

Fungi Contaminated by Radionuclides: Critical Review of Approaches to Modeling

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INTRODUCTION

Data derived from sampling at areas contaminated by the Chernobyl fallout have demonstrated the organic layer of soil to be a major pool of radiocesium in forest ecosystems (Linkov and Schell, 1999, Ruhm et al., 1996). Fungi can directly bind or precipitate radionuclides; they also can indirectly affect radionuclide speciation and mobility in forest soils (Gadd, 1996). Fungi are the greatest living biomass in the decomposing organic layers of forest soil. They are the primary sources of enzymes necessary to degrade the litter. Fungi are one of the most important components of forest ecosystems, since they determine to a large extent the fate and transport processes of radionuclides in forests (Linkov and Von Stackelberg, 1999; Riesen et al., 1999; Linkov and Schell, 1999).

Understanding radionuclide accumulation by fungi is also essential for the development of remediation technologies for contaminated areas. Phytoremediation is emerging as an attractive alternative to high-cost traditional cleaning methods for large areas polluted with low to moderate levels of heavy metals and radionuclides. Recent studies show that phytoremediation of soils contaminated by ¹³⁷Cs may be feasible (Last et al., 1997). Mycorrhizal fungi can maximize radionuclide accumulation and removal from contaminated soil by plants (Entry et al., 1996).

This paper is not intended to provide a comprehensive review of biological processes, but rather to critically review existing modeling techniques. In the recent International Atomic Energy Agency model-model and model-data intercomparisons (BIOMASS, 1999) the radionuclide accumulation in fungi was found to be the most difficult to predict.

Developing reliable radioecological models to describe the radionuclide accumulation in fungi requires an evaluation of many uncertain parameters and variables. These parameters have been found to be especially uncertain as compared to other forest compartments. Many parameters are represented by aggregated values averaged over time, space and species. The uncertainty of these parameters should be considered as an integral part of the modeling process. Excessive model complexity will result in a limited utility of the model, since many uncertain model parameters decrease the overall reliability of model predictions. On the other hand, using over-simplistic models in situations when a better understanding of the ecosystem has already been achieved, might also lead to unrealistic model results that could possibly contradict available experimental data.

2. FATE AND TRANSPORT OF RADIONUCLIDES IN ORGANIC LAYERS AND ROLE OF FUNGI

Many studies (see Linkov and Schell, 1999 for review) report that most of the Chernobyl-released radiocesium can still be found in organic horizons of forest soil. Radiocesium originating from global fallout is also accumulated in these surface layers, even after almost 40 years since deposition (Yoshida and Muramatsu, 1994a). Fungal and microbiological activity is likely to be responsible for the long-term retention of radionuclides in organic layers of forest soil. Fungi are the highest living biomass in the decomposing litter and are mainly situated in the upper part of the organic layer (Reisinger, 1993). Compared to fungal mycelia, above-ground fruit bodies are a minor contribution to the total fungal biomass (Danielsson and Pruden, 1989, Taylor and Alexander, 1990, Olsen, 1994). Cesium was found to be quickly integrated into the nutrient cycle within the organic layer (Rommelt et al., 1990). Similar bioavailability for recently deposited radioactive Cs and stable Cs was found in German forests by Ruhm et al. (1999).

Experimental results indicate that cesium is effectively retained by fungal mycelia. Several authors sterilized forest soil with gamma radiation (Bunzl and Schimmack, 1988, Guillitte et al., 1994), chloroform (Bruckmann and Wolters, 1994), fungicides (Shand et al., 1995) and then examined the change in the amount of easily exchangeable cesium. The results indicate a significant increase in the level of labile cesium after treatment for many soils. Guillitte et al. (1994) estimated that about 40% of the radiocesium was leached from the irradiated samples compared to control samples. Olsen et al. (1990) estimated the fungal biomass in 57 soil samples collected beneath fungal fruit bodies by measuring the ergosterol which is contained only in fungi in soil environment. Based on the biomass measurements and the concentration of radiocesium in the fruit body above ground, the amount of radiocesium in the total fungal biomass was estimated (range: from <10% to >50%; average: 32% of the total radiocesium inventory). This methodology greatly overestimates the biomass (Taylor,

private communication), therefore the total accumulation by fungi is likely to be lower. Fawaris and Johanson (1995) estimated that about 22% of ^{137}Cs in the top 5 cm of a coniferous forest may be bound by fungal mycelia.

According to Griffin (1981), fungi take up nutrients from soil in aqueous solution. Fungi use enzymes to break down macromolecular complexes for uptake. Once broken down, most substances are thought to move into the *hyphae* being bound to specific carrier molecules. This method of transport requires energy and is selective. The uptake rate is not well studied (Harley and Smith, 1983). It is expected to vary with the concentration of ions, moisture content of the soil, the growth rate of the fungus and (in the case of symbiotic fungi) the transpiration rate of the host plant. Fungal hyphae were recently found inside numerous narrow pores of mineral grains in upper soil horizons of coniferous forests in Europe (Jongmans et al., 1997). This observation suggests that fungi may provide a direct link between small pores of minerals and mycorrhizal plant roots that would effectively bypass the bulk soil solution. This view may challenge some of the present models in which the bulk soil solution is assumed to be the medium that nutrients pass through on their way to the plant.

Very little is known about the mechanisms involved in the uptake and retention of radionuclides by fungi. In comparison to plants, the elemental composition of fungal fruit bodies can be characterized by high ^{137}Cs , Cs and Rb concentrations and low Ca and Sr concentrations (Yoshida and Muramatsu, 1998). The concentrations of ^{137}Cs , Cs and Rb for fruit bodies were one order of magnitude higher than those for plants growing in the same forest. High concentrations of Cs and Rb in fungal fruit bodies were also observed in cultivation experiments in flasks using radiotracers (Ban-nai et al., 1994; 1997). The low concentrations for Ca and Sr are consistent with the low concentrations of ^{90}Sr in fungal fruit bodies after the Chernobyl accident (Mascanzoni, 1990). Lower accumulation of radiostrontium in fungi compared with plants was also observed in radiotracer experiments (Ban-nai et al., 1994).

