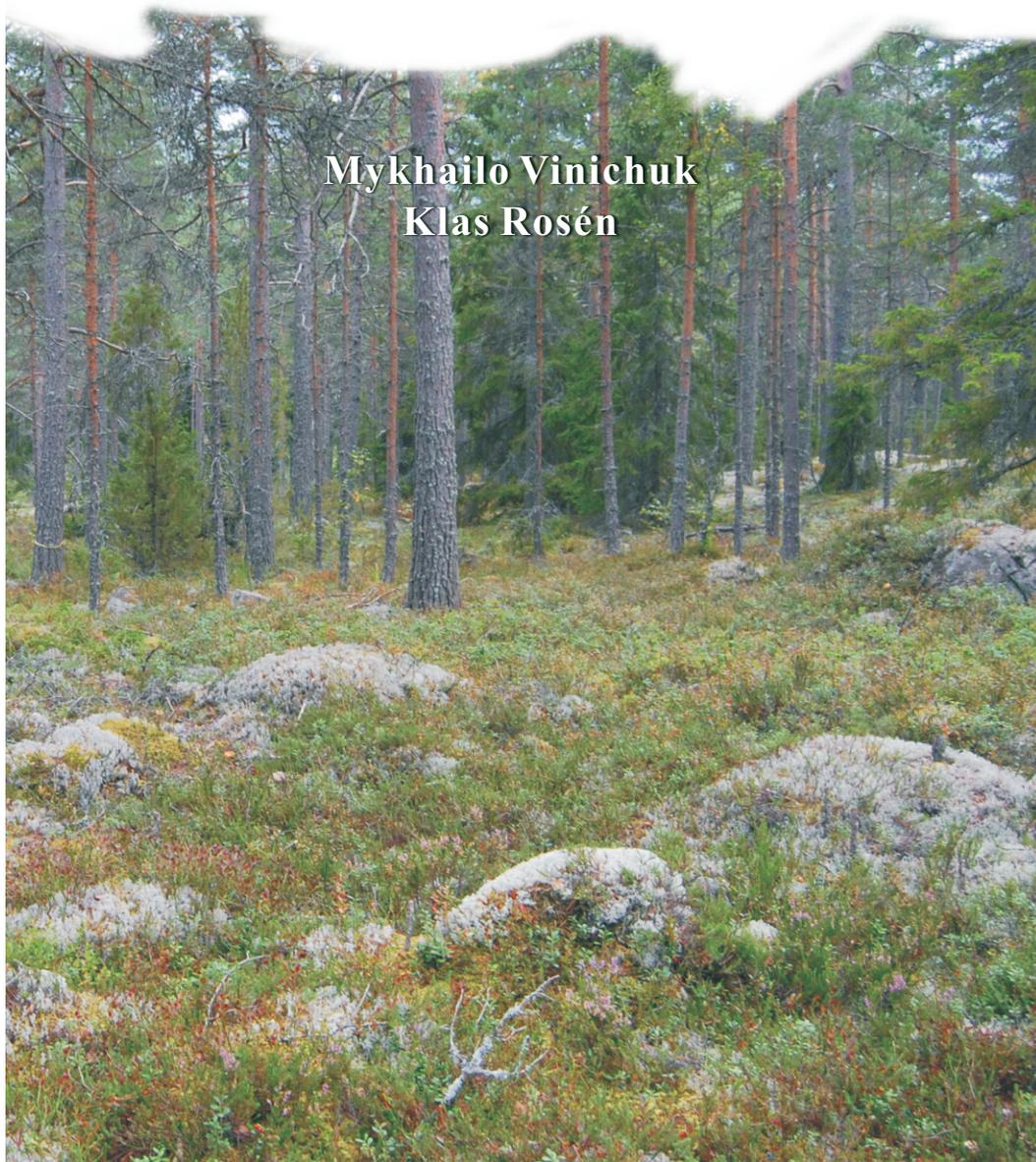


# Cesium ( $^{137}\text{Cs}$ and $^{133}\text{Cs}$ ) and Selected Metals in the Environment

Mykhailo Vinichuk  
Klas Rosén





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## Introduction

This study focuses on the distribution of radiocesium ( $^{137}\text{Cs}$ ) and selected metals in soil fractions and soil fungi of boreal forest ecosystems. The accumulation of selected metals in soil fractions: bulk soil, rhizosphere, soil-root interface and fungal mycelium and sporocarps of mycorrhizal fungi were compared in a Swedish forest. Special attention is given to radiocesium released into the environment as a result of nuclear weapons testing and the Chernobyl accident in 1986, and alkali metals, potassium (K), rubidium (Rb), and cesium ( $^{133}\text{Cs}$ ), whose chemical behavior can be expected to be similar to  $^{137}\text{Cs}$ . The behavior of  $^{137}\text{Cs}$  in forest ecosystems differs from other ecosystems due to the abundance of fungal mycelia in soil, which contribute to the persistence of the Chernobyl radiocesium in the upper horizons of forest soils, as the fungi enhance uptake of these elements into host plants. Even many years after fallout, people in Sweden consume wild fungi and game obtained from these contaminated forests. Substantial research has been conducted in Sweden after the fallout from nuclear weapons testing and the Chernobyl accident and some results presented in this book are published in a series of several articles and book chapters in collaboration with Profs K. J. Johanson, H. Rydin, and Dr. A. Taylor (Vinichuk et al. 2004; 2010a; 2010b; 2011a; 2011b).

Fungi are effective in accumulating a wide range of metals, as well as radioactive isotope  $^{137}\text{Cs}$ . Many trace elements, including some micronutrients, such as mercury (Hg), lead (Pb), cadmium (Cd), copper (Cu), nickel (Ni), and cobalt (Co) are generally considered the most toxic, and numerous studies indicate accumulation of metals by fungal sporocarps (Mietelski et al. 2002; Campos et al. 2012).

However, the contribution of wild growing mycelia and soil fractions, such as the rhizosphere and soil-root interface, with metal accumulation and distribution within forest soil is not well studied. Therefore, we attempted to quantify the

uptake and distribution of selected metals in the soil-mycelium-sporocarps compartments in various transfer steps: bulk soil, rhizosphere, soil-root interface, fungal mycelium, and sporocarps. The relationships between the concentrations of metals studied in bulk soil, soil mycelia, and fungal sporocarps were estimated.

The  $^{137}\text{Cs}$  activity concentration and mass concentration of alkali metals K, Rb, and  $^{133}\text{Cs}$  were also analyzed within individual *Sphagnum* plants (down to 20 cm depth) in boreal ombrotrophic bogs in the northern hemisphere. The distribution of Cs ( $^{133}\text{Cs}$  and  $^{137}\text{Cs}$ ), K, and Rb in the uppermost capitulum and subapical segments of *Sphagnum* mosses were compared to determine the possible mechanisms involved in radiocesium uptake and retention within *Sphagnum* plants.

This book attempts to summarize the knowledge acquired from studies within Sweden and to place them in a larger context, and to update data on metals concentrations in fungal compartments of forest soil, especially in wild growing mycelium.

The discussion of the summarized results addresses the issues of radiocesium ( $^{137}\text{Cs}$ ) activity concentrations, K, Rb, and stable  $^{133}\text{Cs}$  concentrations in soil fractions, fungal compartments and *Sphagnum* plants (Chapter 2); alkali earth metals calcium (Ca) and strontium (Sr) (Chapter 3); transition metals chromium (Cr), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), cadmium (Cd), mercury (Hg), and lead (Pb) (Chapter 4); semimetals arsenic (As) (Chapter 5); and, actinides thorium (Th) and uranium (U) (Chapter 6).

To place the work into a context, a short description of the study area, study design, and methods used is presented.

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**Study Area,  
Design,  
and Methods**

**1**



## Study Area

The K, Rb,  $^{133}\text{Cs}$  concentrations and  $^{137}\text{Cs}$  activity concentrations in soil fractions and fungal compartments were studied in an area located in a forest ecosystem on the east coast of central Sweden (N 60°22'; E 18°13'). The soil was a sandy or clayey till and the humus mainly occurred in the form of mull. A more detailed description of the study area is presented in Vinichuk et al. (2010b).

Sporocarps of ectomycorrhizal fungi *Suillus variegatus* were studied in an area located about 40 km north-west of Uppsala in central Sweden (N 60°08'; E 17°10'). The forest, located on moraine, is dominated by Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*), with inserts of deciduous trees, primarily birch (*Betula pendula* and *Betula pubescens*). The field layer consisted mainly of dwarf shrubs – bilberry (*Vaccinium myrtillus* L.), lingonberry (*Vaccinium vitis-idaea* L.), and heather (*Calluna vulgaris* L.); details about the area and sampling are presented in Dahlberg et al. (1997).

## Study Design

For determining K, Rb, and  $^{133}\text{Cs}$  concentrations in soil fractions and fungal compartments, samples of soil and fungal sporocarps were collected from 10 sampling plots during September to November 2003. Four replicate soil samples were taken, by a cylindrical steel tube with a diameter of 5.7 cm, from around and directly underneath the fungal sporocarps (an area of about 0.5 m<sup>2</sup>), and within each 10 m<sup>2</sup> area to a depth of 10 cm. Soil cores were divided horizontally into two 5-cm thick layers. Sporocarps of 12 different fungal species were collected and identified to species level, and the  $^{137}\text{Cs}$  activity

concentrations in fresh material determined with calibrated HPGe detectors in laboratories at Department of Soil and Environment of Swedish University of Agricultural Sciences. The sporocarps were dried at 35 °C to constant weight and concentrations of K, Rb, and  $^{133}\text{Cs}$  were determined.

