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DETERMINATION OF NATURAL AND ANTHROPOGENIC RADIOACTIVITY IN MUSHROOMS: BIOCONCENTRATION AND DOSE ASSESSMENT

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Abstract. This paper presents studies on natural and anthropogenic radioactivity determination in different mushroom species (*Boletus Aereus*, *Boletus Edulis*, *Boletus Aestivalis*, *Agaricus Arvensis*, *Cantharellus Lutescens*, *Cantharellus Cibarius*, *Leccinum Quercinum*, *Macrolepiota Konradi*, *Lactarius Deliciosus*) collected in Calabria, southern Italy, aimed at determining ^{137}Cs and ^{40}K content in analyzed samples, evaluating the bioconcentration levels and estimating the annual effective dose for ingestion to mushrooms consumers. For all investigated samples, experimental radioactivity results, obtained through High-Purity Germanium (HPGe) gamma spectrometry, were found to be much lower than the specific activity limit set by the international legislation. Information about radionuclides bioaccumulation were given through the calculation of the bioconcentration factor. The effective dose due to the ingestion of mushrooms by adult members of the population was found to be much lower than the recommended level for the public (1 mSv y^{-1}). Results reported in this paper can provide useful information on the environmental risk and can be further used for a radiological mapping of the studied area.

1. Introduction

Natural radioactive elements are present in very low concentrations in Earth's crust (Caridi *et al.* 2016a). Human activities, such as oil and gas exploration, mining, or coal burning in power plants, can enhance their content (Persson and Holm 2011). Technologically Enhanced Naturally Occurring Radioactive Materials (TENORM) consist of materials, usually industrial wastes or by-products enriched with radioactive elements found in the environment, such as uranium (U), thorium (Th), and potassium (K) and any of their decay products (Caridi *et al.* 2017c). Also, the accident at the Chernobyl nuclear power plant (April 1986), and the following radioactive contamination of most of the European territory, initiated extensive research on the environment, including mushrooms (Heinrich 1992).

Mushrooms are a complementary foodstuff, widely consumed in Italy as a delicacy. They typically grow in forests and fields, but almost all ecosystems will favor their growth in the correct substrate medium (Kalač 2001, 2012). The fruiting bodies of mushrooms are generally considered as absorbing mineral constituents, including heavy metals and radionuclides and, thus, their contents can pose a health hazard, mainly in areas heavily contaminated by radioactive fallout (Caridi *et al.* 2017a,b). Mushrooms could then be used as environmental biomonitoring indicators to evaluate the level of the environment contamination, as well as the quality of the ecosystem (Skwarzec and Jakusik 2003).

The analysis of the radioactive content of mushrooms reported in literature was mainly focused, on one side, on radiocaesium, a long-lived anthropogenic radionuclide ($T_{1/2}$ = 30.2 years), chemically analogue of potassium and released into the environment by atmospheric nuclear weapons tests and various accidents involving nuclear materials (Caridi *et al.* 2016d), and, on the other side, on ^{40}K, because mushrooms contain between 1.5 g and 117 g of potassium per kg of dry matter and, thus, its level in many species is considerably higher than that in foods of plant origin (Seeger and Schweinshaut 1981; Júnior *et al.* 2006). Other anthropogenic (^{90}Sr, $^{239+240}$Pu, ^{241}Am, etc.) and natural (uranium and thorium daughter products) radionuclides were less studied, because their content and radiological impact rarely surpass those of ^{137}Cs and ^{40}K (Lux *et al.* 1995; Mietelski *et al.* 2002; Wichterey and Sawallisch 2002).

Based on the aforementioned considerations, the present study was aimed i) at determining ^{137}Cs and ^{40}K content in fruiting bodies of different edible mushroom species (*Boletus Aereus*, *Boletus Edulis*, *Boletus Aestivalis*, *Agaricus Arvensis*, *Cantharellus Lutescens*, *Cantharellus Cibarius*, *Leccinum Quercinum*, *Macrolepiota Konradi*, *Lactarius Deliciosus*) collected in Calabria, southern Italy, ii) at calculating the levels of radionuclides bioconcentration, and iii) at estimating annual effective radiation doses to mushrooms' consumers due to their ingestion, and, thus, the level of their radiotoxicity (Baeza *et al.* 2004).