Several studies (Rommelt et al., 1990; Heinrich, 1993; Sugiyama et al., 1993; Yoshida and Muramatsu, 1994a; 1994b) investigated the differences in the ability of saprophytic and symbiotic fungi to accumulate radiocesium. Rommelt et al. (1990) studied the differences among saprophytic and symbiotic fungi in twenty five different fungal species (21 symbionts and 4 saprophytes), collected at a forest near Siegenburg, Germany, in 1988. Their results indicated higher concentrations of radiocesium for symbiotic species. In view of the pronounced vertical profile of radiocesium, especially during the first years after the Chernobyl accident, these observations can be explained, at least in part, by the different vertical distributions of mycelia in soil. Guillitte et al. (1990) proposed to use the isotopic ratio $^{134}\text{Cs}/^{137}\text{Cs}$ in fungal fruit bodies to localize the exploited soil layer *in situ*. Explicitly referring to the corresponding soil layers, they estimated the ability of different fungal species to take up radiocesium at a Belgian forest site. Other hypotheses include differential retention of radionuclides by fungi (Guillitte et al., 1994).

Clint et al. (1991) and Dighton et al. (1991) investigated the uptake of radiocesium by fungi and their capacity to retain radiocesium within the mycelium after uptake. In laboratory experiments with several basidiomycete species they found that (i) the rate of the cesium influx varied by a factor of 4 between 24 studied species, and (ii) the loss effect of radiocesium following loading proceeds at a much slower rate than the original uptake rate. The authors concluded that fungi can be potential "accumulators" for radiocesium, a consequence of high influx and low efflux rates. They also suggested that much of the absorbed cesium is biologically bound within the tissue and that fungi might have the capacity to hold all the potentially "labile" cesium in soil (Dighton et al., 1991).

Since the biomass of fungal fruit bodies constitutes only a small percentage of the total biomass of fungi, radiocesium is expected to be stored predominantly within mycelia. According to Nikolova et al. (1997), the above ground fungal biomass can accumulate only between 0.01 and 0.1% of the total cesium inventory in different years. The radionuclide concentration in fruit bodies is probably close to that of the fungal parts of mycorrhizae (Nikolova et al., 1997), although the radiocesium concentration varies considerably in different above ground parts of fungal fruit bodies (Heinrich, 1993).

3. TRANSFER FACTORS AND RADIONUCLIDE ACCUMULATION BY FUNGI

3.1 Conventional Transfer Parameters

The quantitative evaluation of activity levels in fungal fruit bodies is a challenging task. The concept of transfer factors and concentration ratios, commonly used in agricultural radioecology, has also extensively been applied to quantify the transfer of radionuclides from soil to fungi. These parameters are commonly expressed as the contamination of plants divided by the contamination of soil. The contamination of plants is usually given as the amount of radioactivity per unit weight, either on a dry weight (Bq/kg dry weight) or a fresh weight basis (Bq/kg fresh weight). The contamination of soil is usually given as the amount of radioactivity per surface area (Bq/m²) or per unit dry weight (Bq/kg dry weight), whereby the latter definition refers to standardized soil depths.

Within the concept of transfer factors, it is assumed that the radionuclide concentration in plants can be described by a linear function passing through the origin. This assumption was criticized to be generally flawed, at least if the concentrations of radionuclides in plants and soils at different sites are simultaneously considered (McGee et al., 1995). Conventionally defined transfer factors are applicable only to equilibrium conditions. Due to the migration of radionuclides in forest soil, such conditions will be attained in natural and semi-natural ecosystems only long time after the contamination event, according to Schell et al. (1996) after about 10-20 years in the case of forest ecosystems. Nevertheless, the concept of transfer factors has been recommended by many agencies: IAEA (IAEA, 1996), IUR (IUR, 1992) and ICRP (ICRP, 1979). As the result, an extensive database of transfer factors for many plants, including mushrooms, has been compiled. The necessity to use dynamic parameters to describe activity levels in plants, especially in forest ecosystems (Schell et al., 1996), is now well accepted by many scientists. IUR is currently collecting these dynamic parameters into a database (FLUX database, Mitchell, 1998). Unfortunately, it will take a long time to design common procedures for the collection of experimental data and the evaluation of appropriate parameters. Therefore, there will probably be no alternative parameters besides transfer factors in the near future. Subsequently, common definitions of transfer factors will be discussed briefly in this section. The next section presents Transfer Factors for specific soil horizons - an approach that promises a significant uncertainty reduction in transfer factors.

Aggregated transfer factors. Aggregated transfer factors (T_{ag}) are defined as the ratio of the activity in plant (Bq/kg fresh weight or Bq/kg dry weight) divided by the total deposition on soil (Bq/m²). The concept of aggregated transfer factors was developed to avoid difficulties related to the radionuclide concentration in soils with a multi-layered structure as is the case for soil horizons in natural and semi-natural environments. Aggregated transfer factors are a useful tool to estimate quickly but only roughly the uptake of radionuclides, notably during the first time after an accidental release. However, aggregated transfer factors suffer from some disadvantages: 1) Because of their definition, they can only give a static picture of activity levels in plants. They are, therefore, only of limited usefulness for dynamic radioecological models. 2) They do not take into account the rooting depth of green plants or the vertical distribution of fungal mycelia in soil, i.e. they do not refer to the specific soil layer, from which nutrients and radionuclides are taken up. It is therefore not surprising that aggregated transfer factors for fungi vary by about two orders of magnitude, even for the same fungal species (Howard et al., 1996).

Transfer factors (concentration ratios) referring to standardized soil depths. Transfer factors referring to standardized soil depths are defined as the ratio of the activity concentration in plant (Bq/kg fresh weight or Bq/kg dry weight) divided by the activity concentration in soil within the uppermost layer of a standardized thickness. This definition was designed especially for agricultural ecosystems. It is based on the assumption that radionuclides are distributed homogeneously within the rooting depth of agricultural plants. Similar to aggregated transfer factors, transfer factors referring to standardized soil depths are of limited usefulness in case of soils with a multi-layered structure and a pronounced vertical profile of activity concentration.

Transfer factors soil solution - plant. Several studies (Horril et al., 1990; Desmet et al., 1991; Tikhomirov et al., 1993; Myttenaere et al., 1993; Schell et al., 1996) suggested that the bioavailability of a given radionuclide in soil, not its concentration, is important for plant uptake. The possibility to calculate transfer factors as the ratio of the activity concentration in plant (Bq/kg fresh weight or Bq/kg dry weight) divided by the activity concentration in soil solution (Bq/l) has been discussed (Desmet et al., 1991). Experimental results for forest soils revealed that the fraction of easily exchangeable radiocesium can be low, but nevertheless radiocesium is highly available for uptake by fungi. This effect can be very pronounced for organic horizons of forest soil. At present, there is no experimental method available to quantify the bioavailable fraction of radionuclides in soil. According to our current understanding of the processes within the organic layers of forest soil, however, most radiocesium in the organic horizons may be available for uptake by fungi. Ruhm et al. (1999) reported that the availabilities for stable ¹³³Cs and radioactive ¹³⁷Cs and ¹³⁴Cs are approximately the same in the organic horizons of a German forest site.

