

Radiocaesium in The Fungal Compartment of Forest Ecosystems

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Abstract

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Fungi in forest ecosystems are major contributors to accumulation and cycling of radionuclides, especially radiocaesium. However, relatively little is known about uptake and retention of ^{137}Cs by fungal mycelia. This thesis comprises quantitative estimates of manually prepared mycelia of mainly ectomycorrhizal fungi and their possible role in the retention, turnover and accumulation of radiocaesium in contaminated forest ecosystems.

The studies were conducted in two forests during 1996-1998 and 2000-2003. One was in Ovruch district, Zhytomyr region of Ukraine (51°30"N, 28°95"E), and the other at two Swedish forest sites: the first situated about 35 km northwest of Uppsala (60°05"N, 17°25"E) and the second at Hille in the vicinity of Gävle (60°85"N, 17°15"E).

The ^{137}Cs activity concentration was measured in prepared mycelia and corresponding soil layers. Various extraction procedures were used to study the retention and binding of ^{137}Cs in O_f/O_h and A_h/B horizons of forest soil. ^{137}Cs was also extracted from the fruit bodies and mycelia of fungi.

The fungal mycelium biomass was estimated and the percentage of the total inventory of ^{137}Cs bound in mycelia in the Ukrainian and Swedish forests was calculated. The estimated fungal biomass in Ukrainian forests varied from 0.07 to 70.4 mg g⁻¹ soil, in Swedish forests between 3.6 and 19.4 mg g⁻¹ soil. Between 0.5 to 50 % of the total ^{137}Cs activity in the 0-10 cm soil profile was retained in the fungal mycelia. The ^{137}Cs activity concentration in mycelia was thus higher than that found in soil, and ^{137}Cs activity concentrations in the fruit bodies was higher than that in the mycelium.

The survey study revealed that a major part, around 50 % of the plant-available ^{137}Cs in forest soil, was retained in the fungal mycelium.

The most probable sources of ^{137}Cs for fungal mycelia and fruit bodies of fungi were found to be water soluble substances, humic matter, hemicellulose and cellulose.

Key words: bioavailability, biochemical fractions, litter, organic matter, ^{137}Cs , residual fraction, transfer factor.

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List of abbreviations

AM	Arbuscular mycorrhizae
Bq m ⁻²	Becquerel per Square Metre (deposition unit)
Bq kg ⁻¹ DW	Becquerel per Kilogram Dry Matter (dry matter)
CEC	Cation Exchange Capacity (meq/100 g air-dried soil)
CNA	Chernobyl Nuclear Accident
CNPP	Chernobyl Nuclear Power Plant
DW	Dry Weight
ECM	Ectomycorrhizal Fungi
FAs	Fulvic Acids
FW	Fresh Weight
HAs	Humic Acids
NPP	Nuclear Power Plant
SD	Standard Deviation
SSEP	Semi-Sequential Extraction Procedure
SOM %	Soil Organic Matter Content (percent)
TF	Transfer Factor (Bq kg ⁻¹ mycelium DW/Bq kg ⁻¹ soil DW)

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The present thesis is based on the following papers, which will be referred to by their Roman numerals:

- I. Vinichuk M. & Johanson K. J. 2003. Accumulation of ^{137}Cs by Fungal Mycelium in Forest Ecosystems of Ukraine. *Journal of Environmental Radioactivity*, 64, 27-43.
- II. Vinichuk M., Johanson K. J. & Taylor A.S.F. 2003. ^{137}Cs in the Fungal Compartment of Swedish Forest Soils. *The Science of the Total Environment*. (In press).
- III. Vinichuk M., Johanson K. J., Rosén K & Nilsson I. 2003. Role of the Fungal Mycelium in the Retention of Radiocaesium in Forest Soils. *Journal of Environmental Radioactivity*. Submitted.
- IV. Vinichuk M., Johanson K. J. & Dolgilevich M. 2003. Sources of ^{137}Cs Uptake by Fungal Mycelium in Forest Ecosystems of Ukraine. *Reports of the National Academy of Sciences of Ukraine*, 1, 180-185. (In Russian). Paper is translated into English in full.
- V. Vinichuk M., Johanson K. J. & Dolgilevich M. Sources of ^{137}Cs Uptake by Fruiting Bodies of Fungi in Forest Ecosystems of Ukraine. 2003. *Reports of the National Academy of Sciences of Ukraine*, 4, 172-176. (In Russian). Paper is translated into English in full.

Papers I, IV and V are reproduced by permission of the journals concerned.

Related paper (In Russian). A summary in English is given.

Vinichuk M., Johanson K. J. & Dolgilevich M. 2003. The Distribution of the Fungal Mycelium in Forest Soil in Connection with Organic Matter Composition. *Reports of the National Academy of Sciences of Ukraine*. (In press).

Introduction

Background

The Chernobyl accident: release of radionuclides and deposition

On 26 April 1986, when an experiment was being conducted at unit 4 in the Chernobyl nuclear power plant, the most serious accident in the history of nuclear industry occurred (UNSCEAR, 1988). The reactor was destroyed after an uncontrolled nuclear reaction causing two explosions. During the following 10 days, large quantities of radionuclides were ejected into the environment. The Chernobyl nuclear accident (CNA) produced plumes of radioactive materials that drifted over parts of the Western USSR and a large part of Europe, particularly Scandinavia.

The total activity released from the reactor has been estimated to be around $12 \cdot 10^{18}$ Bq, including between 6 to $7 \cdot 10^{18}$ Bq of radioactive noble gases (IAEA, 1996). The amount released was estimated as 3.5 ± 0.5 % of the total activity present in the reactor or nearly 100 % of the noble gases and 20-60 % of the volatile radionuclides such as iodine isotopes.

The released radionuclides had a complex composition. The radioactive isotopes of iodine and caesium were of special radiological significance. With their short lifetimes, the radioiodines had a greater radiological impact in the short term; the radiocaesium isotopes, with lifetimes in the order of tens of years, will have a greater radiological impact in the long term (Izrael *et al.* 1987; Kiselev *et al.* 1996). The current estimates of the activity released of these nuclides are ^{131}I ca. $2 \cdot 10^{18}$ Bq, ^{134}Cs ca. $0.06 \cdot 10^{18}$ Bq and ^{137}Cs ca. $0.09 \cdot 10^{18}$ Bq or about 50-60 % of the radioiodine in the reactor core at the time of the accident and 20-40 % of the radiocaesium (IAEA, 1996).

The major part of the radioactive discharge occurred between April 26th and May 6th 1986. Material released into the atmosphere was dispersed and eventually deposited back on the surface of earth, usually by wet deposition. As a result a large area of the Ukraine, Belarus and Russia was contaminated mainly by ^{137}Cs and ^{90}Sr , but also by transuranium radionuclides such as $^{238}, ^{239}, ^{240}\text{Pu}$ and ^{241}Am . It was estimated that 46,500 square kilometres (23 % of total territory) in Belarus, and more than 59,000 square kilometres (almost 1.5 % of the European part of total territory) in Russia and 43,500 square kilometres (7 % of total territory) in the Ukraine were contaminated with more than 37 kBq m^{-2} of ^{137}Cs (Izrael *et al.* 2001).

The regions commonly identified as experiencing the greatest contamination include the 'oblasts' (regions) of Homyel', Mogilev, and Brest in southern and western Belarus; Kyiv, Zhytomyr, and Chernygyv in northern Ukraine and Bryansk in southwestern Russia (*Fig. 1*).

In Sweden, the radiocaesium from Chernobyl was mainly deposited in the central part of the country along the Baltic coast (*Fig. 2*). The plume from the emission at Chernobyl on April 26th reached Sweden on the April 27th, whereupon it started to rain in central Sweden.

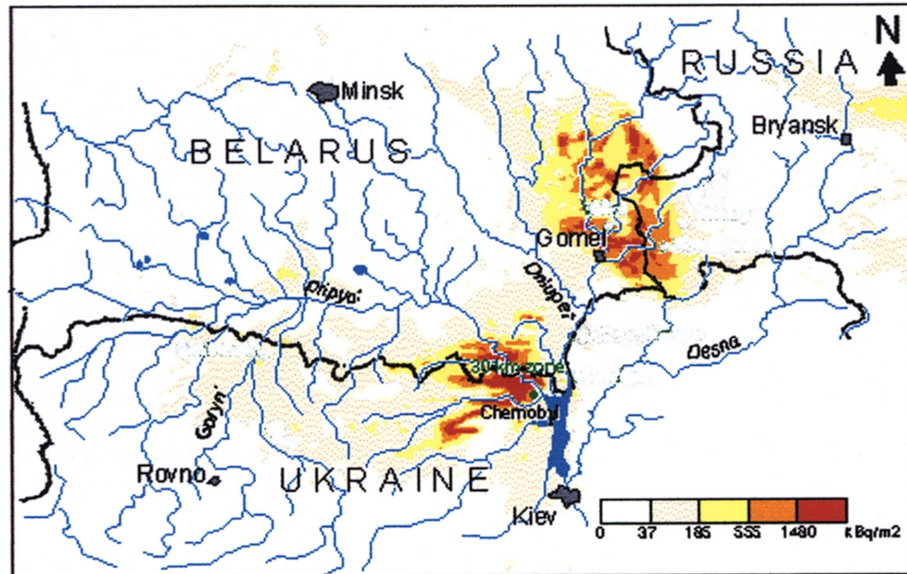


Fig. 1. Areas in Ukraine, Belarus, and Russia contaminated by radiocaesium, from the Chernobyl nuclear power plant accident. (Van der Perk, Gillett & Burema, 2000).

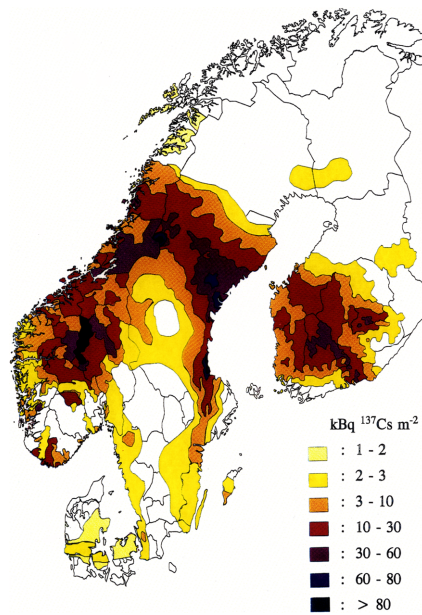


Fig. 2. Ground deposition of ^{137}Cs from Chernobyl in the Nordic countries: Denmark, Finland, Norway and Sweden. (Source: Nordic Radioecology. Ed. H Dahlgaard, 1994).

Particularly in the eastern part, the rain washed out much of the radionuclides which were deposited on the ground or on the vegetation.

During the period May 8th to May 10th, radionuclides emitted from Chernobyl on May 5th once again reached Sweden. It was mainly the southern and the central part of Sweden that was affected by this plume (Persson *et al.* 1986).

Forest ecosystems in Ukraine

The forest area of Ukraine amounts to 10.8 million hectares (ha), of which 9.4 million ha is currently wooded. Compared with some other European countries, the Ukraine is sparsely forested (15.6 % of its territory), with forest concentrated primarily in its western region (Polyakov, 1999; Nijnik & Cornelis van Kooten, 2000) (Fig. 3).

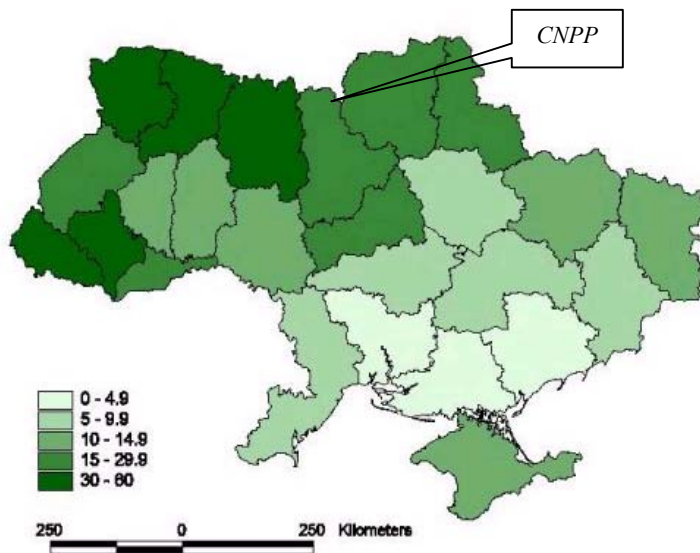


Fig. 3. Percentage of forest cover in Ukraine (Thanks to M. Polyakov (1999) for the map).

For management purposes, federal forests in Ukraine are divided into 2 groups. The area of forest in the first group is 3.4 million hectares including 2.8 million hectares of forest-covered land. This group of forests includes green belts around cities and industrial centres (37.6 %), riparian areas (11.4 %), soil erosion control forests and windbreaks (30.4 %), forest belts along roads and railroads (6.9 %), resort forests, nature preserves and other forests. The second forest group covers 3.7 million hectares including 3.3 million hectares with forest cover, where only limited timber harvest is allowed (Gensiruk, 1992).