A preliminary study on the radioactivity in mushrooms was already carried out and published in (Caridi and Belmusto 2017). In the present article, the study was deepened i) by expanding the number of sampling sites, in order to have a more complete mapping of the environmental risk in Calabria; ii) by evaluating other quantities of specific interest such as the bioconcentration factor; iii) by carrying out a statistical analysis by using the Principal Component Analysis (PCA) method and, finally, iv) by taking into account, even in the case of quantities previously measured for some of the investigated samples, the implementation of the instrumentation available for the measurements, consequent to the accreditation of the test by *ACCREDIA: L'Ente Italiano di Accreditamento* (2021).

2. Materials and Methods

2.1. Samples collection. Different species of mushrooms (*Boletus Aereus*, *Boletus Edulis*, *Boletus Aestivalis*, *Agaricus Arvensis*, *Cantharellus Lutescens*, *Cantharellus Cibarius*, *Leccinum Quercinum*, *Macrolepiota Konradi*, *Lactarius Deliciosus*) were collected in nine sampling sites of the Calabria region, south of Italy: site ID1 (GPS coordinates: 39° 07' 53" N, 16° 04' 05" E), ID2 (39° 14' 22" N, 16° 20' 30" E), ID3 (39° 54' 51" N, 16° 35' 09" E), ID4 (39° 02' 34" N, 16° 45' 30" E), ID5 (38° 02' 41" N, 15° 54' 00" E), ID6 (38° 21' 43" N, 16° 14' 24" E), ID7 (38° 18' 50" N, 16° 11' 59" E), ID8 (38° 10' 06" N, 15° 47' 21" E) and ID9 (38° 06' 03" N, 15° 38' 49" E). The map of the sampling points is shown in Fig. 1. In particular, sites ID1, ID2, ID3, ID4 and ID9 are large retails with samples from

FIGURE 1. The map of the sampling points.

the surrounding hinterland, sites ID5, ID6, ID7 and ID8 are sampling sites in the Italian National Park of Aspromonte (Cirrincione 2012).

2.2. High-Purity Germanium (HPGe) gamma spectrometry analysis. All mushroom samples, after the removal of plant and soil using plastic knife, were dried and inserted in Marinelli containers of 1 L capacity for High-Purity Germanium (HPGe) gamma spectrometry analysis. They were counted for 70000 s and spectra analyzed to obtain the activity concentration of ^{137}Cs and ^{40}K through the evaluation of their γ-lines at 661.66 keV and 1460.8 keV, respectively.

The experimental setup was composed by a positive biased Ortec HPGe detector (GEM) and integrated digital electronics, with full width at half maximum (FWHM) of 1.85 keV, peak to Compton ratio of 64:1 and relative efficiency of 40 % at 1.33 MeV (^{60}Co). The detector was shielded from environmental background, by using lead shields with copper and tin lining. Efficiency and energy calibrations were performed using Eckert and Zigler Nuclitec GmgH traceable multinuclide radioactive standards, number AK 5901, covering the energy range 59.54 keV-1836 keV, customized to reproduce the exact geometries of samples in a water-equivalent epoxy resin matrix.

The Gamma Vision (Ortec) software was used for data acquisition and analysis (Caridi *et al.* 2016c). The activity concentration of ^{137}Cs and ^{40}K was calculated using the following formula (Caridi *et al.* 2016b):

$$C = \frac{N_E}{\varepsilon_E t \gamma_d M} \tag{1}$$

where N_E indicates the net area of the peak at energy E = 661.66 keV and E = 1460.8 keV for ^{137}Cs and ^{40}K, respectively; ε_E and γ_d are the efficiency and yield of the photopeak at these energy values, respectively; M is the dry mass (d. m.) sample (kg) and t is the live time of the apparatus (s). The quality of the gamma spectrometry experimental results was certified by ACCREDIA (Caridi *et al.* 2020b).

