^{137}Cesium in samples of wild-growing *Boletus edulis* Bull. from the Lucca province (Tuscany, Italy) and other Italian and European geographical areas

Laura Betti,[1] Lionella Palego,[2] Antonio Lucacchini,[1] Gino Giannaccini[1]✉

[1]: ⌂*Department of Pharmacy, University of Pisa, Via Bonanno 6, 56126, Pisa, Italy, and: Interdepartmental Center of "Nutraceutical Research and Food for Health", University of Pisa, Italy.*

[2]: *Department of Clinical and Experimental Medicine, University of Pisa, Via Savi 10, 56126, Pisa, Italy.*

Corresponding Author:
✉: Giannaccini Gino, PhD,
Via Bonanno, 6
56126, PISA
ITALY
Ph:+39-050-2219518
e-mail: gino.giannaccini@unipi.it

⌂: The research was conducted at this Department.

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Abstract

This study focussed on the risk assessment for human health in respect to the consumption of the delicious mushroom species *Boletus edulis* Bull. and the content of the artificial radionuclide ^{137}Cs, through the examination of Italian and European samples.

Fresh *B. edulis* carpophores were locally picked-up in 4 distinct green microhabitats of the Lucca province (Tuscany, North-Central Italy), whereas dried non-cultivated samples coming from this same district and 11 other Italian provinces or European countries were purchased from commercial sources. Amounts of ^{137}Cs, reported as Bq kg^{-1} dry-weight (*dw*), were measured by γ-spectrometry.

The radionuclide concentration varied relatively to the gathering site in local samples, resulting 41.8 ± 5.2 Bq kg^{-1}*dw* in carpophores picked up at site 1, Tosco-Emiliani Appennine, and 4-folds lesser, 12.8 ± 1.3 Bq kg^{-1}*dw*, at site 2, Apuan Alps. Moreover, fresh or dried fruiting bodies from the Lucca province displayed among the lowest ^{137}Cs contents in Italy and other European areas. Average ^{137}Cs levels resulted always remarkably below the legal threshold for edible mushrooms, 600 Bq kg^{-1}*dw*, in all analyzed carpophores.

By this investigation, we show that the radionuclide variation in *B. edulis* is related to the distance from Chernobyl accident as well as to multi-factorial features of collection sites. Besides, we report that the consumption of Italian and European *B. edulis* does not represent a major risk for human health in respect to ^{137}Cs radio-contamination.

Keywords: ^{137}Cs, *Boletus edulis*, source, health risk, consumers.

Introduction

Edible mushrooms are consumed since ancient times by humans as a gourmet delicacy owing to their pleasant taste, aroma and unique content of essential microelements, making them exclusive at the nutritional level (Manzi et al, 2001). If the ability at absorbing and/or bioaccumulating trace elements from top-soils gives such dietary attributes, on the other hand, all mushrooms, including edible species, can take up and concentrate soil toxic heavy metals (Kalač and Svoboda, 2000; Zimmermanovà et al., 2001; Falandysz et al., 2002; Kalač et al., 2004; Rudawska and Leski, 2005; Benbrahim et al., 2006; Olumuyiwa et al., 2007; Tuzen et al., 2007; Chudzyński and Falandysz, 2008; Frankowska et al., 2010; Brzostowski et al., 2011; Chudzyński et al., 2011; Giannaccini et al., 2012), as well as long-lived fallout radionuclides (Grueter, 1971, Fraiture et al., 1990; Giovani et al., 1990; Horyna, 1991; Bannai et al., 1997; Skuterud et al., 1997; Kalač, 2001; Druzhinina and Palma-Oliveira, 2004; IAEA, 2006; Masson et al., 2011), presenting therefore a noxious potential for human health. In particular, radionuclides have been detected in edible mushrooms at higher levels than in other foodstuff (Horyna, 1991; Skuterud et al., 1997; IAEA, 2006).

