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Influence of the soil bioavailability of radionuclides on the transfer of uranium and thorium to mushrooms

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Abstract

The soil–mushroom transfer of thorium and uranium was analyzed in two ecologically similar but geographically separated Spanish ecosystems by means of the transfer factor, TF. Uranium TF values were in the range 0.043–0.49, and thorium TF values in the range 0.030–0.62. These values were similar to those of ^{90}Sr, $^{239+240}$Pu, and ^{241}Am found previously in the same ecosystems. Given the low availability of uranium and thorium, the available transfer factors, ATF, were also determined. These were higher than the TF values by one order of magnitude for $^{234,\ 238}$U, and by 2–3 orders of magnitude for $^{228,\ 230,\ 232}$Th. The ATF value of thorium was similar to that of ^{137}Cs, and that of uranium similar to that of ^{40}K. *Hebeloma cylindrosporum* presented the highest uranium and thorium transfer factors, confirming this species as a good bioindicator of a soil's radioactive content.

Keywords: Fungi; Transfer; Uranium; Thorium

1. Introduction

Analyses of the bioaccumulation and transfer of radionuclides from soil to mushroom have centered mainly on radiocaesium, and for this reason other radionuclides, uranium and thorium in particular, have been less studied. The literature data on the radioactive content of mushrooms collected in Spanish ecosystems which are affected only by global fallout (Mietelski et al., 2002; Baeza et al., 2004a) indicate that the mean value of the dose due to thorium isotopes is similar to that of radiocaesium (Baeza et al., 2004b). Data on the soil–mushroom transfer of these radionuclides are practically inexistent. Uranium and thorium transfer to another biological compartment—plants—has been more studied, however. The data available correspond to soil–plant transfer factors in the range 10^{-5}–10^{-2} for thorium and 10^{-3}–10^{-2} for uranium (IAEA, 1994). One might presume that a similar value should be used for soil–mushroom transfer, but this is not coherent with the activity levels detected previously in fruiting bodies. This could be due to differences in how plants and mushrooms take up radionuclides, as is the case for example with strontium (Yoshida and Muramatsu, 1998). And indeed, mushrooms are known to be bioaccumulators of heavy metals (Kalač and Svoboda, 2000; Demirbaş, 2001).

In order to analyze the soil–mushroom transfer of these natural radionuclides, we selected two ecosystems with a high productivity of mushrooms, and collected samples of several species of mature fruiting bodies and the corresponding surface soil. The transfer was analyzed in terms of transfer factors. But the classical concept of transfer factors as the ratio between the content in the fruiting bodies and the content in the soil (ICRU, 2001) has drawbacks: (a) the soil content is not clearly defined—it can be taken to be the total activity deposited in the soil, or the activity of the surface layer of the soil or of another deeper layer; and (b) some radionuclides which are strongly bound to the soil matrix, such as ^{40}K (Baeza et al., 2005), are not 100% available for transfer.

The objective of this work was to study the transfer of uranium and thorium from soil to mushroom. Given the natural origin of these radionuclides in the soil, their degree of association to soil particles was determined by means of

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a sequential extraction procedure, based on that used by Krouglov et al. (1998). The fractions obtained in order to determine the corresponding availabilities wer: exchangeable (extractable with NH_4OAc), dilute-acid soluble (extractable with HCl 1M), concentrated-acid soluble (extractable with HCl 6M), and residual (representing the radionuclides strongly bound to soil particles). With these results, the transfer was then re-estimated on the basis of the so-called ''available transfer factors'', and compared with the classically determined values.

2. Material and methods

Mushrooms were collected from two pinewood ecosystems, located more than 250 km apart, Muñoveros and Bazagona (Spain), and with the same dominant vegetation type—*Pinus pinaster*. Muñoveros is in the province of Segovia (3° 57′ 5.5″ W, 41° 10′ 26.2″ N), about 100 km from Madrid. Its climate is Mediterranean with continental influences. The mean annual precipitation is 520 mm, and the mean annual temperature 12 °C. Bazagona is in the province of Cáceres, western Spain (5° 53′ 53.3″ W, 39° 55′ 21.2″ N). Its climate is Mediterranean with continental and Atlantic influences. The mean annual precipitation is 606 mm, and the mean annual temperature 16.7 °C.

