of the 2009 compendium of plants reported to contain toxic, additive, psychotropic or other substances (EFSA, 2012).

Taking into account that safety of these products is of upmost importance and the lack of information about elemental and radiological composition of these, to protect the consumer against potential health risks, it is advisable to monitor the content of radionuclides and essential and non-essential or toxic elements in *C. islandica* based pharmaceutical products. Indeed, the aim of this study was 1) to provide baseline data on the elemental and radiological composition of *C. islandica* products sold in Italy for human consumption, 2) to compare their metal contents with tolerable intake levels and, subsequently, 3) to evaluate their potential effects on human health. The nutritional value of the essential elements was assessed on the basis of recommended nutrient intakes (EFSA, 2006), while health risks posed by the toxic elements present in these products were estimated using risk estimators (EFSA, 2006; JECFA, 2006, WHO, 2007, 2011a, 2011b).

In the present investigation, the essential elements (K, Ca, P, S, Cl, Mn, Fe, Cu, Zn, Ni, Br, I) and non-essential or toxic elements (Al, Ti, Si, Rb, Sr, As, Cd, Sn, and Pb) were determined by Energy Dispersive Polarised X-Ray Fluorescence Spectrometry; natural radionuclides (^{238}U, ^{234}U, ^{230}Th, ^{210}Po, ^{232}Th, and ^{228}Th) were determined by alpha spectrometry.

2. Materials and methods

2.1. Samples and sample pretreatment

An elemental and radiological characterization was performed on *C. islandica* pharmaceutical and food supplementation products purchased in local specialty shops in Italy (Table 1). Analyses were carried out on 12 samples: 6 samples (3 comminuted herbal substances; 2 tinctures; 1 syrup) were purchased in herbalist shops and 6 samples (3 infusions and 3 decoctions) were prepared in the laboratory from the 3 samples of comminuted herbal substances.

All samples were analyzed within the effective usage period according to the expiration dates shown on the packages. A sample of 0.1 kg of thallus of *C. islandica* was weighed individually and dried in an oven at 105 °C for 24 h until constant weight was achieved. Samples were then weighed again and homogenized.

2.2. Analytical method

2.2.1. Energy dispersive polarised X-ray fluorescence spectrometry

Elemental determinations were performed by Energy Dispersive Polarised X-Ray Fluorescence Spectrometry (EDPXRF) in samples 1 and 2 (comminuted herbal substance). This technique offers several unique advantages over other analytical methods: this is a simultaneous, reliable, sensitive, quantitative multielemental and non-destructive technique, suitable for routine analysis since it requires minimal sample preparation. Also, the equipment cost is much cheaper than the conventional wavelength XRF techniques. This method has previously been used successfully for the characterization of different complex matrices (Desideri et al., 2016a, Stephens and Calder, 2004). Each source was prepared by mixing the sample with Wachs-C 80004005 Mikropulver, a paraffin wax that helps to reduce the sample to tablet form though pressure. The determinations were made with a Spectro-X-LAB2000 (SN DK 949196) Energy Dispersive Polarised X-Ray Fluorescence Spectrometer (EDPXRF).

The quality of the data was ensured by calibrating the instrument with the following certified reference materials (CRM): MURST-ISS-A1 Marine sediment, GBW07310 Stream sediment, GBW08303 Farmland soil, LGC6138 Soil, SRM 12-3-12 Sludge, STD 12-1-12 Fly ash, BCR CRM 144R Sludge, CCRM LKSD1 Lake sediment, CCRM PACS-2 Marine sediment, NIST SRM 2709 Agricultural soil, NIST SRM 2711 Montana soil, NIST SRM 1633b Fly ash, NIST SRM 1575 Pine needles. The averaged analytical standard errors observed and compared to the reported certified materials were below 10% for As and Cd, and below 7% for the other measured elements (Apitz et al., 2009).

The detection limits, in mg $kg^{-1}{}_{dw,}$ were: 120 for Al, 70 for Si, 6 for Mn, 1 for As, Rb, Cd, Sn, Pb Cu, Zn, Ni, Br, and I.