2.3. Bioconcentration factor. The simplified evaluation of soil-to-mushroom (via mycelium) radionuclides accumulation level provides the bioconcentration factor (BCF) (Olszewski *et al.* 2016):

$$BCF\frac{\text{whole mushroom}}{soil} = \frac{\text{Radionuclide activity concentration}_{\text{whole mushroom}}\,(Bq \times Kg^{-1})}{\text{Radionuclide activity concentration}_{soil}\,(Bq \times Kg^{-1})} \tag{2}$$

2.4. The total annual effective dose. The possible radiological risk for human health is expressed by the total annual effective dose E (Sv y^{-1}) due to the mushrooms ingestion, according to Malinowska *et al.* (2006):

$$E(Sv \times y^{-1}) = Y \times C \times d_k \tag{3}$$

where Y = annual intake of mushrooms (kg per person), C = activity concentration (Bq Kg^{-1} d. m.) and d_k = dose coefficient (conversion factor), defined as the dose received by an adult per unit intake of radioactivity, equal to 1.3x10^{-8} Sv Bq^{-1} and 6.2x10^{-9} Sv Bq^{-1} for ^{137}Cs and ^{40}K, respectively ("Decreto Legislativo 31 luglio 2020, n. 101" 2020).

2.5. Statistical analysis. The XLSTAT statistical software for Windows was used for all statistical calculations (*XLSTAT* 2021) . The statistical analysis was conducted starting from multivariate matrix, where variables were the ^{137}Cs and ^{40}K activity concentrations, BCFs, and the effective dose, while the cases were the sampling sites. Mushrooms species were treated as supplementary variables. With the aim of individuating the presence of the relationships among the original variables, an exploratory method (PCA) was performed. The PCA elaboration ensures the reduction of the data dimensionality, whereas the combinations of variables identified by the principal components (PCs) provide the greatest contribution to sample variability. Before the elaboration, the appropriateness of data set was checked by the Kaiser–Meyer–Olkin (KMO) test and the Bartlett test (sphericity test) (Caridi *et al.* 2020a).

3. Results and Discussion

3.1. ^{137}Cs and ^{40}K activity concentration. The specific activity of ^{137}Cs and ^{40}K, for mushrooms collected in all the investigated sampling sites, is reported in Table 1 as mean value ± standard deviation. Several differences can be noted. The highest ^{137}Cs content was found in *Agaricus Arvensis* sample, (73 ± 5) Bq Kg^{-1} d. m., from the sampling site ID5, while in the *Boletus Aereus*, *Agaricus Arvensis*, *Leccinum Quercinum* (site ID6), *Agaricus Arvensis*, *Macrolepiota Konradi* (site ID7), *Boletus Edulis* (site ID8) and *Agaricus Arvensis* (site ID9) the radiocaesium activity concentration was lower than the minimum detectable activity.

Site ID	Species	Specific activity	
		^{137}**Cs**	^{40}**K**
1	*Boletus Aereus*	1.3 ± 0.1	71 ± 3
2	*Cantharellus Cibarius*	0.9 ± 0.4	157 ± 12
3	*Boletus Edulis*	12 ± 1	91 ± 3
4	*Boletus Aereus*	16 ± 2	30 ± 6
	Boletus Aestivalis	12 ± 1	58 ± 5
5	*Boletus Aereus*	24 ± 2	140 ± 11
	Agaricus Arvensis	73 ± 5	66 ± 6
6	*Boletus Aereus*	<0.6	150 ± 12
	Agaricus Arvensis	<0.8	98 ± 8
	Leccinum Quercinum	<0.8	74 ± 7
7	*Agaricus Arvensis*	<0.7	29 ± 19
	Cantharellus Lutescens	2.3 ± 0.4	122 ± 10
	Macrolepiota Konradi	<0.9	130 ± 13
	Lactarius Deliciosus	6 ± 1	93 ± 12
8	*Boletus Edulis*	<0.5	47 ± 11
9	*Agaricus Arvensis*	<0.5	109 ± 9

TABLE 1. ^{137}Cs and ^{40}K specific activities (Bq Kg^{-1} d. m., mean value ± standard deviation) in the investigated samples.

