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SCIENCE DIRECT®

Science of the Total Environment 359 (2006) 255–266

Science of the Total Environment
An International Journal for Scientific Research into the Environment and its Relationship with Humankind

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Distribution of radionuclides in different parts of a mushroom: Influence of the degree of maturity

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Received 22 August 2004; accepted 9 May 2005
Available online 8 September 2005

Abstract

Mushrooms are known to be bioaccumulators of radionuclides, but little is known about their distribution within the fruiting bodies or the influence of the degree of maturity on uptake. We carried out a series of cultures of the species *Pleurotus eryngii* under controlled laboratory conditions to analyze these variables. The maximal uptake of ^{134}Cs and ^{85}Sr was found to occur in mature fruiting bodies, and with the growth of the mushroom the distribution of radionuclides within the fruiting bodies became inhomogeneous. In particular, there was an exponential increase in the percentage of the total activity of ^{134}Cs, ^{85}Sr, and ^{60}Co in the cap + gills as the fruiting bodies matured, accompanied by a complementary decrease in the stem. Radiocaesium, potassium, calcium, $^{239+240}$Pu, ^{234,238}U, ^{228,230,232}Th, and ^{226}Ra were assayed in the cap, gills, and stem of fruiting bodies of *Tricholoma equestre* collected in a natural ecosystem and cultured *P. eryngii*. Potassium and radiocaesium were mainly located in the cap + gills, and ^{226}Ra in the gills. There was a disequilibrium between ^{230,232}Th and ^{228}Th in the different parts of the fungi, probably due to uptake of ^{228}Ra and subsequent decay to ^{228}Th. Finally, the distribution pattern of $^{239+240}$Pu, ^{234,238}U, and ^{230,232}Th seemed to be species dependent.

Keywords: Fungi; Uptake; Natural radionuclides; Man-made radionuclides; Stable elements

1. Introduction

Mushrooms are known to accumulate radionuclides efficiently. Fruiting bodies collected in areas contaminated by the Chernobyl accident have higher activity levels than other consumption products (Horyna, 1991; Mietelski et al., 1993; Skuterud et al., 1997; Skwarzec and Jakusik, 2003). Usually, radiological studies have considered mushrooms as a whole, but there have been a few papers studying the distribution of radionuclides within the fruiting bodies or the influence of the maturity of the fruiting bodies on radionuclide uptake (Muramatsu et al., 1991; Heinrich, 1993). Indeed, the degree of maturity may be expected to influence radionuclide uptake, because the nutritional requirements of mushrooms,

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doi:10.1016/j.scitotenv.2005.05.015

like other living organisms, change with stage of development.

The objective of the present study is to analyze the distribution of radionuclides of natural and artificial origin within the fruiting bodies, and how their degree of development can affect this distribution. To achieve this objective, we decided to culture fruiting bodies of *Plerurotus eryngii*, as representative of the saprophytic nutritional mechanism, under controlled laboratory conditions. This kind of study was chosen because it allowed us to fix the main variables that condition the development of the fruiting bodies, and hence ensure the reproducibility of the results (Baeza et al., 2000). Moreover, it would be more difficult to analyze the influence of maturity on radionuclide uptake in field studies. We designed and carried out various experiments. In the first, we added known activities of ^{134}Cs and ^{85}Sr to the culture, and the fruiting bodies were harvested at different stages of their development—young, mature, and old. In the next group of experiments, we also added ^{60}Co to the culture, and harvested the fruiting bodies at young and mature stages in order to analyze the uptake of ^{134}Cs, ^{85}Sr, and ^{60}Co, separating them into their major visual parts—(a) cap+gills and stem (Experiment C+G/S), and (b) cap, gills, and stem (Experiment C/G/S). In one culture of Experiment C/G/S, we also added ^{239}Pu, and measured the natural content of potassium, calcium, thorium, uranium, and radium in each part.

Given the low activity levels of man-made radionuclides in general, and of ^{137}Cs in particular, in mushrooms collected in Spain (Arrondo, 1988; Baeza et al., 2004b), and the fact that saprophytic fungi present a lower content of this radionuclide than mycorrhyzal fungi (Guillite et al., 1994; Kammerer et al., 1994; Yoshida and Muramatsu, 1994; Baeza et al., 2004b), we collected fruiting bodies of *Tricholoma equestre* in a seminatural ecosystem—a pinewood located in Muñoveros, a small village in the province of Segovia (Spain), which has a high productivity of mushrooms. This fungus was chosen as representative of a mycorrhizal nutritional mechanism, with a ^{137}Cs content 17 times higher than the typical value for saprophytic fungi collected in Spain (Baeza et al., 2004a). The fruiting bodies were separated into cap, gills, and stem, determining the ^{137}Cs, ^{40}K, $^{239+240}$Pu, potassium, calcium, thorium, uranium, and radium contents in each part, and comparing the results with those obtained under laboratory conditions for the saprophytic fungus, *P. eryngii*. Because the species selected have different nutritional mechanisms, the advantage of this comparison is that if we could obtain equivalent results, these would be independent of the nutritional mechanism.

2. Material and methods

2.1. Mushroom culture under laboratory controlled conditions

Mushrooms of the edible species *P. eryngii* were grown under controlled laboratory conditions, following a procedure explained in detail elsewhere (Baeza et al., 2000). It basically consists of two consecutive phases: (a) spread of the mycelium in the substrate from which it will obtain its nourishment, and (b) transferring the substrate with the mycelium to a soil bed for the subsequent formation of the fruiting bodies. The fruiting bodies were harvested by cutting the stem slightly above soil level so as to have no soil particles adhered to the samples to be analyzed. Known activities of ^{134}Cs, ^{85}Sr, ^{60}Co, and ^{239}Pu (about 1000, 17 000, 5000, and 250 Bq, respectively) were incorporated onto the surface of the substrate/mycelium when the mycelium was in the spreading phase, 10 days after its inoculation into the substrate. ^{134}Cs, ^{85}Sr ^{60}Co were added as chlorides, and ^{239}Pu as nitrate. The radionuclides in previously sterilized solutions were conditioned to neutral pH at the time of their addition to the culture medium, so as not to damage the mycelium's growth (Baeza et al., 2000). The method of incorporation was by uniformly distributing many small droplets over the substrate/mycelium. This incorporation route was designed to simulate the radioactive contamination of an ecosystem, in which man-made radionuclides are normally deposited onto the surface layer of the soil, from where they migrate down to the depth at which the mycelium is located (Baeza et al., 2000).