2.2.2. Alpha spectrometry

The determination of natural radioisotopes was carried out by alpha spectrometry. This radiometric technique consists in measurements of the sources of radionuclides obtained from the solution made from the complete dissolution of the sample (Meli et al., 2016). This technique requires lengthy preparation and source counting; however, it has the advantage of being inexpensive, highly sensitive and specific, while providing information on concentration and isotopic ratios of ^{238}U, ^{235}U, ^{234}U, ^{232}Th, ^{230}Th and ^{228}Th.

The method consists of 3 steps: a) sample dissolution, b) source preparation and c) source counting. A detailed description of the alpha emitter determination in the samples was reported in Desideri et al. (2016b).

Table 1
Sample, herbal preparation and provenience of *Cetraria islandica* (L.) Ach. products.

Sample N.	Herbal preparation	Provenience
1	Comminuted herbal substance	Purchased from herbal shop
2	Comminuted herbal substance	Purchased from herbal shop
3	Comminuted herbal substance	Purchased from herbal shop
4	Tincture (Alcohol, 65%)	Purchased from herbal shop
5	Tincture (Alcohol 60%, thallus, 16%)	Purchased from herbal shop
6	Syrup (thallus, 2.5%)	Purchased from herbal shop
7	Infusion from sample 1	Prepared in laboratory (1 g:125 mL)
8	Decoction from sample 1	Prepared in laboratory (1 g:125 mL)
9	Infusion from sample 2	Prepared in laboratory (1 g:125 mL)
10	Decoction from sample 2	Prepared in laboratory (1 g:125 mL)
11	Infusion from sample 3	Prepared in laboratory (1 g:125 mL)
12	Decoction from sample 3	Prepared in laboratory (1 g:125 mL)

2.3. Infusion and decoction preparation

Infusion and decoction are well-known herbal preparations used for treating diseases and aliments. The infusion and the decoction were prepared in the laboratory following these steps: for the infusion, 1.0 g of comminuted herbal substance was added to 125 mL of distilled water, which had previously been brought to a boil and left to stand for 15′. For the decoction, 1.0 g of comminuted herbal substance was added to 125 mL of distilled water, which was then brought to a boil, and left to stand for 15′.

After filtration, 20 mL of every solution was taken and added of a known activity of ^{209}Po as the internal standard yield. The solutions were evaporated and mineralized with conc. HNO_3 and $H_2O_{2,}$ evaporated to dryness and then treated 3 times with conc. HCl. Finally, the residue was dissolved in 1 M HCl for polonium deposition (Desideri et al., 2016b).

2.4. ^{210}Po annual intake and committed effective dose calculation

The largest part of the dose that the average person receives from ingestion comes from the radionuclides of ^{238}U and ^{232}Th series, particularly ^{210}Po. The ^{210}Po annual intake was calculated by taking into account the ^{210}Po concentration in *C. islandica* products and the ingestion rate of those products. The annual committed effective dose for an individual, as a result of ^{210}Po intake, was calculated using the following formula:

$D_{Po-210} = Q \times C_{Po210} \times I$where D_{Po-210} is the annual committed effective dose for ^{210}Po (μSv y^{-1}), C_{Po-210} is the ^{210}Po concentration (Bq $kg^{-1}{}_{dw}$ or Bq L^{-1}), I is the ingestion rate (kgy^{-1} or Ly^{-1}), and Q is the conversion factor. In this investigation, the annual committed effective dose was calculated using the conversion factors 1.2 μSv Bq^{-1} for adults recommended by UNSCEAR (2000).

2.5. Statistical analyses

As far as radionuclides are concerned, for every sample, the concentrations of all radionuclides with their relevant uncertainties were reported; uncertainties of all measurements were calculated taking into account statistical fluctuations of the peaks and backgrounds, and efficiency calibration. As far as stable elements are concerned, for every sample, the mean concentration was reported with the relevant standard deviation of three repeated measures. Finally, for each radionuclide and for each stable element, the arithmetical mean with one standard deviation (SD) was reported.