At each location, an area of about 2 ha was marked out. Within these areas, mature fruiting bodies of several species of mushroom were collected by cutting the stipe slightly above the soil level. Both these ecosystems have high mushroom productivity, and more than 650 g d.w. of mature fruiting bodies of each species were harvested. After collection, they were washed with bi-distilled water to remove any adhered soil particles, and dried at 100 °C until constant weight to determine their dry weight. In our case, we had found in a previous study, using the ergosterol method, that the surface soil (0–5 cm) of the two selected ecosystems was the layer in which most of the mycelium was located (Baeza et al., 2005). Simultaneously with the sampling of the fruiting bodies, surface soil (0–5 cm) was also collected from the two ecosystems by means of a 53 mm diameter, 50 cm long iron cylinder equipped with a piston. The sampling points, about 20 in each ecosystem, were chosen uniformly within the 2 ha area, in clearings within the pinewood, avoiding slopes and places where roots were visible. They were then merged in order to obtain an average value. The soil from these ecosystems was acidic, sandy, of granitic origin, and with a low content of organic matter (see Table 1). In the laboratory, the soil samples were air-dried and sifted through a 2 mm pore sieve to remove stones and foreign objects.

2.1. Sequential extraction procedure

The speciation procedure for the soils was based on the sequential extraction protocol used by Krouglov et al. (1998). The fractions considered were:

Table 1
Soil characteristics (pH, % organic matter, clay, silt, and sand) of surface soil (0–5 cm) in Muñoveros and Bazagona

Location	pH	% Organic matter	% Clay	% Silt	% Sand
Muñoveros	6.6	3.5	1.9	2.9	95.2
Bazagona	5.8	2.7	3	12.9	84.1

(1) Exchangeable (F1): Air-dried soil was attacked at room temperature with NH_4OAc 1M at a solid-to-liquid mass ratio of 1:10. The suspension was stirred rapidly for 10 min and left overnight. The liquid phase was separated from the solid phase by vacuum filtration through Whatman 42 filter paper and then through a 0.47 μm membrane filter. Finally, it was dessicated on a heat-resistant plastic.
(2) Dilute-acid soluble (F2): The solid residue was attacked with HCl 1M under the same conditions as the F1 fraction. Separation was performed as described for the F1 fraction.
(3) Concentrated-acid extractable (F3): The solid residue was attacked by boiling with HCl 6M on a hot plate for 1.5 h at a solid-to-liquid mass ratio of 1:2. Separation was performed as described for the F1 fraction.
(4) Residual (F4): This is the final solid residue, and represents the fraction of radionuclides strongly bound to the soil.

2.2. Radionuclide determination

The procedure used for the sequential extraction of thorium and uranium isotopes from the soil and mushroom samples was based on that used by (Mietelski et al., 2002; Baeza et al., 2004a). Samples were ashed at 600 °C in order to eliminate the organic matter. Then tracers (^{229}Th and ^{232}U) were added, and the ash was digested with HF, HNO_3, and HCl. The resulting solution was passed through a Dowex 1×8 resin column in HNO_3 8M medium, so that thorium was retained but not uranium. Thorium was eluted from the column with concentrated HCl. The uranium fraction was co-precipitated with $Fe(OH)_3$. The precipitate was re-dissolved in HCl 9M, followed by separation in a column with Dowex 1×4 resin. The uranium is retained in the column, and subsequently eluted with HNO_3 8M. Finally, the alpha sources were prepared by 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. Alpha-spectrometry was 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. The quality of the radiochemical separation was evaluated using reference material IAEA-327 (IAEA, 2000), and the results are listed in Table 2.

3. Results

The uranium and thorium content of the different species of mushroom and of the surface soil (0–5 cm) from the two ecosystems are listed in Table 3. All species of mushroom collected were of the prevalently mycorrhyzal nutritional strategy type. It should be noted that: (a) the radionuclides belonging to the uranium series—^{238}U, ^{234}U, and ^{230}Th—were generally in equilibrium in the fruiting bodies of the different species (except *Tricholoma terreum*), but not in the surface soil; (b) the radionuclides belonging to the thorium series—^{228}Th and ^{232}Th—were generally in equilibrium in the fruiting bodies (except in *T. terreum* and *T. equestre*); and (c) the latter radionuclides were in equilibrium in the surface soil layer in Bazagona ecosystem, but not in Muñoveros.