Three sets of experiments were carried out. First, we carried out an experiment to analyze the uptake of ^{134}Cs and ^{85}Sr at three different stages of development (young, mature, and old) easily identifiable by eye in Fig. 1. In order to make the harvest of the *P. eryngii* systematic within each of the aforementioned three

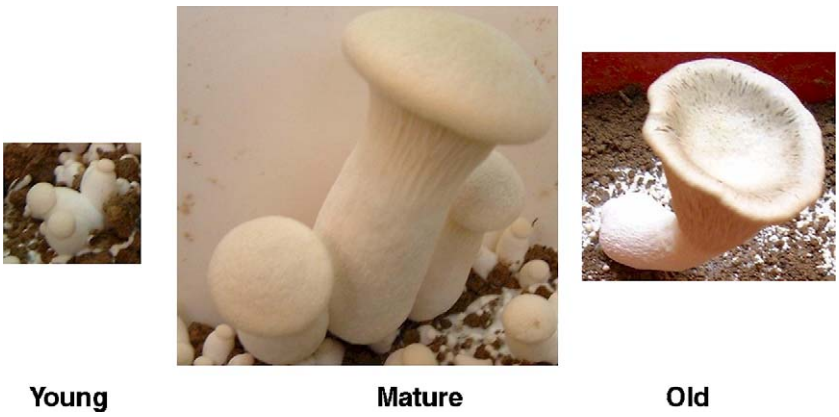

Fig. 1. Different degrees of maturity of fruiting bodies of *P. eryngii*.

developmental stages of the fruiting bodies, we fixed each stage according to the time lapsed after the inoculation of the mycelium, since with our experimental design all the replicates that were produced had very similar rates of development. Mature fruiting bodies were collected 55 days after mycelium inoculation (see Fig. 2), young fruiting bodies were collected 10 days before reaching maturity, and old ones were collected 10 days after reaching maturity. Three replicates were performed for each of these final developmental stages. In the next set of experiments (Experiment C+G/S), we collected the fruiting bodies at different times within the range from young to mature (45 to 55 days after mycelium inoculation), and separated them into cap+gills and stem, given the presumably small mass of the gills. Due to logistics, in 6 replicates we added ^{134}Cs and ^{85}Sr, and in the remaining 13 replicates we also added ^{60}Co. Finally, in Experiment C/G/S we collected the fruiting bodies at the mature stage (55 days after inoculation) and separated them into cap, gills, and stem. In this experiment, we considered 10 replicates.

2.2. Mushrooms collected under field conditions

In order to compare the results of field and laboratory conditions, we collected by cutting the stem slightly above soil level a large amount of visually

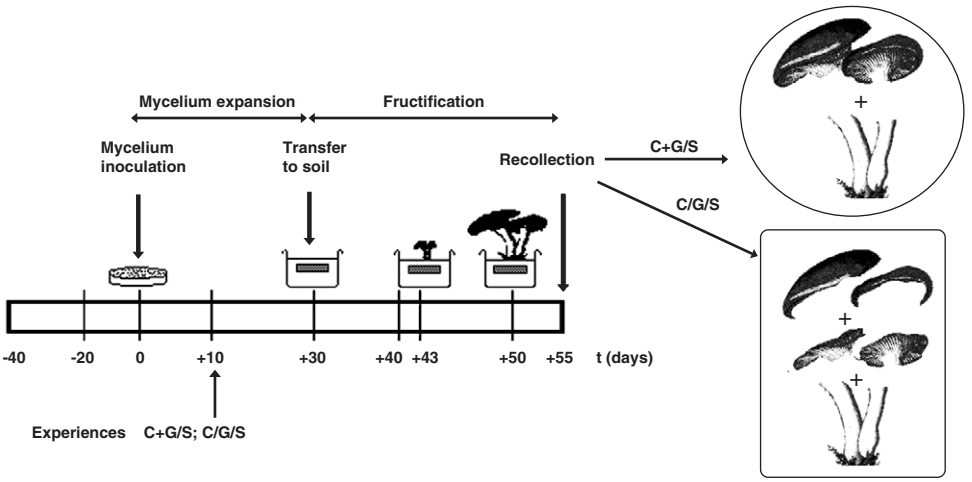

Fig. 2. Chronogram of the culture of *P. eryngii* under controlled laboratory conditions corresponding to experiments C+G/S and C/G/S. Zero time corresponds to the inoculation of mycelium into the substrate.

mature fruiting bodies of *T. equestre* in a pinewood of high mushroom productivity located in Muñoveros, province of Segovia, Spain. The origin of man-made radionuclides in this ecosystem in mainly due to global fallout, being the ^{137}Cs content in the surface layer of the soil 11.7 ± 1.0 Bq/kg d.w. After collection, soil particles were removed from the fruiting bodies by washing with bi-distilled water. They were then separated into cap, stem, and gills, and each part dried separately at 100 °C.