In addition, a selection of dried sporocarps of *Suillus variegatus* (n=51), retained from a study by Dahlberg et al. (1997), on the relationship between  $^{137}\text{Cs}$  activity concentrations and genotype identification, was used. These sporocarps were collected once a week during sporocarp season (end of August through September) in 1994, and were taken from five sampling sites (100 to 1600 m<sup>2</sup> in size) within an area of about 1 km<sup>2</sup>. Among mentioned above sporocarps eight genotypes with 2 to 8 sporocarps each were tested (in total 32 sporocarps): these are referred to as individual genotypes. Sporocarps within genotypes were spatially separated by up to 10-12m. All genotypes were used in the estimation of correlation coefficients, but only genotypes with at least four sporocarps were included in the alkali metal analyses. In addition, 19 Dahlberg's individual sporocarps with unknown genotype (i.e. not tested for genotype identity) were included: these sporocarps consisted of both the same and different genotypes. The combined set of sporocarps refers to all sporocarps: for further details about the sampling and identification of genotypes see Dahlberg et al. (1997). The  $^{137}\text{Cs}$  activity concentration values were corrected to sampling date and expressed as kBq kg<sup>-1</sup> dry weight (DW) for each sporocarp, as reported by Dahlberg et al. (1997).

## Methods

For  $^{137}\text{Cs}$  activity concentrations and concentrations of selected metals in soil fractions and fungal compartments, fungal mycelia were separated with forceps

from the soil samples (30-50 g, 0-5 cm layer depth) under a dissection microscope (magnification X64), small amounts of distilled water were added to disperse the soil. The prepared fraction of mycelium (30-60 mg DW g<sup>-1</sup> soil) was not identified to determine whether the mycelia extracted from the soil samples and the sporocarps belonged to the same species, as it was assumed a majority of the prepared mycelia belonged to the same species as the nearby sporocarps. The method for mycelium preparation is described in Vinichuk & Johanson (2003). Mycelium samples were dried at 35 °C to constant weight for determining <sup>137</sup>Cs activity concentration and metal concentrations.

The soil samples (0-5 cm layer) were partitioned according to the method described by Gorban & Clegg (1996). First, the soil was gently sieved through a 2 mm mesh to give a bulk soil fraction. The remaining soil aggregates containing roots were further crumbled and gently squeezed between the fingers: this was called the rhizosphere fraction. The residue (which is the finest roots with adhering soil particles) was called the soil-root interface fraction. Nine samples of bulk soil fraction and mycelium, 12 samples of fungal sporocarps, and 6 samples of rhizosphere and soil-root interface fraction were analyzed for <sup>137</sup>Cs activity concentration and metal concentrations.

The <sup>137</sup>Cs activity concentrations in bulk soil samples and sporocarps were determined with calibrated HP-Ge detectors, corrected to sampling date, and expressed as Bq kg<sup>-1</sup> DW. The measuring time used provided a statistical error ranging between 5 and 10%. For element analyses, a 2.5 g portion of each sample was analyzed by inductively coupled plasma at the laboratories of ALS Scandinavia (Luleå, Sweden). Plant certified reference material, peach leaves NIST 1547 (NIST, Gaithersburg, USA), which has a matrix sufficiently close to fine roots and fungal material, was used for accuracy assessment: the recoveries were 102.4% for Co, 101.4% for Ni, 103.5% for Cu; 99.4% for Zn, 104.6% for

Cd, and 101.9% for Pb. For soil, CRM SO-2 (heavy metals in soil) was used, but this has no certified values for the metals studied. The detailed measurement procedure is presented in Rodushkin et al. (2008). Bioconcentration ratios (BCR) were defined as the concentration of the element ( $\text{mg kg}^{-1}$  DW) in the specific fraction divided by the concentration of the element ( $\text{mg kg}^{-1}$  DW) in bulk soil for the 0-10 cm soil layer. Element concentrations in the fractions analyzed are reported as  $\text{mg kg}^{-1}$  DW.

For element analyses (K, Rb and  $^{133}\text{Cs}$ ) of *S. variegatus* sporocarps, aliquots of about 0.3 g of each sample were analyzed by inductively coupled plasma technique. Element concentrations are reported as  $\text{mg kg}^{-1}$  DW. The isotopic ratio of  $^{137}\text{Cs}/^{133}\text{Cs}$  was calculated with Equations 1 and 2 (Chao et al. 2008):

$$\frac{^{137}\text{Cs}}{^{133}\text{Cs}} = \frac{A}{C} \times \frac{a}{\lambda \times N} \times 10^3 \quad (\text{Equation 1})$$

where: A is the  $^{137}\text{Cs}$  radioactivity ( $\text{Bq kg}^{-1}$ );  $\lambda$  is the disintegration rate of  $^{137}\text{Cs}$   $7.25 \times 10^{-10} \text{ s}^{-1}$ ; a is the atomic weight of cesium (132.9); N is the Avogadro number, which is  $6.02 \times 10^{23}$ ; and,  $^{133}\text{C}$  and C are the  $^{133}\text{Cs}$  concentration ( $\text{mg g}^{-1}$ ). Equation (1) can be simplified to Equation 2:

$$\frac{^{137}\text{Cs}}{^{133}\text{Cs}} = 3.05 \times 10^{-10} \times \frac{A}{C} \quad (\text{Equation 2})$$

where: A is the  $^{137}\text{Cs}$  activity concentration in  $\text{Bq kg}^{-1}$  and C is the  $^{133}\text{Cs}$  concentration in  $\text{mg kg}^{-1}$ . Thus, the units of the isotope ratio are dimensionless.

The relationships between  $^{137}\text{Cs}$  activity concentrations and metals concentrations in different soil fractions, mycelia, and sporocarps of *S. variegatus* were identified by Pearson correlation coefficients. Correlation coefficients for sporocarps of *S. variegatus* were analyzed in five separate sets of samples: in four sets, all samples had known genotypes and, the last set was a combined set of samples containing both genotypes tested by somatic

incompatibility sporocarps and genotypes that had not been tested. Correlation analyses for genotypes with three or less sporocarps were omitted. All statistical analyses were run with Minitab® 15.1.1.0. software (© 2007 Minitab Inc.); the level of significance was 5% (0.05), 1% (0.01) and 0.1% (0.001).



**Cesium ( $^{137}\text{Cs}$  and  
 $^{133}\text{Cs}$ ) and Alkali  
Metals K and Rb**

**2**



Cesium is an alkali metal and a member of the alkali family in the same group as lithium, sodium, potassium, rubidium, and francium. Stable cesium ( $^{133}\text{Cs}$ ) is the only naturally occurring isotope of cesium and presents in the crust in small amounts. There are a number of artificial radioactive isotopes of cesium. Radioactive cesium ( $^{137}\text{Cs}$ ) is produced in nuclear fission reactions and is of special interest.

### **Cesium ( $^{137}\text{Cs}$ and $^{133}\text{Cs}$ ) and Alkali Metals K and Rb in Forest Soil and Fungi**

Radiocesium ( $^{137}\text{Cs}$ ) has been released into the environment by nuclear weapons testing in the 1950s and 1960s, the Chernobyl accident in 1986, and from the Fukushima Daiichi Nuclear Power Plant (FDNPP) in 2011. As  $^{137}\text{Cs}$  has a long half-life of 30 years and high fission yield, it is still a critical fission product. The study of the cesium radioisotope  $^{137}\text{Cs}$  is important, as the production and emission rates are much higher than other radioisotopes, and as the cesium compounds being water soluble, it moves easily and spreads in the environment, and can result in significant damage to living cells. The results obtained in several experimental studies conducted in Swedish forest ecosystems and peatlands are presented here, along with a discussion on the behavior of cesium isotopes ( $^{137}\text{Cs}$  and  $^{133}\text{Cs}$ ) and their counterparts potassium (K) and rubidium (Rb) in the “soil fractions-soil fungi transfer” system.

The bioavailability of radionuclides controls the ultimate exposure of living organisms and the ambient environment to these contaminants. Thus, the understanding of bioavailability of radionuclides is a key issue in the field of radioecology, both conceptually and methodologically. Soil-fungi-plant transfer is the first step by which  $^{137}\text{Cs}$  enters food chains in forest ecosystems.

The behavior of  $^{137}\text{Cs}$  in forest ecosystems differs substantially from other ecosystems, due to the abundance of fungal mycelia in soil, which contribute to the persistence of Chernobyl radiocesium in the upper horizons of forest soils (Vinichuk & Johanson, 2003). In the microbial biomass in boreal forest soil, fungi are dominant. The mycelium of both saprotrophic and mycorrhizal soil fungi has a central role in both breaking down organic matter and in the uptake of nutrients from soil into plants via the formation of symbiotic mycorrhizal associations (Read & Perez-Moreno, 2003). The mycelium mobilizes nutrients from organic substrates through the action of extracellular catabolic enzymes, thus facilitating nutrient uptake into the host plant (Leake & Read, 1997), and are efficient at taking-up and accumulating microelements (Smith & Read, 1997). As a result, the fruit bodies of fungi are able to accumulate significant amounts of trace elements, both metals and metalloids. This ability also results in the accumulation of non-essential elements and radionuclides, particularly  $^{137}\text{Cs}$ , and can have important consequences for the retention, mobility, and availability of these elements in forest ecosystems (Steiner et al. 2002).