3. Results and discussion

3.1. Elemental and radiological characterization

3.1.1. Stable elements

Table 2 shows, the measured concentrations (mg $kg^{-1}{}_{dw}$) of the elements in samples 1 and 2 considered with the relevant standard deviation of repeated measures (3).

With regard to the essential and toxic elements, their concentrations were of the same order of magnitude in samples 1 and 2; sample 1 showed the highest concentrations of the elements. The variation of elemental content between the two samples was mainly attributable to differences in the mineral composition of the substrate in which the plants had been grown. As far as toxic elements are concerned, Al ranged from 2017 to 2903 mg $kg^{-1}{}_{dw}$, Rb and Sr ranged from 6.2 to 6.8 mg $kg^{-1}{}_{dw}$ and from 9.7 to 11 mg $kg^{-1}{}_{dw}$ respectively; Pb ranged from 9.0 to 9.5 mg $kg^{-1}{}_{dw}$, Ti and Si ranged from 70 to 111 mg $kg^{-1}{}_{dw}$ and from 5464 to 8725 mg $kg^{-1}{}_{dw}$ respectively. As, Cd, Sn were always < 1.0 mg $kg^{-1}{}_{dw}$.

The heavy metal contents found in this investigation are consistent with those reported by EMA (EMA, 2014) in the Assessment report on *C. islandica* (As: 0.76 mg kg^{-1}, Pb: 30 mg kg^{-1}, Cd: 0.30 mg kg^{-1}). Heavy metal contamination and trace element composition might be caused not only by external sources (atmospheric source), but these metals may be also released from materials (stainless steel, galvanized steel, and Al) in inappropriately handled tools and equipment during the processing and conservation steps of herb.

3.1.2. Radioactive elements

It is well known that *C. islandica* concentrates natural radionuclides of atmospheric origin, particularly ^{210}Pb and ^{210}Po (EMA, 2014; WHO, 2009); therefore, we expected to observe high levels of these radioisotopes in the collected samples.

Table 3 shows the activity concentrations found for radionuclides of the ^{232}Th and ^{238}U series measured by alpha spectrometry in samples 1–3. Samples 1 and 2 were not markedly different from one another, whereas sample 3 showed activity concentrations of all radionuclides that were lower than those of samples 1 and 2. ^{232}Th and ^{228}Th concentrations ranged from 0.150 to 0.485 and from 0.31 to 1.49 Bq $kg^{-1}{}_{dw}$ respectively (mean value of 0.349 ± 0.176 and 1.07 ± 0.66 Bq $kg^{-1}{}_{dw}$ respectively). ^{238}U and ^{234}U activity concentrations ranged from 0.148 to 0.376 Bq $kg^{-1}{}_{dw}$ (mean value 0.287 ± 0.122 Bq $kg^{-1}{}_{dw}$) and 0.155 to 0.421 Bq $kg^{-1}{}_{dw}$ activity concentration (mean value 0.324 ± 0.147 Bq $kg^{-1}{}_{dw}$) respectively. ^{230}Th concentrations ranged from 0.195 to 0.564 Bq $kg^{-1}{}_{dw}$ (mean value of 0.412 ± 0.193 Bq $kg^{-1}{}_{dw}$). ^{210}Po ranged from 132 to 489 Bq $kg^{-1}{}_{dw}$ (mean value of 350 ± 191 Bq $kg^{-1}{}_{dw}$).

Table 3 shows the ratio between members of the ^{238}U and ^{232}Th series. These ratios were calculated by dividing the mean activity concentrations reported in the same table of the above-mentioned radionuclides. Radionuclides of the ^{238}U series were not in in secular equilibrium: equilibrium breaks were not found between ^{234}U and ^{238}U, ^{230}Th and ^{234}U, but a very high ratio ^{210}Po/^{238}U (mean value: 1219 ± 222) was found due to the accumulation of airborne radionuclides. The radionuclides of ^{232}Th series were not found to be in secular equilibrium: an equilibrium break was found between ^{232}Th and ^{228}Th (^{228}Th/^{232}Th mean value = 3.07 ± 0.71) and was probably due to the accumulation of ^{228}Ra. The samples analyzed show a narrow variation in the ratios between the radionuclides of the ^{232}Th series and those of the ^{238}U series (^{232}Th/^{238}U, ^{228}Th/^{230}Th). These ratios are mainly > 1, indicating a small enrichment of the members of the ^{232}Th series over those of the ^{238}U series.