2.3. Radionuclide determination

The mushroom samples were weighed both fresh and after drying at 100 °C. They were then ashed at 400 °C and put into 52 mm diameter, 13 mm deep, Petri dishes for assay. The gamma spectrometric analyses were carried out using an n-type germanium detector with a 25% relative efficiency, a 1.87 keV resolution for the 1332 keV ^{60}Co peak, and a peak-to-Compton ratio of 57.5:1. This detector was coupled to a Compton effect suppressing device (López et al., 1998) which enables a reduction of the background, due largely to the Compton scatter of photons from ^{40}K present in the samples, by a factor of 2 in the 661.6 keV energy region of the ^{137}Cs emission. This device was connected for the assay of ^{85}Sr in *Pleurotus eryngii* and for the γ-spectrometry of the radionuclides systematically assayed in *T. equestre*, and disconnected for the assay of ^{134}Cs and ^{60}Co in the mushrooms grown under controlled laboratory conditions since they are cascade emitters of γ-rays.

The procedure used for the simultaneous extraction of plutonium, thorium, and uranium isotopes from the fungi samples is based on an optimization of various methods proposed by other workers (Dregge and Boden, 1984; Iranzo et al., 1992; LaRosa et al., 1992; Mietelski et al., 2002). It consists of the following phases:

i) Calcining the sample at 600 °C in order to eliminate the organic matter.
ii) Addition of tracers (^{242}Pu, ^{229}Th, and ^{232}U).
iii) Digestion of the ash with HF, HNO_3, and HCl.
iv) Reduction of iron and plutonium by adding hydrazine, so that plutonium passes to Pu(III).
v) Adjustment of the oxidation state of plutonium by adding HNO_3 and $NaNO_2$, so that plutonium passes to Pu(IV).
vi) Separation of Pu, U and Th in a column with Dowex 1×8 anion exchange resin. The sample is passed through in HNO_3 8 M, so that plutonium and thorium are retained but not uranium. Thorium is eluted from the column with concentrated HCl, and then plutonium with a combination of dilute HCl and hydroxylamine.
vii) For the uranium fraction, uranium is co-precipitated with $Fe(OH)_3$. The precipitate is redissolved in HCl 9 M, followed by a separation in a column with Dowex 1×4 resin. In this case, uranium is retained in the column and subsequently eluted by adding HNO_3 8 M.
viii) The alpha sources were prepared by the method of co-precipitation with NdF_3 (Sill, 1987).

The determination of ^{228}Th included a correction for the activity of this radionuclide due to the use of ^{232}U as tracer. The mean chemical yields for the overall process for plutonium, thorium, and uranium were [74 ± 17 (S.D.)], [62 ± 12 (S.D.)], and [37 ± 10 (S.D.)], respectively. The main advantage this procedure presents is that plutonium, thorium, and uranium are extracted from the same matrix. The recovery of uranium is lower than that of plutonium, but it is compensated by the greater concentration of uranium in environmental samples.

The procedure used in the radium extraction is based on that proposed by (Baeza et al., 1998). The sample was acid digested, then ^{133}Ba was added as tracer, and its radium content was subsequently co-precipitated with MnO_2. Then it was dissolved in HNO_3 5 M, and the uranium and thorium present were extracted with TBP (tributylphosphate). Finally, radium was precipitated as $Ba(Ra)SO_4$. The recovery was determined by gamma spectrometry. The alpha spectrometric analyses were carried out using silicon detectors with a mean efficiency of 23.2%, and a resolution of 38.7 keV for a source-detector distance of 6 mm.

We periodically participate in intercomparison exercises sponsored by different national and international bodies. Indeed, some of these have served to establish the reference activity levels for new matrices (Povinec et al., 2002). Nevertheless, we checked the validity of the radiochemical procedure used in the

Table 1
Values of the activity levels, expressed in Bq/kg-dry, determined for ^{137}Cs, $^{239+240}$Pu, ^{234,238}U, and ^{228,230,232}Th in the IAEA 327 soil (IAEA, 2001)

Radionuclide	Measured	Recommended value	95% Confidence interval
^{137}Cs	24.7 ± 2	24.9	24.6–25.2
$^{239+240}$Pu	0.59 ± 0.06	0.58	0.56–0.60
^{234}U	33 ± 3	31.9	30.4–33.4
^{238}U	36 ± 4	32.8	31.4–34.2
^{228}Th	36 ± 8	38.2	37.2–39.2
^{230}Th	33 ± 7	34.1	32.4–35.8
^{232}Th	38 ± 9	38.7	37.2–39.2

Reference date for decay corrections, 31st December 1994.

present study by applying it to an IAEA soil, IAEA 327 (IAEA, 2001), that possesses the activity levels recommended for many of the radionuclides of interest in the present study. In Table 1, we give in a comparative form the activities that we determined and the IAEA recommended values. As one observes, we succeeded in reproducing the activity levels of the said radionuclides very satisfactorily.

2.4. Atomic absorption

During the procedure for the determination of alpha emitters, after the digestion step, the volume of the sample was adjusted to 100 mL, and a 1 mL aliquot was taken to determine the calcium and potassium content. The concentrations of these elements were determined by atomic absorption using a flame spectrophotometer, following international standard methods (ISO, 1993, 1986). The sources used were two hollow cathode lamps, one selective for potassium and the other for calcium. The working wavelengths were 766.5 nm for potassium and 422.7 nm for calcium. The flame was acetylene-air.

3. Results and discussion

3.1. Influence of the degree of maturity

We carried out experiments in which we harvested the fruiting bodies at different stages of their growth—young, mature, and old. These stages are identified visually in Fig. 1. Thus, when the fruiting body is young, the stem is larger than the cap. As it matures, the cap develops so that both parts, cap and stem, are of approximately the same size. Finally, when it ages, the edges of the cap turn up. In these experiments, we only considered the uptake of ^{134}Cs and ^{85}Sr, because they are chemical analogues of potassium and calcium which are essential elements in the development of living organisms. The uptake was quantified in terms of the percentage of the activity initially added to the culture detected in the fruiting bodies of the fungi. The mean values of the uptake and the standard deviations of the three replicates for each degree of maturity considered are given in Table 2. In all three stages considered, ^{134}Cs was taken up preferentially to ^{85}Sr, as had been observed in a previous work on mature fruiting bodies (Baeza et al., 2000). The content of ^{134}Cs was maximal when the fruiting body was mature, i.e., there was an increase in the content as the fruiting body grew to its mature form, and a decrease as it aged to the old form. The latter observation may imply a partial return and translocation of the radionuclides from the fruiting body into the mycelium, as has been observed previously for some nutrients (Boswell et al., 2002).