The bioaccumulation of radionuclides, has been recorded throughout Europe following the Chernobyl accident on 26th April 1986 which caused large scale diffusion of radioactivity mostly in the Central and Northern Europe (de Meijer et al., 1988; Battiston et al., 1989; Borio et al., 1991; Heinrich, 1993; Kammerer et al., 1994). Different countries and ecosystems were heterogeneously affected by important amounts of radionuclide contamination. Among radionuclides, the most analyzed in mushrooms is radiocesium and, in particular, the ^{137}Cs chemical species. Basically, there are two reasons for this. The first is related to its environmental impact: ^{137}Cs is a long-lived anthropogenic radionuclide ($T_{1/2}$ = 30.2 years), released into the environment by atmospheric nuclear weapon tests and various accidents involving nuclear materials (UNSCEAR, 2000). The second is that it is a chemical analogue of the essential nutrient potassium, so it can be efficiently taken up and assimilated by living organisms and introduced into the alimentary chain (Chino et al., 2011;Yasunari et al., 2011).

Several factors are supposed to influence results on ^{137}Cs content in mushrooms from European countries affected by the Chernobyl fallout: indeed, beside the inhomogeneous distribution of the radioactive dust all over Europe, and the relative correspondence of the radionuclide contents in carpophores with soil amounts (UNSCEAR, 2000), the decrease of the radionuclide range in macrofungi has been found to differ 1-to-3 orders of magnitude within a same area (Gentili et al., 1991; Mietelski et al., 1994; Mietelski et al., 2010). Moreover, Marzano and co-authors (2001) have reported that, despite more than 10 years had passed from the Chernobyl accident, the concentration of ^{137}Cs inside *Boletus* spp. in Italy and Europe was still highly relevant, a finding also supported by the survey of Giovani and co-authors (2004) who discovered that ^{137}Cs concentration in several mushrooms species, and particularly tree symbionts, did not significantly decrease over the long term. This implies that ^{137}Cs is continuously re-circulated in biological systems for many years following a pulse of contamination and that ecological factors, as climate, habitat and geochemical characteristics of the territory, can differentially affect radio-contaminant levels in mushrooms.

In this study, we measured the ^{137}Cs levels in fruiting body samples of a highly consumed edible mushroom species, *B. edulis* Bull., in respect to collection origins. Indeed, beside being among the most collected macrofungi in Italy, *Boletus* spp. species also display a high affinity for soil Cs (Duff and Rumsey, 2008).

The main objectives of the present work were therefore: i) to first estimate ^{137}Cs content in local *B. edulis* samples in order to assess the radionuclide contamination on the basis of the different microhabitat; ii) to compare ^{137}Cs levels in local *B. edulis* with those measured in samples from commercial sources of various origin. In both cases, the monitoring was conducted to evaluate whether *B. edulis* consumption might pose a health hazard. For these scopes, mushroom samples were harvested in 4 green unpolluted sampling sites, different for soil composition and ecosystem, distant from any human activity and industry, within the Lucca province, Tuscany, North-Central Italy. This district is almost exceptional for the heterogeneity of climates, geology and ecosystems, thus representing a model for the variety of under-woods, tree forests, and fields, depending on the distance from sea levels. To our knowledge, green areas of this province have never been evaluated for the degree of radiocesium contamination in mushrooms growing there. For comparisons, purchased dried samples of wild-growing *B. edulis* originating from this same province (Lucca) and several other Italian districts or European countries were also examined.

Materials and Methods

Mushroom samples and sampling sites

Fruiting bodies of *B. edulis* were collected on Sept-June 2014 in different areas of the Lucca province, Italy. Four different sites, located in green, unpolluted areas well-known for their unique geographical and geological characteristics (Giannaccini et al., 2012), differing in distance or height above sea level, vegetation, or climate, were chosen for the investigation (Table 1). The peculiarity of the Lucca province is mirrored by the site-specific content of trace elements in the fruiting bodies of edible wild-growing mushrooms collected there (Giannaccini et al., 2012). At each site, three sample pools were obtained, consisting into three to six fruit bodies per sample pool. Collection was carried out in agreement with the Italian and regional rules (LR n.16/22 mars 1999, Tuscany Region).