There are some 25 indigenous tree species in the Ukraine. Deciduous species include oak (mostly *Quercus robur* (L)) and beech (*Fagus sylvatica* (L)), which are the most common and valuable ones, as well as birch (*Betula pendula* (Roth)) and *Betula pubescens* (Ehrh)), alder (*Alnus glutinosa* (L) Gaerth)), aspen (*Populus*

tremula (L)) and other deciduous species. Pine (mostly Scots pine *Pinus sylvestris* (L)), spruce, mostly Norway spruce (*Picea abies* (L) Karst)), and fir (*Abies lasiocarpa* (Hooker) Nutt)) are the most common coniferous species. The average age of trees in the forest is 40 years, with young stands accounting for 55 % of the area (Gensiruk & Nizhnik, 1995).

In Sweden the dominant tree species are Norway spruce (46 %) and Scots pine (39 %) (Grönare skog. Skogsstyrelsens Förlag, 1999). The productive forest area in Sweden is 23.5 millions hectares.

Contamination of the forest ecosystems

The CNA had a negative impact on forest ecosystems in Ukraine in 1986. A pine forest close to the CNA (a few km from the reactor) received very high amounts of radiation, up to 100 Gy of β -radiation coming from radionuclides intercepted close to the meristem tissue and the trees died forming the “red forest”. Pine is one of the most radiosensitive plants. In the rest of the forested terrestrial area in Ukraine the main problem is the transfer of radionuclides via various pathways to man. In all nearly 2.3 millions hectares of forest was highly contaminated (Nijnik & Cornelis van Kooten, 2000). In Zhytomyr region the greatest areas of forests heavily contaminated with ^{137}Cs are located in Ovruchsky and Narodichsky districts, close to the Belarussian border. In these districts up to 36 % of the forest area was contaminated with a radiocaesium levels of more than 550 kBq m².

Forest occupies 30-40 percent of the most contaminated area in Ukraine and coniferous forest is the most common type, as it is in Sweden. Most of the fallout was intercepted in the canopy of the coniferous forest. During the first year after the deposition the intercepted radionuclides were washed out from the canopy to the ground or on the vegetation in the field layer. Contamination of the ground thus occurred over a prolonged period in contrast to the situation in arable soils.

The experience gained in the aftermath of the Chernobyl nuclear accident has demonstrated that food pathways starting in forests are important sources of radionuclides intake as a result of the consumption of forest products such as berries, mushrooms and game. Since the Chernobyl accident, it has been recognised that agriculturally produced foods are not the only important types of food that contribute to the amount of radiation humans take in. Rural populations within the former Soviet Union produce or gather much of their own diet (Beresford *et al.* 2001). There is a common tradition of collecting edible fungi and berries from the forest. According to Mehli (1998) the mean annual intake rates of fungi in four Russian and two Ukrainian rural settlements ranged from 1.2 to 14 kg fresh weight (FW). Since forest fungi accumulate high levels of ^{137}Cs , they have been found to be the most notable contributors to the internal dose (Bakken & Olsen, 1990; Mietelski *et al.* 1994; Amundsen *et al.* 1996; Shutov *et al.* 1996; Skuterud *et al.* 1997; Barnett *et al.* 1999; Kalač 2001). The consumption of fungi amongst certain populations can provide up to 81 % of daily intake of ^{137}Cs (Beresford *et al.* 1998) and approximately 98 % the daily intake of ^{134}Cs was found to be associated with the readily digestible fraction of the mushrooms (Baezda *et al.* 2003).

This is because ^{137}Cs deposition and retention in semi-natural ecosystems, especially forests, is often higher than in neighbouring agricultural areas (Bunzl *et al.* 1989; Rosén *et al.* 1999). More important, however, is the fact that ^{137}Cs in forest soils often remains highly bioavailable for uptake for a longer period than in agricultural soils (Valcke & Cremers, 1994), and shows low leaching rates (Tikhomirov & Scheglov, 1994; Belli & Tikhomirov, 1996).

Our studies focused on ^{137}Cs behaviour in the fungal compartment of forest ecosystems due to the following main reasons: (i) relatively long half-life (30 y) and high bioavailability; (ii) fungi are a dominant component of the soil microflora in forest soils; (iii) fungi are important in recycling of ^{137}Cs in forests; (iv) radiocaesium accumulates in fungi and they act as a sink for ^{137}Cs .

Ecology of fungi

Fungi are characterised by a distinctive filamentous, multinucleate vegetative structure known as a mycelium. It consists of a branching system of walled tubes, the hyphae, which contain protoplasm and continually extend by apical growth and lateral branching.

Usually, mycorrhizal infection enhances plant growth by increasing nutrient uptake through providing access to nutrient-rich sites spatially separated from the plant roots. In this way fungi increase the bioavailability of nutrients to the host plants and transport nutrients from distant sources to mycorrhizal roots (Marschner & Dell, 1994). Due to the large surface to volume ratio of the hyphae, fungi have a large contact area with the soil environment, and cell metabolism in fungi occurs closer to the surrounding environment in contrast to the vascular plants. Consequently much of fungal biological activity occurs either outside the cell or just at the cell surface (Park, 1968).

A very high proportion of ectomycorrhizal roots and root clusters (70-90%) is usually located in pores between soil particles and is not in direct contact with the soil itself (Read, 1992). Roots situated in such soil pores, although poorly placed to act as nutrient-absorbing organs, are ideally situated to provide nutrients for their mycorrhizal fungi, the mycelia of which appear to be the primary absorptive structure. Rousseau *et al.* (1994) found that while extramatrical mycelia accounted for less than 20% of the total nutrient absorbing surface mass, they contributed nearly 80% of the absorbing surface area of pine seedlings.

To optimise the use of their acquired nutrients, basidiomycetes have developed special organs, strands and rhizomorphs, where hyphae are aggregated longitudinally in varying degrees of complexity to form organs of mycelial migration and food transport (*Fig 4*). Within these rhizomorphs, where often large diameter hyphae surrounded by thinner, often hydrophobic hyphae, the fungi transport water, carbohydrates and nutrients to overcome spatial and temporal heterogeneity in their environment (reviewed by Boddy, 1999).

The total fungal biomass consists of the above-ground part, the fruit body, and the mycelial mats connecting the fruit body to mycorrhiza, and the mycelial “strands”, which spread out in the surrounding soil.

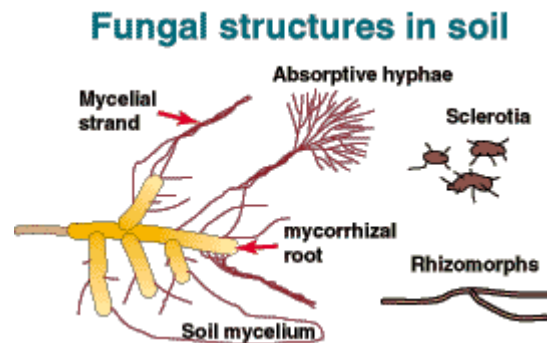


Fig. 4. Diagram of ECM fungus hyphae (Brundrett, <http://www.ffp.csiro.au/research/mycorrhiza/ecm>).

The major part of the fungal biomass is located below the soil surface, and only around 5 % can be seen above the soil surface as fruit bodies (Olsen, 1994) during the mushroom season.

Usually, most of the underground fungal biomass is located in the upper organic layers, where the mycelia are more or less evenly distributed within the soil profile (Olsen, 1994; Reisinger, 1994; Smith & Read, 1997).

Fungi producing large fruit bodies that dominate in forests mainly belong to the basidiomycetes group and are involved in the formation of ectomycorrhiza (Read, 1992). The ectomycorrhizal fungi (ECM) may produce large quantities of hyphae on the host root, comprising some 20-30 % of the total volume of the root. Many ECM fungi also have a sheath, or mantle, of fungal tissue that may completely cover the absorbing root (usually the fine feeder roots).

Coniferous trees colonised by ECM dominate in most of the boreal and temperate biomes of the world, particularly on acidic soils (Steiner *et al.* 2002). In spite of the fact that the diversity of plant species in such forests is low, there is a great diversity of fungal symbionts associated with them (Smith & Read, 1997). Ectomycorrhizal fungi (ECM) (Taylor *et al.* 2000) colonise the vast majority of the fine roots of boreal forest trees.

The hyphae of vesicular-arbuscular mycorrhizae initially grow between cortical cells, but soon penetrate the host cell wall and grow within the cell. The general term for all mycorrhizal types where the fungus grows within cortical cells is endomycorrhiza. Endomycorrhizas are more common and they are found in most plants, including many important crop plants.

The hyphae of ericoid mycorrhizae fungus can penetrate cortical cells, however no arbuscules are formed.

Fungi with a saprotrophic mode of nutrition are called decomposers. By breaking down dead organic material, decomposing fungi continue the cycle of nutrients through ecosystems. While many saprotrophic fungi can utilise cellulose and hemicellulose, there are far fewer, mainly basidiomycetes, that can attack lignin and decompose wood.

Fungi as ^{137}Cs accumulators

Fungi have a great capacity to accumulate mineral nutrients, as well as ^{137}Cs , as observed after the Chernobyl disaster. Radiocaesium was also released after the accident with the graphite reactor at Windscale, Sellafield, in Great Britain as far back as 1957 (Carter *et al.* 1988) and at the Kyshtym accident in the southern part of Ural Mountains (USSR) (Medvedev, 1980). Later, during the 1960s and 1970s research was conducted on uptake and accumulation of ^{137}Cs found in the worldwide fallout from nuclear weapon tests (Grueter, 1971). In order to better understand the mechanisms by which radiocaesium is transported through the environment and made available for plants, the behaviour of radiocaesium in soils has been studied since the Chernobyl disaster in 1986. Collected data, derived from Chernobyl fallout have shown that fungi are a major accumulator of radiocaesium (Gillett & Crout, 2000) and are important in the food chain, contributing to the human radiation intake (Bakken & Olsen, 1990; Amundsen *et al.* 1996; Shutov *et al.* 1996; Barnett *et al.* 1999; Kalač 2001).

Fungi show considerable variability in radiocaesium intake. The fruit bodies of the symbiotic basidiomycetes have been shown to accumulate ^{137}Cs more effectively than saprophytic species (Guillitte *et al.* 1994; Yoshida & Muramatsu 1994; Amundsen *et al.* 1996). ^{137}Cs uptake is determined by the nutrition mechanism of the mushroom. Studies by Baezda *et al.* (2003) showed that mycorrhizal mushrooms accumulate more ^{137}Cs than saprophytes, while the reverse applies, for example, for ^7Be . Therefore some difficulties arise when quantifying radiocaesium within the fungal biomass. Gillett & Crout (2000) reported that ^{137}Cs transfer ratios (defined as the ratio of fungal activity to soil deposit) varied between <0.001 and $> 10 \text{ m}^2 \text{ kg}^{-1}$ among all species and over three orders of magnitude for individual species (*e.g.* *Boletus badius*). Consequently, the radiocaesium content in fungal fruit bodies of different species also varied considerably. For many mycorrhizal basidiomycetes species, Olsen (1994) found variation in the radiocaesium content in fungal fruit bodies of more than 50 (in some cases up to 100) times higher than in other plants sampled on the same site.

According to Guillitte *et al.* (1990, 1994) and Yoshida & Muramatsu, (1994), ^{137}Cs levels in mushrooms were considerably affected by the soil layers in which mycelia were growing. Studies by Römmelt *et al.* (1990) indicate that mushrooms take up nutrients preferably from organic layers of forest soils. By being included into the organic horizon, radiocaesium is integrated very quickly into the nutrient cycling of the forest ecosystems.

On the whole, the ^{137}Cs levels in the mushrooms depend on mycelium habitat and depth (Guillite *et al.* 1994; Yoshida & Muramatsu, 1994; Rühm *et al.* 1997), forest type and fruit body location (Andolina & Cuillitte, 1990) and other factors, such as soil clay content, soil moisture. Studies of ^{137}Cs distribution in the soil layers within the soil profile and in the mycelia from corresponding soil layers may improve understanding of the role of fungi in uptake and turnover of radionuclides. The hypothesis is that distribution of the mycelia is one of the main reasons for the different levels of radiocaesium in fruit bodies of different species grown on the same site (Giovani *et al.* 1990). However, it is impossible to determine *in situ* the

soil section in which the mycelia are located and ideally, such a soil layer should be taken into consideration, *e.g.* for correct interpretation of transfer factors for fungal species (Guillitte *et al.* 1990).

Fungi- mediated translocation of ^{137}Cs

There is much evidence that highly developed and diverse forest microbiota contribute to the long-term retention and translocation of radiocaesium in the top soil organic layer. Fungi were shown to be very important agents for the recycling of nutrients as well as radiocaesium in the upper layers of forest soils (Dighton *et al.* 1991; Thiry & Myttenaere, 1993; Guillitte *et al.* 1994). Further, it was shown that fungi, being one of the most important components of the forest soil ecosystem, are involved in immobilisation and translocation of ^{137}Cs (Tegen *et al.* 1991; Brückmann & Wolters, 1994; Wirth *et al.* 1994; Rafferty *et al.* 1997). In organic-rich layers of forest soil, fungi can directly bind or accumulate radionuclides, and in so doing affect its mobility. Thus Cs may be accumulated and redistributed within the fungal thallus, making it temporarily unavailable to the other components in the ecosystem. As pointed out by Steiner *et al.* (2002) (and references therein) fungi may provide a direct link between small pores of minerals and mycorrhizal plant roots that would effectively bypass the bulk soil solution. Lindahl *et al.* (2002) showed that during the decomposition of plant litter, nutrients are transferred between two major organic pools, that is from plant matter to the fungal mycelia. Only a small fraction is likely to be released as inorganic ions to the soil solution. This means that the bulk soil solution is not the medium that nutrients pass through on their way to the plant, as was assumed earlier.