3.2 Transfer Factors for Specific Soil Horizons

In the context of dynamic radioecological models transfer factors (concentration ratios), defined as the ratio of the radiocesium concentration in fungal fruit bodies divided by the radiocesium concentration of the specific soil layer exploited by the mycelium, proved to be useful. This definition of transfer factors was proposed in the late 80s.

Unfortunately, mycelia are very difficult to localize *in situ*. Byrne et al. (1988) and Guillitte et al.

(1990) proposed to use the isotopic ratio $^{134}\text{Cs}/^{137}\text{Cs}$ for that purpose. The approach is based on the idea that the isotopic ratio in fungal fruit bodies should reflect the isotopic ratio of that soil horizon, from which radiocesium is predominantly taken up. The time-dependent isotopic ratio $^{137}\text{Cs}/^{134}\text{Cs}$ turned out to be a "fingerprint" of the different layers of forest soil at several sampling sites, a consequence of the mixing of the residual ^{137}Cs from the global fallout from atmospheric nuclear tests with ^{134}Cs and ^{137}Cs from the Chernobyl fallout. Hence, the location of fungal mycelia in forest soil can be determined by comparing the isotopic ratios $^{137}\text{Cs}/^{134}\text{Cs}$ in fruit bodies with the corresponding values of different soil horizons. This basic idea was developed in an operational tool by Ruhm et al. (1997) who determined the mycelium location for 14 fungal species in German forests.

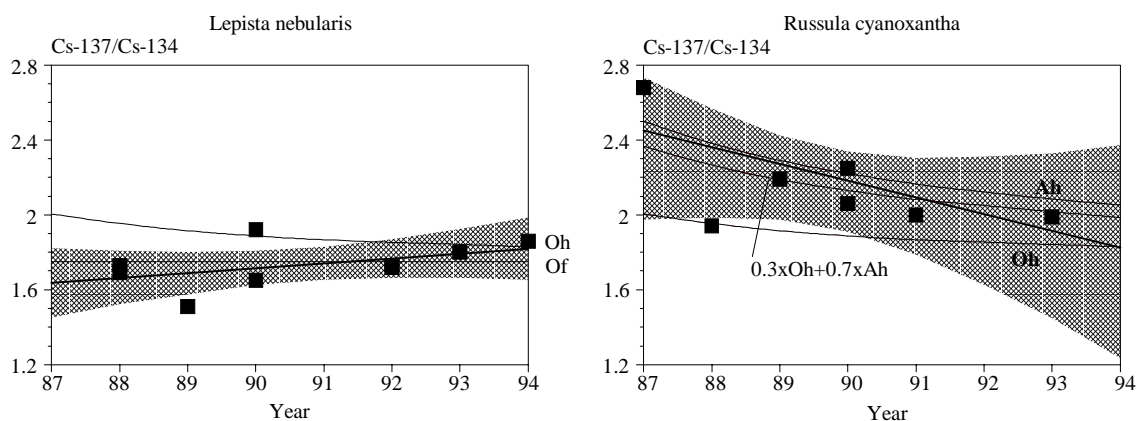


Figure 1. $^{137}\text{Cs}/^{134}\text{Cs}$ ratios as functions of time in two mushroom species, decay-corrected for May 1, 1986. The black boxes denote measurements. The thick full line represents linear regression curves and the shaded areas are the corresponding 95 percent confidence bands. The predictions of a compartment model for the isotopic ratios of different horizons are shown for comparison.

As an illustration, Figure 1 presents the isotopic ratios $^{137}\text{Cs}/^{134}\text{Cs}$ as functions of time measured in samples of *Clitocybe nebularis* and *Russula cyanoxantha* together with the 95 percent confidence bands. The $^{137}\text{Cs}/^{134}\text{Cs}$ ratios for different soil horizons are also shown for comparison. Obviously, *Clitocybe nebularis* has a superficial mycelium located in the L and/or Of horizon. The $^{137}\text{Cs}/^{134}\text{Cs}$ ratios in samples of *Russula cyanoxantha* are significantly higher and indicate that this symbiotic species is supplied with radiocesium from both Oh and Ah horizon.

Unfortunately, ^{134}Cs is a short-lived radionuclide and the application of Ruhm's methodology is very difficult now. Current research projects, however, deal with the question, whether the ratio $^{137}\text{Cs}/\text{stable Cs}$ can alternatively be used to localize fungal mycelia *in situ*. In organic soil layers, where the fraction of cesium fixed within mineral particles is likely to be very small, the ratios radiocesium/stable Cs in fruit bodies were close to those of the soil layers, from which certain species of fungi take up radiocesium (Ruhm et al., 1999). If mycelia colonize deeper horizons, where usually the concentration of mineral particles is increased, the ratios radiocesium/stable cesium in fruit bodies can be higher compared with the ratio in the corresponding soil layer (Tsukada et al. 1998, Yoshida and Muramatsu, 1998). This trend is indeed expected, if a significant fraction of stable cesium is enclosed in mineral particles and thus not available for uptake by fungi.

4. UNCERTAINTY AND VARIABILITY OF TRANSFER FACTORS

The detailed mechanisms involved in radionuclide and heavy metal fixation by fungi are not yet well understood. In spite of this limited knowledge, simple models can be developed to reproduce the measured and predict the future radionuclide concentration in the organic layer compartments and fungal fruit bodies. These models use aggregated parameters, such as residence times for radionuclides in soil compartments and transfer factors. Reducing uncertainty in these parameters is highly desirable for predictive modeling of environmental contamination, human dose and risk assessment, and evaluation of the remedial policies in contaminated ecosystems (Linkov et al., 1997).