The uptake of radiocaesium by fungi is variable and affected by the fungi's environment. Many fungal species accumulate more  $^{137}\text{Cs}$  than vascular plants do, and  $^{137}\text{Cs}$  activity concentrations in many fungi are 10 to 100 times higher than in plants (Ros n et al. 2011), indicating their substantial contribution to  $^{137}\text{Cs}$  cycling in forest systems.

Fungi are principally important in radiocesium migration in nutrient poor and organic rich soils of forest systems (Rafferty et al. 1997). The presence of single strains of saprotrophic fungi in organic matter considerably enhances the retention of Cs in organic systems, with  $\approx 70\%$  of the Cs spike being strongly (irreversibly) bound (remains non-extractable) (Parekh et al. 2008), compared to only  $\approx 10\%$  in abiotic (sterilized) systems.

Generally, fungal mycelium contains a substantial amount of radiocesium: up to 50% of the total  $^{137}\text{Cs}$  may be located within the upper 0-10 cm layers in Swedish and Ukrainian forest soils (Vinichuk & Johanson, 2003, Vinichuk et al. 2004). In terms of the total radiocesium within a forest ecosystem, fungal sporocarps contain a relatively little and may only account for about 0.5 % (McGee et al. 2000) or even less – 0.01 to 0.1% (Nikolova et al. 1997) of the total radiocesium deposited within a forest ecosystem. Based on the calculation of the total vegetation biomass and through relationships between fungal biomass in both sporocarps and mycelia in soil, the total  $^{137}\text{Cs}$  activity located in fungi is estimated as 0.1% in bog, 2% in pine swamp, and 11% in forest (Vinichuk et al. 2013). However, these estimates are based on the assumption that radionuclide concentration in fungal sporocarps is similar to of the concentration in the fungal parts of mycorrhizae (Nikolova et al. 1997). Although activity concentration in sporocarps is probably higher than in mycelium (Vinichuk & Johanson, 2003, Vinichuk et al. 2004), sporocarps constitute only about 1% of the total mycelia biomass in a forest ecosystem. Due to the high levels of  $^{137}\text{Cs}$  in sporocarps, their contribution to the internal dose in humans may be high through consumption of edible mushrooms (Kalač, 2001). Consequently, the consumption of sporocarps of edible fungi (Skuterud et al. 1997), or of game animals that consume large quantities of fungi with high  $^{137}\text{Cs}$  contents (Johanson & Bergström, 1994), represents an important pathway through which  $^{137}\text{Cs}$  enters the human food system. However,  $^{137}\text{Cs}$  activity concentration in edible fungi species has neither decreased since the late 1990s (*S. variegatus*) nor significantly increased (*Cantharellus* spp.) (Mascanzoni, 2009; Ros  et al. 2011).

Although fungi are important for  $^{137}\text{Cs}$  uptake and migration in forest systems, the reasons and mechanisms for the magnitude higher concentration of

radiocesium in fungi than in plants remains unclear (Bystrzejewska-Piotrowska & Bazala, 2008). In addition to radiocesium, fungi effectively accumulate potassium (K), rubidium (Rb) and stable cesium ( $^{133}\text{Cs}$ ) (Gasó et al. 2000), and the concentrations of  $^{137}\text{Cs}$ ,  $^{133}\text{Cs}$ , and Rb in fungal sporocarps can be one order of magnitude higher than in plants growing in the same forest (Vinichuk et al. 2010b).

The chemical behavior of the alkali metals, K, Rb, and  $^{133}\text{Cs}$  can be expected to be similar to  $^{137}\text{Cs}$  due to similarities in their physicochemical properties, e.g. valence and ion diameter (Enghag, 2000). Potassium is a macronutrient and an obligatory component of living cells, which depend on  $\text{K}^+$  uptake and flux to grow and maintain life. Although potassium is not a permanent structural organic component of plants, potassium uptake is usually higher than any other macronutrient. In radioecology, cesium is assumed to behave similarly to potassium. There is evidence fungi cannot distinguish between cesium and potassium, therefore, cesium can occupy potassium-binding sites when potassium is deficient (Zichner, 2000). As it accumulates within cells, potassium is the most important ion for creating membrane potential and excitability.

Radioactive ( $^{137}\text{Cs}$ ) and stable ( $^{133}\text{Cs}$ ) cesium and K are assumed to assimilate in a similar fashion with the elements passing through the biological cycle together (Chao et al. 2008). The influx of Cs into cells and its use of K transporters are reviewed by White & Broadley (2000), and potassium transport in fungi is reviewed by Rodríguez-Navarro (2000).

Rubidium (Rb) is another rarely studied alkali metal, which is consistently biomagnified in diverse food webs (Campbell et al. 2005) and may be an essential trace element for organisms, including fungi. This element accumulates in large amounts in certain fungal species (Campos et al. 2012), and fungal fruit bodies

have substantially higher Rb concentrations than plants (Yoshida & Muramatsu, 1998). The concentrations of K, Rb, and  $^{133}\text{Cs}$  have been analyzed in fungal sporocarps (Baeza et al. 2005; Vinichuk et al. 2010b; 2011a) and a relation between the uptake of Cs and K has been found (Bystrzejewska-Piotrowska & Bazal, 2008). The content of K, Rb, and  $^{133}\text{Cs}$  in the fungi's environment appears important and radiocesium uptake in fungi is affected by the presence of K, Rb, and  $^{133}\text{Cs}$  (Gyuricza et al. 2010; Terada et al. 1998).

However, there is scarce information on the concentration and distribution of Rb in fungi, particularly in the mycelial part, and its behavior in food webs originating in the forest. Rubidium is often used in studies on K uptake, as it appears to emulate K (Marschner, 1995): both K and Rb have the same uptake kinetics and compete for transport along concentration gradients in different soil and organisms compartments (Rodríguez-Navarro, 2000). However, in fungal sporocarps, the relationships between these alkali metals and  $^{137}\text{Cs}$ , when taken up by the fungi, and the underlying mechanisms are insufficiently understood, as Cs does not always have high correlation with K, and it is suggested there is an alternative pathway for Cs uptake into fungal cells (Yoshida & Muramatsu, 1998).

The correlations between  $^{137}\text{Cs}$  and these alkali metals suggest the mechanism of fungal uptake of  $^{133}\text{Cs}$  and  $^{137}\text{Cs}$  is different from K, and that Rb has an intermediate behavior between K and  $^{133}\text{Cs}$  (Yoshida & Muramatsu, 1998). However, this interpretation is based on a few sporocarp analyses from each species, and comprising different ectomycorrhizal and saprotrophic fungal species. In spite of the fact that fungal accumulation of  $^{133}\text{Cs}$  is reported as species-dependent, there are few detailed studies of individual species (Gillet & Crout, 2000), and the variation in  $^{137}\text{Cs}$  levels within the same genotype of fungal sporocarps can be as large as the variation among different genotypes (Dahlberg et al. 1997).

Another way of interpreting and understanding the uptake and relations between  $^{137}\text{Cs}$ ,  $^{133}\text{Cs}$ , K, and Rb in fungi is to use the isotopic (atom) ratio  $^{137}\text{Cs}/^{133}\text{Cs}$ . Although  $^{133}\text{Cs}$  and  $^{137}\text{Cs}$  are the same chemically, atom abundance and isotopic disequilibrium differ, and among other factors, the uptake of  $^{133}\text{Cs}$  and  $^{137}\text{Cs}$  by fungi depends on whether equilibrium between the two isotopes is achieved. Within forest ecosystems, equilibrium between stable  $^{133}\text{Cs}$  and  $^{137}\text{Cs}$  in the bioavailable fraction of soils is reported (Karadeniz & Yaprak, 2007), but in cultivated soils, equilibrium between fallout  $^{137}\text{Cs}$  and stable  $^{133}\text{Cs}$  among exchangeable, organic bound and strongly bound fractions has not reached, even 20 years after most of the  $^{137}\text{Cs}$  was deposited on the soils (Tsukada, 2006).

The mechanisms involved in nutrient uptake by fungi in forest soils, in particular, the role of fungi in  $^{137}\text{Cs}$  transfer between soil and fungi require better understanding. Although transfer of radioactive cesium from soils to plants through fungi is well researched, there is still limited knowledge on the transfer of stable  $^{133}\text{Cs}$  and other alkali metals (K and Rb) through fungi. However, alkali metals have a potential role in predicting radiocesium behavior, and there is a relationship between  $^{133}\text{Cs}$  and other alkali metals (K and Rb) during uptake by fungi (Vinichuk et al. 2011b).

To be able to explore the mechanisms governing the uptake of radionuclides ( $^{137}\text{Cs}$ ), data are required on the uptake of stable isotopes of alkali metals (K, Rb,  $^{133}\text{Cs}$ ) by fungal species, and the behavior of the three alkali metals K, Rb, and  $^{133}\text{Cs}$  in bulk soil, fungal mycelium, and sporocarps. Therefore, an attempt was made to quantify the uptake and distribution of the alkali metals in the soil-mycelium-sporocarp compartments, and to study the relationships between K, Rb, and  $^{133}\text{Cs}$  in the various transfer steps (Vinichuk et al. 2010a, 2010b, 2011a, 2011b). The sporocarps of ectomycorrhizal fungi *Suillus variegatus* were analyzed to determine whether i) Cs ( $^{133}\text{Cs}$  and  $^{137}\text{Cs}$ ) uptake was correlated

with K uptake; ii) intraspecific correlation of these alkali metals and  $^{137}\text{Cs}$  activity concentrations in sporocarps was higher within, rather than among, different fungal species; and, iii) the genotypic origin of sporocarps affected uptake and correlation.