Table 2
Measured activity levels, reference values, and confidence intervals of ^{234,238}U and $^{228,\ 230,232}$Th, expressed in Bq/kg d.w., in the reference material IAEA-327 (IAEA, 2000)

Radionuclide	Measured	Reference value	Confidence interval
^{234}U	33±6	31.9	30.4–33.1
^{238}U	33±6	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	37.2–40.2

The transfer of radionuclides from soil to the fruiting bodies of mushrooms has usually been quantified in terms of the transfer factor (TF) defined as the ratio between the activity level in the fruiting bodies and that of the total fraction of the soil (Heinrich, 1993; Amundsen et al., 1996; ICRU, 2001). In our case, we considered the surface layer of soil (0–5 cm) because in the selected ecosystems most of the mycelium was found in this layer (Baeza et al., 2005). This classical form of the TF is defined in Eq. (1):

$$TF = \frac{A_M(mushroom)}{A_S(surface\ soil)}, \quad (1)$$

where A_M(mushroom) and A_S(surface soil) are the activity levels detected in the fruiting bodies and in the corresponding surface soil, respectively. Both quantities are expressed in Bq/kg d.w. The TF values for uranium are listed in Table 4. Since ^{234}U–^{238}U were in equilibrium in the soil and in the fruiting bodies, the TF values for these two radioisotopes were quite similar, and can therefore be

Table 3
Activity levels of ^{234,238}U, and ^{228,230,232}Th, expressed in Bq/kg d.w., detected in the fruiting bodies of the species of mushroom analyzed and in surface soil collected in Muñoveros and Bazagona ecosystems

	Samples	^{234}U	^{238}U	^{228}Th	^{230}Th	^{232}Th
Muñoveros	*Amanita muscaria* (M, NE)	2.4±0.3	2.3±0.3	4.5±0.7	2.1±0.4	3.6±0.6
	Hebeloma cylindrosporum (M, NE)	3.3±0.3	3.2±0.3	7.4±0.4	4.0±0.3	6.9±0.4
	Tricholoma equestre (M, E)	0.94±0.05	0.91±0.05	2.62±0.10	0.94±0.05	1.44±0.07
	Tricholoma pessandatum (M, NE)	1.62±0.18	1.58±0.14	2.9±0.4	1.41±0.22	2.4±0.2
	Tricholoma terreum (M, E)	1.6±0.4	1.9±0.5	7.60±0.10	3.4±0.6	5.2±0.8
	Surface soil	6.7±0.5	7.0±0.5	19.4±0.3	17.9±0.5	11.1±0.3
Bazagona	*Lactarius deliciosus* (M, E)	1.21±0.24	1.3±0.3	1.82±0.20	1.10±0.14	1.65±0.20
	Rhizopogon rosseoulus (M, E)	0.81±0.02	0.94±0.20	1.8±0.3	1.07±0.20	1.07±0.20
	Russula cessans (M, NE)	6.5±0.8	6.8±0.8	10.7±2.3	6.7±1.5	6.7±1.5
	Surface soil	19±3	22±3	61±4	32.3±2.2	54±4

M means mycorrhizal; E means edible, NE means not edible.

Table 4
Soil-to-mushroom classical, TF, and available, ATF, transfer factors for ^{234,238}U for the different species collected in Muñoveros and Bazagona ecosystems

	Species	^{234}U		^{238}U	
		TF	ATF	TF	ATF
Muñoveros	*Amanita muscaria*	0.36±0.05	6.0±1.3	0.33±0.05	4.5±1.0
	Hebeloma cylindrosporum	0.49±0.06	8.2±1.6	0.46±0.05	6.3±1.2
	Tricholoma equestre	0.140±0.013	2.3±0.4	0.130±0.012	1.8±0.3
	Tricholoma pessandatum	0.24±0.03	4.0±0.8	0.23±0.03	3.1±0.6
	Tricholoma terreum	0.24±0.06	4.0±1.2	0.27±0.07	3.8±1.2
Bazagona	*Lactarius deliciosus*	0.064±0.016	0.67±0.21	0.059±0.016	0.75±0.23
	Rhizopogon rosseoulus	0.043±0.007	0.41±0.11	0.043±0.011	0.54±0.16
	Russula cessans	0.34±0.07	3.6±1.0	0.31±0.06	3.9±0.9