Uncertainty and variability should be clearly distinguished in environmental applications. Uncertainty arises when a quantity has a single true value that is not known exactly. Parameter uncertainty is usually considered to arise from empirical uncertainty due to statistical variation, inherent randomness, approximation or other sources. For example, transfer factors referring to a specific soil layer with clear boundaries could be determined quite well, if the fungal mycelia were uniformly distributed within this soil layer. But even in this case, the measurement uncertainty related to the sampling and counting procedure still exists. Model uncertainty arises when it is unclear how to describe the relationship between two or more model parameters. The many different

definitions of transfer parameters soil-fungi are an example of model uncertainty. Up until now, there is no general agreement whether transfer parameters should refer to the total radionuclide inventory of soil, activity concentration within a certain soil depth or the activity concentration of the soil layers exploited by the mycelium. In contrast, variability arises when a quantity has many true values. For example, transfer parameters for different fungal species are variable and the thickness of the organic horizons is also variable. In contrast to uncertainty, variability reflects nature and can not be reduced.

Both uncertainty and variability contribute to the wide ranges that have been reported for the transfer factors for fungi. In this paper we investigate uncertainty and variability in the transfer factors for *Xerocomus badius* and *Boletus edulis*, the two most frequently studied fungal species. *Xerocomus badius* tends to develop mycelia in the holorganic layer (Guillitte et al., 1994). In contrast, *Boletus edulis* tends to explore deeper soil horizons. Even though fungal mycelia can be widely distributed within the soil column (mycorrhizae of *Xerocomus badius* were found at a soil depth of 3 m down in the soil (A. Taylor, private communication)), there should be a soil layer, from which radionuclides and nutrients are primarily taken up. Guillitte et al. (1990) estimated that the mycelium of *Xerocomus badius* is located in the Of layer, while the mycelium of *Boletus edulis* explores the Ah1 horizon. Ruhm et al. (1997) determined the location of the mycelium of *Xerocomus badius* using the isotopic ratio $^{137}\text{Cs}/^{134}\text{Cs}$. They found that it is located in the L/Of/Oh horizons (Ruhm et al., 1997), while the mycelium of *Boletus edulis* was estimated to be in the Oh/Ah horizons (Ruhm, private communication). Below sources of uncertainty and variability are presented and limits to which they can be reduced are discussed.

4.1 Conceptual Uncertainty of Transfer Parameters

A large variation in values of transfer factors has been reported. Even within a single fungal species transfer factors may vary by three orders of magnitude, interspecies variations are even higher (Howard et al., 1996; IAEA, 1996). Subsequently, several approaches, which have been proposed to reduce the uncertainty of transfer factors, will briefly be discussed.

Standardized sampling conditions. A working group of the International Union of Radioecologists defined standard conditions for experimental studies (IUR, 1982). They recommend to take soil cores with a thickness of 20 cm for all agricultural plants and 10 cm for meadows, respectively. However, a compilation of transfer factors for a wide variety of agricultural plants by IUR (Frissel, 1992) still shows confidence intervals of about two orders of magnitude. Recently, Frissel (1998) proposed a classification of transfer factors according to soil types to reduce the uncertainty in estimates of transfer factors. The nutrient status was proposed as a basis for a such classification. Possible classifications of forest soils have also been discussed (Linkov and Schell, 1999), but the utility of this approach is still unclear. Nevertheless, standardized sampling and reporting protocols are very important to establish a reliable data basis of transfer parameters from soil to plant.

Time dependency of transfer factors. Transfer factors are an equilibrium concept. Radionuclide redistribution in soil after an acute deposition, notably vertical migration, generally leads to time-dependent transfer factors, if conventional definitions are used. Transfer factors are expected to peak when the maximum of the vertical radiocesium distribution reaches the rooting zone resp. the soil layer with maximal mycelium density. Many experiments have been designed to study these temporal changes, tabulate time-dependent transfer factors and use them for modeling. Recently, Gillett and Crout (2000) demonstrated statistical evidence for the time dependency of aggregated transfer factors, based on an extensive literature review and experimental data sets. This conclusion has been arrived at, although individual studies often report different time series for different species.

Each of the definitions for transfer parameters soil-fungi presented above has its own limitations. Nevertheless, the variability of transfer factors can significantly be reduced, if explicitly referring to the soil horizons exploited by fungal mycelia. For *Xerocomus badius* and *Boletus edulis*, we have collected papers that either report transfer factors related to the organic layers or give other information that allows an easy calculation of these transfer factors, e.g. activity concentration in fungi and activity concentration in the organic layers or in the top few cm of forest soil. Data are compiled in Table 1. The uncertainty associated with the average value for the transfer factor is less than one order of magnitude, even though the reviewed studies report data for different types of ecosystems, locations and radionuclide deposition levels. Despite this potential reduction of uncertainty, most publications still report transfer factors related to the total radionuclide deposition in forests rather than the fraction located in the organic layer compartments.

4.2 Variability of the Structure of the Organic Layer and Mycelium Location

The idea to refer transfer parameters to those soil layers, where fungal mycelia are located, appears simple, its implementation is very difficult. The primary reason is that the organic layers can have a complex structure and different horizons may be difficult to identify. Moreover, even for a certain species fungal mycelia

can exploit different horizons depending on soil and ecosystem characteristics.

Table 1. Radiocesium¹ transfer factors (Bq/kg dw plant/Bq/kg dw organic layer²) for *Xerocomus badius* and *Boletus edulis*.

Reference	<i>Xerocomus badius</i>					<i>Boletus edulis</i>				
	mean	SD	min	max	N	mean	SD	min	max	N
Rohleder, 1967	5.8		2	8						
Seeger, 1981 ³	24		2	81	8	0.7		0.04	1.5	7
Bem et al., 1990 (1984)						2				
Bem et al., 1990 (1986-1988)	17.5					1.2				
Elstner, 1987			10	20		1	0.1			
Gerzabek et al., 1988	17.9	13.9		53	26	1.8	1.8		11.5	104
Heinrich et al., 1989						0.81				
Heinrich, 1992	17.6		2.9	16.1	13	0.74		1	2	33
Guillitte et al., 1990	18.5		6.8	27.5	91	1.6		1.4	1.8	35
Block, 1993	11.5		5.9	38		1.9		0.6	4.7	
Zagrodzki, 1994	20	15	1	56	20					
Kammerer, 1994	20	14	7	50	9	2.7	1.3	1.8	3.6	3
Reisinger, 1994	3.8	3	0.8	10.3	10	3.3	2.3	1.2	6.2	7
Amundsen, 1996						1.4				
Nifontova, 1997						0.8	0.8			
Shutov, 1996, Shutov, private commn.	20	31			24	2.8	4.5			446
Barnett et al., 1997	4.8		0.1	36	31	4.2		0.1	43	24
Ruhm, 1998	17.2				21					
Ruhm, private communication, 1998						2		1.8	2.2	3
Ruhm, Yoshida, 1999	22.4	2.3								

¹If reported, the value for ¹³⁷Cs was used. Otherwise the total radiocesium concentrations were used in calculations.