Worth of note, as can be seen from an inspection of Table 1, a different specific activity of ^{137}Cs was measured for the same mushroom species coming from different sampling sites. As a matter of fact, several factors can affect the ^{137}Cs content in mushrooms. First, the radionuclide quantity deposited onto soil is closely related to the range of the contents, especially to the maximum content (United Nations Scientific Committee on the Effects of Atomic Radiation 2000). The Chernobyl fallout was inhomogeneous all over the countries affected and, therefore, areas with different radioactive contamination can be found within a given country (Mietelski *et al.* 1996). In addition, considering a specific sampling site, the ^{137}Cs content can also vary from one species of mushroom to another, according to the type of nutritional mechanism and the habitat of the mycelium (Yoshida and Muramatsu 1994).

Regarding to the radioisotope ^{40}K (0.012 % of natural potassium), the highest content was found in *Cantharellus Cibarius*, (157 ± 12) Bq Kg^{-1} d. m. in the sampling site ID2, while the *Agaricus Arvensis* in the sampling site ID7 had the lowest specific activity, (29 ± 19) Bq Kg^{-1} d. m. As potassium is an essential nutrient, its range of variation is limited. Although caesium is a chemical analogue of potassium, no correlation between ^{137}Cs and ^{40}K was found, suggesting different uptake mechanisms for these two elements (Mietelski *et al.* 1994). All these experimental results, shown in the 3D-plot of Fig. 2, put into evidence a very low contamination level, compared to the specific activity limit set by the international legislation at 600 Bq Kg^{-1} for ^{137}Cs and at 1258 Bq Kg^{-1} for ^{40}K, respectively ("Council Regulation (EC) No 733/2008" 2008).

3.2. Bioconcentration and dose assessment. The experimental results of ^{137}Cs and ^{40}K activity concentrations in mushrooms, together with those referred to the sampling sites

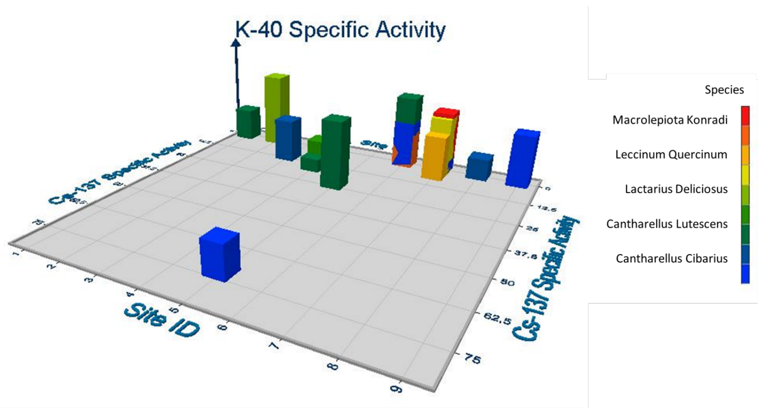

FIGURE 2. Mean concentration 3D map of ^{137}Cs and ^{40}K into the investigated sites.

topsoil, allowed for calculating the bioconcentration factors (BCFs) (Table 2). The evalua-

Site ID	**Species**	**BCF**	
		^{137}Cs	**^{40}K**
1	*Boletus Aereus*	-	-
2	*Cantharellus Cibarius*	-	-
3	*Boletus Edulis*	-	-
4	*Boletus Aereus*	-	-
	Boletus Aestivalis	-	-
5	*Boletus Aereus*	0.25	0.37
	Agaricus Arvensis	0.77	0.18
6	*Boletus Aereus*	-	0.17
	Agaricus Arvensis	-	0.11
	Leccinum Quercinum	-	0.08
7	*Agaricus Arvensis*	-	0.06
	Cantharellus Lutescens	0.04	0.27
	Macrolepiota Konradi	-	0.29
	Lactarius Deliciosus	0.1	0.21
8	*Boletus Edulis*	-	0.08
9	*Agaricus Arvensis*	-	-

TABLE 2. The bioconcentration factors (BCF) for ^{137}Cs and ^{40}K in the investigated samples.

tion was carried out only in those cases for which the soil analysis was possible, i.e. for sampling sites in the Italian National Park of Aspromonte, for which the topsoil ^{137}Cs and ^{40}K specific activity values, previously measured (Caridi *et al.* 2017a,b), were (95 ± 7) Bq Kg^{-1} d. m. and (375 ± 40) Bq Kg^{-1} d. m. for the site ID5, (< 0.3) Bq Kg^{-1} d. m. and (891

± 95) Bq Kg^{-1} d. m. for the site ID6, (60 ± 5) Bq Kg^{-1} d. m. and (451 ± 52) Bq Kg^{-1} d. m. for the site ID7, (< 0.4) Bq Kg^{-1} d. m. and (613 ± 81) Bq Kg^{-1} d. m. for the site ID8, respectively.