There have been several reports of the upward mobilisation of ^{137}Cs in forest soils (Brückmann & Wolters, 1994; Rafferty *et al.* 1997; Drissner *et al.* 1998) which suggest that mycorrhizal fungi mediate the transport of radiocaesium from soil to the plant. However, the transport mechanism of such mediation is more complex than simple ion exchange in the soil solution. With such fungi-mediated translocation, the radiocaesium transport occurs simultaneously with the fungal infection of fresh litter material. The decomposition of forest litter was accompanied by an increase in ^{137}Cs activity, mainly due to import of ^{137}Cs by invading decomposing fungi. Recent studies (Fukuyama & Takenaka, 2003) have confirmed the effect of microbial activity on the upward migration of ^{137}Cs . Steiner *et al.* (2002) suggest that this effect would lead to a significant net transfer of radiocaesium from heavily contaminated colonised material to fresh material with low contamination. A similar phenomenon has been demonstrated with basidiomycetous fungi that can take up phosphorus from the soil and transport it to the woody resources, which the fungi use as sources of energy (Wells, & Boddy, 1995; Wells *et al.* 1990). As reported by Wirth *et al.* (1994), mycelia and plant roots even retard the downward migration of ^{137}Cs by upward translocation. By transporting nutrients into the upper horizons, the forest microflora thus significantly accelerates recycling of ^{137}Cs in forest ecosystems (Brückmann & Wolters, 1994).

It was found that the density of hyphae in soil affects the transport activity of ^{137}Cs , however, the effect of hyphae density on radiocaesium uptake needs to be a subject

of future investigation (Driscoll *et al.* 1998). Furthermore, knowledge of the mechanisms and processes involved in the mobilisation, translocation and uptake of radionuclides is still limited. The precise role of mycorrhizal fungi, which may also act on the Cs soil-to-plant transfer, has been poorly documented so far (Kruyts & Delvaux, 2002).

Fungi and decomposition of organic matter

In boreal forest ecosystems, as in most terrestrial ecosystems, fungi play a very important role in the transformation of SOM. Having the highest biomass in the decomposing organic layers, fungi serve as the most important sources of enzymes to degrade forest litter. In the acid forest soils, fungi are believed to be the main decomposer organisms (Swift *et al.* 1979). Fungi are almost exclusively responsible for the decomposition of woody plant tissues (Tanesaka *et al.* 1993). The most significant activity of fungi is their special ability to break down polysaccharides such as cellulose, which make up 40-60% of the content of wood. By using enzymes, fungi break down macromolecular complexes and make them available for plant uptake (Griffin, 1981). Smaller molecules (simple sugars, amino acids, etc) which are in solution and in the water surrounding the hyphae can be directly absorbed by the hyphae, but larger insoluble polymers (cellulose, starch and proteins) must undergo some digestion before they can be used (Swanson, 1972). By producing various acidic compounds, like citric, acetic and some other organic acids, fungi are actively involved in the decomposition and humification of organic residues. Many saprotrophic fungi can also utilise hemicellulose, and some, mainly basidiomycetes, can attack lignin (Swanson, 1972; Ingold, 1979). There is increasing evidence that not only saprotrophic but also some symbiotic ectomycorrhizal (ECM) fungi release enzymes enabling them to take part directly in decomposition activities (Smith & Read 1997). According to Haider & Domsch (1969) the basidiomycetes are the most efficient lignin decomposers in soil.

Potassium, Rubidium and Caesium uptake by fungi

The chemical behaviour of radiocaesium is expected to be similar to that of stable caesium, potassium and rubidium, which all belong to group I (the alkali metals) in the periodic table. A key factor for recognising the high plant availability of radiocaesium in biological systems is its chemical similarity to potassium (Nylén 1996 and references therein). Radiocaesium absorbed by roots follows transport systems similar to those of potassium (Shaw & Bell, 1989). Laboratory experiments (Buysse, *et al.* 1996; Smolders *et al.* 1997) provide evidence that a potassium concentration above 1 mM in the external solution influences radiocaesium absorption, which may slightly increase or decrease depending on plant species. At a potassium concentration below *ca.* 1 mM, the root uptake of radiocaesium increases significantly (Cline & Hungate, 1960; Smolders *et al.* 1996). Generally, the concentration ratios of ¹³⁷Cs, stable Cs and Rb for mushrooms were at least one order of magnitude higher than those for vascular plants growing in the same forest (Ban-nai *et al.* 1997; Yoshida & Muramatsu, 1998). The corresponding ratio for K was found to be around four times higher and Cs uptake did not correlate with potassium uptake in mushrooms in contrast to vascular plants (Ismail, 1994; Yoshida & Muramatsu, 1998). Potassium

concentration in mushrooms varied within a narrow range, while the concentration of Cs had a very wide range. The suggestion is that the mechanism of Cs uptake is different from that of K (Yoshida & Muramatsu, 1998). Rb showed intermediate behaviour between that of K and Cs, and it is supposed that Rb might be partly taken up by mushrooms by the same mechanism as Cs. According to Vogt & Edmonds (1980), potassium was concentrated at significantly higher levels in fruit bodies than in the forest floor in all ecosystems investigated.

Fungi as a sink for ^{137}Cs

The role of the fungal mycelium in keeping ^{137}Cs in the upper layers of soil might be important in terms of temporary immobilisation of radiocaesium in the forest ecosystems. Prevention of leaching of ^{137}Cs from soil by the fungal mycelium will result in an increased retention of it in the upper soil horizons. Studies (Nikolova *et al.* 2000) showed that a substantial fraction of the ^{137}Cs in the forest soil may in some way be associated with soil organisms, probably with the fungal compartment. Efflux studies (Dighton *et al.* 1991) indicate that more than 40 % of the Cs taken up is retained within the fungal hyphae. Many studies (Clint *et al.* 1991; Dighton *et al.* 1991; Brückmann & Wolters, 1994; Guillitte *et al.* 1994; Paper I showed that fungal mycelia may effectively retain at least temporarily significant levels of radiocaesium. Thus fungal mycelia were found to be a sink for radiocaesium (Olsen *et al.* 1990). However, the results obtained were mainly based on indirect methods for measuring soil microbial biomass, which have some limitations and disadvantages (Martens, 1995).

Role of organic matter in retention and turnover of ^{137}Cs in forest soil

Organic matter content in forest soils is often the dominant part in the upper soil layers and seems to be important for the retention and bioavailability of ^{137}Cs . However the retention mechanisms of radiocaesium in forest soils with a high organic matter content remain unclear. With an organic matter content in soil of 10-40 % or more, the fixation levels of ^{137}Cs associated with the SOM were shown to be up to 50 % (Valcke & Cremers, 1994). Later studies (Lofts *et al.* 2002) showed however that organic matter might play only a minor role in binding Cs, even in highly organic soils. In contrast to a mineral soil, caesium cannot be fixed physico-chemically in the organic layer (Lieser & Steinkopff, 1989), since soil organic matter has no well-defined capacity to bind reversibly exchangeable ions (Flaig *et al.* 1975). Humic substances sorb ^{137}Cs only weakly or non-specifically (Stevenson, 1982; Valcke & Cremers, 1994).

Further studies on the physiochemical state of ^{137}Cs in the substrates are required to understand and quantify the role of microbial (fungal) radiocaesium retention (Yoshida & Muramatsu, 1994). The mechanisms that support low mobility and high bioavailability of ^{137}Cs are poorly understood, taking into account that the clay content in upper horizon of forest organic soils is usually very low (Lieser & Steinkopff, 1989). It is not known if specific binding of ^{137}Cs to some fungal biomolecules or superstructures occurs.

The reason for the lack of understanding of the fungal mycelium's role in the turnover of radionuclides seems to be that it is the most difficult part of the forest ecosystem to experimentally examine and manipulate. Associated with mycorrhizal roots, mycelia cannot be easily extracted from soil and examined in a non-destructive manner. Therefore there are still few data in the literature concerning the accumulation of radionuclides by fungal mycelia in forest soils, mainly due to the complexity of the forest soil system, where many biomolecules are involved. Difficulties arise associated with preparation of mycelia as well as the other potential caesium binders among the biomolecules.

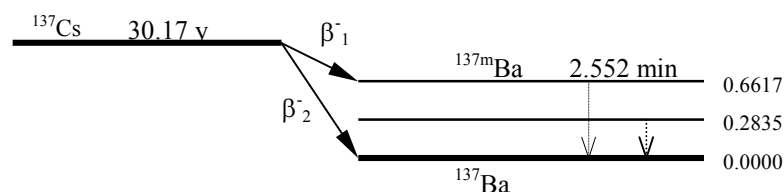
Cs isotopes and radiometry

Because of high ^{137}Cs abundance, long physical half-life and biological mobility, ^{137}Cs is a critical component of the nuclear fuel cycle as well as of the ambient environment.

Table 1. Fission yields of ^{137}Cs (Crouch, 1977)

Nuclide	Fission yield, %	
	Thermal	Fast
^{235}U	6.21	6.12
^{239}Pu	6.64	6.50
^{238}U		5.93
^{232}Th		6.73

The radioactive isotope ^{137}Cs is produced in nuclear fission and is one of the more significant fission products. The fission yield is relatively high, about 6 atoms per 100 fissions, independent of the type of fission in uranium or plutonium (Table 1).



Radiation type	Energy (keV)	Intensity (%)
^{137}Cs		
β_1^-	max. 511.6 (avg. 173.5)	94.4
β_2^-	max. 1173.2 (avg. 415.4)	5.6
Total β	avg. 186.6	100.0
$^{137\text{m}}\text{Ba}$		
γ_1	661.6	89.9

Fig. 5. The decay scheme of ^{137}Cs (Adapted from Environmental health criteria for selected radionuclides. World Health Organization, Geneva, 1983).

It has a radioactive half-life of 30.17 y and its beta decay is accompanied by a gamma ray of moderate energy. Figure 5 shows the decay scheme and lists the primary transition energies.

Together with ^{137}Cs , ^{134}Cs was also an important constituent of the radioactive material released in the accident. In terms of the activity (*e.g.* in Bq or Ci), its release was around 56 % of that of ^{137}Cs . Because of its much shorter half-life (2.06 y) the deposition levels of ^{134}Cs have declined rapidly compared to ^{137}Cs . Some 12 years after the accident, the residual deposition levels (in terms of activity) of ^{134}Cs are only about 1 % of those of ^{137}Cs .

The energies of β - and γ rays of ^{134}Cs decay (MeV): β - 0.089 (27 %), 0.66 (70 %), γ 0.605 (98 %), 0.795 (85 %) (Eisenbud, 1987).

^{135}Cs with its long half-life ($3 \cdot 10^6$ y) is a pure β - emitter and may be important from the viewpoint of long-term storage of radioactive waste.

Aims

The general aim was to study the behaviour of radiocaesium in Chernobyl-affected forest ecosystems of Ukraine and Sweden and to consider the role of fungi in radiocaesium retention and turnover. The study presented here focused particularly on the fruit bodies of fungi and fungal mycelia. The specific aims were:

- to quantify the vertical distribution of the fungal biomass in the upper 10 cm of the upper forest soil profiles by manually preparing the major fraction of fungal mycelia,
- to quantify the ^{137}Cs activity in the soil, fruit bodies as well as in the prepared fungal mycelia in the same soil profile,
- to quantify the amount of total ^{137}Cs activity bound to the fungal biomass in the upper layers of forest soil,
- to quantify the relative importance of soil organic matter, fungal mycelia and fruit bodies for the binding of ^{137}Cs in the forest floor,
- to quantify the relative importance of different chemical components of soil organic matter for the binding of ^{137}Cs ,
- to determine the possible sources of ^{137}Cs uptake by fungal mycelia and fruit bodies of fungi in forest ecosystems.

Materials & Methods

Sampling area

The studies were conducted in two forest ecosystems. One was in Ovruch district, Zhytomyr region of Ukraine (51°30"N, 28°95"E), and the other at two Swedish forest sites: the first situated about 35 km northwest of Uppsala (60°05"N, 17°25"E) and the second at Hille in the vicinity of Gävle (60°85"N, 17°15"E).

The study area in Ukraine was located more than 70 km west of the Chernobyl Nuclear Power Plant (Paper I). The ground deposition of ^{137}Cs in these forest regions was between 30 to 750 kBq m². The major type of soil in the study area was a soddy podzolic soil. Organic matter content in the soil profiles varied from 5.4 to ca. 70 % for the top 1-3 cm layers and decreased to 0.2 % at a depth of 10 cm. Thickness of the organic horizons did not exceed 2-3 cm. The mean clay content of the soil was 5.7 % (min 2.0 - max 19.7), and the mean CEC 7.8 meq/100g (min 1.0 – max 18.8).