3.2. Distribution of radionuclides within the fruiting body

3.2.1. Laboratory conditions: ^{85}Sr, ^{134}Cs, and ^{60}Co

As has been shown previously, radionuclide uptake to fungi depends closely on the degree of maturity. The internal distribution of the radionuclides may therefore also be a function of maturity. Table 3 lists for each of the 19 replicates of the cultures of *P. eryngii* the percentages of the total activity of ^{85}Sr, ^{134}Cs, and ^{60}Co detected in the two parts considered in Experiment C+G/S (cap+gills and stem). As can be observed, the two parts did not have the same percentage of incorporated activity, so that the distribution of radionuclides in the

Table 2
Mean value and standard deviation of the percentage of ^{85}Sr and ^{134}Cs taken up by the fruiting bodies of *Pleurotus eryngii* for each one of the degrees of maturity considered: young, mature, and old

Degree of maturity	^{85}Sr (%)	^{134}Cs (%)
Young	0.14 ± 0.07	0.052 ± 0.011
Mature	0.35 ± 0.19	1.21 ± 0.06
Old	0.15 ± 0.02	0.45 ± 0.11

Table 3
Percentages of total activity of ^{85}Sr, ^{134}Cs, and ^{60}Co detected in Cap+Gills (C+G) and Stem (S) of fruiting bodies harvested in Experiment C/G/S

N	Fraction	m	^{85}Sr (%)	^{134}Cs (%)	^{60}Co (%)	N	Fraction	m	^{85}Sr (%)	^{134}Cs (%)	^{60}Co (%)
1	C+G	1.6114	10 ± 5	82 ± 12	NA	11	C+G	3.7146	DL	77 ± 3	64.1 ± 0.9
	S	1.8160	90 ± 12	18 ± 5	NA		S	3.8146	DL	23.3 ± 1.1	35.9 ± 0.5
2	C+G	1.0735	DL	79 ± 11	NA	12	C+G	0.2632	4.6 ± 2.3	23 ± 3	32.4 ± 1.1
	S	0.5690	DL	21 ± 6	NA		S	1.7374	95 ± 8	77 ± 7	67.6 ± 2.2
3	C+G	0.7789	DL	79 ± 13	NA	13	C+G	3.8976	DL	81.9 ± 1.9	68.5 ± 0.8
	S	0.4430	DL	21 ± 8	NA		S	2.6116	DL	18.1 ± 0.6	31.5 ± 0.4
4	C+G	1.4668	DL	89 ± 9	NA	14	C+G	1.5057	5 ± 3	80.1 ± 2.3	56.8 ± 1.0
	S	0.7023	DL	11 ± 5	NA		S	1.3744	97 ± 6	19.9 ± 1.0	43.2 ± 0.8
5	C+G	1.2727	DL	94 ± 12	NA	15	C+G	0.0670	3.0 ± 2.0	10 ± 4	9.1 ± 0.4
	S	0.1886	DL	6 ± 5	NA		S	1.4085	97 ± 6	90 ± 13	90.9 ± 2.0
6	C+G	1.0723	DL	86 ± 14	NA	16	C+G	8.3881	58 ± 9	85.7 ± 2.2	75 ± 3
	S	0.7168	DL	14 ± 8	NA		S	3.6818	42 ± 9	14.3 ± 0.8	24.7 ± 1.9
7	C+G	1.5057	5 ± 3	80.1 ± 2.3	56.8 ± 1.0	17	C+G	1.9136	14.9 ± 1.4	48 ± 4	35 ± 4
	S	1.3744	95 ± 8	19.9 ± 1.0	43.2 ± 0.8		S	4.7453	85 ± 3	51.6 ± 2.4	65 ± 4
8	C+G	1.6427	3 ± 2	71 ± 6	47 ± 3	18	C+G	0.1924	7.2 ± 2.0	17 ± 4	18.5 ± 0.8
	S	2.0368	97 ± 6	29 ± 4	53 ± 3		S	1.3680	93 ± 8	83 ± 9	81.5 ± 1.8
9	C+G	2.4985	DL	82 ± 9	53 ± 3	19	C+G	0.0812	DL	30 ± 21	33 ± 6
	S	1.6370	DL	18 ± 5	47 ± 3		S	0.5211	DL	70 ± 31	67 ± 8
10	C+G	0.7474	DL	75 ± 14	58 ± 6						
	S	0.4870	DL	25 ± 8	43 ± 4						

N=identification number of each replicate; m=mass of the C+G or S, expressed in g d.w.; DL=detection limit; NA=not added.

fruiting bodies was clearly heterogeneous, confirming the findings of other workers (Muramatsu et al., 1991; Heinrich, 1993). To analyze these data in more detail, we considered the degree of maturity for each sample of fruiting bodies. This variable can be quantified by means of an adimensional factor M, defined by Eq. (1).

$$M = \frac{\text{Mass Cap} + \text{Mass Gills (g d.w.)}}{\text{Mass Stem (g d.w.)}}. \quad (1)$$

As can be observed in Fig. 1, when the fruiting body is at the initial stages of development (young) the stem is larger than the cap+gills, so that the M value at this stage would be <1. But for mature or old fruiting bodies, the two parts are of about the same size, if not larger, than the stem, so that the M value in this case would be around unity or >1.