Soil-to-mushroom BCF gave information about the radionuclides accumulation level. In the case of plants or mushrooms, the BCF value describes their capability to accumulate a specific chemical element starting from its original concentration in topsoil. For all the analyzed mushroom samples, BCF values turned out in the range 0.04-0.77 for ^{137}Cs and 0.06-0.37 for ^{40}K, indicating ^{137}Cs and ^{40}K concentrations lower than their activities in soil.

The highest values of BCF were observed in the site ID5 (0.77 for ^{137}Cs and 0.37 for ^{40}K), the sampling site rich in podsoils characterized by high permeability of ions transfer, high acidity, and low humus content. Transfer of radionuclides from soil to mushrooms depends on soil properties. The amount of organic matter in soil as well as its pH have a strong influence on metals content in mushrooms (Calmon *et al.* 2009; Melgar *et al.* 2009). The results showed that the bioconcentration of ^{137}Cs was a little more effective than ^{40}K. The effective dose was estimated by Eq. (3) very conservatively, assuming the worst scenario: all species of mushroom can be considered as edible, they are eaten raw and the entire radionuclide content in the mushroom can be assimilated by man. The mass of consumed samples was fixed at about 850 g per person per year, for each species (Tagliavini and Tagliavini 2003).

Site ID	**Species**	**Effective dose**
1	*Boletus Aereus*	0.39
2	*Cantharellus Cibarius*	0.84
3	*Boletus Edulis*	0.61
4	*Boletus Aereus*	0.33
	Boletus Aestivalis	0.44
5	*Boletus Aereus*	1.05
	Agaricus Arvensis	1.15
6	*Boletus Aereus*	0.80
	Agaricus Arvensis	0.56
	Leccinum Quercinum	0.39
7	*Agaricus Arvensis*	0.16
	Cantharellus Lutescens	0.67
	Macrolepiota Konradi	0.69
	Lactarius Deliciosus	0.56
8	*Boletus Edulis*	0.26
9	*Agaricus Arvensis*	0.58

TABLE 3. The estimated total annual effective dose (μSv y^{-1}) for the mushrooms ingestion, due to ^{137}Cs and ^{40}K radionuclides.

The estimated total annual effective dose for the mushrooms ingestion, due to ^{137}Cs and ^{40}K radionuclides, is reported in Table 3. It ranges from 0.16 μSv y^{-1} (*Agaricus Arvensis* in site ID7) to 1.15 μSv y^{-1} (*Agaricus Arvensis* in site ID5), much lower than the recommended level for the public (1 mSv y^{-1}) (Eckerman *et al.* 2012). In addition, despite the very conservative criteria used in the foregoing estimations of the dose by ingestion, mushrooms are not eaten raw. They are generally cooked and consumed immediately or preserved. As reported in literature (Aumann *et al.* 1989), some cooking procedures can significantly reduce the radiocaesium content: washing of about 20 %, boiling of 40-87 %, salt of 36-63 %, frying of about 70 %, and finally, pickling of 59-73 %.

3.3. Statistical features. A PCA was conducted to examine the differences in activity concentrations of ^{137}Cs and ^{40}K in the analyzed mushrooms as well as to investigate the intercorrelations between experimental and calculated parameters and sampling sites. PCA data revealed that 88.51 % of information regarding the radionuclides compositional variability for the nine sites could be described by two varifactors (Fig. 3).