In Scots pine stands the major part of annual litter fall consists of needles (52-58 %), branches (11-33 %) and cones (25-50 %). In mixed stands the major part of the tree litter fall consists of leaves (54-79 %), branches (31-34 %) and conifer needles (16-25 %) (Kremenetska, 2000).

The dominant trees were Scots pine (*Pinus sylvestris* (L)) with some intermixture of birch (*Betula pubescens* (Ehrh)). The stand was approximately 30-50 years old. In the field layer, the most common plants were bilberry (*Vaccinium myrtillus*), lingonberry (*Vaccinium vitis-idaea*) and heather (*Calluna vulgaris*). The ground was partly covered by mosses and lichens.

In Sweden soil samples were collected from two sites (Paper II). The first site was located at Stalbo near Heby, about 35 km northwest of Uppsala. Soil samples were taken from a bog with slow-growing Scots pine (*Pinus sylvestris* (L)) and a very high organic matter content, and from a rocky area close to the bog with a medium organic content. The ground deposition of ^{137}Cs in the 0 to 10 cm layer was about 30 kBq m². The dominant trees were Scots pine from 60 to 100 years old. In the field layer the most common species were bilberry (*Vaccinium myrtillus*), lingonberry (*Vaccinium vitis-idaea*) and heather (*Calluna vulgaris* (L) Hull) and in the bog also cloudberry (*Rubus chamaemorus* (L)), crowberry (*Empetrum hermaphroditus*) and wild rosemary (*Ledum palustre* (L)). Mosses and lichens mostly covered the ground.

The second site in Sweden located at Hille, about 10 km north of Gävle was an 80- to 100-year-old stand consisting of a mixture of Norway spruce (*Picea abies* (L) Karst) and Scots pine (*Pinus sylvestris* (L)). The upper 10 cm of the soil had a rather high organic content – from 29 to 96%.

In the field layer the dominant plants were bilberry and lingonberry and a moss layer covered the ground. The ground deposition of ^{137}Cs in the upper 10 cm was about 100 kBq m².

Sampling

We started sampling in Ukraine (Paper I) by collecting fruit bodies of certain fungi species and then soil samples were collected to a depth of 10 cm from 4-5 spots within an area of about 0.5 m² around and directly underneath the fruit body. Samples of forest soil and fruit bodies were collected simultaneously during the period from July to October 1995-1998.

Soil samples for semi-sequential extraction (SSE) (Paper III) were collected during summer and autumn 1995 using steel bore (diameter 5.0 cm, height 10 cm). In the laboratory soil cores were divided in two fractions, one organic rich fraction (O_f/O_h horizons) and one organic poor fraction (A_f/B horizons). The soil samples were air dried at room temperature and then milled.

In 1996, 6 different species of fruit bodies and 60 corresponding soil samples were collected and analysed. The soil cores were sectioned horizontally directly in the forest into 2 cm layers. Additionally, in 1996 samples of leaf litter and other litter and soil were taken at the five experimental plots for analysis of SOM. The soil samples were taken from the depths 0-2, 2-4 and 4-6 cm (O) and from the mineral soil layer of 6-12 cm (A). Composite samples were prepared to represent each experimental plot. The leaf litter was collected using open containers with a cross section of 0.25 m² (0.5 x 0.5 m). Five containers were put under the trees in each experimental plot. Composite samples of leaf litter were prepared to represent each plot, dried at a temperature of 90 °C and milled.

In 1997, using a cylindrical steel bore with a diameter of 5.35 cm, six species of fruit bodies and 120 corresponding soil samples were collected. The soil samples were sliced into 1-cm thick sections directly in the forest.

In 1998, four species of fruit bodies and 24 corresponding soil samples were collected.

The sampled fruit bodies were identified to species. ¹³⁷Cs activity in soil samples and fruit bodies was determined. Then all soil samples and fruit bodies were dried at room temperature, the DW was determined and samples were ground to a powder before the semi-sequential extraction procedure. An aliquot of the soil samples was ignited at 550 °C for determination of SOM.

Samples from the Swedish forest soil (Paper II) were taken during September to October 2000 from 3-4 spots at each site in an area of about 1 m² to the depth of 10 cm using a cylindrical steel bore with a diameter of 5.8 cm. All cores were sectioned horizontally, in the forest, into 1-cm thick layers and the slices from the 3 to 4 sub-samples were pooled together. In the laboratory, ¹³⁷Cs activity concentrations were determined in the fresh soil samples from each layer. Afterwards, an aliquot of soil (4-5 g) was taken to determine the dry mass. The samples were dried at 105°C to constant weight. The mycelia were extracted from the rest of the fresh soil.

Preparation of mycelia

The mycelia from each layer of the soil profile were prepared under microscopic examination (magnification 64 times) using tweezers and by adding distilled water

to the soil (Paper I and II). It was not a pure fraction of mycelia as it contained hyphae, strands, rhizomorphs, sclerotia and also some small mycorrhizal rootlets. A rough estimation of the composition of the fungal fraction obtained was done at the same magnification. The mycelia fractions were stored cold until radiometric measurement was carried out. The major part of the mycelia, at least that visible under microscope with the magnification we used, was extracted from soil. The prepared mycelia taken from the Ukrainian forest was not identified to species. However we assumed that the prepared mycelia belonged to the identified fruit bodies of fungi, since soil samples were taken directly underneath fruit bodies, and the structure of the isolated mycelia within a soil profile seemed to be rather similar to each other and usually different from mycelia from other soil profiles. We cannot exclude the possibility that other species contributed to the presented biomass, however, at least the major part of the mycelia belong to the same species as the fruit bodies (Paper I). Mycelia prepared from Swedish forest soils were identified to species in accordance with Agerer (1987, 1991 and 1998) (Paper II). With the exception of site 2, it was obvious that a single fungal taxon formed the majority of the fungal structures within a sample. These were identified as being formed by *Tylospora spp.*, (site 1), *Piloderma fallax* (site 3) and *Hydnellum peckii* (site 4). At site 2, *Cenococcum geophilum* and *P. fallax* occurred in more or less equal quantities. After determination of the ^{137}Cs activity concentration in the mycelia, the samples were dried at 30 °C to constant weight for determination of DW.

Semi-sequential extraction of the ^{137}Cs from fungi and the upper soil layers

Fruit bodies. Extraction of ^{137}Cs from fruit bodies was done using water at room temperature and water at 80 °C (Paper III).

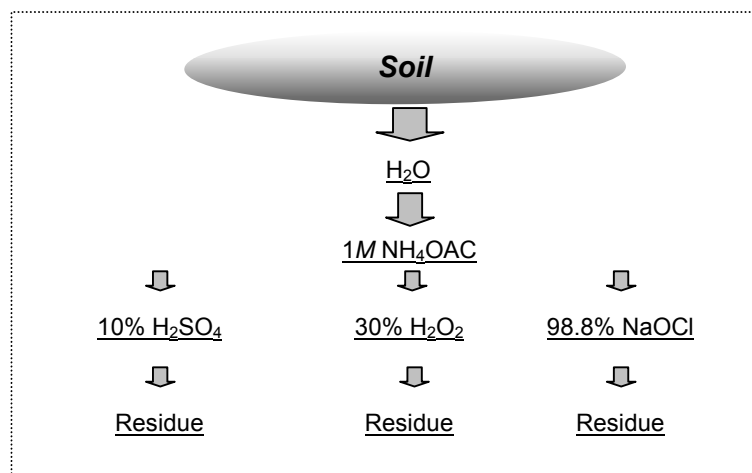


Fig. 6. Simplified scheme of semi-sequential extraction of ^{137}Cs from soil (Adapted from Stevenson, (1982) and Kononova, (1963)). ^{137}Cs activity was determined in all fractions obtained and soil residues.

Mycelia. 0.1 - 0.5 g of freshly prepared mycelium from soil was treated with distilled water (10 - 20 ml) followed by extraction with 1 M NH_4OAc .

Soil. The semi-sequential extraction of ^{137}Cs from organic-rich (O_f/O_h) and organic-poor (A_h/B) soil layers was done in several steps (*Fig. 6*) and fully described in paper III.

Biochemical analysis procedure

The biochemical analyses of leaf litter and soil are described in papers IV and V and a general scheme is shown in figure 7.

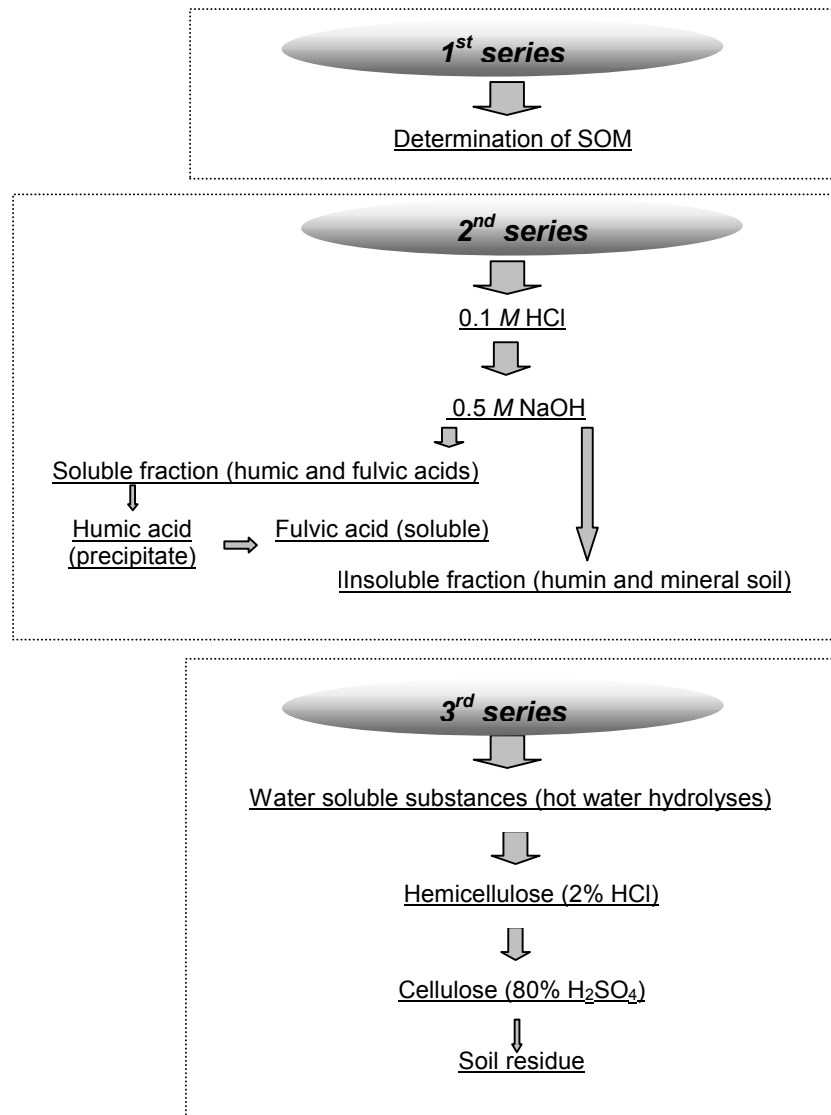


Fig. 7. Simplified scheme of biochemical analysis (Adapted from Stevenson, (1982) and Kononova, (1963)). ^{137}Cs activity was determined in all fractions obtained and soil residues.

The total SOM was determined by dry ashing of each sample at 550 °C.

Humic substances, *i.e.* humic acids (HAs), fulvic acids (FAs) and humins (+ mineral soil) were extracted and determined in accordance with Stevenson (1982): 40 g of an acid-washed (0.1 M HCl) soil sample was treated with 200 ml of 0.5 M NaOH solution, shaken for 12 hours before centrifugation, leaving a soluble fraction (HAs and FAs) in liquid phase and an insoluble fraction (humins and mineral soil) in solid phase. The dark-coloured supernatant liquor was decanted off, filtered through glass wool to remove suspended plant material and adjusted to a pH of about 1.0 with concentrated HCl.

The solution was left alone to allow the HAs to settle. The procedure was repeated, and the mixture shaken for one hour. Finally, the humic acids were separated from the fulvic acids by centrifugation. The residual organic matter (humins), not dissolved in aqueous acidic or basic media is left insoluble. Humins likely contains glomalin, significant amounts of aliphatic hydrocarbons, fatty acids/esters, waxes and trapped HAs and FAs (Hayes & Clapp, 2001). Large amounts of lignin oxidation products is present here, although lignin is also present in the humic acid fraction, and to some extent in the fulvic acid fraction (Kögel-Knabner, 1992).

In another series of samples, water-soluble substances, hemicellulose, cellulose and soil residue were determined.

Water soluble substances were isolated by hot water extraction, followed by evaporation of an aliquot of the extract and weighing of the dry residue.

The dry residue after hot extraction was subjected to hydrolysis with 2 % HCl. During hydrolysis hemicellulose was destroyed and sugars formed. Using Bertran's method (Kononova, 1963) the amount of reducing sugars was determined. The method is based on the ability of sugars to reduce Cu^{2+} ions. The amount of Cu^{2+} reduced is equivalent to that of the sugars. The hemicellulose content is equivalent to the sugars' content multiplied by a factor of 0.9.