Fig. 3 shows the dependence of the percentages of the total activity of ^{85}Sr, ^{134}Cs, and ^{60}Co detected in the cap+gills and stem on the degree of maturity, M. For each of these radionuclides, the percentage of their total activity in the stem decreases with increasing M, with a complementary increase of the percentage in the cap+gills. This seems to indicate that, as the fruiting bodies mature, the activity is translocated from the stem to the cap+gills. To quantify this trend, we fitted exponentials to the dependence of the percentage of the total activity in the stem on the degree of maturity (see Fig. 3).

$$A_S(^{85}\text{Sr})\% = (108.1 \pm 1.7) \times e^{-(0.39 \pm 0.18) \times M}$$
$$r = -0.82 \quad (2)$$

$$A_S(^{134}\text{Cs})\% = (45.9 \pm 1.2) \times e^{-(0.405 \pm 0.0.017) \times M}$$
$$r = -0.80 \quad (3)$$

$$A_S(^{60}\text{Co}) = (80.9 \pm 1.1) \times e^{-(0.527 \pm 0.017) \times M}$$
$$r = -0.93. \quad (4)$$

The percentage of the activity in the cap+gills can be fitted by the respective complementary equations.

The different accumulation of the radionuclides in the cap+gills, and stem can also be described by using the discrimination ratio, DR, defined as:

$$\text{DR} = \frac{A_{C+G}\ (\text{Bq})}{A_S\ (\text{Bq})} \quad (5)$$

where A_{C+G} is the activity of the cap+gills, and A_S is the activity of the stem, both expressed in Bq. A value of DR>1 therefore indicates that the fruiting bodies accumulate this radionuclide in the cap+gills better

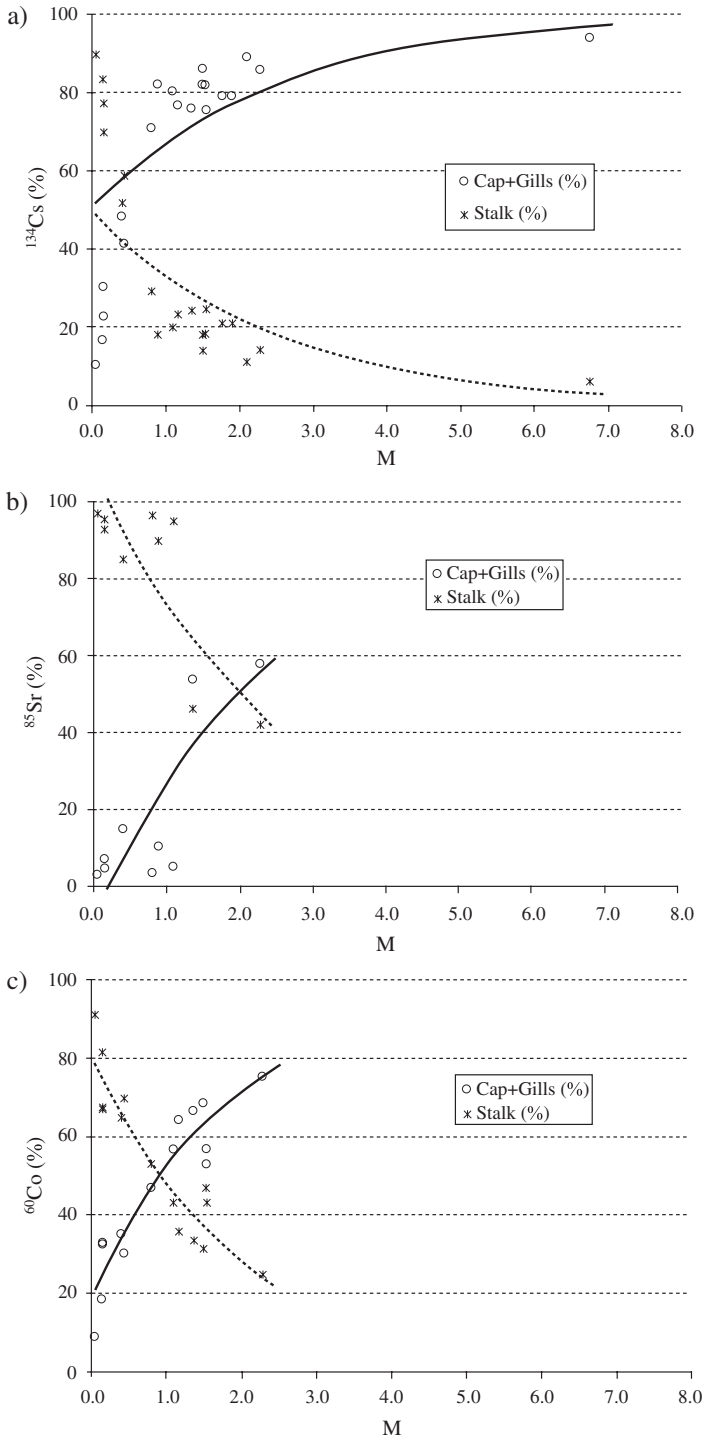

Fig. 3. Dependence on the degree of maturity, *M*, of the percentage of the total activity of (a) ^{134}Cs, (b) ^{85}Sr, and (c) ^{60}Co, detected in the Cap+Gills and the Stem of fruiting bodies of experiment C+G/S.