The activity concentrations found for ^{210}Po in liquid products of *C. islandica* (samples 4–12) are shown in Table 4. The ^{210}Po activity concentrations ranged from 0.195 to 0.274 Bq L^{-1} (mean value: 0.214 ± 0.053 Bq L^{-1}) for products purchased from herbalist shops (tinctures and syrup) and from 0.047 to 0.073 Bq L^{-1} (mean value: 0.060 ± 0.009 Bq L^{-1}) for infusions and decoctions prepared in the laboratory (1 g:125 mL). The ^{210}Po extraction percent in infusions and decoctions are also reported, with values ranging from 1.25 to 3.87%.

Regarding radioactive elements, Table 5 shows a comparison of the concentration ranges of radionuclides in *C. islandica* (Bq $kg^{-1}{}_{dw}$) found by the authors with the reference values and concentration ranges for leafy vegetables reported by UNSCEAR (2000) in the world (in Bq $kg^{-1}{}_{dw}$).The concentration ranges of ^{238}U, ^{230}Th, ^{232}Th and ^{228}Th found in *C. islandica* were in line with those reported by UNSCEAR (2000) for leafy vegetables. The ^{210}Po concentration range for *C. islandica* (132–489 Bq $kg^{-1}{}_{dw}$) was much higher than those reported by UNSCEAR (2000) for leafy vegetables both in the world (0.04–74.0 Bq $kg^{-1}{}_{dw}$) and as a reference value (1.00 Bq $kg^{-1}{}_{dw}$).

Table 2
Concentration (mg $kg^{-1}{}_{dw}$) of essential (major and trace) and non-essential or toxic elements in *Cetraria islandica* (L.) Ach. comminuted herb (samples 1 and 2).

Element	Sample 1	±	Sample 2	±
Major and trace				
K	2245	225	1938	181
Ca	16,557	828	17,823	1342
P	450	45	834	89
S	357	36	228	30
Fe	833	58	521	22
Cl	182	18	154	12
Mn	28	2	19	1
Ni	2.2	0.2	1.7	0.3
Cu	4.9	0.3	3.7	0.4
Zn	32.2	2.3	30.2	1.1
Br	1.7	0.2	1.1	0.1
I	3.6	0.4	4.5	0.8
Non-essential or toxic				
Al	2903	290	2017	191
As	<1		<1	
Rb	6.8	0.7	6.2	1.4
Sr	11.0	1.1	9.7	1.8
Cd	<1		<1	
Sn	<1		<1	
Pb	9.5	0.7	9.0	0.7
Ti	111	8	70	9
Si	8725	611	5464	633

Table 3
Activity concentration (Bq $kg^{-1}{}_{dw}$) with relevant uncertainty of natural radionuclides of the ^{238}U and ^{232}Th series in *Cetraria islandica* (L.) Ach. comminuted herb (samples 1–3) determined by alpha spectrometry, radionuclide ratios, mean and standard deviation (SD).

Radionuclide	Sample 1	±	Sample 2	±	Sample 3	±	Mean	±SD
^{238}U	0.376	0.056	0.338	0.051	0.148	0.022	0.287	0.122
^{234}U	0.421	0.065	0.397	0.059	0.155	0.023	0.324	0.147
^{230}Th	0.564	0.090	0.477	0.071	0.195	0.029	0.412	0.193
^{210}Po	489	49	429	43	132	15	350	191
^{232}Th	0.485	0.073	0.411	0.061	0.150	0.023	0.349	0.176
^{228}Th	1.49	0.223	1.41	0.211	0.31	0.046	1.07	0.66
^{234}U/^{238}U	1.12		1.17		1.04		1.13	0.06
^{230}Th/^{234}U	1.34		1.20		1.26		1.27	0.07
^{210}Po/^{238}U	1300		1269		892		1219	222
^{228}Th/^{232}Th	3.07		3.43		2.07		3.07	0.71
^{232}Th/^{238}U	1.29		1.21		1.01		1.22	0.14
^{232}Th/^{230}Th	2.64		2.95		1.59		2.60	0.71