After determination of hemicellulose, the sample residue was subjected to hydrolysis in 80 % H_2SO_4 . Cellulose content was determined by Bertran's method. The residue after hydrolysis with 80 % H_2SO_4 was determined as the difference between total organic matter and the sum of all fractions: water-soluble, hemicellulose and cellulose.

Radiometry

The ^{137}Cs activity concentrations in soil and fruit bodies of fungi samples were determined using well-calibrated HP Ge detectors at the Department of Radioecology and Department of Soil Sciences, the Swedish University of Agricultural Sciences, Uppsala. The standard deviation due to the random decay of ^{137}Cs was usually below 5%. The ^{137}Cs activity concentration was expressed as Bq kg^{-1} DW. The ^{137}Cs activity in biochemical fractions obtained and mycelium samples were determined by using a NaI(Tl) crystal scintillation detector (Packard Co) at the same departments. The measuring time was 300 minutes giving a standard deviation of about 5-10%. A measurement of ^{137}Cs activity concentration in various liquid extracts was also done at the laboratory of the Radiation

Protection and Radioecology Institute, Hannover University, Germany using HP Ge detectors. All results were decay-corrected to the date of sampling.

Results & Discussion

Estimation of fungal biomass in the soil profile

The fungal biomass was calculated as mg g^{-1} dry weight of the soil from which the mycelium was extracted, or as mg cm^{-3} (Papers I and II). Calculation of the fungal biomass as mg cm^{-3} seems to be more relevant in this case since soil bulk density increases with depth. In the Ukrainian forest the estimated fungal biomass varied from 0.1 to 70.4 mg g^{-1} soil. The arithmetic mean of the weight of fungal mycelium in the soil profile to 10 cm was 7.3 mg g^{-1} soil; and 59 % of total fungal biomass was located in the upper 0 - 4 cm layers. By using another approach the fungal biomass was calculated as 9.8 mg cm^{-3} (arithmetic mean) with a range of 0.1 – 120.9 mg cm^{-3} . At the sites, with *Lactarius vellereus*, *L. necator*, *Suillus luteus*, *Cantharellus cibarius* and *Xerocomus subtomentosus*, a major fraction of the biomass of the mycelium was found in the first 3-4 cm of the soil profile (Fig. 8). At other sites of *Boletus edulis*, *Paxillus involutus*, and *Sarcodon imbricatus*, the biomass of the mycelium was more deeply located and rather homogeneously distributed within the upper 10 cm of the soil profile.

The largest fungal biomass was found at the sites of *Lactarius necator*, *L. vellereus*, *Cantharellus cibarius*, *Xerocomus subtomentosus*, *Amanita muscaria* and *Sarcodon imbricatus* (Table 2).

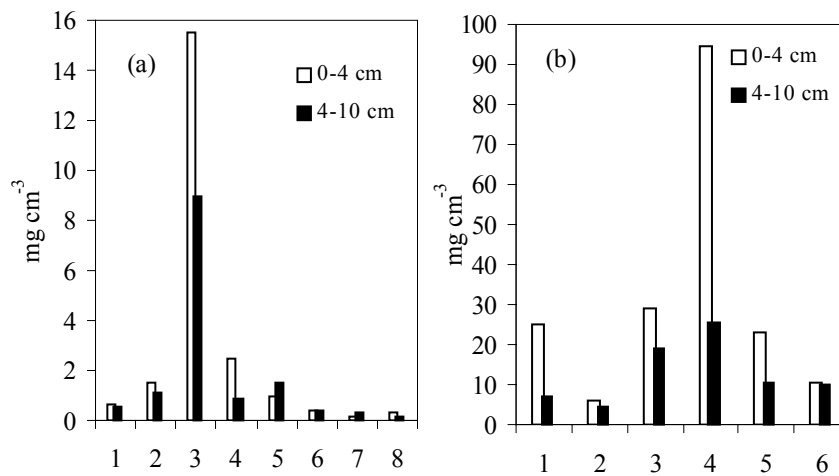


Fig. 8. Distribution of the fungal biomass within the soil profile at depths of 0-4 and 4-10 cm, mg cm^{-3} (Ukrainian forest sites). a - 2 cm intervals: 1 - *Lactarius vietus*, 2 - *Boletus edulis*, 3 - *Lactarius vellereus*, 4 - *Suillus luteus*, 5 - *Suillus granulatus*, 6 - *Paxillus involutus*, 7 - *Amanita muscaria*, 8 - *Boletus edulis*. b - 1 cm intervals: 1 - *Cantharellus cibarius*, 2 - *Leccinum aurantiacum*, 3 - *Amanita muscaria*, 4 - *Lactarius necator*, 5 - *Xerocomus subtomentosus*, 6 - *Sarcodon imbricatus*.

Table 2. The fungal biomass as a mean for 0-10 cm of the forest soil profile

Sites		mg g ⁻¹	mg cm ⁻³
Ukrainian forest sites (2 cm intervals)	<i>Lactarius vellereus</i>	9.29	11.56
	<i>Boletus edulis</i>	1.14	1.30
	<i>Suillus luteus</i>	0.96	1.50
	<i>Suillus granulatus</i>	0.62	1.28
	<i>Paxillus involutus</i>	0.36	0.39
	<i>Lactarius vietus</i>	0.29	0.58
	<i>Boletus edulis</i>	0.15	0.23
	<i>Amanita muscaria</i>	0.13	0.26
Ukrainian forest sites (1 cm intervals)	<i>Lactarius necator</i>	21.5	52.94
	<i>Xerocomus subtomentosus</i>	9.40	15.36
	<i>Amanita muscaria</i>	7.46	22.83
	<i>Sarcodon imbricatus</i>	7.36	10.09
	<i>Cantharellus cibarius</i>	4.10	15.58
	<i>Leccinum aurantiacum</i>	1.67	5.03
Swedish forest sites (1 cm intervals)	<i>Tylospora spp.</i>	3.31	0.17
	<i>Cenococcum geophilum and Piloderma fallax</i>	4.45	0.45
	<i>Piloderma fallax</i>	11.56	1.47
	<i>Hydnellum peckii</i>	10.26	1.37

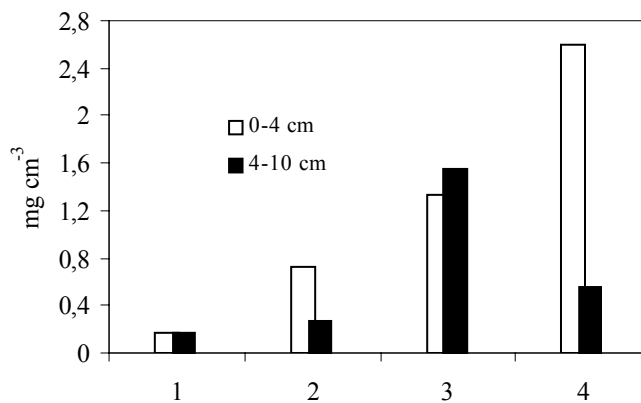


Fig. 9. Distribution of the fungal biomass within the soil profile at the depths of 0-4 and 4-10 cm, mg cm⁻³. 1 *Tylospora spp.*; 2 *Cenococcum geophilum* and *Piloderma fallax*; 3 *Piloderma fallax*; 4 *Hydnellum peckii*. Swedish forest sites.

In the Swedish forests fungal biomass was also calculated as mg g⁻¹ or mg cm⁻³. The mycelium from the peat soil at the *Tylospora spp.* site was rather homogeneously distributed in the profile, with a maximum in the 7 - 9 cm layers. In contrast, mycelium at the *Hydnellum* site was most prolific in the upper 2 cm and at the *Piloderma* site it was found mainly in the 3 to 6 cm layers. *Tylospora*

from nutrient poor peat soil was mostly (ca. 80%) recovered as part of ectomycorrhizal root tips and the actual mycelium was poorly developed in comparison with mycelia from the other sites where mycelia were well developed and mostly recovered (ca. 80%) as single hyphae and rhizomorphs. The mycelium biomass extracted from the Swedish forest is shown in Fig. 9.

The results obtained give a reasonable estimation of the biomass, which varied in the soil profile (0-10 cm depth) from 0.07 to 70 mg mycelium per g soil, with a mean value of 7.3 mg g⁻¹ soil in the whole soil profile. Estimations of FDA-active fungal biomass determined by the fluorescein diacetate (FDA) method at other sites showed results - between 4 - 5 and 12 - 16 mg mycelium per g soil in pine needle litter and in the fermentation layer, respectively (Söderström, 1979; Berg & Söderström, 1979). FDA method allows to measure the metabolically active soil fungal biomass (Söderström, 1977). Olsen et al. (1990) obtained a higher value - about 54 mg mycelium per g soil with variation from 12 to 138 mg g⁻¹ soil in the upper 3 cm of the forest floor. The fungal biomass was estimated by using the ergosterol method, based on the fact that most of the dominating species of fungi in soil have a cell membrane, with a fairly constant amount of ergosterol; a sterol which is not present in the membranes of other organisms in soil (Olsen, 1973). Therefore such method gives reliable estimates of the total fungal biomass in soil.

The ratio of ¹³⁷Cs activity concentration in mycelia to that in soil

The ¹³⁷Cs activity concentrations in soil layers as well as in the mycelium showed a considerable decrease with depth at both locations (Papers I and II). However, the

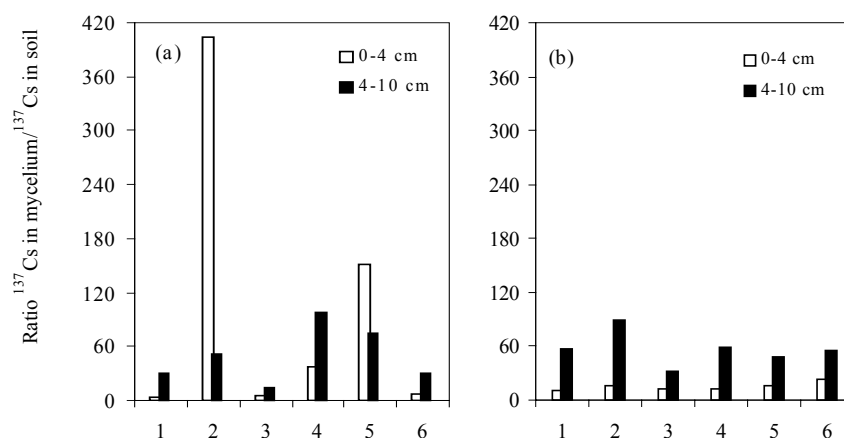


Fig. 10. Ratios of ¹³⁷Cs activity concentration in mycelium, kBq kg⁻¹ to ¹³⁷Cs activity concentration in corresponding layers of soil, kBq kg⁻¹: a - 2 cm intervals: 1 *Boletus edulis*; 2 *Lactarius vellereus*; 3 *Suillus granulatus*; 4 *Paxillus involutus*; 5 *Amanita muscaria*; 6 *Boletus edulis*. b - 1 cm intervals: 1 *Cantharellus cibarius*; 2 *Leccinum aurantiacum*; 3 *Amanita muscaria*; 4 *Lactarius necator*; 5 *Xerocomus subtomentosus*; 6 *Sarcodon imbricatus*. Ukrainian forest.

¹³⁷Cs activity concentration in soil layers decreased with depth more rapidly than that in mycelium prepared from the corresponding layers of soil.

The observed variability of ^{137}Cs activity concentrations in mycelium and soil between different sites also varied considerably. For the Ukrainian forests the range was from 17 to 13 440 Bq kg^{-1} in soil and from 1 840 to 384 000 Bq kg^{-1} in the corresponding mycelium in the 0-4 cm layer. In the Swedish forest the range was from 2 130 to 13 440 Bq kg^{-1} and from 510 to 23 490 Bq kg^{-1} in the 0-4 cm layer respectively.

The highest ^{137}Cs levels were found in mycelium in the upper 4 cm at sites of *Xerocomus subtomentosus*, *Amanita muscaria*, *Lactarius necator*, *Paxillus involutus* and *Sarcodon imbricatus* species. A deeper distribution of ^{137}Cs in mycelium was found at, for example, the *Boletus edulis* site. However, mycelium was not determined to species, and another species of fungi might have been mixed into the prepared mycelia samples. As reported by Olsen *et al.* (1990) in addition to the expected morphological type of mycorrhiza, underneath a large number of fruit bodies at least two other types are frequently encountered. At all sites studied, ^{137}Cs activity concentrations in mycelium biomass were several times higher than those in corresponding soil layers, which indicates the high ability of fungal mycelium to accumulate radiocaesium.

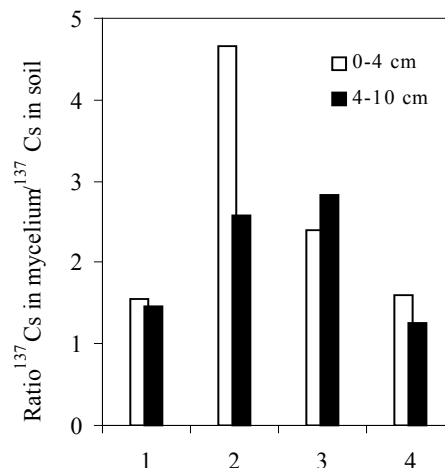


Fig. 11. Ratio of ^{137}Cs activity concentration in mycelium, kBq kg^{-1} to ^{137}Cs activity concentration in corresponding layers of soil, kBq kg^{-1} . 1 *Tylospora spp.*; 2 *Cenococcum geophilum* and *Piloderma fallax*; 3 *Piloderma fallax*; 4 *Hydnellum peckii*. Swedish forest sites.