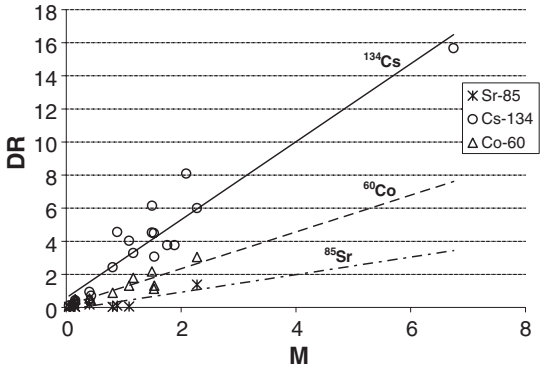

Fig. 4. Evolution of the discrimination ratios, DR, for ^{134}Cs, ^{85}Sr, and ^{60}Co with the degree of maturity, M.

than in the stem. In Fig. 4, it can be observed that DR was dependent on the degree of maturity. For all radionuclides, DR increased with increasing M, indicating that, as the fruiting bodies matured, the ^{134}Cs, ^{85}Sr, and ^{60}Co contents in the cap+gills were greater than in the stem. A linear regression between the two variables gave the following equations:

$$\mathrm{DR}\left(^{85}\mathrm{Sr}\right) = -(0.15\pm0.14) + (0.53\pm0.05)\times M \quad r = 0.85 \tag{6}$$

$$\mathrm{DR}\left(^{134}\mathrm{Cs}\right) = (0.6\pm2.5) + (2.6\pm0.3)\times M \quad r = 0.95 \tag{7}$$

$$\mathrm{DR}\left(^{60}\mathrm{Co}\right) = (0.11\pm0.13) + (1.11\pm0.03)\times M \quad r = 0.92. \tag{8}$$

Comparing the DR values between radionuclides, one observes that the rate of this increase was highest for ^{134}Cs, followed by ^{60}Co, and finally ^{85}Sr.

Given the dependence of the distribution of radionuclides on the degree of maturity, we show in Fig. 5 the mean percentages of the total activity of these radionuclides in cap+gills and in stem (Experiment C+G/S), taking into account only the mature fruiting bodies. One observes that, in this case, the order of the accumulation of these radionuclides in each part was:

- Cap+Gills: ^{134}Cs > ^{60}Co ≈ ^{85}Sr.
- Stem: ^{85}Sr ≈ ^{60}Co > ^{134}Cs.

The results obtained for ^{134}Cs are comparable to those reported for ^{137}Cs in mushrooms collected in the field (Muramatsu et al., 1991; Heinrich, 1993), in which ^{137}Cs is detected preferentially in the cap+gills of the species that are analyzed.

In Experiment C/G/S, we considered three parts within the fruiting bodies–cap, gills, and stem–and considered only mature fruiting bodies. As can be seen in Fig. 5, all three parts had a similar percentage of the total activity. Because the mass of the gills was smaller than the other two parts of the fruiting bodies,

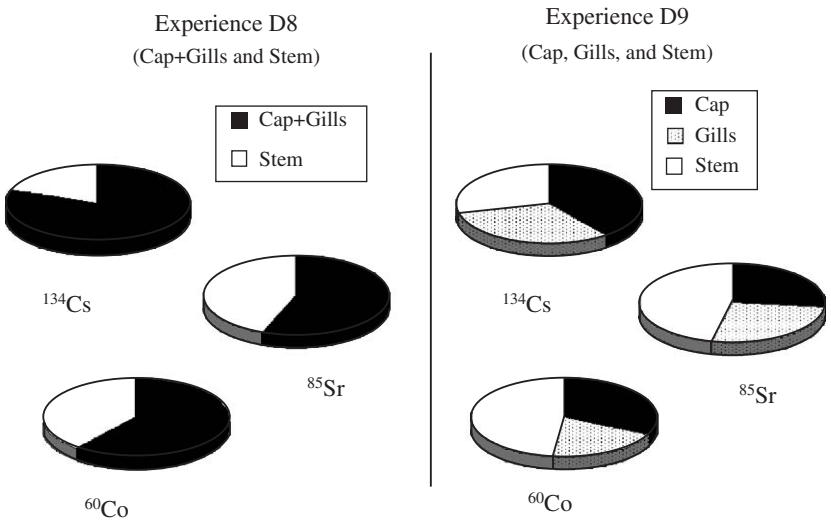

Fig 5. Mean values of the percentage of the total activity of ^{85}Sr, ^{134}Cs, and ^{60}Co detected in each part of the fruiting body studied in experiments C+G/S and C/G/S. Only mature fruiting bodies were considered in the calculations.

Table 4
Content of potassium and calcium (g/kg d.w.), and activity level of ^{137,134}Cs, ^{40}K, ^{60}Co, $^{239(+240)}$Pu, ^{234,238}U, ^{228,230,232}Th, ^{226}Ra (Bq/kg d.w.), and mass (g d.w.), detected in the whole fruiting bodies, and the percentage of the total content of each radionuclide detected in the Cap, Gills, and Stem part of mature fruiting bodies of *Pleurotus eryngii* harvested under laboratory conditions, and *Tricholoma equestre* collected in the field