The fruiting bodies of wild-growing, non-cultivated *B. edulis* coming from the Lucca province or from 11 other Italian provinces, Spain and eastern European countries, were instead purchased from local food stores as dried samples. Only confections clearly indicating the country of collection as well as the natural growth of mushrooms were considered for the study. For each country, a pool of *B. edulis* was prepared from 4 dried carpophores for each of 3 confections purchased during the years 2013-2014. Figure 1 reports the European map indicating radio-contamination across Europe in terms of multiples of the normal dose as occurred one week after the accident; the Figure also shows the *B. edulis* collection sites with their main geographical coordinates.

Samples preparation and measurement techniques

Before analysis, freshly collected samples were weighed, carefully cleaned in order to remove soil particles and vegetation residues; after cleaning, fungi were disaggregated, dried at 110°C and weighted to appraise dry weight. Dried purchased samples were instead only cleaned, disaggregated and weighted. Subsequently, all fungi samples, from the fresh or the dried source, were pulverized in a furnace at 600°C for 5 h. The concentration of ^{137}Cs activity was measured in the residue by an automatic γ counter (WIZARD^2, PerkinElmer) using activated thallium and a sodium iodide (NaI) crystal detector (47% efficiency for ^{137}Cs; efficiency=CPM/DPM x 100%, window 15 keV–2000 keV). Calibration of detectors was performed using commercial calibration sources and counting time was preset to obtain a counting statistical uncertainty of better than 5% (2σ) for ^{137}Cs measurements. Sample measurements were always carried out together blanks and calibration standards evaluations. The radionuclide concentrations were reported as Bq kg^{-1} dry weight (*dw*).

Calculation and data analysis

Descriptive and inferential analyses were carried out using the Graph-Pad Prism program (version 5, San Diego, CA, USA). Data are presented as mean ± SD of n=3 mushroom sample pools; ANOVA tests were used for comparisons between mushrooms collected: i) within the Lucca province; ii) within Italian regions; iii) within European countries, followed by the Bonferroni Post-hoc test. The statistical threshold was set up at $P = .05$.

Results and Discussion

Thirty years after the Chernobyl accident the radioelement ^{137}Cs is the most important radionuclide still present in European soils. By this investigation, conducted 28 years after this nuclear fallout, we precisely monitored the presence of the radionuclide ^{137}Cs in *B. edulis* from local green habitats (Lucca province) in comparisons with samples found in the trade from various origins, Italian or from other European countries. As shown in Figure 1, the Lucca province (geographic coordinates: 43.5°N, 10°E) was moderately affected by radiation (zone 2: 1-5 multiples of background levels) 1 week after the Chernobyl disaster (Chernobyl: 51°N, 30° E; zone 6-7: 100^+ multiples of normal rate). Local *Boletus* collected in the Lucca province displayed in fact low radio-caesium concentrations but differed among the different sites, despite the narrow area investigated. In fact, as reported in Table 2, ^{137}Cs values obtained in the Appennines ecosystem were higher than those detected in the same species from other areas of the Lucca province. In particular, the highest ^{137}Cs concentration was detected in mushrooms collected at sites 1 (Tosco-Emiliani Appennines), 41.8 ± 5.2 Bq kg^{-1} *dw*, while the lowest concentration was measured in mushrooms collected at site 2 (Apuan Alps), 12.8 ± 1.3 Bq kg^{-1} *dw* (ANOVA, Bonferroni post-hoc, $P < .001$). Fruiting bodies gathered at sites 1 and 3 (40.5 ± 4.5 Bq kg^{-1} *dw*) resulted similar in their ^{137}Cs composition (ANOVA, Bonferroni post-hoc, $P > .05$), as observed for sites 2 and 4 (17.2 ± 1.8 Bq kg^{-1} *dw*, $P > .05$). On the whole, these data, as previously supposed/observed (Gentili et al., 1991; Mietelski et al., 1994; Mietelski et al., 2010), provide considerations beyond the simple monitoring of ^{137}Cs contents in edible mushrooms: the reported variance of ^{137}Cs distribution in *B. edulis* collected within the Lucca province indicates that factors other than the geographical distance from the Chernobyl accident have influenced the radionuclide permanence in ecosystems. Amongst these, there is the contribution of meteorological factors, rainfalls and precipitations occurring the subsequent days after the Chernobyl accident