²If reported, radiocesium concentrations in Oi or Of horizons were used for *Xerocomus badius* and in Oh or A horizons for *Boletus edulis*. Otherwise the total concentration in the organic layer or in the top reported soil layer were used for the calculations.

³Stable Cs. Data for soil concentration were taken from Ruhm et al. (1999).

In principal, the isotopic ratio ¹³⁴Cs/¹³⁷Cs could be used to identify the location of the mycelium, but the ¹³⁴Cs concentration is now too low at many forest sites to implement this methodology.

Nevertheless, Table 1 and data by Yoshida et al. (1994) show that the range of variation of transfer factors soil-fungi (concentration ratios) can be reduced substantially, if referring to the whole organic layer instead of the traditional soil depth of 20 cm or the total deposition. The range of uncertainty of aggregated transfer factors could probably be reduced significantly, if the area-related inventory of only the organic layers rather than the total soil deposition is taken as a basis (Barnett et al., 1997).

4.3 Variability among different fungal species

The biological differences between different fungal species have often been cited as the primary source of variability of transfer parameters. To overcome this difficulty, several scientists proposed to group mushrooms according to their biological characteristics: parasitic, mycorrhizal and saprophytic (Rommelt et al., 1990; Heinrich, 1993; Sugiyama et al., 1993; Yoshida and Muramatsu, 1994a; 1994b). The danger of naive grouping according to the living habit is shown in our review. Activity levels of radiocesium in *Xerocomus badius* and *Boletus edulis* that should be grouped together according to grouping scheme mentioned above vary by almost two orders of magnitude and exhibit completely different time pattern of contamination.

Recent studies revealed that the total amounts of P, K, Ca, Mg, and the microelements Fe, Zn, Mn, and Al in mycorrhizae vary only within a factor of 4 for 17 different fungal species (Kottke et al., 1998). Autoradiography and quantitative image analysis show that basidiomycetes can accumulate radionuclides in melanized regions of the mycelium (Gray et al., 1996), while undifferentiated hyphae and mycelia of other species (Gray et al., 1995) leak radiocesium to surrounding areas. Interestingly enough, among 17 studied fungal species (Kottke et al., 1998) *Xerocomus badius* has the highest total amount of macro- and microelements,

notably potassium, phosphorus and zinc. It is also one of the strongest accumulators of magnesium, calcium and iron. The high storage capacity of *Xerocomus badius* appeared to be connected to both activity of the hyphal sheath and the frequent occurrence of vacuolar bodies, where deposition can occur (Kottke et al., 1998). Aumann et al. (1989) found that *Xerocomus badius*, in contrast to *Boletus edulis*, contains pileus pigments that bind potassium and cesium ions absorbed from the environment by mycelia and transport them to the fruit body. These findings might explain the higher radiocesium uptake by *Xerocomus badius* compared to *Boletus edulis* (see also Table 1).

Our review indicates that the interspecies variability (i.e. different ability of different species to take up radiocesium) may be overestimated. The differences between transfer factors for *Xerocomus badius* and *Boletus edulis* reported in Table 1 can partially be explained by methodological biases in the determination of the soil layer colonized by the mycelium. It is likely that the transfer factor for *Boletus edulis* is in fact higher than that reported in Table 1. These fungi are likely to develop a significant portion of their mycelium below the organic layer, where the radiocesium concentration is much lower. Therefore, the denominator that is used to calculate transfer factors is likely to be overestimated. The difference in transfer factors for many fungal species is probably closer to the factor of 4 that Kottke et al. (1998) reported for the element accumulation by a variety of fungal species.

The conclusion mentioned above is supported by experimental data for 79 species of Japanese mushrooms (Yoshida et al., 1994). Even though individual measurements of radiocesium levels scatter considerably, the average transfer factors calculated for species with similar mycelium locations were found to be within a narrow range from 5.5 to 13. Transfer factors for different fungal species were found to vary from about 4 to 20 in cultivation experiments with contaminated agar culture (Ban-nai et al., 1994). Therefore, grouping of different fungal species according to the location of their mycelia is expected to reduce the uncertainty of transfer parameters. Moreover, a such grouping is biologically meaningful and useful for predictive modeling.

4.4 Spatial variability

Spatial heterogeneity in radionuclide distribution and the role of fungi are in the list of top priorities for future research in forest radioecology (Linkov and Schell, 1999). The structure of the organic layers as well as the radiocesium deposition in forests can be highly heterogeneous in space. This fact may also influence the experimental determination of transfer parameters soil-fungi. Bunzl et al. (1997) studied small and large scale spatial variability of radiocesium in grasslands. They found that the coefficient of variation for the small scale variability (within 10 m²) is in the range from 40 to 80% and for the larger scale variability up to 90% for different fractions of radiocesium. This finding is consistent with the results of Dahlberg et al. (1997), who found that 60% of the wide range of ¹³⁷Cs activity concentration in sporocarps of *Suillus variegatus* (13.6 - 182 kBq/kg) can be explained by the small-scale within-population variations, while remaining 40% can be due to larger-scale variability among populations.

Our review indicates that transfer factors vary less than the measured activity concentrations, provided transfer factors refer to the radionuclide concentration of that soil horizon, where the mycelium is located (Table 1). Nevertheless, Barnett et al. (1997) found that the variability for the transfer factors is similar to the variations in radionuclide concentrations in fresh mushrooms for several species in the UK.

5. CONCLUSIONS AND RECOMMENDATIONS

Even though a considerable lack of knowledge still exists about the mechanisms and processes involved in radionuclide uptake and retention by fungi, experimental data clearly suggest that fungi are one of the key compartments responsible for the effective retention of radionuclides and heavy metals in the organic layers of forest soil. Fungi are considered to be responsible for the spatial re-distribution of radionuclides. A basidiomycete mycelial cord was reported to transport ³²P more than 50 cm during 5 days in the field. Nikolova et al. (1997) observed a local enrichment of radiocesium by fungal clusters in Scandinavian forests, indicating a horizontal transport of radiocesium in forest soil by fungal mycelia. Gray et al. (1995) reported explicit evidence for such translocations in microcosm laboratory experiments.