The ratios of ^{137}Cs activity concentrations in mycelium (kBq kg^{-1}) to ^{137}Cs activity concentrations in corresponding soil layers (kBq kg^{-1}) were calculated (Fig. 10 and Fig. 11).

A large range of ratios - from 1.6 to ca. 400 was observed for the analysed sites of fungi. Most of the Ukrainian sites, however, had ratios between 20-100. Some sites such as those with *Lactarius vellereus* and *Amanita muscaria* showed a higher ratio at the upper layer of the soil profile (0-4 cm). The majority of the analysed

sites, however, had higher ratios in the deeper (4-10 cm) layer, which may indicate that vertical redistribution of ^{137}Cs activity within fungal biomass occurs.

In the Swedish forests a high ^{137}Cs activity concentration in mycelium in the upper layer was found at the *Cenococcum geophilum* site.

The ratio of ^{137}Cs activity concentration in fruit bodies to that in mycelia

The highest ^{137}Cs activity concentrations in the sampled fruit bodies in the Ukrainian forest were found at the sites of *Paxillus involutus* - 862 kBq kg^{-1} , *Xerocomus subtomentosus* - 117 kBq kg^{-1} and *Sarcodon imbricatus* - 98 kBq kg^{-1} DW (Papers I and II) (Fig. 12).

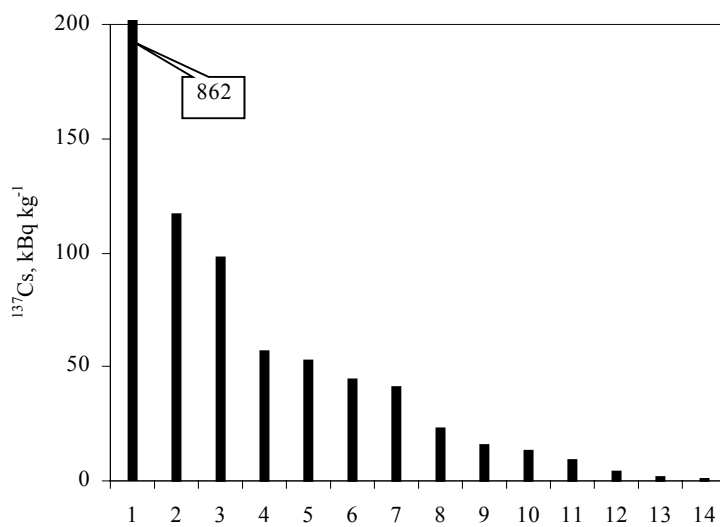


Fig. 12. ^{137}Cs activity concentrations in fruit bodies of fungi: 1 *Paxillus involutus*; 2 *Xerocomus subtomentosus*; 3 *Sarcodon imbricatus*; 4 *Lactarius vietus*; 5 *Lactarius necator*; 6 *Suillus granulatus*; 7 *Boletus edulis*; 8 *Suillus luteus*; 9 *Cantharellus cibarius*; 10 *Amanita muscaria*; 11 *Amanita muscaria*; 12 *Lactarius vellereus*; 13 *Boletus edulis*; 14 *Leccinum aurantiacum*; Ukrainian forest sites.

The ratios (kBq kg^{-1} in fruit bodies divided by kBq kg^{-1} in mycelium) were calculated for sites sampled in the Ukrainian forest. The ratio calculated for 18 analysed species of fungi varied considerably from 0.1 to 65.8, and can be ranked in the following order:

- < 1.0 - 3 species
- 1.0 – 5.0 - 8 species
- 5.0– 50.0 – 6 species
- > 50.0 - 1 species

The fungal species showed a wide variation in their capability to concentrate radiocaesium within their fruit bodies. Most of the analysed species (15) showed a

ratio > 1. Some of the sites, such as those of *Xerocomus subtomentosus*, *Lactarius vietus*, *Sarcodon imbricatus* *Paxillus involutus* showed the highest ratios – 65, 24, 22 and 22 respectively. At the *Boletus*, *Leccinum* and *Amanita* sites with mycelium located more or less homogeneously the ratios were low (0.08, 0.12 and 1.8), thus the ^{137}Cs activity concentrations in the mycelium at the first two sites were higher than in the fruit bodies (Table 3). In the Swedish forests, fruit bodies of fungi were not sampled and analysed due to two of the taxa included in this study (*Tylospora* spp. and *P. fallax*) belong to a group of fungi that does not produce visible sporocarps; instead they form thin, crust-like structures (resupinate sporocarps) on the underside of woody debris. *Cenococcum geophilum* does not produce any type of sporocarp.

Table 3. The ratio between ^{137}Cs activity concentration in fruit bodies of fungi, kBq kg^{-1} to that in mycelium (as a mean for the whole profile), kBq kg^{-1} . Ukrainian forest

Thickness of the soil layers	Species of fungi	Ratio
1 cm	<i>Lactarius vietus</i> Fr	23.7
	<i>Paxillus involutus</i> (Batsch.)	21.6
	<i>Suillus granulatus</i> Fr.	12.2
	<i>Suillus luteus</i> Fr.	9.1
	<i>Xerocomus subtomentosus</i> Fr.	3.1
	<i>Suillus variegatus</i> Fr.	2.6
	<i>Boletus edulis</i> Fr.	2.3
	<i>Amanita muscaria</i> Fr.	1.8
	<i>Lactarius vellereus</i> Fr	1.7
	<i>Inocybe fastigiata</i> Fr.	1.4
	<i>Boletus edulis</i> Fr.	0.2
	<i>Boletus edulis</i> Fr.	0.1
	2 cm	<i>Xerocomus subtomentosus</i> Fr.
<i>Sarcodon imbricatus</i> Fr.		22.2
<i>Cantharellus cibarius</i> Fr.		5.0
<i>Lactarius necator</i> Fr.		4.9
<i>Amanita muscaria</i> Fr.		1.0
<i>Leccinum aurantiacum</i> (Bull.St.Am)		0.1

Since most of the fruit bodies show higher activity concentrations than the prepared mycelia, we assumed that ^{137}Cs was being pumped out from soil, thereby retarding downward migration, which might be reasonable from the viewpoint of fungal physiology. Vogt & Edmonds (1979) reported that nitrogen and potassium were concentrated in significant levels in fruit bodies (higher than 1% of the dry weight of the fruiting bodies) compared to the forest floor in all the ecosystems studied, while magnesium, phosphorus, manganese and sodium make up less than 1 % of the dry weight.

Total ^{137}Cs activity associated with fungal biomass

One of the main aims of the present study was to quantify ^{137}Cs activities bound to the mycelium (Papers I and II). The amount of radiocaesium activity incorporated

into the fungal mycelium in the soil layer 0-10 cm in the Ukrainian forest varied considerably from 0.6 to 50 % as a mean for the whole soil profile.

The highest content of ^{137}Cs was found in the sites with *Lactarius necator* (ca. 50 %), *Lactarius vellereus* (28 %), *Sarcodon imbricatus* (ca. 20 %), *Suillus variegatus* (19 %), *Boletus edulis* (ca. 15 %). High percentages were also found in mycelium at the sites of *Xerocomus subtomentosus* (17 %) and *Amanita muscaria* (12 %). At the *Lactarius necator* site 57 % of ^{137}Cs activity was associated with the mycelia in the 0-4 cm layer and 45 % in the 4-10 cm layer of forest soil. At the *Xerocomus subtomentosus* site, 23 and 13 % of radiocaesium was found in mycelia in the 0-4 and 4-10 cm layers respectively.

For the Swedish forest we found that between 0.7 and 2.5 % of the total ^{137}Cs activity (0-10 cm) was located within the fungal mycelium. Well-developed *Hydnellum peckii* mycelium retains up to 3.5 % of total activity within whole soil profile and 9 % in the 0-1 cm layer. The mycelia of *Tylospora spp.* and *Piloderma fallax*, however, which do not produce visible sporocarps, showed only weak ability to accumulate ^{137}Cs . Less than 1 % of ^{137}Cs activity was found in mycelium of *Tylospora spp.* extracted from the peat soil, mainly due to poorly developed mycelia resulting in a smaller amount of prepared mycelium biomass. Only 2.3 % ^{137}Cs was associated with the mycelia at the site of *Cenococcum geophilum*, a group which does not produce any type of sporocarp either.

Many factors are involved in determining interspecific differences in ^{137}Cs levels in fruit bodies of fungi. It has been suggested that the most important one is location of the mycelium and the ecophysiological behaviour of fungi (Yoshida & Muramatsu, 1994; Rühm *et al.* 1997; Steiner *et al.* 2002). We conclude that well-developed mycelia may retain considerable amounts of radiocaesium activity. Several sites at least, such as those of *Lactarius necator*, *L. vellereus*, *Xerocomus subtomentosus* and *Sarcodon imbricatus* showed high percentages of radiocaesium activity associated with mycelia. They also had a large fungal biomass and ^{137}Cs activity concentrations at the depth 0-4 cm. However, the *Paxillus involutus* and *Boletus edulis* sites, for example, showed rather different ^{137}Cs activity concentrations in mycelia, 348 and 87 kBq kg^{-1} , respectively, while they had more or less the same fungal biomass and ^{137}Cs activity concentration in the upper 0-4 cm layers. The patterns of mycelium biomass distribution within the soil profile differed only slightly. However, due to the low fungal biomass in this layer (0.4 and 0.3 mg cm^{-3} respectively) only 0.1 – 1.0 % of ^{137}Cs activity was associated with mycelia.

Semi-sequential extraction of the ^{137}Cs from fungi and the upper soil layers

Extraction of the ^{137}Cs from fruit bodies of fungi with distilled water at room temperature resulted in a release of between 42 and 68 % and between 70 and 90 % with hot (80 °C) water (Paper III). Generally, more ^{137}Cs seemed to be extracted from fruit bodies of mycorrhizal fungi, than from saprotrophic species and parasitic fungi (Table 4).

Fruit bodies showed low retention of ^{137}Cs and, as suggested by Fraiture, Guillitte & Lambinon, (1990), this is probably due to loss of impermeability of the cell

membrane, resulting in outflow of cytoplasm. Obviously fruit bodies of fungi have only a few strong binding sites for ^{137}Cs . Therefore they will probably locally play an important role mostly in the short-term cycling of the element.

Table 4. ^{137}Cs extracted from fruit bodies of fungi, % of the total activity

Number of samples analyzed	H ₂ O room T°	Number of samples analyzed	80° C	Insoluble fraction
Mycorrhizal fungi				
28	68.2(10.5)*	28	90.9(6.3)	9.1
Saprophytes				
1	52.6	1	70.1	29.9
Parasites/Saprophytes				
2	42.3(25.9)	2	72.0(19.9)	28.0

* Mean (SD)

The water-soluble fraction of ^{137}Cs for fungal mycelium samples was found to be around 29 % with a range of 11 - 41 %. Additionally 24 % of the ^{137}Cs activity from mycelium was released by ammonium acetate extraction. In total about 53 % of the ^{137}Cs activity was released from the mycelium. Thus mycelium seems to contain more binding sites for ^{137}Cs compared to fruit bodies. One possible explanation of the binding of ^{137}Cs to the mycelium may be the fact that chitin, which is generally very resistant to hydrolysis by acids and alkali, is an important constituent in mycelium tissue (Greenland & Oades, 1975), and particularly in filamentous fungi (Greenland & Hayes, 1981). The metal-binding abilities of fungal mycelium (Gadd & White, 1989) support the hypothesis that mycelium may also contain binding sites for ^{137}Cs (Paper III). However as reported by Shand *et al.* (1995), 88-95 % of ^{137}Cs was extracted with 1M ammonium acetate from the mycelium of soil fungi, grown in liquid culture, which indicates a weak binding.

The ^{137}Cs from the upper forest soil layer was extracted with H₂O, 1 M NH₄OAc and 10% H₂SO₄ (second series of soil) (Table 5).

Table 5. Semi-sequential extraction of ^{137}Cs from forest soil, % of the total activity

Number of samples analyzed	Summary fraction H ₂ O and 1 M NH ₄ OAc	Number of samples analyzed	10% H ₂ SO ₄	Insoluble fraction
O _f /O _h layer				
12	12.4(1.4)*	10	30.2(3.9)	57.5
A _h /B layer				
19	22.7(3.6)	9	38.1(12.4)	39.2

Mean (SD)

In the organic-rich and organic-poor layers of forest soil we found 12 % and 23 % of the ^{137}Cs as NH₄OAc exchangeable fractions. Experimental data for forest soils (Fawaris & Johanson, 1995; Andolina & Guillitte, 1990) and for uncultivated soils

from Norway, Byelorussia and Ukraine Oughton *et al.* (1992) showed similar results.