## K, Rb and $^{133}\text{Cs}$ Concentrations in Soil Fractions and Fungal Compartments

From the concentrations of K, Rb, and stable cesium ( $^{133}\text{Cs}$ ) in soil fractions and fungal compartments, the bioconcentration ratio (BCR) at each step of transfer in the soil-fungi system can be calculated, and differences in uptake between elements and their relationships can be determined. This may be the main reason for the different K, Rb, and  $^{133}\text{Cs}$  concentrations observed in sporocarps of various fungal species (Vinichuk et al. 2011b). The concentrations of K, Rb, and  $^{133}\text{Cs}$  in bulk soil are similar to those found in rhizosphere fraction, although the values for all three elements are slightly higher in the rhizosphere fraction (Table 2.1).

**Table 2.1** Mean concentrations of K, Rb, and  $^{133}\text{Cs}$  ( $\text{mg kg}^{-1} \text{DW} \pm \text{standard deviation}$ ) in soil fractions and fungi<sup>1</sup> (Vinichuk et al. 2010b, 2011b).

Element	Bulk soil	Rhizosphere	Soil root interface	Fungal mycelium	Fruit bodies
K	642±215 <sup>a</sup>	899 ±301 <sup>a</sup>	3 215±843 <sup>b</sup>	2 867±728 <sup>b</sup>	43 415±20 436
Rb	3.9±2.7 <sup>a</sup>	5.4±4.4 <sup>a</sup>	6.8±1.7 <sup>a</sup>	13.8±6.9 <sup>b</sup>	254±274
$^{133}\text{Cs}$	0.3±0.2 <sup>a</sup>	0.4±0.3 <sup>a</sup>	0.2±0.05 <sup>a</sup>	0.8±0.8 <sup>a</sup>	5.7±7.1 <sup>b</sup>

<sup>1</sup>Means within rows with different letters (a or b) are significantly different ( $p < 0.001$ ).

Potassium concentrations are higher in both the soil-root interface and fungal mycelium fractions than in the bulk soil and rhizosphere fraction. Fungal sporocarps accumulate much greater amounts of K, Rb, and  $^{133}\text{Cs}$  than mycelium. For example, K concentrations in fungal sporocarps collected from the same plots

where soil samples and mycelium were extracted are about 15 times higher than K concentrations found in mycelium (Vinichuk et al. 2010b). The concentrations of Rb in fungal sporocarps are about 18-fold higher than in corresponding fungal mycelium, and those of  $^{133}\text{Cs}$  are about 7-fold higher (Table 2.1).

Thus, potassium concentration increases in the order bulk soil < rhizosphere < fungal mycelium < soil-root interface < fungal sporocarps and is higher in the soil-root interface fraction and fungi than in bulk soil. The high concentrations of K in fungal sporocarps may reflect a demand for this element as a major cation in osmoregulation and that K is an important element in regulating the productivity of sporophore formation in fungi (Tyler, 1982).

Rubidium in mycelium is about 3.5-fold higher than in bulk soil and about 2.5-fold higher than in rhizosphere: the concentrations of Rb increase in the order bulk soil < rhizosphere < soil-root interface < fungal mycelium < fungal sporocarps and are slightly higher in the soil-root interface fraction than in bulk soil. Thus, fungi appear to have high preference for Rb, as the accumulation of Rb by fungi, and especially fungal sporocarps, is well pronounced. Rubidium concentrations in sporocarps are more than one order of magnitude higher than in mycelium extracted from soil of the same plots where fungal sporocarps are sampled. Fungi have the ability to accumulate Rb: mushrooms accumulate at least one order of magnitude higher concentrations of Rb than plants growing in the same forest (Yoshida & Muramatsu, 1998).

Concentrations of stable cesium vary among soil fractions but the differences are not significant (Vinichuk et al. 2010b). Stable  $^{133}\text{Cs}$  is generally evenly distributed within bulk soil, rhizosphere, and soil-root interface fractions, indicating no  $^{133}\text{Cs}$  enrichment in these forest compartments. Thus, cesium concentrations increase in the order soil-root interface < bulk soil < rhizosphere <

fungal mycelium < fungal sporocarps, and are only significantly higher in fungal sporocarps, than in bulk soil. Concentrations of  $^{133}\text{Cs}$  in sporocarps are nearly one order of magnitude higher than concentrations of  $^{133}\text{Cs}$  in soil mycelium.

The behavior of radioactive  $^{137}\text{Cs}$  appears similar to  $^{133}\text{Cs}$ : the activity concentrations of  $^{137}\text{Cs}$  increase in the order soil < mycelium < fungal sporocarps (Vinichuk & Johanson, 2003; Vinichuk et al. 2004). The differences between fungal species in their preferences for uptake of radioactive  $^{137}\text{Cs}$  or stable  $^{133}\text{Cs}$  may reflect the location of the fungal mycelium relative to the location of cesium within the soil profile (Rühm et al. 1997). Unlike  $^{137}\text{Cs}$ , stable  $^{133}\text{Cs}$  originates from soil; therefore, the amount of unavailable  $^{133}\text{Cs}$  in soil, compared to the total amount of  $^{133}\text{Cs}$ , is presumably higher than that of  $^{137}\text{Cs}$ . As a result, stable  $^{133}\text{Cs}$  is less available for uptake, as it is contained in mineral compounds and is difficult for fungi or plants to access: the concentration ratio of stable  $^{133}\text{Cs}$  in mushrooms is reported to be lower than for  $^{137}\text{Cs}$  (Yoshida & Muramatsu, 1998). The difference in behavior between naturally occurring and radioactive forms of  $^{133}\text{Cs}$  may also derive from their disequilibrium in the ecosystem (Horyna & Řanad, 1988).

### **Concentration Ratios of K, Rb, and $^{133}\text{Cs}$ in Soil Fractions and Fungi**

The concept of bioconcentration ratios (BCR, defined as the concentration of the element ( $\text{mg kg}^{-1}$  DW) in a specific fraction or fungi divided by the concentration of the element ( $\text{mg kg}^{-1}$  DW) in bulk soil) is widely used to quantify the transfer of radionuclides from soil to plants/fungi. This approach allows the estimation of differences in uptake of the elements. The elemental concentration ratio has a similar pattern to their content in the respective fraction, but the enrichment of all three elements in fungal material is more evident, particularly in sporocarps (Table 2.2).

**Table 2.2** Bioconcentration ratios in bulk soil, mean values  $\pm$  standard deviation).  
Adapted from Vinichuk et al. (2010b).

Element	Rhizosphere	Soil root-interface	Fungal mycelium	Fruit bodies
K	1.7 $\pm$ 0.4	6.1 $\pm$ 1.9	5.1 $\pm$ 1.4	68.9 $\pm$ 23.1
Rb	1.3 $\pm$ 0.4	2.7 $\pm$ 1.1	3.9 $\pm$ 1.1	122.7 $\pm$ 172.2
Cs	1.1 $\pm$ 0.5	0.8 $\pm$ 0.3	2.1 $\pm$ 0.9	39.7 $\pm$ 67.6

Thus, for all three alkali metals, the levels of K, Rb,  $^{133}\text{Cs}$ , and  $^{137}\text{Cs}$  in sporocarps are at least one order of magnitude higher than the levels in fungal mycelium (Table 2.2, Vinichuk et al. 2010b). The concentration ratios for each element vary considerably among fungal sporocarps of sampled species. The saprotrophic fungus *Hypholoma capnoides* grown in boreal forest has the lowest values and the mycorrhizal fungus *Sarcodon imbricatus* has the highest. The concentration ratios of sporocarps ( $\text{mg kg}^{-1}$  DW in fungi)/( $\text{mg kg}^{-1}$  DW in bulk soil) are presented in Table 2.3.

*Sarcodon imbricatus* accumulates nearly 100 000 Bq  $\text{kg}^{-1}$  of  $^{137}\text{Cs}$ , giving TF values (defined as  $^{137}\text{Cs}$  activity concentration ( $\text{Bq kg}^{-1}$  DW) in fungi divided by  $^{137}\text{Cs}$  deposition ( $\text{kBq m}^{-2}$ )) of about 22 (Vinichuk & Johanson, 2003). The sporocarps of *Sarcodon imbricatus* have distinctively higher bioconcentration ratios of Rb and  $^{133}\text{Cs}$  than other species that have been analyzed. The mycorrhizal fungus *Cantharellus tubaeformis*, which grows on living or rotten wood, is another species with relatively high concentration ratios, particularly for K and Rb and accumulates several tens of thousands Bq  $\text{kg}^{-1}$  of  $^{137}\text{Cs}$  (Kammerer et al. 1994). Among the fungi with moderate concentration ratios for each element are *Boletus edulis*, *Tricholoma equestre*, *Lactarius scrobiculatus* and *Cortinarius* spp.