Table 4
Activity concentration with relevant uncertainty (Bq L^{-1}) of ^{210}Po in *Cetraria islandica* liquid products (samples 4–12), mean and standard deviation (SD), extraction percentage (%).

Sample (N)	Activity	±	Extraction
Tincture (4)	0.195	0.029	
Tincture (5)	0.173	0.026	
Syrup (6)	0.274	0.041	
Mean ± SD	*0.214*	*0.053*	
Infusion from sample 1 (7)	0.064	0.012	1.58
Decoction from sample 1 (8)	0.073	0.013	1.45
Infusion from sample 2 (9)	0.047	0.008	2.00
Decoction from sample 2 (10)	0.054	0.010	1.25
Infusion from sample 3 (11)	0.057	0.010	3.87
Decoction from sample 3 (12)	0.065	0.012	2.57
Mean ± SD	*0.060*	*0.009*	*2.12*

Table 5
Comparison of the radionuclide concentration ranges in *Cetraria islandica* (Bq $kg^{-1}{}_{dw}$) (by authors) with the reference values and concentration ranges for leafy vegetables in the world reported by UNSCEAR (2000) in Bq $kg^{-1}{}_{dw}$.

Radionuclide	*Cetraria islandica* (L.) Ach.	Leafy vegetables	Leafy vegetables
	Concentration range (Bq $kg^{-1}{}_{dw}$) by authors	Reference value (Bq $kg^{-1}{}_{dw}$) by UNSCEAR	Concentration range in the world (Bq $kg^{-1}{}_{dw}$) by UNSCEAR
^{232}Th	0.15–0.48	0.15	0.04–0.23
^{228}Th	0.31–1.49	0.15	–
^{238}U	0.15–0.38	0.20	0.06–22
^{230}Th	0.19–0.56	0.20	0.06–3.8
^{210}Po	132–489	1.00	0.04–74

This difference is due to lichens' accumulation of airborne natural radionuclides such as ^{210}Pb and ^{210}Po.

3.2. Health benefits from Iceland moss consumption

In order to assess the potential health benefits of the essential elements found in Iceland moss, namely K, Ca, Cl and P (major) and Cu, Zn, Mn, Fe and I (trace), it was essential to know the amount of the herb that is consumed. In our investigation, we considered the average daily consumption of 4–6 g (dry weight) of the herb, as indicated by WHO (2009). Table 6 shows the mean concentration of several essential elements, daily intake (mg day^{-1}) from a 4–6 g daily herb consumption and a comparison with recommended nutrient intakes. The Population Reference Intake (PRI) and Adequate Intake (AI) (EFSA, 2006) were used in this study to evaluate benefits for consumers.

Regarding the major elements, a daily herb consumption of 4–6 g resulted in a daily intake of K and Cl (calculated from the mean concentrations) of 8.36–12.5 and 0.67–1.01 mg respectively, which represents 0.21–0.32, 0.029–0.043% of AI respectively. A daily intake of 2.57–3.85 and 68.8–103 mg was calculated for P and Ca respectively, which represents 0.367–0.551 and 7.0–10% of PRI.

Among trace elements, a daily herb consumption of 4–6 g accounted for a daily intake (calculated from the mean concentrations) of 2.71–4.06, 0.125–0.187, 0.017–0.026, and 0.094–0.141 mg of Fe, Zn, Cu and Mn, respectively which represents 27.1–40.6, 1.04–1.16, 1.89–2.83 and 3.48–5.22%, respectively of AI (Cu) or of PRI (Fe, Zn and Mn).