Table 5
Soil-to-mushroom classical, TF, and available, ATF, transfer factors for ^{228,230,232}Th for the different species collected in Muñoveros and Bazagona ecosystems

	Species	^{228}Th		^{230}Th		^{232}Th	
		TF	ATF	TF	ATF	TF	ATF
Muñoveros	*Amanita muscaria*	0.23±0.04	38±7	0.12±0.03	195±86	0.32±0.05	188±57
	Hebeloma cylindrosporum	0.381±0.021	62±8	0.223±0.018	371±128	0.62±0.04	360±95
	Tricholoma equestre	0.136±0.006	22±3	0.053±0.003	87±30	0.130±0.007	75±20
	Tricholoma pessandatum	0.149±0.021	24±4	0.079±0.012	131±49	0.216±0.019	125±34
	Tricholoma terreum	0.392±0.008	64±7	0.190±0.03	316±120	0.47±0.07	272±81
Bazagona	*Lactarius deliciosus*	0.030±0.004	3.0±0.4	0.034±0.005	4.9±0.8	0.031±0.004	16±3
	Rhizopogon rosseoulus	0.030±0.005	3.0±0.6	0.033±0.007	4.7±1.0	0.027±0.006	14±3
	Russula cessans	0.18±0.04	17±4	0.21±0.05	30±7	0.17±0.04	88±22

averaged. The mean value of the averaged TF for uranium considering both ecosystems was [0.23±0.15 (SD)], and the range was 0.043–0.47. *Amanita muscaria* and *Hebeloma cylindrosporum* presented the highest TF values, and *Lactarius deliciosus* the lowest.

The TF values for thorium are listed in Table 5. The mean values were: ^{228}Th [0.19±0.14 (SD)], range 0.030–0.392; ^{230}Th [0.12±0.08 (SD)], range 0.033–0.223; and ^{232}Th [0.25±0.21 (SD)], range 0.027–0.62. One observes that the ^{228}Th, ^{230}Th, and ^{232}Th transfers behaved differently in the two ecosystems as a consequence of the different equilibria in the respective soils. In Muñoveros, the TF values were generally highest for ^{232}Th followed by ^{228}Th and ^{230}Th, whereas in Bazagona, the values were similar for all three radionuclides. With respect to the uranium series, the TF values for ^{230}Th were lower than those for ^{234}U and ^{238}U. However, the ^{228}Th and ^{232}Th TF values were of the same order of magnitude as those of uranium. Also, *H. cylindrosporum* presented the highest TF values for the thorium isotopes.

The TF values for uranium and thorium transfer from soil to mature fruiting bodies of mushroom (Tables 4 and 5), within the range 10^{-2}–10^{-1}, were higher than those that have been reported for plants (IAEA, 1994), indicative again of their heavy metal bioaccumulating character. In the two selected ecosystems, the transfer of other radionuclides (^{137}Cs, ^{40}K, ^{226}Ra, ^{90}Sr, $^{239+240}$Pu, and ^{241}Am) had also been analyzed in previous work (Baeza et al., 2005, 2006). The results are shown along with the present results for uranium and thorium in the upper part of Fig. 1. In this figure, medians are used instead of means because of the asymmetry in the ^{137}Cs accumulation (Baeza et al., 2004b). The other radionuclides presented a symmetrical distribution, and therefore their medians and means are practically the same. Compared to the uranium and thorium TF values, those of ^{137}Cs were generally higher, those of ^{40}K, ^{226}Ra, and ^{90}Sr were similar or a little higher, and those of $^{239+240}$Pu and ^{241}Am were similar.