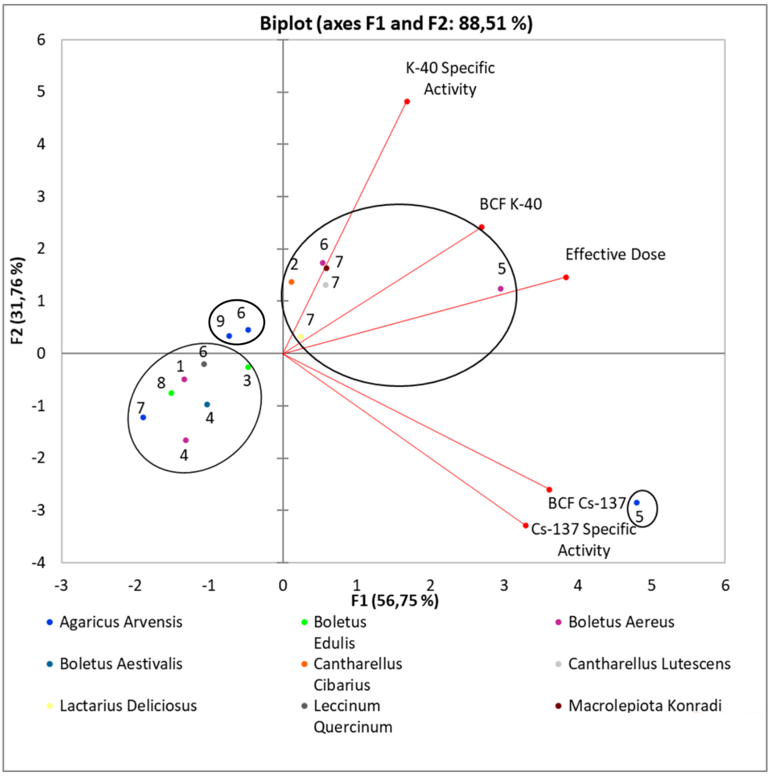

FIGURE 3. 2D plots of the first two PCs obtained through PCA elaboration.

Results of the PCA allow to group the analyzed samples in four clusters, reported in the bi-plot of Fig. 3, that seem to be comparable to each other in terms of ^{137}Cs and ^{40}K activity concentrations and BCFs, and effective dose. Specifically, four groups (labelled from I to IV) were recognized. Group I, containing samples *Cantharellus Cibarius* (site ID2), *Boletus Aereus* (sites ID5 and ID6), *Lactarius Deliciosus* (site ID7), *Cantharellus Lutescens* (site ID7) and *Macrolepiota Konradi* (site ID7), and group IV, containing *Agaricus Arvensis* (site

ID5), refer to samples that turned out to be positively correlated, as far as PC1 is concerned, with ^{137}Cs and ^{40}K activity concentrations, BCFs, and effective dose. Regarding PC2, samples in group I exhibit a positive correlation with values of ^{40}K activity concentration, BCF and effective dose, and a negative correlation with other variables. The opposite occurs for samples in group IV. Going on, group II, containing *Agaricus Arvensis* (sites ID6 and ID9), and group III, containing samples *Agaricus Arvensis* (site ID7), *Boletus Edulis* (sites ID3 and ID8), *Boletus Aereus* (sites ID1 and ID4), *Leccinum Quercinum* (site ID6), and *Boletus Aestivalis* (site ID4), are referred to samples that turned out to be negatively correlated, as far as PC1 is concerned, with ^{137}Cs and ^{40}K activity concentrations, BCFs, and effective dose. Regarding PC2, mushrooms in group II exhibit a positive correlation with values of ^{40}K activity concentration, BCF and effective dose, and a negative correlation with other variables. The opposite occurs for samples in group III. Differences observed between different species of mushrooms can then be due to the different contamination of the selected sampling site, nutritional mechanism, mycelium depth, climate and bioavailability of the radionuclide.

4. Conclusions

This paper shows that investigated mushrooms (*Boletus Aereus*, *Boletus Edulis*, *Boletus Aestivalis*, *Agaricus Arvensis*, *Cantharellus Lutescens*, *Cantharellus Cibarius*, *Leccinum Quercinum*, *Macrolepiota Konradi*, *Lactarius Deliciosus*), coming from different sampling sites of the Calabria region, southern Italy, accumulate ^{137}Cs and ^{40}K at different levels. In particular, after about thirty years since the Chernobyl nuclear accident, the radiocesium specific activity found in the investigated samples is very low compared to the limit set by the international legislation.

To evaluate any possible health risk due to the samples ingestion, the total annual effective dose for adult members of the population was estimated. It was found to be much lower than the recommended level from the ICRP. Data reported in this article provide useful information on the environmental risk of the studied area and can be further used for a radiological mapping.

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