(Abraham et al., 2000). Indeed, the Lucca district is defined by microclimates and variable climatology features, implying the uneven distribution of the radiation dose there: samples collected in high-altitude Tuscan-Emilian Apennines and proximity, places usually presenting greater annual rates of precipitations, displayed a higher concentration of ^{137}Cs. Winds after the accident could also have contributed to the inhomogeneous maintain of the radionuclide in ecosystems within this same province (Yamauchi, 2012). Beside climatic factors, ^{137}Cs retention and redistribution in environment can additionally depend on the different soil geological/organic matter composition and/or on the diverse forest density (Walling and He, 1993; Garten et al, 2000; Vilic et al., 2005; Semizhon et al., 2009; Vinichuk et al., 2010; Gaspar and Navas, 2013; Söderlund et al., 2016). The Lucca province is also characterized by various microhabitats and heterogeneous soils, suggesting a differential retain of the radionuclide also on the basis of these parameters. This would explain why limestone hill site 2 shows the lowest radioactivity values, or why, at sandstone site 4, a plateau in Tuscan-Emilian Apennines, the observed radiation levels in *B. edulis* samples were 2-to-4-folds lower than sandstone mountain sites 1 and 3 (see Table 1 and 2). Table 3 reports instead the radiocesium concentrations obtained in dried purchased samples from Italy and other European countries. For the sake of clarity, this Table also shows the geographical coordinates and the diverse zones of radio-contamination, these last obtained from Fig. 1, relative to all sites. The significantly highest value in Italy was obtained in samples from Gorizia, 132.9 ± 14 Bq kg^{-1} *dw* (ANOVA, Bonferroni post-hoc, $P < .001$), a province of Friuli Venezia Giulia, a region located exactly in the Eastern part of Northern Italy (45.9 °N, 13.6° E), on the border with Croatia. The Gorizia province is also positioned inside the zone 3 of radio-contamination (5-10 multiples of the background levels, Fig.1). This value was followed by a province of Veneto, Treviso (zone 2, 1-5 multiples of normal rates, Fig. 1; 45.6°N, 12°E): 88.7 ± 9.3 Bq kg^{-1} *dw*, and by a province of Trentino Alto Adige, Trento (zone 3, Fig.1; 46°N, 11°E): 72.2 ± 6.8 Bq kg^{-1} *dw*, two regions slightly located to the west in respect of Friuli Venezia Giulia, thus more distant from the Ukrainian Chernobyl site. Samples coming from Verona (45°N, 10.6°E), Padova (45.5°N, 11.9°E) and Vicenza (45.5°N, 11.3°E), three provinces situated in close proximity to Treviso (Veneto), all inside the zone 2 of radio-contamination, displayed ^{137}Cs levels comparable to the southwestern Tuscany districts Arezzo and Pistoia, thus supporting what above observed for the Lucca province. Extending the evaluation to *B. edulis* samples from other regions located at further western as Lombardy, or further south as Emilia Romagna and Tuscany, these resulted less interested by the radionuclide contamination. Samples from these areas displayed low values, very similar to each other: Bologna (Emilia Romagna), Arezzo and Pistoia (Tuscany) (lat. 43-44 °N; long. 10.6-11.5° E; zone 2) showed ^{137}Cs levels accounting for by 47-49 Bq kg^{-1} *dw*; samples from Cremona (Lombardy), at a further longitude west (10° E; zone 2), reported the lowest mean levels, 25.6 ± 2.3 Bq kg^{-1} *dw*. Thus, Italian provinces progressively positioned to the south and/or to the west, showed a tendency to intermediate-to-lower ^{137}Cs values inside carpophores. Dried samples from the Lucca province contained the lowest ^{137}Cs values in Tuscany, being quite comparable to the average amount obtained in fresh samples directly collected in the same district. This is an important safety observation, since the Lucca province is frequented by people coming worldwide during summertime and early autumn, and the collection and/or consumption of *B. edulis* is very popular among both local habitants and tourists.