Due to the natural origin of uranium and thorium in the soil, they were assumed not to be 100% available for transfer, in analogy with other natural radionuclides such as ^{40}K and ^{226}Ra. Therefore, for the surface (0–5 cm) soil samples, a sequential extraction procedure based on that used by Krouglov et al. (1998) was carried out. The results are shown in Fig. 2. One observes in the figure that the thorium and uranium were mainly associated with the residual fraction of the soil. Only a very small percentage of these radionuclides were associated with the exchangeable and dilute-acid fractions. Therefore, the greater part of these radionuclides in the soil was not available for transfer to the fruiting bodies. ^{234}U and ^{238}U were in equilibrium in both ecosystems, and presented the same pattern: residual > concentrated-acid soluble ⩾ dilute-acid soluble > exchangeable. Nevertheless, there was a difference: in the Bazagona ecosystem the percentage of uranium associated with the concentrated-acid fraction was higher than in Muñoveros. This may reflect differences in soil texture—although both soils were sandy, in Bazagona the silt content was higher than in Muñoveros. The thorium radioisotopes were even more strongly attached to the residual fraction. The pattern was: residual > concentrated-acid soluble ⩾ dilute-acid soluble > exchangeable. In any case, the thorium in the soils was very unavailable, with less than 1% associated with the dilute-acid and exchangeable fractions. In these fractions, in the Muñoveros ecosystem ^{228}Th presented the highest percentages (0.61±0.07%), followed by ^{232}Th (0.17±0.04%) and ^{230}Th (0.06±0.02%), and the order was reversed in the Bazagona ecosystem, (0.99±0.07%), (0.70±0.06%), and (0.20±0.02%) for ^{228}Th, ^{230}Th, and ^{232}Th, respectively.

The classical definition of TF has the limitation that the radionuclides present in the soil are not 100% capable of being transferred to the fruiting bodies, but are associated in different degrees to the soil particles. This can be partially solved using a definition of transfer factor based on the percentage of radionuclides that may be accessible to exchange reactions instead of on the total fraction of the soil. In the present study, our working hypothesis, also used in previous work (Fesenko et al., 2001; Baeza et al., 2006), was that the radionuclides associated with fractions