**Table 2.3** Elemental bioconcentration in fungi for fungal sporocarps.  
Adapted from Vinichuk et al. (2010b).

Sampling plots according to Vinichuk et al. (2010b)	Species	Concentration ratios		
		K	Rb	$^{133}\text{Cs}$
4	<i>Boletus edulis</i>	62.7	77.4	37.4
6	<i>Cantharellus tubaeformis</i>	1045	110	15.5
7	<i>Cortinarius armeniacus</i>	67.5	69.6	19.2
5	<i>C. odorifer</i>	71.8	70.9	34.7
8	<i>C. spp.</i>	90.9	157.2	14.8
8-10	<i>Hypholoma capnoides</i> <sup>1</sup>	26.6	13.1	6.9
1	<i>Lactarius deterrimus</i>	29.9	17.2	2.6
3	<i>L. scrobiculatus</i>	67.8	26.2	3.7
6	<i>L. trivialis</i>	77.5	126.9	52.2
5-7	<i>Sarcodon imbricatus</i>	102	676	259
2	<i>Suillus granulatus</i>	58.6	41.4	14.7
10-11	<i>Tricholoma equestre</i>	66.6	75.4	15.4

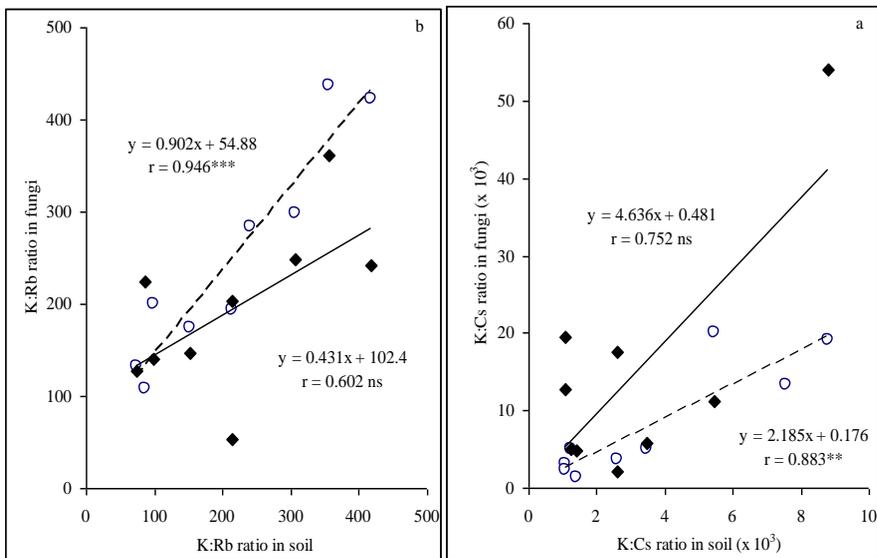
<sup>1</sup>Saprophyte, all other analyzed fungal species are ectomycorrhizal (Vinichuk et al. 2010b)

Thus, the levels of K, Rb,  $^{133}\text{Cs}$ , and  $^{137}\text{Cs}$  in sporocarps are at least one order of magnitude higher than the levels in fungal mycelium, indicating biomagnification through the food web in forest ecosystems. The saprotrophic fungus *Hypholoma capnoides* has the lowest CR values and the mycorrhizal fungus *Sarcodon imbricatus* has the highest CR values.

### Relationships between K, Rb, and $^{133}\text{Cs}$ in Soil and Fungi

Although correlation analysis may be not definitive, it is a useful approach for elucidating similarities or differences in uptake mechanisms of cesium ( $^{137}\text{Cs}$  and  $^{133}\text{Cs}$ ), K, and Rb: close correlation between elements indicates similarities in uptake mechanisms. However, no significant correlations between K in soil and in either mycelium ( $r = 0.452$ , ns) or in sporocarps ( $r = 0.338$ , ns) have been identified, and sporocarp Rb and  $^{133}\text{Cs}$  concentrations are unrelated to soil

concentrations. However, in mycelium both elements are correlated with soil concentrations (Rb:  $r=0.856$ ,  $p=0.003$ ; Cs:  $r=0.804$ ,  $p=0.009$ ). The K: $^{133}\text{Cs}$  ratio in soil and fungal components has the following pattern: the K:Cs ratio in mycelium is closely positively correlated ( $r=0.883$ ,  $p=0.01$ ) to the K: $^{133}\text{Cs}$  ratio in soil (Figure 2.1a), but is relatively weak and not-significantly correlated to soil in fungal sporocarps. There is a close positive correlation ( $r=0.946$ ,  $p=0.001$ ) between the K:Rb ratio in soil and in fungal mycelium (Figure 2.1b): this relationship is also apparent between soil and sporocarps, but is weak and non-significant ( $r=0.602$ , ns: Figure 2.1b). No significant correlations have been identified among the concentrations of the three elements in fungi, soil pH, or soil organic matter content (data not shown).



**Figure 2.1** Ratio of (a) K: $^{133}\text{Cs}$  and (b) K:Rb in fungal sporocarps (◆, solid line) and soil mycelium (○, dotted line) in relation to the soil in which they are growing (Vinichuk et al. 2010b). \*\*  $p=0.01$ , \*\*\*  $p=0.001$ .

In an attempt to estimate the relationships between the concentrations of K, Rb, and  $^{133}\text{Cs}$  in soil, mycelia, and fungal sporocarps, the competition between

these elements in the various transfer steps has been investigated (Vinichuk et al. 2010b). The lack of a significant correlation between K in soil and in either mycelium or sporocarps indicates a demand for essential K within fungi, regardless of the concentration of this element in the soil. Irrespective of fungal species, K concentration in fungi appears to be controlled within a narrow range (Yoshida & Muramatsu, 1998), and supports the claim K uptake by fungi is self-regulated through internal nutritional requirements of the fungus (Baeza et al. 2004).

In fungal sporocarps, when there is no relationship between uptake of Cs and K, Rb concentrations are related to concentrations of stable Cs, and the concentrations of K and Rb only moderately related (Table 2.4).

**Table 2.4** Correlation coefficients among K, Rb, and Cs concentration in fruit bodies of fungi.

	K	Rb
Rb	0.51*	
Cs	0.26	0.91**

\*  $P < 0.05$ , \*\*  $P < 0.01$

The relationships observed between K:Rb and K: $^{133}\text{Cs}$  ratios in fungal sporocarps and soil mycelia, with respect to the soil in which they are growing (Figure 2.1), also indicate differences in fungal uptake of these alkali metals. Although correlation analyses are not the best tool for analyzing uptake mechanism, the closest positive correlations between K:Rb ratios in fungal mycelium and in soil indicate similarities in the uptake mechanism of these two elements by fungi, although the relationships between K: $^{133}\text{Cs}$  ratios in soil mycelium and in soil are less pronounced. Yoshida & Muramatsu (1998) suggest there may be an alternative pathway for  $^{133}\text{Cs}$  uptake into cells and the mechanism of  $^{133}\text{Cs}$  uptake by fungi is similar to that for Rb, as  $^{133}\text{Cs}$  does not have good

correlation with K. The high efficiency of Rb uptake by fungi indicates Rb, but not  $^{133}\text{Cs}$ , eventually replaces essential K, due to K limitation (Brown & Cummings, 2001), and Rb has the capacity to partially replace K, whereas  $^{133}\text{Cs}$  does not (Wallace, 1970, and references therein). Forest plants apparently discriminate between  $\text{K}^+$  and  $\text{Rb}^+$  in soils, and a shortage of  $\text{K}^+$  favors uptake of the closely related  $\text{Rb}^+$  ion (Nyholm & Tyler, 2000), whereas, increasing  $\text{K}^+$  availability in the system decreases  $\text{Rb}^+$  uptake (Drobner & Tyler, 1998). These results provide new insights into the use of transfer factors or concentration ratios.

### **The Isotopic (Atom) Ratios $^{137}\text{Cs}/\text{K}$ , $^{137}\text{Cs}/\text{Rb}$ and $^{137}\text{Cs}/^{133}\text{Cs}$ in Fungal Species**

The isotopic ratios of  $^{137}\text{Cs}/\text{K}$ ,  $^{137}\text{Cs}/\text{Rb}$ , and  $^{137}\text{Cs}/^{133}\text{Cs}$  in the fungal sporocarps belonging to different species have been used to interpret the distribution of  $^{137}\text{Cs}$  and the alkali metals in fungi and to provide better understanding of the uptake mechanisms. Measurements of trace levels of stable  $^{133}\text{Cs}$  can provide information about the biological behavior of  $^{137}\text{Cs}$  and to obtain better estimates, the isotopic ratios for fungal sporocarps have been calculated by Vinichuk et al. (2011b) and compared with estimates calculated in similar studies by Yoshida & Muramatsu (1998). The mean values of isotopic ratios of  $^{137}\text{Cs}/\text{K}$ ,  $^{137}\text{Cs}/\text{Rb}$ , and  $^{137}\text{Cs}/^{133}\text{Cs}$  in the fungal sporocarps, and range and correlation coefficients between concentration ratios  $^{137}\text{Cs}/^{133}\text{Cs}$  and K, Rb, and  $^{133}\text{Cs}$  are presented in Table 2.5.