Transfer factors and concentration ratios are now being used for aggregated descriptions of radionuclide uptake from soil by fungi. A wide range of transfer factors (more than four orders of magnitude) has been reported for fungi, if activity levels in soil refer to the deposition density (aggregated transfer factors Tag) or the radionuclide concentration in the top 20 cm of soil. Attempts to narrow down this variation by introducing an explicit time dependence of transfer factors or grouping different species according to genus and soil properties can result in a limited reduction of variation. Even though the resulting reduction is small – probably not more than one order of magnitude – such a grouping has been recommended for predictive modeling when developing regulatory policies. This paper clearly demonstrated the danger of a naive species aggregation. Activity levels in *Boletus edulis* and *Xerocomus badius*, which should be grouped together according to simple grouping schemes, differ considerably. The absolute values of radionuclide concentration differ by 2-3 orders of

magnitude. The change of contamination over time is also quite different for the two species: no change or a slight increase for *Boletus edulis* and a clear decrease for *Xerocomus badius*.

The present paper further supports the hypothesis that the uncertainty in transfer parameters soil-fungal fruit bodies can be reduced if these parameters are based on the soil layer, where the mycelia develop and from which nutrients are taken up (Guillitte et al., 1990; Linkov, 1995; Schell et al., 1996; Ruhm et al., 1998). The grouping of fungi according to the mycelial location in soil is expected to reduce the uncertainty of transfer parameters (concentration ratios) to about one order of magnitude. A further reduction without species-specific transfer parameters might be impossible, since interspecies differences probably contribute about one order of magnitude to the observed uncertainty.

In the context of predictive modeling, fungi should be considered as a part of the complex processes within the organic layer (Linkov, 1995; Schell et al., 1996). Transfer parameters, explicitly referring to the soil layer exploited by their mycelia, should be used to quantify the uptake of radionuclides (Ruhm et al., 1997; 1998). For screening purposes, the use of aggregated transfer factors may be sufficient (Barett et al., 1997, Gillett and Crout, 1998).

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REFERENCES

1. Amundsen, G. Gulden, and Per Strand. *Accumulation and long term behaviour of radiocesium in Norwegian fungi*. Sci of the Total Env. 184:163-171; 1996.
2. D.C. Aumann, G.Clooth, B.Steffan and W.Steglich, *Complexation of Cesium-137 by the Cap Pigments of the bay Boletus*. Angew. Chem. Int. Ed. Engl. 28, 453-454 (1989).
3. T.Ban-nai, S.Yoshida and Y.Muramatsu, *Cultivation experiments on uptake of radionuclides by mushrooms*. Radioisotopes. 43, 77-82 (1994; in Japanese).
4. T.Ban-nai, Y.Muramatsu, S.Yoshida, S.Uchida, S.Shibata, S.Ambe, F.Ambe and A.Suzuki, *Multitracer studies on the accumulation of radionuclides in mushrooms*. J. Radiat. Res. 38, 213-218 (1997).
5. C.L.Barnett, B.A.Dodd, P.Eccles, N.A.Beresford, B.J.Howard and J.C.Frankland, *Radionuclide contamination of fruitbodies of macrofungi in England & Wales: A survey of contamination levels and dietary intake habits*. Project No. T07051w1, MAFF Contract No. RP0425. Final report to Ministry of Agriculture, Fisheries and Food. Institute of Terrestrial Ecology, Grange-over-Sands (1997).
6. H. Bem, Lasota, W., Kusmierek, E., Witusik, M. *Accumulation of Cs-137 by mushrooms from Rogozno area of Poland over the period 1984-1988*. J. radioanal. Nucl. Chem Letters, 145:39-46; 1990.
7. BIOMASS. Materials of the IAEA BIOMASS Working Group; 1999.
8. K. Bunzl and W. Schimmack. *Effect of microbial biomass reduction by gamma-irradiation on the sorption of Cs-137, Sr-85, Ce-139, Co-57, Cd-109, Zn-65, Ru-103, Tc-95m and I-131 by soils*. Radiat Environ Biophys 27:165-176; 1988.
9. K.Bunzl, W.Schimmack, M.Belli and M.Riccardi, *Sequential extraction of fallout radiocesium from soil: Small scale and large scale spatial variability*. J. Radioanal. And Nuclear Chem. 226, 47-53 (1997).
10. A.R. Byrne. *Radioactivity in fungi in Slovenia, Yugoslavia, following the Chernobyl accident* J. Environ. Radioactivity 6:177-183; 1988.
11. G.Clint, A.Harrison and D.Howard, *The Release of Caesium-137 from plant litters and effects of microbial activity on this process*. In: *Transfer of Radionuclides in Natural and Semi-Natural Environments* (eds. G.Desmet, P.Nassimbeni and M.Belli), Elsevier Applied Science, London, New York (1990).
12. Dahlberg, I.Nikolova and K.-J.Johanson, *Intraspecific variations in ¹³⁷Cs activity concentration in sporocarps of *Suillus variegatus* in seven Swedish populations*. Mycol. Res. 5, 545-551 (1997).
13. R.M.Danielsson and M.Pruden, *Ecto mycorrhizal Status of Urban Spruce*. Mycologia 81, 335-41 (1989).
14. G.M.Desmet, L.R.Van Loon and B.J.Howard, *Chemical speciation and bioavailability of elements in the environment and their relevance to radioecology*. Sci. Total Environ. 100, 105-124 (1991).
15. J.Dighton, G.M.Clint and J.Poskitt, *Uptake and accumulation of Cs-137 by upland grassland soil fungi: a potential pool of Cs immobilization*. Mycol. Res. 95, 1052-1056 (1991).
16. E.F.Elstner, R.Fink, W.Holl, E.Lengfelder and H.Ziegler. *Radioactivity in fungi, mosses and soil samples of defined biotopes in S.W. Bavaria*. Oecologia 73: 553-558; 1987
17. E.F.Elstner, R.Fink, W.Holl, E.Lengfelder and H.Ziegler, *Radioactivity in fungi, mosses and soil samples of defined biotopes in S.W. Bavaria two years after Chernobyl*. Oecologia 80, 173-177 (1989).
18. J.A.Entry, N.C.Vance, M.A.Hamilton, D.Zabowski, L.A.Watrud and D.Adriano, *Phytoremediation of Soils Contaminated with Low Concentrations of Radionuclides*. Water, Air and Soil Pollution 88, 167-176 (1996).
19. B.H.Fawaris and K.J.Johanson, *Fractionation of Caesium (Cs-137) in Coniferous Forest Soil in Central Sweden*.