Radionuclide	*Pleurotus eryngii*				*Tricholoma equestre*			
	Whole	% Cap	% Gills	% Stem	Whole	% Cap	% Gills	% Stem
K	12.77 ± 0.20	62.8 ± 1.5	16.6 ± 0.8	20.6 ± 0.8	31.6 ± 0.3	38.2 ± 0.4	20.1 ± 0.4	41.7 ± 0.6
Ca	84.6 ± 1.2	60.1 ± 1.4	4.42 ± 0.24	35.5 ± 1.0	3.78 ± 0.13	22.0 ± 2.2	24.6 ± 2.0	54 ± 3
Cs[a]	(1.55 ± 0.03) · 10^6	73.5 ± 2.4	22.3 ± 1.0	4.1 ± 0.4	34.7 ± 1.2	28.2 ± 2.3	47 ± 3	24.8 ± 2.0
^{40}K	DL[b]	DL	DL	DL	1260 ± 30	35.0 ± 1.8	19.8 ± 1.4	45.2 ± 2.0
^{60}Co	(10.10 ± 0.08) · 10^6	60.9 ± 0.8	34.1 ± 0.5	4.99 ± 0.15	DL	DL	DL	DL
Pu[c]	2.67 ± 0.10	52 ± 3	22.7 ± 1.6	25.1 ± 1.6	(36.1 ± 0.26) · 10^{-3}	19 ± 3	17 ± 3	64 ± 8
^{238}U	0.63 ± 0.05	58 ± 9	21 ± 4	21 ± 3	0.91 ± 0.05	25.7 ± 2.2	14.6 ± 1.6	60 ± 5
^{234}U	0.64 ± 0.05	55 ± 9	22 ± 4	23 ± 4	0.94 ± 0.05	24.4 ± 2.1	15.6 ± 1.7	60 ± 5
^{232}Th	0.56 ± 0.05	68 ± 11	16 ± 3	16 ± 3	1.44 ± 0.07	33 ± 3	18.3 ± 1.9	48 ± 4
^{230}Th	0.54 ± 0.05	64 ± 11	19 ± 4	17 ± 3	0.94 ± 0.05	30 ± 4	19.4 ± 2.3	50 ± 5
^{228}Th	4.12 ± 0.17	59 ± 4	28.6 ± 2.0	12.3 ± 0.8	2.62 ± 0.10	41 ± 3	28.0 ± 2.0	31.2 ± 2.3
^{226}Ra	(5.1 ± 0.5) · 10^{-3}	24 ± 6	44 ± 8	32 ± 6	(21.0±0.9) · 10^{-3}	7.6 ± 0.8	52 ± 4	40 ± 3
Mass	5.5892	49.4	21.9	28.7	74.1	34.7	17.1	48.2

[a] ^{134}Cs for *P. eryngii*, and ^{137}Cs for *T. equestre*.
[b] DL = detection limit.
[c] ^{239}Pu for *P. eryngii*, and $^{239+240}$Pu for *T. equestre*.

this part presented the highest specific activity in the fruiting bodies. With the activity of the cap and the gills considered together, these results are coherent with those obtained in Experiment C+G/S. This was the situation suggested by Heinrich (1993) in observing the autoradiography of fruiting bodies. Lastly, for mature fruiting bodies, the accumulation of the radionuclides studied in each part considered in Experiment C/G/S was in the order:

- Cap: ^{134}Cs > ^{60}Co > ^{85}Sr.
- Gills: ^{134}Cs > ^{85}Sr > ^{60}Co.
- Stem: ^{85}Sr ≈ ^{60}Co > ^{134}Cs.

3.2.2. Laboratory conditions: ^{239}Pu, uranium, thorium, radium, potassium, and calcium

In one culture of *P. eryngii*, designed to perform an experiment of the type Experiment C/G/S, we also added a known activity of ^{239}Pu, and harvested the fruiting bodies when they were mature, determining the distribution of radionuclides of natural origin (uranium, thorium, and radium) and of the added radionuclides (^{134}Cs, ^{60}Co, and ^{239}Pu), and the potassium and calcium content (see Table 4). In this culture, ^{85}Sr was below the detection limit. The cap presented the highest percentage of the total activity of the fruiting bodies for all the radionuclides con-

Table 5
Discrimination ratios, DR, of the radionuclides considered, and of potassium and calcium, for *Pleurotus eryngii* and *Tricholoma equestre*

Radionuclide	*P. eryngii*	*T. equestre*	Radionuclide	*P. eryngii*	*T. equestre*
K^+	3.85 ± 0.18	1.400 ± 0.024	^{238}U	3.9 ± 0.7	0.67 ± 0.06
Ca^{2+}	1.81 ± 0.07	0.87 ± 0.07	^{234}U	3.4 ± 0.6	0.67 ± 0.06
Cs[a]	23.1 ± 2.5	3.0 ± 0.3	^{232}Th	5.3 ± 1.0	1.07 ± 0.11
^{40}K	DL[b]	1.21 ± 0.07	^{230}Th	4.9 ± 0.9	0.98 ± 0.11
^{60}Co	19.0 ± 0.5	DL	^{228}Th	7.1 ± 0.5	2.21 ± 0.18
Pu[c]	2.97 ± 0.23	0.57 ± 0.08	^{226}Ra	2.1 ± 0.5	1.50 ± 0.14

[a] ^{134}Cs for *P. eryngii*, and ^{137}Cs for *T. equestre*.
[b] DL = detection limit.
[c] ^{239}Pu for *P. eryngii*, and $^{239+240}$Pu for *T. equestre*.

sidered except ^{226}Ra, for which the highest percentage of activity was in the gills.

To compare these results with those of the previous section, we considered the discrimination ratios, DR, defined by Eq. (5) and given in Table 5. As can be seen, all the radionuclides presented a DR value greater than unity, thus indicating that *P. eryngii* accumulated them preferentially in the cap+gills relative to the stem. The DR values for potassium and calcium were coherent with those found in other studies (Muramatsu et al., 1991; Heinrich, 1993). The ratio DR(^{134}Cs)/DR(K) was 6.0 ± 0.7, indicating that caesium was accumulated preferentially over potassium in the cap+gills. The ratio DR(Ca)/DR(K) was 0.47 ± 0.03, indicating that potassium was accumulated better than calcium. The uranium and plutonium DR values were very close given the associated uncertainties, with the mean value of the ratio DR(Pu)/DR(U) being 0.82 ± 0.17. Finally, it was striking that the DR values for ^{230}Th and ^{232}Th were very similar, but lower by a factor 1.3–1.4 than the ^{228}Th value. The main difference between their patterns of distribution was that the percentage of ^{228}Th detected in the gills was greater than that of ^{230,232}Th. This may suggest that the ^{228}Th content in the fruiting bodies is due to the partial uptake of this radionuclide not as thorium, but as ^{228}Ra, and therefore with an increased percentage in the gills, as occurred with ^{226}Ra. ^{228}Ra would subsequently decay to ^{228}Ac and ^{228}Th, as suggested by a previous study (Baeza et al., 2004a).