The activity concentrations of  $^{137}\text{Cs}$  in fungal sporocarps are about 13 to 16 orders of magnitude lower than mass concentrations of K, 10 to 13 orders of magnitude lower than mass concentrations for Rb, and 8 to 9 orders of magnitude lower than mass concentrations for  $^{133}\text{Cs}$ . In fungal sporocarps collected in Sweden, isotopic (atom) ratios are two-three orders of magnitude lower than in

fungal sporocarps collected in Japan, which reflects the level of  $^{137}\text{Cs}$  concentrations in mushrooms. The median value for all fungi species is 4 151 Bq kg<sup>-1</sup> DW in Swedish forests and 135 Bq kg<sup>-1</sup> DW in Japanese forests. The isotopic (atom) ratios of  $^{137}\text{Cs}/\text{K}$ ,  $^{137}\text{Cs}/\text{Rb}$ , and  $^{137}\text{Cs}/^{133}\text{Cs}$  vary in both datasets and appear independent of specific species of fungi. These ratios might reflect the isotopic ratios in the soil horizons from which radiocesium is predominantly taken up and be a possible source of the variability in isotopic ratios in fungal fruit bodies. Rühm et al. (1997) used the isotopic ratio  $^{134}\text{Cs}/^{137}\text{Cs}$  to localize fungal mycelia in *in situ* species; alternatively, the isotopic (atom) ratio  $^{137}\text{Cs}/^{133}\text{Cs}$  can be used to localize fungal mycelia *in situ*. However, this approach is only appropriate for organic soil layers, which contain virtually no or very little clay mineral to which cesium can bind. The isotopic ratios  $^{137}\text{Cs}/^{133}\text{Cs}$  in fruit bodies of fungi are similar to the ratios found in organic soil layers of forest soil (Rühm et al. 1997; Karadeniz & Yaprak, 2007).

**Table 2.5** Isotopic (atom) ratios of  $^{137}\text{Cs}/\text{K}$ ,  $^{137}\text{Cs}/\text{Rb}$ ,  $^{137}\text{Cs}/^{133}\text{Cs}$ , correlation coefficients between isotopic ratios  $^{137}\text{Cs}/^{133}\text{Cs}$  and mass concentrations of K, Rb, and  $^{133}\text{Cs}$  in fungal sporocarps. The result of the comparison between our data (Vinichuk et al. 2011b, Sweden) and data from Yoshida & Muramatsu (1998), Japan.

Data set	n	Isotopic ratios		
		$^{137}\text{Cs}/\text{K}$	$^{137}\text{Cs}/\text{Rb}$	$^{137}\text{Cs}/^{133}\text{Cs}$
Vinichuk et al. (2011b), Sweden	12	14.4(1.54–45.4) x10 <sup>-13</sup>	7.8(0.55–30.9) x10 <sup>-10</sup>	4.9(0.30–15.1) x10 <sup>-8</sup>
Yoshida & Muramatsu (1998), Japan	29	5.2(0.15–23.0) x10 <sup>-16</sup>	3.4(0.14–18.2) x10 <sup>-13</sup>	4.1(1.53–5.94) x10 <sup>-9</sup>
		Correlation coefficients		
		$^{137}\text{Cs}/^{133}\text{Cs}:\text{K}$	$^{137}\text{Cs}/^{133}\text{Cs}:\text{Rb}$	$^{137}\text{Cs}/^{133}\text{Cs}:\text{Cs}$
Vinichuk et al. (2011b), Sweden	12	0.25	–0.35	–0.31
Yoshida & Muramatsu (1998), Japan	29	0.12	0.39	0.26

<sup>1</sup>n = number of sporocarps analyzed

The relationships observed between the concentration ratios  $^{137}\text{Cs}/^{133}\text{Cs}$  and K, Rb, and  $^{133}\text{Cs}$  in fungal sporocarps vary widely and are inconsistent (Table 2.5).

The concentration of K, Rb, and  $^{133}\text{Cs}$  in sporocarps appears independent of the  $^{137}\text{Cs}/^{133}\text{Cs}$  isotopic ratio, suggesting differences in fungal uptake of these alkali metals and complex interactions between fungi, their host, and the environment.

### **K, Rb, and Cs ( $^{137}\text{Cs}$ and $^{133}\text{Cs}$ ) in Sporocarps of a Single Species**

Most results presented in this Chapter are already published (Vinichuk et al. 2011a), and based on sporocarp analysis of different ectomycorrhizal and saprotrophic fungal species. As fungal accumulation of  $^{137}\text{Cs}$  is suggested to be species-dependent (Kammerer et al. 1994)  $^{137}\text{Cs}$  activity concentration and mass concentration of K, Rb, and  $^{133}\text{Cs}$  in fungal sporocarps belonging to the mycorrhizal fungus *Suillus variegatus* were analyzed. Sporocarps were collected in the forest area located in Harbo (Heby county), about 40 km north-west of Uppsala in central Sweden (N 60°08'; E 17°10'). *S. variegatus* form *mycorrhiza* with Scots pine and predominantly occur in sandy, acidic soils. This fungus has a marked ability for accumulating radiocesium (Dahlberg et al. 1997) and, as it is an edible mushroom, the high radiocesium content presents some concern regarding human consumption.

The concentrations of K (range 22.2-52.1 g kg<sup>-1</sup>) and Rb (range 0.22-0.65 g kg<sup>-1</sup>) in sporocarps of *S. variegatus* vary within relatively narrow ranges, whereas, the mass concentration of  $^{133}\text{Cs}$  has a range of 2.16 to 21.5 mg kg<sup>-1</sup> and the activity concentration of  $^{137}\text{Cs}$  ranges from 15.8 to 150.9 kBq kg<sup>-1</sup>. Both  $^{133}\text{Cs}$  and  $^{137}\text{Cs}$  have wider ranges than K or Rb within sporocarps from the same genotype or across a combined set of sporocarps (Table 2.6, Vinichuk et al. 2011a). The means the  $^{137}\text{Cs}/^{133}\text{Cs}$  isotopic ratio in the combined set of sporocarps was  $2.5 \times 10^{-7}$  (range  $8.3 \times 10^{-8}$  to  $4.4 \times 10^{-7}$ ). The  $^{137}\text{Cs}/\text{Cs}$  isotopic ratios from identified genotypes were site-genotype dependent: the ratio values

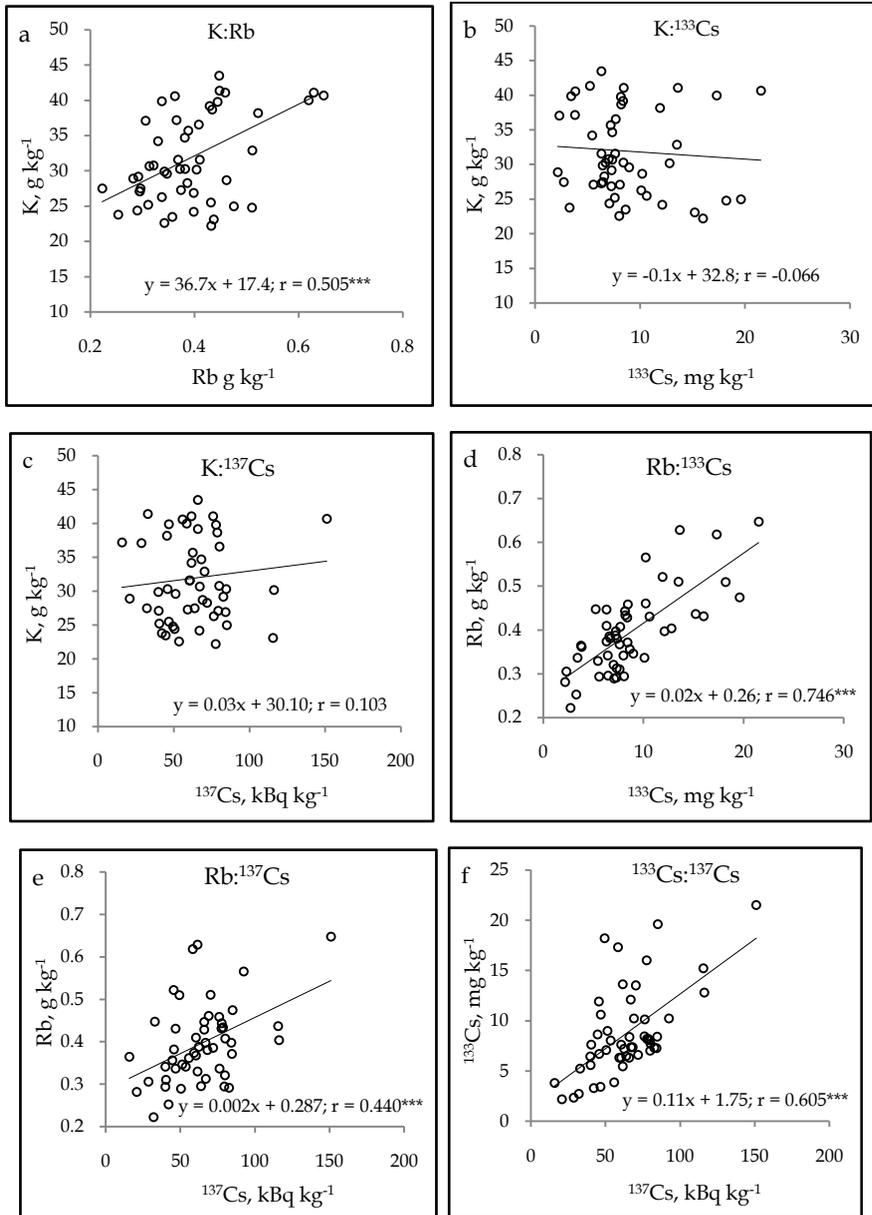
of genotypes at site 4 were about two-times higher than the ratios of genotypes at site 2 (Table 2.7, Vinichuk et al. 2011a).

Similarly, in another study (Vinichuk et al. 2004), the concentrations of K in sporocarps of *S. variegatus* were not related to the concentrations of  $^{137}\text{Cs}$  ( $r=0.103$ ) or  $^{133}\text{Cs}$  ( $r=-0.066$ ) in a combined data set (Figure 2.2: c, b). However, the concentrations of K and Rb were significantly correlated in the combined dataset ( $r=0.505$ , Figure 2.2: a).