- Sci. Total Environ. 170, 221-228 (1995).
20. M.Frissel, *A Preliminary Classification of Soil Ecosystems as a Means to Estimate Expected Soil to Plant Transfer of Radionuclides*. IUR Newsletter 30, 8-13 (1998).
 21. M.J.Frissel, *An update of the recommended soil-to-plant transfer factors of Sr-90, Cs-137 and transuranics*. VIIIth Report of the Working Group Soil-to-Plant Transfer Factors, IUR (1992).
 22. G.M Gadd. *Influence of microorganisms on the environmental fate of radionuclides*. Endeavour 20:150-156;1996.
 23. M.Gerzabek, E.Haunold and O.Horak, *Radioaktivität in Pilzen*. Bodenkultur 39, 37-52 (1988).
 24. S.N.Gray, J.Dighton, S.Olsson and D.H.Jennings, *Real-time measurements of uptake and translocation of ¹³⁷Cs within mycelium of Schizophyllum commune Fr. By autoradiography followed by quantitative image analysis*. New Phytol. 129, 449-465 (1995).
 25. D.H.Griffin, *Fungul Physiology*. John Wiley & Son, New York. 1981.
 26. A.G.Gillett and N.M.J.Crout, *A review of ¹³⁷Cs transfer to fungi and consequences for modeling environmental transfer*. J. Environ. Radioactivity 48, 95-121 (2000).
 27. Guillitte, A.Fraiture and J.Lambinson, *Soil-fungi radiocaesium transfers in forest ecosystems*. In: *Transfer of Radionuclides in Natural and Semi-Natural Environments* (eds. G.Desmet, P.Nassimbeni and M.Belli), Elsevier Applied Science, London, New York (1990).
 28. Guillitte, J.Melin and L.Wallberg, *Biological pathways of radionuclides originating from the Chernobyl fallout in a boreal forest ecosystem*. Sci. Total Environ. 157, 207-215 (1994).
 29. J.L.Harley and S.E.Smith, *Mycorrhizal Symbiosis*. Academic Press, New York; 1983.
 30. G.Heinrich, H.J.Muller, K.Oswald and A.Gries, *Natural and artificial radionuclides in selected styrian soils and plants before and after the reactor accident in Chernobyl*. Biochem. Physiol. Pflanzen 185, 55-67 (1989).
 31. G.Heinrich, *Uptake and transfer factors of ¹³⁷Cs by mushrooms*. Radiat. Environ. Biophys. 31, 39-49 (1992).
 32. G.Heinrich, *Distribution of radiocaesium in the different parts of mushrooms*. J. Environ. Radioactivity 18, 229-245 (1993).
 33. A.D Horril, V.H Kennedy; T.R. Harwood. *The concentration of Chernobyl derived radionuclides in species characteristic of natural and semi-natural ecosystems*. In: Desmet, G. et al., eds. *Transfer of Radionuclides in Natural and Semi-Natural Environments*. Elsevier; 1990.
 34. B.J.Howard, K.Hove, P.Strand and V.Pronevich, *Aggregated Transfer Coefficients*. In: *Proc. International Conference One Decade After Chernobyl*. IAEA-SM-339/149, 247-258 (1996).
 35. IAEA Handbook; 1996
 36. IUR, Report on a Workshop on Measurement of Soil to Plant Transfer Factors for Radionuclides. EURATOM-Ital. Report, Wageningen, Netherlands (1982).
 37. A.G.Jongmans, N.Vanbreemen, U.Lundstrom, P.A.W.Vanhees, R.D.Finlay, M.Srinivasan, T.Unestam, R.Giesler, P.A.Melkerud and M.Olsson, *Rock-eating fungi*. Nature 389, 682-683 (1997).
 38. L.Kammerer, L.Hiersche and E.Wirth, *Uptake of radiocaesium by different species of mushrooms*. J. Environ. Radioactivity 23, 135-150 (1994).
 39. Kottke, X.M.Qian, K.Pritsch, I.Haug and F.Oberwinkler, *Xerocomus badius – Picea abies, an ectomycorrhiza of high activity and element storage capacity in acid soil*. Mycorrhiza 7, 267-275 (1998).
 40. M.M.Last, W.A.Norvell and L.V.Kochian, *Potential for Phytoextraction of ¹³⁷Cs from a Contaminated Soil*. Plant and Soil 195, 99-106 (1997).
 41. Linkov, *Radionuclide Transport in Forest Ecosystems: Modeling Approaches and Safety Evaluation*. PhD Dissertation, University of Pittsburgh, USA (1995).
 42. Linkov, I. and von Stackelberg, K. 1999. *Pilot elicitation of expert judgments on model parameters and research needs in forest radioecology* in: Linkov, I., W.R. Schell, eds. "Contaminated Forests: Recent Developments in Risk Identification and Future Perspectives." Kluwer, Amsterdam.
 43. Linkov and W.R.Schell (eds.), *Contaminated Forests: Recent Developments In Risk Identification and Future Perspectives*, Kluwer, Amsterdam (1999).
 44. Linkov, B.Morel and W.R.Schell, *Remedial policies in radiologically-contaminated forests: environmental consequences and risk assessment*. Risk Analysis 17, 67-75 (1997).
 45. D.Mascanzoni, *Uptake of ⁹⁰Sr and ¹³⁷Cs by mushrooms following the Chernobyl accident*. In: *Transfer of Radionuclides in Natural and Semi-Natural Environments* (eds. G.Desmet, P.Nassimbeni and M.Belli), Elsevier Applied Science, London, New York (1990).
 46. E.J.McGee, K.J.Johanson, M.J.Keating, H.J.Synnott and P.A.Colgan, *An evaluation of ratio systems in radioecological studies*. Health Phys. 70, 215-221 (1996).
 47. NG. Mitchell. *Fruit Models and Data Requirements*. Journal of Environmental Radioactivity (in press).
 48. C Myttenaere; WR Schell, Y Thiry; L. Sombre; C. Ronneau, J. Van der Stegen de Schrieck. *Modeling of the Cs-137 cycling in forest: recent developments and research needed*. Sci. Total Env; 136:77-91; 1993.
 49. MG Nifontova. *Dynamics of long-lived radionuclides in mosses and lichens*. Russian Journal of Ecology [Russian J. Ecol.]; Ekologiya , vol. 28, no. 4, pp. 240-244; no. 4, pp. 273-277, Aug 1997
 50. Nikolova, K.J.Johanson and A.Dahlberg, *Radiocaesium in Fruitbodies and Mycorrhizae in Ectomycorrhizal Fungi*. J. Environ. Radioactivity 37, 115-25 (1997).