The “cellulose” and “hemicellulose” complex is supposed to be decomposed by hydrolysis with 10 % sulphuric acid followed by gentle boiling for one hour (Allen, 1974). Our data show that this treatment resulted in a release of 30 % of ^{137}Cs from the organic-rich soil and 38 % from the organic-poor soil.

About 58 and 39 % of the ^{137}Cs activity remains in soil after extraction with sulphuric acid from organic-rich and organic-poor soil, respectively.

The treatment of soil with 30 % hydrogen peroxide resulted in release of about 11 and 15 %, respectively of the ^{137}Cs -activity from the organic-rich soil and organic-poor soil (Table 6).

Table 6. *Semi-sequential extraction of ^{137}Cs from forest soil by 30 % H_2O_2 , % of the total activity*

Number of samples analyzed	Summary fraction H_2O and 1 M NH_4OAc	Number of samples analyzed	30% H_2O_2	Insoluble fraction
12	12.4(1.4)*	O _f /O _h layer 6	10.5(6.5)	77.1
19	22.7(3.6)	A _h /B layer 4	14.5	62.8

*Mean (SD)

Treatment of soil with 98.8 % sodium hypochlorite released around 27 % of ^{137}Cs activity in both types of forest soils (Table 7).

Table 7. *Semi-sequential extraction of ^{137}Cs from forest soil by 98.8 % NaOCl , % of the total activity*

Number of samples analyzed	Summary fraction H_2O and 1 M NH_4OAc	Number of samples analyzed	98.8% NaOCl	Insoluble fraction
12	12.4(1.4)*	O _f /O _h layer 6	27.3(10.6)	60.3
19	22.7(3.6)	A _h /B layer 4	26.8(2.5)	50.5

*Mean (SD)

The high yield obtained with 30 % H_2O_2 and 98.8 % NaOCl fractions cannot be completely related to ^{137}Cs release from the organic matter since oxidation of organic components with 30 % hydrogen peroxide or 98.8 % sodium hypochlorite also leads to a release of some mineral soil-bound ^{137}Cs . Therefore, we can assume that ^{137}Cs fixed to minerals can also be solubilised by the treatment.

In the third soil series the humic substances were extracted from soil with 0.5 M NaOH and fractionated on the basis of solubility to the following components: humic acids, fulvic acids and residual organic compounds resistant to most chemical treatments.

Humic substances seem to sorb the radiocaesium only weakly. Our findings show that around 11 % of the total ^{137}Cs activity was found in the humic acid fraction, and 5 % in the fulvic fraction. This left 46 % of the ^{137}Cs activity fraction in the residual fraction. The results are summarised and showed in the table 8.

Table 8. ^{137}Cs in humic acid, fulvic acid and residual fraction

	Number of samples analyzed	%
0.1 M HCl soluble fraction	21	38.1(7.0)*
Humic acid	19	10.9(2.1)
Fulvic acid	12	5.2(0.5)
Residual fraction	20	45.9(5.6)

*Mean (SD)

Based on the results we suggest that the plant-available fraction of ^{137}Cs in the forest soils studied may be about 20 % of the total ^{137}Cs deposition. If we assume that 20 % of the total ^{137}Cs inventory in soil is located within the fungal mycelium and about 50 % of this pool is soluble, this fungal pool will contribute about 50 % of the total exchangeable ^{137}Cs activity in the soil.

About 50 % of the ^{137}Cs inventory was insoluble in spite of different extraction methods applied in these studies. Since these fractions were about 10 to 18 % higher in the organic-rich soils compared to organic-poor soils it seems reasonable to conclude that ^{137}Cs binds strongly to some organic components in the soil. Based on the result of the semi-sequential extraction procedure we suggest that “cellulose”, “hemicellulose”, humic acids and fulvic acids are minor ^{137}Cs binders. Since ^{137}Cs might be associated with the residual fraction, this could explain to some extent the ^{137}Cs binding in the soil.

Possible sources of ^{137}Cs uptake by fungal mycelium and fruit bodies in forest ecosystems

In order to determine which of the biochemical fractions of SOM might serve as a possible ^{137}Cs source for the fungi, water-soluble substances, hemicellulose, cellulose, humic substances as well as insoluble residual fraction (Papers IV and V) were extracted from the soil. The chemical composition of forest litter and the upper soil layer are presented in table 9.

Within the soil profile the insoluble (residual) fraction was most pronounced in the upper layers and decreased with depth. This fraction was found to be a major part of the organic matter in forest soil. Of the total content of organic matter in soil in the 0-6 cm layer, 110 mg cm³ or 63-65 % was found in the residual fraction.

The content of humic substances increased within the soil profile from 10.5 % at a depth of 0-2 cm up to 28.8 % at a depth of 6-12 cm, with a mean of 15.8 % for the whole profile.

Table 9. *Chemical composition of the upper soil layer (mean for whole profile 0-12cm)*

	%	mg cm ⁻³
Organic matter	56.2	
Water soluble fraction	6.7(0.7)*	9.0
Hemicellulose	9.4(0.5)	13.5
Cellulose	6.5(0.6)	10.2
Humic substances	15.8(1.2)	17.5
Insoluble (residual) fraction	61.6(1.8)	88.4

*Mean (SD)

Water-soluble fractions were found to be 6.3-6.7 % and showed only slight changes with depth. Hemicellulose fractions were also more or less constant in the upper layers (9.5-10.2 %) and decreased slightly with depth to 8.0 % (mean value 9.4 %). The cellulose fraction content decreased significantly with depth from 9.8 to 3.2 %, with a mean of 6.5 %.

The ¹³⁷Cs activity concentrations in all extracted organic fractions were determined and are shown in table 10.

Table 10. *¹³⁷Cs activity concentration in the extracted fractions of forest soil (0-12 cm)*

	kBq kg ⁻¹	Bq cm ⁻³
Water soluble fraction	23	0.2
Acid soluble fraction	433	-
Hemicellulose	835	7.7
Cellulose	31	0.2
Humic substances	22	0.4
Insoluble (residual) fraction	538	52.7

The highest ¹³⁷Cs activity concentrations in specific layers were found in hemicellulose 1 750 kBq kg⁻¹ (6-12 cm). In the insoluble residual fraction most activity (1 120 kBq kg⁻¹) was found at the depth of 4-6 cm and in the acid-soluble fractions (1 210 kBq kg⁻¹) in deeper layers 6-12 cm. The means for the whole soil profile were 835, 538 and 434 kBq kg⁻¹ respectively (Table 9). The water-soluble fraction and residual fraction showed the highest ¹³⁷Cs activity concentration at depths of 2-4 and 4-6 cm. As for cellulose fractions, most activity was found in the layer 4-6 cm.

Residual fraction (lignin) undergoes relatively slow decomposition in soil. A large amount of ¹³⁷Cs bound to this residual fraction - 52.7 Bq cm⁻³ seems to be due to

both the high content of this fraction in soil and its relatively high ^{137}Cs activity concentration.

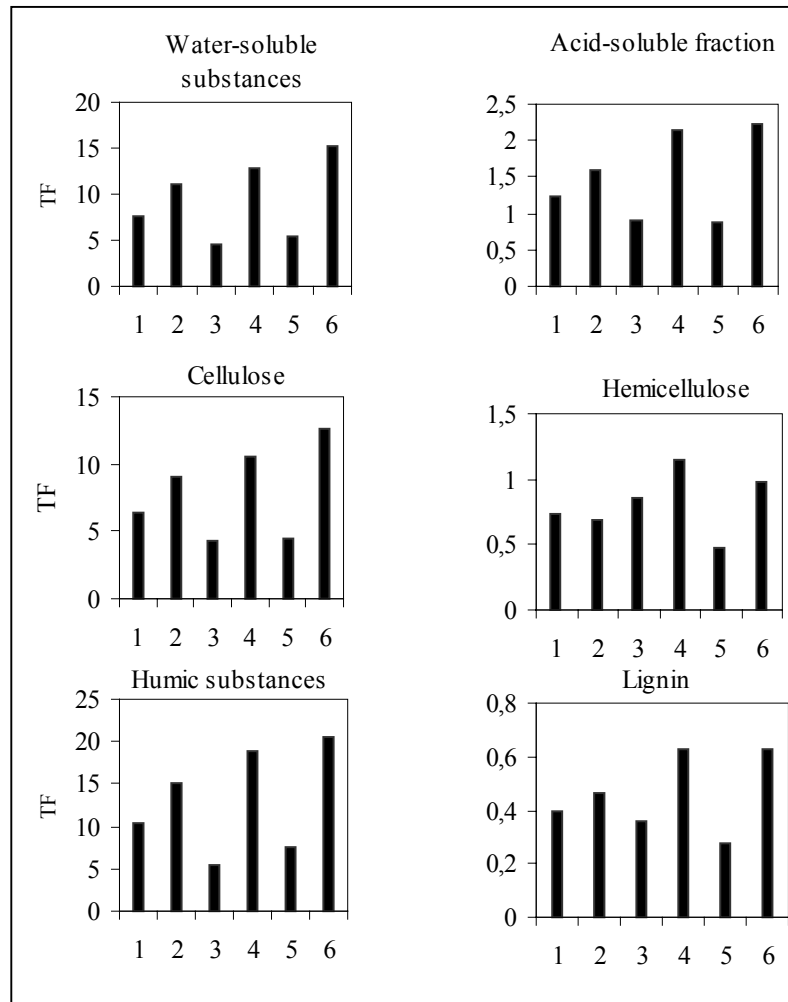


Fig. 13. Transfer factors (TF), defined as kBq kg^{-1} in mycelium (DW)/ kBq kg^{-1} in extracted fractions (DW) in corresponding layers of forest soil. Mean values for the whole profile are shown. 1 *Cantharellus cibarius*; 2 *Leccinum aurantiacum*; 3 *Amanita muscaria*; 4 *Lactarius necator*; 5 *Xerocomus subtomentosus*; 6 *Sarcodon imbricatus*. Ukrainian forest.

As shown earlier the highest ^{137}Cs activity concentration in mycelia was usually found in the 1-4 cm layer. The *Xerocomus* and *Lactarius* spp. showed a mainly superficial distribution with the highest levels of activity in the 0-1 cm layer – 82 and 112 kBq kg^{-1} DW respectively.

Using transfer factors (TF) we investigated the possible correlation between ^{137}Cs activity concentration in fungal mycelium and the extracted biochemical fraction in

corresponding layers of the soil profile (Fig. 13). Such an approach might be useful for identification of possible sources from which the fungal mycelium of the species studied takes up radiocaesium. The mean values of TF for the whole soil horizon (0-12 cm) are presented (Fig. 13).

Our data indicate that there are species differences in ability to accumulate radiocaesium. Highest transfer factors were observed for instance at sites of *Lactarius necator* and *Sarcodon imbricatus*. For these species of fungi high levels of radiocaesium activity concentrations both in fruit bodies and mycelium were found – 53 and 98 kBq kg⁻¹ in fruit bodies and 37 and 13 kBq kg⁻¹ in mycelium.

The fungi studied were mycorrhizal species; however, some species normally considered to be facultative mycorrhizal species (*Xerocomus* spp, *Paxillus involutus*, etc.) remain dependent on dead organic matter, even when they produce mycorrhiza (Guillitte *et al.* 1990). We found well-developed mycelia in most of species of fungi studied in the upper layers of forest soil that are rich in organic matter. The content of SOM in these layers varied from 1 to 27 %. Consequently, about 70 % of the total mycelium biomass was found in these layers. The major part – more than 90 % of water-soluble fractions, cellulose, hemicellulose, residual fraction and about 80 % of humic substances were also located in the upper, 0-6 cm, horizons of the soil profile.

Lignin, which consists of residues of organic compounds, is resistant to most microbiological and chemical processes and is decomposed especially by basidiomycetes (Swanson, 1972). The hemicellulose is next to cellulose the most abundant compounds in plant wall cells. Our results did not show that these fractions were ¹³⁷Cs bioavailable sources for soil fungi since transfer factors for these fractions were quite low. The lignin- and hemicellulose-decomposing fungi are more active only in the early stage of decomposition. Further breakdown of lignin is probably carried out by another agent than primary lignin-decomposing basidiomycetes, while actinomycetes, are more active in the later stages of hemicellulose decomposition (Swanson, 1972).

Our results indicate that ¹³⁷Cs is probably more actively taken up by mycelium from the cellulose compounds, since the fungi include basidiomycetes which are particularly active as cellulose decomposers. Thus, cellulose fractions might be considered as one of the sources of radiocaesium biological uptake for fungi. However the highest values of transfer factors (1.0-66.3) were found for water-soluble fractions and newly formed humic substances. These two fractions are considered to be readily available for mycelium uptake.

We suggest that the sources of radiocaesium for uptake by fungal mycelium are located in the upper layers of the forest soil profile. Uptake seems to occur in the A_h horizon at a depth, in our case, of around 4-6 cm with a moderate content of organic matter – about 50 %. The highest values of transfer factors for these layers were found for the water-soluble fraction – 39.7; cellulose – 30.1 and humic substances – 66.3 (*Sarcodon imbricatus* site, table 11).

Accumulation of ¹³⁷Cs in fruit bodies of *Cantharellus cibarius*, *Leccinum aurantiacum*, *Amanita muscaria*, *Lactarius necator*, *Xerocomus subtomentosus*, and *Sarcodon imbricatus* owing to forest litter decay was studied (Paper V).