**Table 2.6** Potassium, rubidium and cesium ( $^{133}\text{Cs}$ ) mass concentrations and  $^{137}\text{Cs}$  activity concentrations in sporocarps of *S. variegatus* (DW) from identified and unknown genotypes, where  $n$  = number of sporocarps of each genotype analyzed,  $M$  = mean,  $SE$  = standard deviation,  $CV$  = coefficient of variation.  
Adapted from Vinichuk et al. (2011a).

Site-genotype <sup>1</sup>	n	K			Rb			$^{133}\text{Cs}$			$^{137}\text{Cs}$		
		g kg <sup>-1</sup>		%	g kg <sup>-1</sup>		%	mg kg <sup>-1</sup>		%	kBq kg <sup>-1</sup>		%
		M	SD	CV	M	SD	CV	M	SD	CV	M	SD	CV
Sporocarps with identified genotypes													
2-1	8	30.6	8.06	26.4	0.47	0.12	24.7	12.1	4.23	35.1	67.3	35.1	52.2
2-2	6	28.0	6.99	25.0	0.50	0.07	13.8	16.6	2.19	13.2	75.9	23.2	30.6
4-3	4	28.5	2.13	7.5	0.39	0.16	4.0	6.6	0.44	6.7	68.9	11.7	17.0
4-4	3	33.6	8.60	-	0.30	0.04	-	3.0	0.60	-	39.1	9.38	-
4-5	2	38.9	2.40	-	0.36	0.02	-	3.8	0.04	-	35.7	28.2	-
4-6	2	35.2	8.84	-	0.37	0.11	-	3.7	2.16	-	26.8	8.54	-
7-7	5	33.7	5.79	17.2	0.34	0.06	17.9	6.7	0.80	12.0	71.4	9.30	13.0
6-8	2	25.4	1.34	-	0.31	0.03	-	8.7	2.16	-	63.3	18.3	-
Sporocarps with unknown genotypes													
	19	33.4	6.69	20.0	0.38	0.08	20.3	7.7	1.97	25.5	66.0	21.3	32.3
Combined set of sporocarps (identified and unknown genotypes)													
	51	31.9	6.79	21.3	0.40	0.09	23.6	8.7	4.36	50.1	63.7	24.2	38.0

<sup>1</sup>Site numbering according to Dahlberg et al. (1997), the second figure is a running number of the study's different genotypes according to Vinichuk et al. (2011a).



**Figure 2.2** Relationship between  $^{137}\text{Cs}$  and K, Rb, and  $^{133}\text{Cs}$  concentrations in sporocarps in the combined set of all *S. variegatus* sporocarps (a-f). K:Rb (a); K: $^{133}\text{Cs}$  (b); K: $^{137}\text{Cs}$  (c); Rb: $^{133}\text{Cs}$  (d); Rb: $^{137}\text{Cs}$  (e); and,  $^{133}\text{Cs}$ : $^{137}\text{Cs}$  (f). \*\*\*  $p=0.001$ .

Rubidium is strongly correlated with stable  $^{133}\text{Cs}$  ( $r=0.746$ ) and moderately correlated with  $^{137}\text{Cs}$  ( $r=0.440$ ) and K ( $r=0.505$ : Figure 2.2: d, e, a). Both  $^{133}\text{Cs}$  and  $^{137}\text{Cs}$  were significantly correlated in the combined dataset (Figure 2.2: f).

**Table 2.7**  $^{137}\text{Cs}/^{133}\text{Cs}$  isotopic (atom) ratios in sporocarps of *S. variegatus* from identified genotypes, with unknown genetic belonging, and the two combined groupings,  $\times 10^{-7}$ . *M* = mean, *CV* = coefficient of variation. Adapted from Vinichuk et al. (2011a).

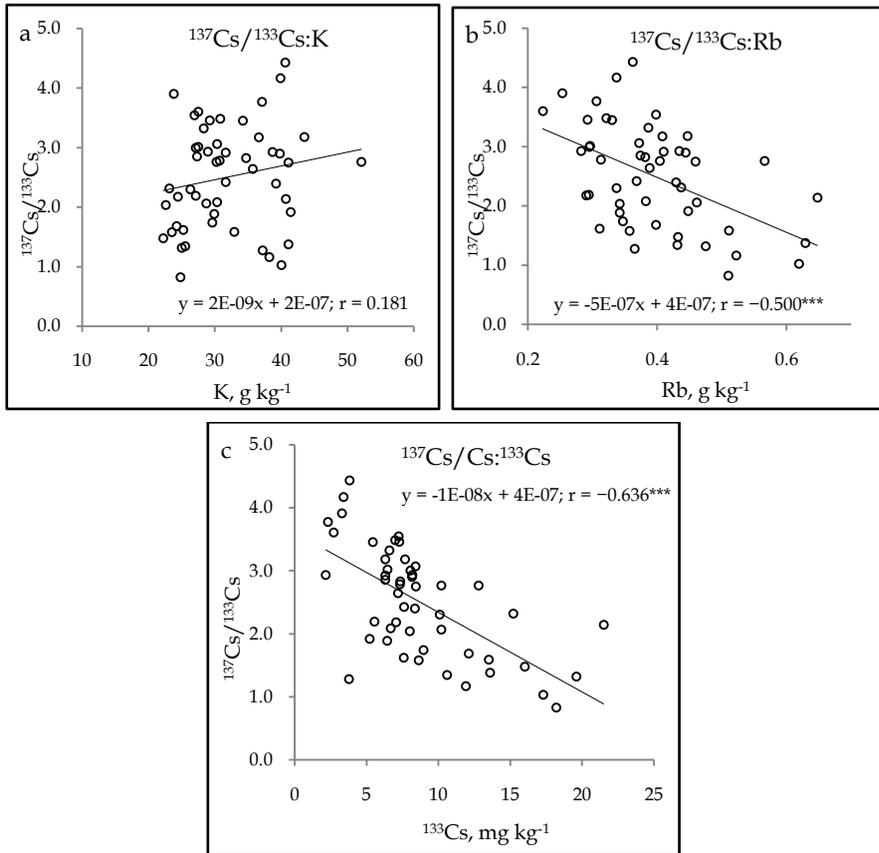
Parameters	Site-genotype <sup>1</sup>								Unidentified genotypes	Combined set of sporocarps
	2-1	2-2	4-3	4-4	4-5	4-6	7-7	6-8		
M	1.67	1.43	3.16	3.95	2.86	2.43	3.27	2.24	2.62	2.50
CV (%)	97.1	36.4	10.4	5.1	78.1	29.5	9.2	3.9	20.0	34.6

<sup>1</sup>Site numbering according to Dahlberg et al. (1997), the second figure is a running number of the study's different genotypes according to Vinichuk et al. (2011a).

The  $^{137}\text{Cs}/^{133}\text{Cs}$  isotopic ratio in the combined dataset was not correlated to K concentration, but correlated moderately and negatively with both  $^{133}\text{Cs}$  ( $r=-0.636$ ) and Rb ( $r=-0.500$ ) concentrations (Figure 2.3: a, c, b).

Thus, the study of *S. variegatus* revealed no significant correlation between  $^{133}\text{Cs}$  mass concentration or  $^{137}\text{Cs}$  activity concentration and the concentration of K in sporocarps, either within the whole population or among the genotypes.

Potassium,  $^{133}\text{Cs}$ , and  $^{137}\text{Cs}$  within the four genotypes were also not correlated, with one genotype exception (Table 2.8). This exception was conditional due to one single value. Three of four sporocarp genotypes analyzed had high correlation between K and Rb: the fourth was only moderately correlated (Table 2.8).



**Figure 2.3** Relationship between the  $^{137}\text{Cs}/^{133}\text{Cs}$  isotopic (atom) ratios ( $\times 10^{-7}$ ) and K, Rb, and  $^{133}\text{Cs}$  mass concentrations in the combined set of *S. variegatus* sporocarps, (a)  $^{137}\text{Cs}/^{133}\text{Cs}:K$ ; (b)  $^{137}\text{Cs}/^{133}\text{Cs}:Rb$ ; and, (c)  $^{137}\text{Cs}/^{133}\text{Cs}:^{133}\text{Cs}$ . \*\*\*  $p=0.001$ .

The correlations between  $^{137}\text{Cs}$  and K and Rb and  $^{133}\text{Cs}$  in the four genotypes were inconsistent (Table 2.8). Potassium, Rb,  $^{133}\text{Cs}$ , and  $^{137}\text{Cs}$  were correlated in genotype 2-1 (due to one single value), whereas, no or negative correlations were found between the same elements/isotopes for the other three genotypes. In two (2-2 and 4-3) of four genotypes, the  $^{137}\text{Cs}/^{133}\text{Cs}$  isotopic ratio was not correlated with  $^{133}\text{Cs}$ , K, or Rb; however, there was a negative correlation with

Rb in one genotype (2-2) and positive correlation with  $^{133}\text{Cs}$  in another (4-3) (Table 2.8.).

Mass concentration of  $^{133}\text{Cs}$  and activity concentration of  $^{137}\text{Cs}$  have different relations in fungal sporocarps. In three of four genotypes, there was a high correlation, two of which were significant ( $r=0.908^{**}$  and  $r=0.979^{*}$ ), but there was no correlation in the fourth genotype ( $r=-0.263$ , Table 2.8), whereas, correlation between  $^{137}\text{Cs}$  and  $^{133}\text{Cs}$  within the whole population was only moderate ( $r=0.605^{***}$  Figure 2: f).