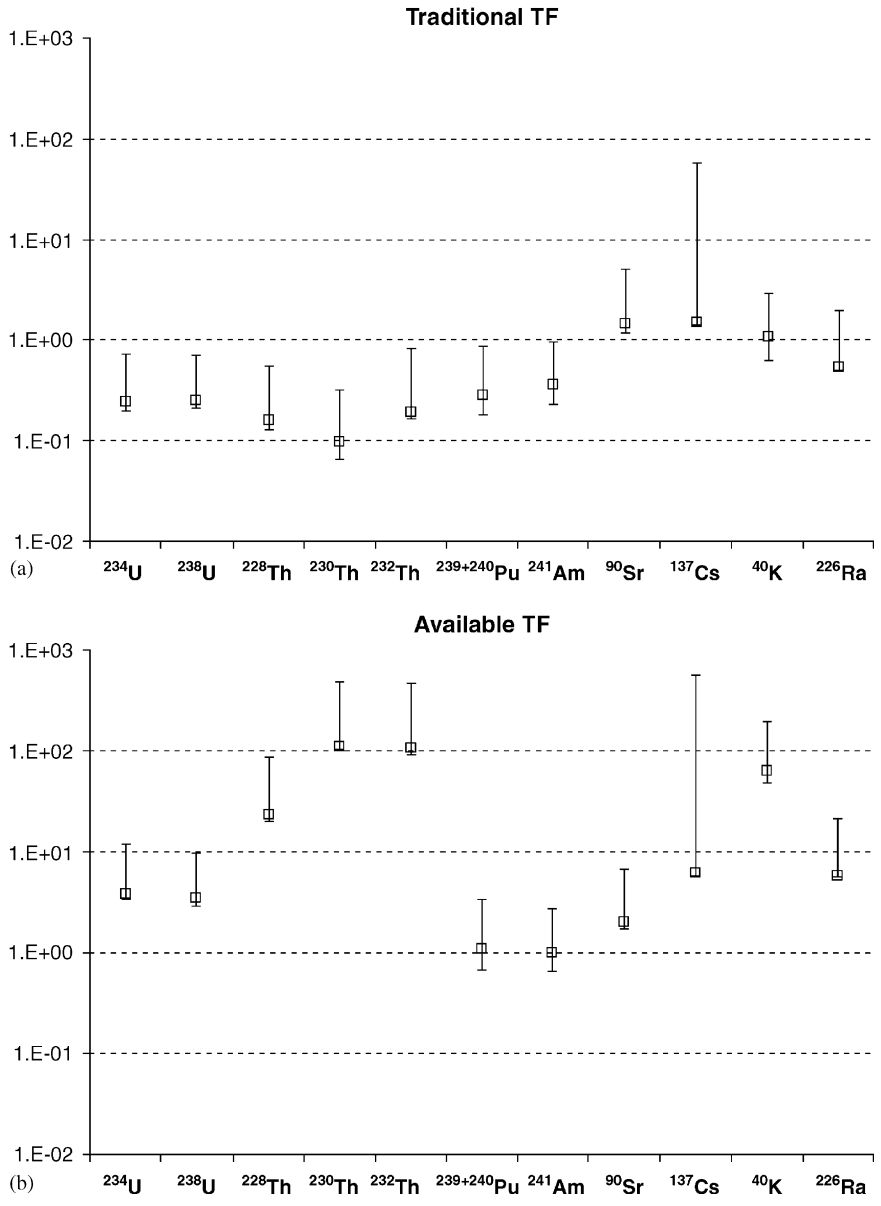

Fig. 1. Median and range of: (a) classical transfer factors, TF, and (b) available transfer factors, ATF, for $^{234,\ 238}$U, $^{228,\ 230,\ 232}$Th, $^{239+240}$Pu, ^{241}Am, ^{90}Sr, ^{137}Cs, ^{40}K, and ^{226}Ra for several species of mushroom collected (Baeza et al., 2005, 2006).

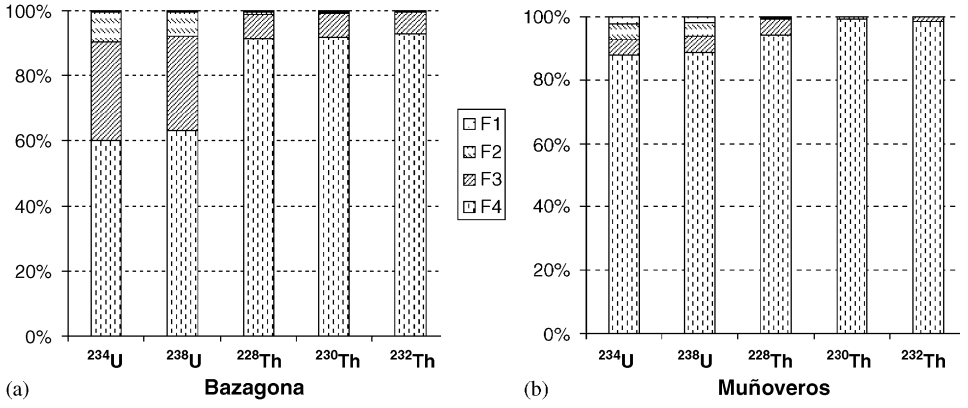

Fig. 2. Percentages of association of $^{234,\ 238}$U and $^{228,\ 230,\ 232}$Th with the exchangeable (F1), dilute-acid soluble (F2), concentrated-acid soluble (F3), and residual fractions (F4) obtained in the sequential extraction procedure for the surface layer (0–5 cm) of soils from (a) Bazagona and (b) Muñoveros (Spain).

strongly bound to soil particles (concentrated-acid and residual) were not accessible to exchange reactions. Therefore, the available fraction (AF) would be defined as

$$AF(\%) = 100 - \textit{Concentrated acid fraction}(\%) - \textit{Residual fraction}(\%). \quad (2)$$

The available transfer factor (ATF) is defined by the ratio between the content in the fruiting bodies and that fraction in the soil that is capable of being transferred (see Eq. (3)). This definition has been used in previous work on the soil–mushroom transfer of man-made radionuclides (Baeza et al., 2005, 2006):

$$ATF = \frac{A_M(mushroom)}{A_S(surface\ soil)\,AF(\%)}100\%. \quad (3)$$

The ATF values for uranium and thorium are listed in Tables 4 and 5. Thorium presented ATF values higher than those for the uranium isotopes, suggesting that there is a preferential incorporation of thorium into the fruiting bodies as against that of uranium. *H. cylindrosporum* also presented the highest ATF values for both uranium and thorium. These results, together with the high affinity for radiocaesium of this species and another species of the genus *Hebeloma* (Yoshida and Muramatsu, 1994; Baeza et al., 2004b), seems to confirm that the *Hebeloma* genus may be a good bioindicator of radionuclides in the environment.