Table 11. Transfer factors (TF), defined as ^{137}Cs , kBq kg^{-1} in mycelium (DW)/ ^{137}Cs , kBq kg^{-1} in extracted fractions (DW) in corresponding layers of the forest soil. *Sarcodon imbricatus* site. Ukrainian forest.

Depth, cm	Water soluble fraction	Acid-soluble fraction	Hemicellulose	Cellulose	Humic substances	Residual fraction
0-2	1.4	1.1	1.6	2.5	2.3	0.5
2-4	18.1	1.7	0.6	17.7	12.9	0.6
4-6	39.7	6.1	1.7	30.1	66.3	1.2
6-10	1.5	0.02	0.01	0.5	0.9	0.1

It was found that the humic substances, the water-soluble substances and to some extent hemicellulose and cellulose fractions might be important sources of ^{137}Cs for fruit bodies of fungi. The transfer factors for the above-mentioned biochemical fractions were found to be 2.6, 2.4, 2.4 and 0.9 respectively. The acid-soluble fraction and residual fraction were found to be less important sources of ^{137}Cs uptake by fruit bodies of fungi. This is probably related to the fact that fungi are less important in decomposition of lignin since only some of them, mainly basidiomycetes, can attack lignin. Concerning cellulose, fungi are important decomposers mainly in the initial stages of forest litter decay.

Distribution of the fungal mycelium in connection with organic matter composition

The distribution of mycelia of the ECM fungi *Piloderma fallax* and *Hydnellum peckii* in the forest soil in connection with organic matter composition was examined (related paper). It was found that biomass of *Hydnellum peckii* mycelium correlated well with biochemical fractions obtained in this study. The correlation coefficients between mycelium biomass and fractions of soil organic matter were as follows: cellulose ($r = 0.85$), hemicellulose ($r = 0.68$), water-soluble fractions ($r = 0.56$). The biomass of *Piloderma fallax* mycelium showed only weak correlation with the content of water-soluble fractions ($r = 0.41$). No correlation was found at the *Piloderma fallax* site for cellulose, hemicellulose and organic matter content in soil.

Final Discussion

One of the aims of this study was to quantify the vertical distribution of the fungal biomass in the upper 10 cm of forest soil profiles. The manual extraction method used in this study allowed determination of the spatial distribution of the fungal mycelium of some fungal species and quantification of ^{137}Cs activities bound to these mycelia. Unfortunately this method is restricted to species that have distinctive hyphal structures. Despite these constraints this method can provide information about ^{137}Cs activities bound to the mycelia, especially in fungal species

that do not produce visible sporocarps, such as *Tylospora* spp. and *P. fallax*, and also in those that do not produce any type of sporocarp, such as *C. geophilum*.

The estimated fungal biomass in the Ukrainian forest varied in the soil profile from 0.07 to 70.44 mg mycelium per g soil, with a mean value of about 7 mg in the whole soil profile. Studies by Söderström (1979) and Berg & Söderström (1979) found the metabolically active soil fungal biomass of between 4 - 5 and 12 - 16 mg mycelia per g soil in pine needle litter and the fermentation layer, respectively. Olsen et al. (1990) estimated the total fungal biomass by using the ergosterol method and found about 54 mg mycelium per g soil with variation from 12 to 138 mg g⁻¹ soil to a depth of 3 cm.

For the Swedish forest our estimation of average fungal biomass was 3.6, 14.3, 16.0 and 19.4 mg g⁻¹ dry weight of soil in the upper 4 cm for the four sites respectively. However, the variation of fungal biomass over years and seasons is well documented (Söderström, 1979) and may vary by several orders of magnitude. In addition, although the fungi included in this study formed the greatest part of the mycelia in the soil samples, there was a small amount of fungal structures that were not included in the biomass estimates because they could not be identified.

To quantify the ¹³⁷Cs activity concentration in the soil, in fruit bodies as well as in the prepared fungal mycelium in the same soil profile was the next aim of this study. The ¹³⁷Cs activity concentrations in soil layers as well as in the mycelium showed a considerable decrease with depth in both the Ukrainian and Swedish forests. However activity in soil layers decreased with depth more rapidly than that in mycelia prepared from the corresponding layers of soil.

The ¹³⁷Cs activity concentrations in mycelia were always highest in the upper 4 cm of the soil profile. *Xerocomus subtomentosus* and *Sarcodon imbricatus* are examples of species that seem to have the highest ¹³⁷Cs levels in the upper 4 cm. *Paxillus involutus*, *Xerocomus subtomentosus* and *Sarcodon imbricatus* were species in which we found very high ¹³⁷Cs activity concentrations in both fruit bodies and mycelia of fungi. In further studies, these types of species should be used in preference for ¹³⁷Cs studies. Guillitte et al. (1990), using the ¹³⁷Cs/¹³⁴Cs ratios in fruit bodies and in corresponding soil layers, showed that the mycelia of *Xerocomus badius* were located in the O_f/O_h horizon. Similar results have been reported by Rühm et al. (1997).

Ratios of ¹³⁷Cs activity concentration in mycelium to those in corresponding layers of soil indicate the high ability of fungal mycelia to accumulate radiocaesium since the ¹³⁷Cs activity concentrations in mycelia of all species studied were several times higher than those in corresponding soil layers. The ratios of ¹³⁷Cs activity concentration in fruit bodies to those in mycelia demonstrate a similar tendency. Most of the fruit bodies show higher activity concentrations than the mycelia prepared from the same sites. Therefore we suggest that ¹³⁷Cs is transported out from the soil thorough mycelia to fruit bodies, retarding downward migration.

Estimation of the ¹³⁷Cs activity associated with mycelia has usually been based on the assumption that ¹³⁷Cs activity concentrations are the same in underground fungal biomass as well as in fruit bodies of fungi (Olsen et al. 1990). However, our results show that this may be a questionable assumption, since ¹³⁷Cs activity

concentrations in fruit bodies usually were higher than those in mycelia for the same species of fungi. The mean ratio of ^{137}Cs activity concentration in fruit bodies to that in mycelia in corresponding layers was found to be about 10. Studies of nutrient concentrations in basidiocarps (Vogt & Edmonds, 1980) in an *Abies amabilis* ecosystem showed wide variation in the capability to concentrate potassium within different parts of the fungi of various species. The epigeous and hypogeous sporocarps concentrated 14-37 times more potassium in sporocarps than sclerotia or mycelia. A high potential to store and take up potassium was found in, for example, *Xerocomus badius* - *Picea abies* mycorrhizae (Kottke *et al.* 1998).

The next aim of study was to quantify the amount of total ^{137}Cs activity bound to the fungal biomass in the upper forest soil layer (0-10 cm). For the Ukrainian forest we found that between 0.6 to 50 % of the total ^{137}Cs activity in the soil was located within the fungal mycelia. In the Swedish forest we estimated that between 0.3 and 1.8 % of the total ^{137}Cs activity within the 0-10 cm soil profile was found in the fungal compartment. The highest percentage however was recorded for *H. peckii* in the 0-1 cm soil layer – 9 %. Many authors who used indirect methods for estimation of the fungal biomass reported that the forest microflora, particularly fungi mycelia, contained a significant fraction, up to 20 - 30 %, of the total inventory of ^{137}Cs in the soil. It may be assumed that our method of extracting the mycelia from the soil profile resulted in an underestimation of the fungal biomass of the individual species in soil due to the difficulties in extracting mycelium from the organic matter. However our method gives an immediate direct estimation of fungal biomass, which is a major advantage over indirect methods that may overestimate the total biomass (Martens, 1995; Steiner *et al.* 2002).

Estimation of the relative importance of soil organic matter, fungal mycelium and fruit bodies for the binding of ^{137}Cs in the forest floor was the next aim of this study. Based on the extraction method employed it was shown that the plant-available fraction of ^{137}Cs was about 12 % of the total ^{137}Cs activity in the upper soil layer and 23 % in the deeper layer, which confirms results obtained in other studies. The hydrolysis with sulphuric acid resulted in a release of 30 % of ^{137}Cs from the organic-rich soil and 38 % from the organic-poor soil. Oxidation of organic components with hydrogen peroxide or sodium hypochlorite also leads to a release of mainly organic-bound ^{137}Cs . However extraction with hypochlorite was found to be more efficient in extracting organic material compared with hydrogen peroxide.

We assume that between 10 and 25 % of the soil ^{137}Cs inventory ($\text{H}_2\text{O} + 1\text{ M NH}_4\text{OAc}$ extractable) is supposed to be more or less directly available for fungal uptake. Additionally at least 25 % might be available after fungal break down of organic material. We suggest that cellulose, hemicellulose, humic acids and fulvic acids are minor ^{137}Cs binders since about 50 % of the total ^{137}Cs activity was still in the insoluble residue fraction. Since these fractions were about 10 to 18 % higher in the organic-rich soils compared to organic-poor soils it seems reasonable to conclude that ^{137}Cs binds strongly to some organic components in the soil. Some part of the ^{137}Cs might be associated with the residue lignin fraction since lignin occurs in large amounts in plants and might explain the ^{137}Cs association, at least,

in the organic rich soil layer. However, the mechanism of such an association is not clear. The glomalin, probably being an important component of humin (Hayes & Clapp, 2001), was found in the hyphae of arbuscular mycorrhizal fungi at a level of 60 mg cm^{-3} (Rillig *et al.* 2001). There is also indication that glomalin is sorbed to the soil (colloidal) surface (Wright, 1999), which would explain its resistance to decomposition. Glomalin takes several years to decades to turnover in the soil, whereas the turnover time of the fungal hyphae is of the order of days or weeks.

The fruit bodies of fungi obviously are not responsible for strong binding of ^{137}Cs therefore they will probably locally play a certain role in the short-term cycling of ^{137}Cs . The mycelia instead bind more ^{137}Cs than fruit bodies, which might be explained by presence of chitin in mycelia cell walls (Greenland & Oades, 1975), particularly in filamentous fungi (Greenland & Hayes, 1981). Parts of these fungal cell walls are very resistant in soils, due to the presence of substantial amounts of melanins in the walls (Alexander 1979, Martin & Haider, 1986), which play a significant role for protection against lysis, *e.g.* by enzymes (Bell & Wheeler, 1986). The water and 1 M ammonium acetate extractable fraction for mycelia was found to be around 50 % of the total ^{137}Cs activity in the mycelia. Since fungal mycelia show high ^{137}Cs activity concentrations and relatively high ability to retain ^{137}Cs , they will probably play an important role in long-term cycling of ^{137}Cs , in spite of the fact that the turnover rate of most mycelial structures appears to be a few weeks.

Conclusions

Summaries of the most important findings are as follows:

- The estimated fungal biomass in the Ukrainian forest varied in the soil profile from 0.07 to 70.4 mg mycelium per g soil, with a mean of about 7 mg in the total soil profile 0-10 cm. For Swedish soils our estimation of average fungal biomass was 3.6, 14.3, 16.0 and 19.4 mg g^{-1} dry weight of soil in the upper 4 cm for the four sites respectively.
- Between 0.5 to 50 % of the total ^{137}Cs activity in the 0-10 cm soil profile were found in the fungal mycelium in Ukrainian forest. Between 0.3 and 1.8 % of the total ^{137}Cs inventory of the upper 10 cm of the soil was found within fungal mycelium in the Swedish forest. The highest percentage, 9 %, was observed in the surface 0-1 cm layer at the *H. peckii* site. Usually, the ^{137}Cs activity concentrations in mycelium were higher than those found in soil, and ^{137}Cs activity concentrations in the fruit bodies were higher than those in the mycelium.
- The exchangeable fraction of ^{137}Cs was found to be between 12 and 23 % in the organic-rich and the organic-poor layer. Hydrolysis with sulphuric acid resulted in an additional release of 30 and 38 % of ^{137}Cs respectively, whereas 30 % hydrogen peroxide seemed to be less effective in releasing the ^{137}Cs activity from the forest soil. Use of 98.8 % sodium hypochlorite resulted in

release of around 27 % of ^{137}Cs in both types of soil. However 46 % of the ^{137}Cs activity was still left unsolved in the residue fraction of soil.

- The cellulose, hemicellulose, humic acids and fulvic acids were found to be minor ^{137}Cs binders since about 50 % of the total ^{137}Cs activity was still left in the insoluble residue fraction. Some part of the ^{137}Cs might be associated with residual fraction, at least, in the organic-rich soil layer. However the mechanism of such an association is not clear. The fruit bodies of fungi also showed weak ability to retain ^{137}Cs and therefore may be involved in a short-term radiocaesium cycling. The fungal mycelium was found to be one of the possible major binding sites for ^{137}Cs and therefore may be involved in a long-term cycling of ^{137}Cs . The water and 1 M ammonium acetate extraction released about 53 % of the total ^{137}Cs activity from the mycelia. Our data indicate that a major part - around 50 % of the plant-available ^{137}Cs in forest soil - has already been incorporated into the living biomass, mainly in the fungal mycelium.
- It is suggested that main sources of ^{137}Cs uptake for fungal mycelium and fruit bodies during the decomposition process of forest litter are water-soluble fractions, humic substances, hemicelluloses and celluloses. Acid-soluble fractions are probably less important as a source of radiocaesium for soil fungi.

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