Table 6
Element mean concentration (mg kg^{-1}) in samples 1 and 2, its daily dose (mg day^{-1}) from a consumption of 4–6 g herb in divided doses (WHO, 2009) and comparison with risk estimators (PTWI = provisional tolerable weekly intake, PTMI = provisional tolerable monthly intake; UL = tolerable upper intake level) for a 70 kg adult (EFSA, 2006; JECFA, 2006, WHO, 2011a, 2011b) and with recommended nutrient intakes (PRI = population reference intake; AI = adequate intake) (EFSA, 2006).

Element	Mean concentration	Daily dose for 4–6 g consumption	Daily dose from risk estimators	Daily recommended nutrient intakes
Al	2460	9.84–14.8	10 (from PTWI)	
Total As	<1	0.004–0.006	0.15 (inorganic) (from PTWI)	
Pb	9.25	0.037–0.055	0.250 (from PTWI)	
Cd	<1	0.004–0.006	0.058 (from PTMI)	
Zn	31.2	0.125–0.187	25 (from UL)	12 (PRI)
Cu	4.3	0.017–0.026	5 (from UL)	0.9 (AI)
I	4.05	0.016–0.024	0.6 (from UL)	0.15 (AI)
Fe	677	2.71–4.06		10 (PRI)
Mn	23.5	0.094–0.141		2.7 (PRI)
K	2091	8.36–12.5		3900 (AI)
Ca	17,190	70–100	2500 (from UL)	1000 (PRI)
P	642	2.57–3.85		700 (PRI)
Cl	168	0.67–1.01		2300 (AI)

3.3. Potential health hazards resulting from consumption of C. islandica products

3.3.1. Stable elements

Lichens are known to naturally contain bitter and potentially toxic acids, as well as concentrated heavy metals that are taken up from the environment, particularly from the atmosphere, as widely reported in the literature. In April 2009, EFSA published on its website a Compendium of botanicals reported to contain naturally occurring substances of possible concern for human health when used in food and food supplements (EFSA, 2012) and *C. islandica* is on the list. Nevertheless, the medicinal use of Iceland moss has been documented in the monographs of many national and European pharmacopoeias and by WHO, when the lead content is limited to 10.0 mg kg^{-1}(European Directorate for the Quality of Medicines and HealthCare, 2013; WHO, 2007, 2009). In Italy, Iceland moss is also included in the list of herbal preparations allowed in food supplements (Italian Ministry of Health, 2012). The limit of lead indicated for food supplements is 3 mg kg^{-1} (EC, 2008). Lead was present in the samples of *C. islandica* investigated in the present study (samples 1 and 2); its content was always <10 mg kg^{-1}, the limit for medicinal herbs, but >3 mg kg^{-1}, the limit for food supplements. In the samples investigated, the Cd level was lower than the limit (1.0 mg kg^{-1}) indicated by European legislation for cadmium in food supplements (EC, 2008). The maximum limit of cadmium (0.3 mg kg^{-1}) recommended by WHO (2007) for herbal medicines is lower than the detection limit (1.0 mg kg^{-1}) for this element in the present investigation; therefore, pre-concentration techniques will be applied in future studies to reach a lower detection limit. In any case, high contamination levels, if present, would have been detected with our measurements. WHO has not established recommended limits for As, but this element was always <1 mg kg^{-1}.

In this study, the Provisional Tolerable Weekly or Monthly Intakes (PTWI and PTWM) recommended by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2006; WHO, 2011a, 2011b) for an average adult body weight of 70 kg and the Tolerable Upper Intake Level (UL) recommended by European Food Safety Authority (EFSA, 2006) were used for the elements analyzed in our investigation to determine any potential toxicity risks to consumers from *C. islandica* products.