The use of ATF has the advantage that it is easier to identify the radionuclides that are preferentially taken up by mushrooms. In the lower part of Fig. 1, the ATF values obtained in this study are compared with ^{137}Cs, ^{40}K, ^{226}Ra, ^{90}Sr, $^{239+240}$Pu, and ^{241}Am ATF values for the several species of mushroom collected in the same ecosystems (Baeza et al., 2005, 2006). It can be seen that the transfer of uranium and thorium based on the available fraction was similar to that of radiocaesium and higher than that of other artificial radionuclides. The thorium ATF values were at the same level as ^{40}K and, for some species of mushroom, also as ^{137}Cs, and higher than ^{226}Ra. In the uranium case, the situation was the reverse. These results suggest that mushrooms are good bioindicators of uranium and thorium because they were able to accumulate what little of these radionuclides was available in the soil.

4. Conclusions

Eight mushroom species were collected in two selected ecosystems, both pinewoods and with sandy granitic soil. At these locations, we also sampled the surface soil (0–5 cm), because in a previous work it had been found that this layer of soil contained most of the mycelium. The soil and mushroom samples were assayed for uranium and thorium, both radionuclides of natural origin. The transfer of these radionuclides from the soil to the fruiting bodies was quantified in terms of the classical definition of the TF. The TF values indicated that transfer was at a low level, of the same order as artificial radionuclides, $^{239+240}$Pu and ^{241}Am, but higher than the soil–plant TF values. The TF values for ^{234}U and ^{238}U were equal within the experimental error at both selected locations. ^{230}Th also belongs to the series $(4n+2)$, but its TF values were lower than those for uranium, mainly due to the lower availability of ^{230}Th in soil. The ^{228}Th and ^{232}Th TF values depended on the state of equilibrium of the thorium series $(4n)$ in the surface soil: in Bazagona, ^{228}Th and ^{232}Th were in equilibrium and their TF values were similar, but in Muñoveros, they were in disequilibrium, and the ^{232}Th TF value was higher than the ^{228}Th TF value.

The definition of TF has the drawback that it does not take into account the availability of radionuclides for transfer. This is especially important in the case of radionuclides of natural origin, which are presumably attached to soil particles. To study this association, a sequential extraction procedure was applied to the surface soil samples from the two locations. This procedure considered separately the exchangeable, the dilute-acid soluble, the concentrated-acid soluble, and the residual fractions. We considered that the fraction of radionuclides capable of transfer to the fruiting bodies would be that which was not strongly bound to the soil particles, i.e., that in the exchangeable and dilute-acid soil fractions. This available fraction was found to be very small for both uranium and thorium, reflecting their natural origin, and of the same order of magnitude for uranium and thorium at the two selected locations. The ATF values were consequently higher than the TF values, the difference being one order of magnitude for $^{234,\ 238}$U (Table 4), and usually 2–3 orders of magnitude for $^{228,\ 230,\ 232}$Th (Table 5).

The uranium and thorium ATF values were compared with ATF values for other radionuclides of anthropogenic and natural origin. While the available transfer of thorium was similar to that of caesium, that of uranium was greater than ^{90}Sr, $^{239+240}$Pu, and ^{241}Am. This suggests that, despite the low availability of thorium and uranium in the soil, the mushrooms were able to transfer what little was available to their fruiting bodies. Given the medians and ranges of the available transfer for all the species studied, the efficiencies of the radionuclide transfers were ranked as follows:

$$^{228,230,232}\mathrm{Th} \approx {}^{40}\mathrm{K} \geqslant {}^{137}\mathrm{Cs} \geqslant {}^{234,238}\mathrm{U} \approx {}^{226}\mathrm{Ra} \geqslant {}^{90}\mathrm{Sr} \geqslant {}^{239+240}\mathrm{Pu} \approx {}^{241}\mathrm{Am}.$$

There are species such as *H. cylindrosporum* for which the transfer of radiocaesium is known to be particularly high. Since this species has also presented high transfer factors for the other radionuclides, it can be used as a good bioindicator of the radioactive content of the ecosystem in which it grows.

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