The evaluation of samples of dried *B. edulis* from other European countries confirmed the spread of the radioactive dust over the European continent. The highest value reported in Romania (46°N, 25°E) , 80.1 ± 5.5 Bq kg^{-1} *dw*, is essentially due to the proximity of this central European country to the accident site. Romania is also located within the 3 and 4 zones of radio-contamination (Fig. 1). The lowest concentration was detected in mushrooms from Spain (40°N, 3°W; radio-contamination zone 1), a Western Europe country: 17.5 ± 1.6 Bq kg^{-1} *dw* (ANOVA, Bonferroni post-hoc, $P < .001$); Bulgaria (43°N, 25°E) and Serbia

(44°N, 21°E) reported instead comparable, intermediate values (ANOVA, Bonferroni post-hoc, $P > .05$), 35.7 ± 3.3 vs. 32.9 ± 2.8 Bq kg^{-1} *dw*, respectively. Both countries lies on radio-contamination zone 3 (Fig. 1). Therefore, in a much larger scale (area) than the comparatively very narrow and more diversified Lucca province, European data support that: i) the passage of the cloud or the degree of geographical exposition, ii) the weather/rainfall conditions on the days following the date of April 26th, 1986, and iii) the geochemical composition of soils or habitat features, were all concurrent factors for the distribution and contamination of ^{137}Cs inside the continent.

It is also worth noting that ^{137}Cs contents in fruiting bodies collected in the Italian Lucca province (sites 2-4) were similar to those reported in Spain, both significantly lower than those coming from the other Italian or European regions (ANOVA, Bonferroni post-hoc, $P < .001$). The relevant heterogeneity of ^{137}Cs values in *B. edulis* carpophores in relation to their original geographical area, even with local impact, are in good agreement with those obtained in previous works and other Italian regions (Nonnis-Marzano et al., 2001) or European countries (Kalač, 2001).

Moreover, as a whole, our study did not produce alarming results for mushroom consumers. The harvesting and distribution of foodstuff is regulated by national and international legislation (EC, 2008; Hamada and Ogino, 2012). In EU, the limit of radiocesium was established at 600 Bq kg^{-1} dw for agricultural production (EC, 2008). Thus, we overall reveal a low risk for human health and the appropriateness for a continuous consumption of *B. edulis* in Italy and Europe, which are therefore marketable (Pettenella et al, 2007; Voces et al, 2012).

Our survey also opens up towards future studies on the bioavailability of ^{137}Cs in edible mushrooms, for comparison with other natural trace elements (Falandysz, 2008; Giannaccini et al, 2012). Furthermore, the relatively higher variance of natural trace elements reported in *B. edulis* from the Lucca province (Giannaccini et al, 2012) in respect to that obtained here for the artificial radionuclide, presumably suggests diverse adaptive mechanisms.

To summarize, the significant origin-related differences observed for ^{137}Cs amounts in present samples should be considered to enhance local products, thus favoring those coming from particular provinces/regions than others within a same country, in respect to a multi-factorial survey. Tuscany, and especially the areas named Alta Versilia and Garfagnana in the province of Lucca, showed the lowest local values, underlining the attractiveness, prestige and excellence of the distribution and use of food products coming from this place.

Conclusion

On the basis of present results, it can be concluded that ^{137}Cs contamination in *B. edulis* growing in Europe does not represent a risk for the population health, suggesting a safe consumption of this food. The analysis of samples from the Lucca province, other Italian regions and different European countries provides an useful model for a deeper appraise of many of the influencing factors of the radionuclide accumulation and diffusion in the environment and alimentary chain, such as rainfalls but also other climatic/habitat factors. The low values reported in some parts of the Lucca province, the Alta Versilia and Garfagnana areas, implies the nutritional relevance of *B. edulis* carpophores collected there. It is also important to underline that the potential health hazard due to the consumption of mushrooms is also based on the effective ingestion dose and therefore to the consumption rate of this foodstuff which much depends on the country gastronomic tradition.

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Disclosure Statement

The authors declare no conflict of interest.