The Joint FAO/WHO Expert Committee on Food Additives has designated a PTWI of 70 mg (10 mg day^{-1}, 1 mg kg^{-1} body weight per week) for Al from all sources (JECFA, 2006). For inorganic As, a PTWI of 1.05 mg (0.15 mg day^{-1}, 0.015 mg kg^{-1} body weight per week) is indicated (WHO, 2011a). For Cd from all sources, the Joint FAO/WHO Expert Committee on Food Additives has established a PTMI of 1.75 mg (0.058 mg day^{-1}, 0.025 mg kg^{-1} body weight per month) (WHO, 2011b), and for Pb, a PTWI of 1.75 mg (0.25 mg day^{-1}, 0.025 mg kg^{-1} body weight per week). The European Food Safety Authority (EFSA, 2006) has designated a UL of 2500, 25, 5 and 0.6 mg day^{-1} for Ca, Zn, Cu and I, respectively.

Table 6 shows the mean concentration of every element in *C. islandica* comminuted herb (samples 1 and 2), the daily dose for a consumption of 4–6 g of herb per day in divided doses (WHO, 2009) and a comparison with the risk estimators.

For toxic elements, a 4–6 g daily consumption of *C. islandica* with a Pb mean concentration of 9.25 mg $kg^{-1}{}_{dw}$ corresponds to a daily intake of 0.037–0.055 mg. This intake only represents approximately 14.8–22.2% of the tolerable daily intake (0.250 mg) for Pb.

For Cd, considering a content that does not exceed the detection limit (1 mg $kg^{-1}{}_{dw}$), a 4–6 g daily consumption corresponds to a daily intake of 0.004–0.006 mg. This intake represents approximately 6.7–10.5% of the tolerable daily intake for Cd. A 4–6 g daily consumption of *C. islandica* with an Al mean concentration of 2460 mg $kg^{-1}{}_{dw}$ corresponds to a daily intake of 9.84–14.8 mg. This intake alone represents approximately 98.4–148% of the tolerable daily dose for Al. For As, considering a content that does not exceed the detection limit (1 mg $kg^{-1}{}_{dw}$), a 4–6 g daily consumption corresponds to a daily intake

Table 7
Daily dosage, ^{210}Po daily and annual intake (Bq day^{-1} and Bq y^{-1}) from consumption of *Cetraria islandica* products (over 300 days per year) and relevant committed effective dose (μ Svy^{-1}) for adults; comparison with annual intake and committed effective dose (μ Svy^{-1}) for adults from diet reported by UNSCEAR (2000).

Sample (N)	Daily dosage (g or mL)	Daily intake (Bq day^{-1})	Annual intake (Bq y^{-1})	Committed dose from lichen (μ Svy^{-1})
Comminuted herb substance	g			
Sample 1	4–6	1.96–2.93	714–1071	857–1285
Sample 2	4–6	1.72–2.57	628–942	753–1130
Sample 3	4–6	0.53–0.79	193–289	232–347
Mean		*1.40–2.10*	*511–766*	*613–920*
Liquid products	mL			
Tincture (4)	30	0.006	1.75	2.10
Tincture (5)	30	0.005	1.56	1.87
Syrup (6)	40	0.011	3.29	3.95
Infusion from sample 1 (7)	250	0.016	4.80	5.76
Decoction from sample 1 (8)	250	0.018	5.47	6.56
Infusion from sample 2 (9)	250	0.012	3.52	4.22
Decoction from sample 2 (10)	250	0.013	4.05	4.94
Infusion from sample 3 (11)	250	0.014	4.27	5.12
Decoction from sample 3(12)	250	0.016	4.87	5.84
Mean		*0.012*	*3.73*	*4.48*
From diet (UNSCEAR, 2000)			*58*	*70*

of 0.004–0.006 mg. This intake represents approximately 2.7–4.0% of the tolerable daily intake for inorganic arsenic. As far as arsenic is concerned, it was assumed that the species of metal found in the herb was inorganic As. It should be noted that the As that was measured was the total As and not inorganic As; therefore, the estimates shown are overestimated. In order to assess the real risk of As-mediated toxicity, chemical composition and biochemical properties of the individual As species must be considered.