51. R.A.Olsen, E.Joner and L.R.Bakken, *Soil fungi and the fate of radiocaesium in the soil ecosystem - a discussion of possible mechanisms involved in the radiocaesium accumulation in fungi, and the role of fungi as a Cs-sink in the soil*. In: *Transfer of Radionuclides in Natural and Semi-Natural Environments* (eds. G.Desmet, P.Nassimbeni and M.Belli), Elsevier Applied Science, London, New York (1990).
52. R.A.Olsen, *The transfer of radiocaesium from soil to plants and fungi in seminatural ecosystems*. In: *Nordic Radioecology: The transfer of radionuclides through nordic ecosystems to man* (ed. Dahlgaard), Elsevier; (1994).
53. Reisinger, *Radiocäsium in Pilzen (Radiocesium in fungi)*. Bibliotheca mycologica, Berlin, 1994.
54. T.K.Riesen, S.Fessenko and K.Higley, *Perspectives in Forest Radioecology..* In: *Contaminated Forests: Recent Developments In Risk Identification and Future Perspectives* (eds. I.Linkov and W.R.Schell), Kluewer, Amsterdam (1999).
55. R.Rommelt, L.Hiersche, G.Schaller and E.Wirth, *Influence of soil fungi (Basidiomycetes) on the migration of Cs 134 + 137 and Sr 90 in coniferous forest soil*. In: *Transfer of Radionuclides in Natural and Semi-Natural Environments* (eds. G.Desmet, P.Nassimbeni and M.Belli), Elsevier Applied Science, London, New York (1990).
56. K.Rohleder, *Zur radioaktiven Kontamination von Speisepilzen*. Deutsche Lebensmittel-Rundschau 5, 135-138 (1967).
57. W.Ruhm, L.Kammerer, L.Hiersche and E.Wirth, *Migration of ¹³⁷Cs and ¹³⁴Cs in Different Forest Soil Layers*. J. Environ. Radioactivity 33, 63-75 (1996) and Erratum in J. Environ. Radioactivity 34, 103-106 (1997).
58. W.Ruhm, L.Kammerer, L.Hiersche and E.Wirth, *The ¹³⁷Cs/¹³⁴Cs Ratio in Fungi as an Indicator of the Major Mycelium Location in Forest Soil*. J. Environ. Radioactivity 35, 129-148 (1997).
59. W.Ruhm, M.Steiner, L.Kammerer, L.Hiersche and E.Wirth, *Estimating Future Radiocaesium Contamination of Fungi on the Basis of Behaviour Patterns Derived from Past Instances of Contamination*. J. Environ. Radioactivity 39, 129-147 (1998).
60. W.Ruhm, S.Yoshida, Y.Muramatsu, M.Steiner and E.Wirth, *Distribution Patterns for Stable ¹³³Cs and their Implications with Respect to the Long-Term Fate of Radioactive ¹³⁴Cs and ¹³⁷Cs in a Natural Ecosystem*. J. Environ. Radioactivity 45, 253-270 (1999).
61. W.R.Schell, I.Linkov, C.Myttenaere and B.Morel, *A dynamic model for evaluating radionuclide distribution in forests from nuclear accidents*. Health Phys. 70, 318-335 (1996).
62. W.R.Schell, I.Linkov, V. Rimkevich, V. Chistic and A. Lutsko, A.M. Dvornik and T.A. Zhuchenko. *Model-directed sampling in Chernobyl forests: general methodology and 1994 sampling program*. Sci. Total Environ. 180, 229-235 (1996).
63. W.R.Schell, I.Linkov, E.Belinkaia and B.Morel, *Application of a dynamic model for evaluating radionuclide concentration in fungi*. In: Proc. of 1996 Int. Congress on Radiation Protection, Vienna, p.2-752 (1996).
64. R.Seeger and P.Schweinschaut, *Vorkommen von Caesium in Höheren Pilzen*. Sci. Total Environ. 19, 253-276 (1981).
65. V.N.Shutov, G.Ya.Bruk, L.N.Basalaeva, V.A.Vasilevitskiy, N.P.Ivanova and I.S.Kaplun, *The role of mushrooms and berries in the formation of internal exposure doses to the population of Russia after the Chernobyl Accident*. Radiat. Prot. Dosim. 67, 55-64 (1996).
66. H.Sugiyama, H.Terada, K.Isomura, H.Tsukada and H.Shibata, *Radiocaesium uptake mechanisms in wild and culture mushrooms*. Radioisotopes 42, 683-690 (1993, in Japanese).
67. A.F.S.Taylor and I.J.Alexander, *Ecomycorrhizal Synthesis with Tylospora Fibrillosa, a member of the Corticiaceae*. Mycol. Res. 95, 381-384 (1990).
68. F.A Tikhomirov.; A.I. Shcheglov, VP Sidorov. *Forests and forestry: radiation measures with special reference to the Chernobyl accident zone*. Sci. of the Total Environment 137:289-305; 1993
69. H.Tsukada, H.Shibata and H.Sugiyama, *Transfer of radiocaesium and stable caesium from substrata to mushrooms in a pine forest in Rokkasho-mura, Aomori, Japan*. J. Environ. Radioactivity 39, 149-160 (1998).
70. S.Yoshida and Y.Muramatsu, *Accumulation of radiocaesium in basidiomycetes collected from Japanese forests*. Sci. Total Environ. 157, 197-205 (1994a).
71. S.Yoshida and Y.Muramatsu, *Concentrations of radiocaesium and potassium in Japanese mushrooms*. Environ. Sci. 7, 63-70 (1994b).
72. S.Yoshida, Y.Muramatsu and M.Ogawa, *Radiocaesium concentrations in mushrooms collected in Japan*. J. Environ. Radioactivity 22, 141-154 (1994).
73. S.Yoshida and Y.Muramatsu, *Concentrations of alkali and alkaline earth elements in mushrooms and plants collected in a Japanese pine forest, and their relationship with ¹³⁷Cs*. J. Environ. Radioactivity 41, 183-205 (1998).
74. P. Zagrodzki, JW Mieltski, M. Krosniak; B. Petelenz. *Accumulation of cesium and radiocaesium in forest litter in selected regions of Poland and its influence on litter-to-mushroom transfer factor*. Biol Trace Elem Res; v. 43-45, p273-7; 1994.