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Table 1. Characteristics of mushrooms' sampling sites inside the Lucca province (Tuscany, Italy).

	Site 1	Site 2	Site 3	Site 4
Location	Tosco-Emiliani Appennines	Mountainside of the Apuan Alps	Near Tosco-Emiliani Appennines	Tosco-Emiliani Appennines
Altitude	1200 m	700 m	1200 m	700 m
Oreography	Mountain	Hill	Mountain	Plateau
Lithology	Sedimentary sandstone rocks	Limestone rocks, metamorphic rocks	Sedimentary sandstone rocks	Sedimentary sandstone rocks
Vegetation	*Fagus sylvatica* L., *Picea abies* Karst., *Abies alba* Mill.; under-wood: *Vaccinium myrtillus*	*Castanea sativa* Mill; under-wood: *Ericaceae*, *Vaccinium myrtillus*	*Fagus sylvatica* L., *Picea abies* Karst., *Abies alba* Mill.; under-wood: *Vaccinium myrtillus*	*Castanea sativa* Mill; under-wood: *Ericaceae*, *Vaccinium myrtillus*

Table 2. Concentrations of radiocesium ^{137}Cs in samples of *Boletus edulis* harvested at the four distinct site of the Lucca province (43°N, 10°E), Tuscany, Italy, located inside the radio-contamination zone 2 (Fig. 1).

	Sampling area	^{137}Cs (Bq/kg dw)
Site 1	Appennines	41.8 ± 5.2$^{(***)}$
Site 2	Side of Apuan Alps	12.8 ± 1.3
Site 3	Near Appennines	40.5 ± 4.5
Site 4	Near Appennines	17.2 ± 1.8

Data are the Mean ± SD of n=3 measurements, as indicated in the Material and Methods Section. (***): ANOVA, Bonferroni post hoc test, $P < .001$, significantly higher values at sites 1-3 than at sites 2-4. ANOVA, Bonferroni post hoc test, $P > .05$, comparable values at site 1 vs. site 3 or at site 2 vs. site 4.

Table 3. Concentrations of radiocesium ^{137}Cs in different purchased samples of edible *Boletus edulis* from different countries.

Country	Province	^{137}Cs (Bq/kg dw)	G. coord.	Zone
Italy	Gorizia	132.9 ± 14.0$^{(***)}$	45.9°N, 13.6°E	3
	Treviso	88.7 ± 9.3	45.7°N, 12°E	2
	Trento	72.2 ± 6.8	46°N, 11°E	3
	Bologna	49.5 ± 5.2	44°N, 11° E	2
	Arezzo	48.0 ± 5.1	43°N, 11.5°E	2
	Pistoia	47.9 ± 4.8	43°N, 10.6°E	2
	Verona	43.4 ± 4.2	45°N, 10.6°E	2
	Padova	43.4 ± 4.5	45.5°N, 11.9°E	2
	Vicenza	30.8 ± 3.2	45.5°N, 11.3°E	2
	Cremona	25.6 ± 2.3	45°N, 10°E	2
	Lucca	34.6 ± 3.5	43.5°N, 10°E	2
Romania	unsp.	80.1 ± 5.5	46°N, 25°E	3-4
Serbia	unsp.	35.7 ± 2.8	44.5°N, 20°E	3
Bulgaria	unsp.	32.9 ± 3.3	43°N, 25°E	3
Spain	unsp.	17.5 ± 1.6$^{(§§§)}$	40°N, 3°W	1
Ukraine	Chernobyl	-	51° N, 30°E	6-7

Data are the Mean ± SD of n=3 measurements, as indicated in the Material and Methods Section.

G. coord.: Geografical coordinates- for European countries, a mean value is reported; dose of radio-contamination, as in Fig. 1.

$^{(***)}$: ANOVA, Bonferroni post hoc test, P < .001, significantly higher values in *B. edulis* from Gorizia, Treviso e Trento vs. other Italian collection sites.
$^{(§§§)}$: ANOVA, Bonferroni post hoc test, P < .001, significantly lower values in *B. edulis* from Spain vs. other collection sites in Europe. Unsp.: the province of these countries is unspecified.