3.2.3. Field conditions

To assess the results obtained under laboratory conditions, we also divided mature fruiting bodies of *T. equestre* ($M=1.07$) into cap, gills, and stem. This species is edible and has a mycorrhizal nutritional mechanism. It was collected in a seminatural ecosystem—a pinewood in Muñoveros (Segovia) with a high productivity of mushrooms. The potassium, calcium, ^{137}Cs, ^{40}K, $^{239+240}$Pu, ^{234,238}U, ^{228,230,232}Th, and ^{226}Ra content of the whole fruiting body, and the percentage of the total activity detected in each part were determined (see Table 4). The respective discrimination ratios, DR, are listed in Table 5. The activity levels for the whole fruiting body of each radionuclide considered were similar to those found in previous studies in Spain (Mietelski et al., 2002; Baeza et al., 2004a,b). The potassium content was of the same order of magnitude as that found under laboratory conditions, but the calcium content was significantly lower. This is because the potassium content inside fungus cells is controlled but the calcium uptake is species dependent (Jennings, 1995).

With respect to the ^{137}Cs content, we observed that this radionuclide was accumulated mainly in the gills, followed by the cap and the stem. But ^{40}K and natural potassium presented a different pattern, accumulating mainly in the stem, followed by the cap and the gills. The percentages of ^{40}K and natural potassium in the different parts were very similar, with the mean ratio of the percentage of ^{40}K and potassium in each part of the fruiting bodies being [0.99 ± 0.09 (S.D.)]. The DRs for ^{137}Cs, ^{40}K, and natural potassium were greater than unity, as previously observed in laboratory conditions for *P. eryngii*. This species may therefore accumulate caesium and potassium preferentially in the cap+gills. The DR(^{137}Cs)/DR(^{40}K) ratio for this species was also greater than unity (2.5 ± 0.3). Radiocaesium was therefore accumulated preferentially over potassium in the cap+gills. This has also been observed for another two species of the genus *Tricholoma* (Bystrzejewska et al., 2003).

The calcium percentage was highest in the stem, followed by gills and cap which had similar percentages. This result differs from that under laboratory conditions mainly because of the different nutritional mechanism of the two species, and in particular the different calcium uptake. The ratio DR(Ca)/DR(K) was (0.62 ± 0.05), showing an accumulation of potassium relative to calcium in the cap+gills, as was also the case under laboratory conditions for *P. eryngii*.

For ^{234,238}U and $^{239+240}$Pu, the greatest percentage of the total activity was found in the stem of the fruiting bodies, followed by cap and gills. This pattern is different to that observed for *P. eryngii*, probably again because of the nutritional mechanism. The DRs for uranium and plutonium were both below unity. But the mean ratio DR(Pu)/DR(U) was (0.85 ± 0.14), very similar to that obtained under laboratory conditions. This may therefore suggest that uranium and plutonium follow a similar process of distribution within the fruiting body, regardless of the nutritional mechanism.

The percentages of ^{232}Th and ^{230}Th in each fraction considered were very similar, and showed a

similar pattern to that of plutonium and uranium (stem > cap > gills). But, unlike plutonium and uranium, the percentage of the total activity reflected the percentage of the dry mass of each fraction. Indeed, the ratios between the percentages of total activity and mass were [1.01 ± 0.06 (S.D.)] and [1.01 ± 0.14 (S.D.)] for ^{232}Th and ^{230}Th, respectively. This suggests that these radionuclides are fairly evenly distributed in the fruiting bodies of *T. equestre*. These patterns are different from what we observed in the laboratory for *P. eryngii*. Their uptake may therefore be species dependent, although further studies would be required to confirm it. The pattern presented by ^{228}Th was different from that of the other thorium isotopes. The greatest percentage was found in the cap, followed by stem and gills which were similar in degree given their uncertainties. This may be due to a partial uptake of ^{228}Th as ^{228}Ra. Finally, as was also the case under laboratory conditions, ^{226}Ra was accumulated mainly in the gills, followed by the stem and cap.

4. Conclusions

The degree of maturity of fruiting bodies plays an important role in the uptake and distribution of radionuclides within the fruiting bodies of a given species. The incorporation of ^{85}Sr and ^{134}Cs was found to be maximal for mature fruiting bodies. As the fruiting bodies matured, the percentage of the total activity of ^{85}Sr, ^{134}Cs, and ^{60}Co grew exponentially in the cap + gills, with a complementary decrease in the stem. The use of discrimination factors–the ratio between the activity of the cap + gills and that of the stem–showed the accumulation of these radionuclides in the cap + gills to increase as the fruiting bodies matured. This may be indicative of a translocation of these radionuclides during the process of development of these parts of the fruiting body.

We also compared the distribution patterns (cap, gills, and stem) of other radionuclides ($^{239+240}$Pu, ^{234,238}U, ^{228,230,232}Th, and ^{226}Ra) and of potassium and calcium in fungi–*P. eryngii*–grown under laboratory conditions with fungi–*T. equestre*–collected in a natural ecosystem. For both species, the discrimination factors showed the radiocaesium and potassium to mainly be located in the cap + gills, with these parts accumulating more radiocaesium than potassium. ^{226}Ra was mainly found in the gills. The different patterns of ^{228}Th and ^{230,232}Th seemed to indicate that the former is not only taken up directly as thorium, but as the radioactive antecessor ^{228}Ra. The distribution pattern of $^{239+240}$Pu, ^{234,238}U, and ^{230,232}Th were different in the two species, suggesting that their accumulation in each part of the fruiting bodies may be species dependent, although further research is needed to clarify the issue.

Acknowledgements

The present work was made possible by financing from Spain's Nuclear Safety Board (Consejo de Seguridad Nuclear) and ENRESA under the project entitled "Study of the transfer of radioactivity to fungi. Interactions and consequences. 2nd phase".

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