With regard to the essential elements, the comparison between their intake and the relevant UL reported by EFSA (EFSA, 2006) shows that a consumption of 4–6 g per day of *C. islandica* herb with a mean Ca level 17.2 mg kg^{-1}$_{dw}$ corresponds to a daily intake of 0.07–0.10 g. This intake represents approximately 2.7–4.1% of the tolerable daily Ca intake (2.5 g). A 4–6 g daily consumption of *C. islandica* with a Cu mean concentration of 4.3 mg kg^{-1}$_{dw}$ corresponds to a daily intake of 0.017–0.026 mg, which represents approximately 0.34–0.50% of the tolerable daily Cu intake (5 mg). A 4–6 g daily consumption of *C. islandica* with a Zn mean concentration of 31.2 mg kg^{-1}$_{dw}$ corresponds to a daily intake of 0.12–0.19 mg. This intake represents approximately 0.5–0.7% of the tolerable daily Zn intake (25 mg). A 4–6 g daily consumption of *C. islandica* with an iodine mean concentration of 4.05 mg kg^{-1}$_{dw}$ corresponds to a daily intake of 0.016–0.024 mg. This intake represents approximately 2.7–4.0% of the tolerable daily I dose (0.6 mg).

3.4. Radionuclide intake and radiation dose estimation

Table 7 shows daily dosage reported by WHO (2009) in the Monograph and ^{210}Po daily and annual intake from products of *C. islandica* (comminuted herb, tincture, syrup, infusion and decoction).The mean ^{210}Po annual intake from liquid product (samples 4–12) ingestion was 3.73 ± 1.36 Bq y^{-1}. ^{210}Po annual intake did not exceed 58 Bq y^{-1}, the annual intake from diet (milk, meat, fish and grain products, fruits, root and leafy vegetables, drinking water) in the world reported by UNSCEAR (2000); however, ^{210}Po annual intake from liquid *C. islandica* products did represent a significant fraction (about 6%) of annual intake from diet (UNSCEAR, 2000). The ^{210}Po annual intake range from comminuted herb (samples 1–3) ingestion was 511–766 Bq y^{-1}. These values were much higher than the annual intake from diet reported by UNSCEAR (2000). Table 7 shows the annual committed effective dose for an individual resulting from radionuclide intake from ingestion of comminuted herb (samples 1–3) and liquid products (samples 4–12) of *C. islandica*: it was calculated using conversion factors of 1.2 μSv Bq^{-1} for adults recommended by UNSCEAR (2000). A comparison with the committed effective dose from ingestion reported by UNSCEAR (UNSCEAR, 2000) is also shown in Table 7. The committed effective dose due to ^{210}Po from ingestion of liquid products accounts for 6.4% of dietary (UNSCEAR, 2000) and 0.19% of natural radiation exposure in Italy. Regarding the samples of comminuted herb, the daily consumption of 4–6 g determines a mean annual committed effective dose of 613–920 μSvy^{-1}, accounting for 25–38% of natural radiation exposure in Italy. These values are about 10 times higher than those reported by UNSCEAR (2000) for the committed effective dose from diet (70 μSvy^{-1}). However, it should be specified that to correctly evaluate the committed effective dose in this investigation, an extremely conservative scenario was selected. In fact, it was based on the continuous consumption of the herb as a food supplement during the year, whereas, the medicinal uses were restricted to limited periods of the year for the treatment of ailments. Moreover, it should also be pointed out that the values shown here are based on single randomly selected commercially available, off-the-shelf samples, and do not necessarily represent all available *C. islandica* products.

4. Conclusions

The contamination of herbal medicines by radionuclides and toxic elements such as heavy metals could pose serious health risks to consumers due to the growing popularity of alternative medicine. The samples of *C. islandica* products investigated in this study were not completely devoid of contaminants; however, the consumption of these herbal medicines determines a daily intake of heavy metals that is well below the tolerable dose, while this is not the case for ^{210}Po. These findings highlight the need for the monitoring of heavy metal and ^{210}Po levels in medicinal plants to guarantee the safety and proper quality of these popular herbal remedies. Indeed, the continuous monitoring of radioactive and stable contaminants in *C. islandica* products, can ensure that their contents do not exceed recommended levels, thus safeguarding the health of consumers.

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