**Table 2.8** Correlation coefficients between concentrations of potassium, rubidium, and cesium ( $^{133}\text{Cs}$  and  $^{137}\text{Cs}$ ) in genotypes of *S. variegatus* with more than four sporocarps (Vinichuk et al. 2011a).

	$^{137}\text{Cs}$	K	Rb	$^{133}\text{Cs}$
Genotype2-1 (8 sporocarps)				
K	0.502			
Rb	0.626*	0.966***		
$^{133}\text{Cs}$	0.908**	0.745*	0.837**	
$^{137}\text{Cs}/^{133}\text{Cs}$		-0.172	-0.058	0.240
Genotype2-2 (6 sporocarps)				
K	-0.472			
Rb	-0.658	0.928**		
$^{133}\text{Cs}$	-0.263	-0.138	0.159	
$^{137}\text{Cs}/^{133}\text{Cs}$		-0.352	-0.608	-0.586
Genotype4-3 (4 sporocarps)				
K	-0.531			
Rb	0.177	0.696		
$^{133}\text{Cs}$	0.979*	-0.569	0.182	
$^{137}\text{Cs}/^{133}\text{Cs}$		-0.488	0.163	0.930
Genotype 7-7(5 sporocarps)				
K	-0.562			
Rb	-0.472	0.987**		
$^{133}\text{Cs}$	0.699	-0.528	-0.404	
$^{137}\text{Cs}/^{133}\text{Cs}$		-0.115	-0.155	-0.345

\*  $p=0.05$ ; \*\*  $p=0.01$ ; \*\*\*  $p=0.001$

The data obtained for *S. variegatus* supports results from other studies (Ismail, 1994; Yoshida & Muramatsu, 1998) on different species of fungi; suggesting cesium ( $^{137}\text{Cs}$  and  $^{133}\text{Cs}$ ) and K are not correlated in mushrooms. Thus, correlation analysis may be a useful, although not definitive, approach for determining similarities or differences in the uptake mechanisms of cesium ( $^{137}\text{Cs}$  and  $^{133}\text{Cs}$ ) and K.

The concentration of K in sporocarps appears independent of the  $^{137}\text{Cs}/^{133}\text{Cs}$  isotopic ratio in both the whole population (Figure 2.2) and among the genotypes, with one exception (Table 2.8). The lack of correlation between  $^{137}\text{C}$  (or  $^{133}\text{Cs}$ ) and K in fungi may be due to the incorporation of K being self-regulated by the nutritional requirements of the fungus, whereas, incorporation of  $^{137}\text{Cs}$  is not self-regulated by the fungus (Baeza et al. 2004).

Although K and cesium ( $^{133}\text{Cs}$  and  $^{137}\text{Cs}$ ) concentrations did not correlate within *S. variegatus*, both  $\text{K}^+$  and  $\text{Cs}^+$  ions may compete for uptake by fungi. In experiments under controlled conditions and with sterile medium (Bystrzejewska-Piotrowska & Bazala, 2008), the competition between  $\text{Cs}^+$  and  $\text{K}^+$  depends on  $\text{Cs}^+$  concentration in the growth medium and on the path of  $\text{Cs}^+$  uptake. The addition of monovalent cations of  $\text{K}^+$ ,  $\text{Rb}^+$ , and  $\text{NH}_4^+$  reduces the uptake of Cs by the hyphae of basidiomycete *Hebeloma vinosophyllum* grown on a simulated medium (Ban-Nai et al. 2005). Radiocesium transport by arbuscular mycorrhizal (AM) fungi decreases if K concentration increases in a compartment only accessible to AM (Gyuricza et al. 2010), and a higher Cs: K ratio in the nutrient solution increases uptake of Cs by ectomycorrhizal seedlings (Brunner et al. 1996). A noticeable (20-60%) and long-lasting (at least 17 years) reduction in  $^{133}\text{Cs}$  activity concentration in *in situ* fungal sporocarps after a single K fertilization of  $100 \text{ kg ha}^{-1}$  in a Scots pine forest is reported by Ros n et al. (2011).

The relation between  $^{137}\text{Cs}$  and K, and Rb and  $^{133}\text{Cs}$  within *S. variegatus* (Figure 2.2) was similar to earlier findings on different species of fungi (Yoshida & Muramatsu, 1998). Rubidium concentration in sporocarps was positively correlated with  $^{133}\text{Cs}$  and  $^{137}\text{Cs}$ , but generally negatively correlated with  $^{137}\text{Cs}/^{133}\text{Cs}$  isotopic ratio, i.e. a narrower  $^{137}\text{Cs}/^{133}\text{Cs}$  ratio in sporocarps results in higher Rb uptake by fungi. This ratio may reflect the soil layers explored by the mycelia (Rühm et al. 1997). Fungi have a higher affinity for Rb than for K and cesium (Ban-Nai et al. 2005; Yoshida & Muramatsu, 1998), and Rb concentrations in sporocarps can be more than one order of magnitude greater than in mycelium extracted as fungal sporocarps from soil in the same plots (Vinichuk et al. 2011a). Soil mycelia consist of numerous fungal species and the intraspecific relationships between soil mycelia and sporocarps has not yet been estimated; however, the development of molecular methods with the ability to mass sequence environmental samples in combination with quantitative PCR may enable these analyses to be conducted.

In terms of  $^{133}\text{Cs}$  and  $^{137}\text{Cs}$  behavior, there would be no biochemical differentiation, but differences in atom abundance and isotopic disequilibrium within the system. Fungi have large spatiotemporal variation in  $^{133}\text{Cs}$  and  $^{137}\text{Cs}$  content in sporocarps of the same species and different species (de Meijer et al. 1988), and the variation in K, Rb,  $^{133}\text{Cs}$ , and  $^{137}\text{Cs}$  concentrations within a single genotype appear similar, or lower, than the variation within all genotypes. The results for  $^{137}\text{Cs}$  and alkali elements in a set of samples of *S. variegatus*, collected during the same season and consisting of sporocarps from both different and the same genotype, indicate the variability in concentrations is similar to different fungal species collected in Japan over three years (Yoshida & Muramatsu, 1998).

The relatively narrow range in K and Rb variation and the higher  $^{133}\text{Cs}$  and  $^{137}\text{Cs}$  variations may be due to different mechanisms being involved. The differences in correlation coefficients between  $^{137}\text{Cs}$  and the alkali metals varied among and within the genotypes of *S. variegatus*, suggesting interspecific and intrapopulation variation in the uptake of K, Rb, stable  $^{133}\text{Cs}$  and,  $^{137}\text{Cs}$  and, that their relationships can be explained by factors other than genotype identity. As the variability in  $^{137}\text{Cs}$  transfer depends on the sampling location of fungal sporocarps (Gillett & Crout, 2000), the interaction factors for *S. variegatus* might include the spatial pattern of soil chemical parameters, heterogeneity of  $^{137}\text{Cs}$  fallout, mycelia location, and heterogeneity due to abiotic and biotic interactions increasing over time (Dahlberg et al. 1997).

Within the combined set of sporocarps, the concentration of Rb and  $^{137}\text{Cs}$  activity concentration in *S. variegatus* sporocarps were normally distributed but the frequency distribution of  $^{133}\text{Cs}$  and K was not: asymmetry in  $^{137}\text{Cs}$  frequency distributions is reported in other fungal species (Baeza et al. 2004; Ismail, 1994). According to Gillett & Crout (2000), the frequency distribution of  $^{137}\text{Cs}$  appears species dependent: high accumulating species tend to be normally distributed and low accumulating species tend to be log-normally distributed. However, lognormal distribution is the default for concentration of radionuclides and is unlikely to be a species-specific phenomenon, as it also occurs in soil concentrations; implying normal distribution is not expected, even if large set of samples are analyzed.

### **Possible Mechanisms of $^{137}\text{Cs}$ and alkali Metals Uptake by Fungi**

There is a lack of information about the mechanisms involved in the uptake and retention of radionuclides by fungi, and there are few studies of uptake mechanisms and affinity for alkali metals in fungi are scarce, although some

results are reviewed by Rodríguez-Navarro (2000). Fungal fruit bodies can be characterized by high  $^{137}\text{Cs}$ ,  $^{133}\text{Cs}$ , and Rb concentrations and low calcium (Ca) and strontium (Sr) concentrations, compared to plants (Yoshida et al. 1998). In a laboratory experiment with a wood-inhabiting mushroom *Pleurotus ostreatus* (Fr.) Kummer Y-1 (Terada et al. 1998),  $^{137}\text{Cs}$  uptake by mycelia decreases as the concentration of  $^{133}\text{Cs}$ , K, or Rb in the media increases, and K uptake by mycelia decreases as the concentration of  $^{133}\text{Cs}$  increases. In an experiment with pure cultures of mycorrhizal fungi (Olsen et al. 1990) some species preferred Cs to K. In experiments with yeast (Conway & Duggan, 1958), K had preference over Cs and the affinity for alkali metal uptake decreased in the order  $\text{K}^+ < \text{Rb}^+ < \text{Cs}^+$  then  $\text{Na}^+$  and  $\text{Li}^+$ , with a relative ratio of 100:42:7:4:0.5. Fungi (mycelium and sporocarps) have higher affinity for uptake of Rb and K than for Cs, and based on the CR values for fungal sporocarps (Table 2.3), alkali metals can be ranked in the order  $\text{Rb}^+ > \text{K}^+ > \text{Cs}^+$ , with a relative ratio of 100:57:32, which is within the range of 100:88:50 derived by Yoshida & Muramatsu (1998).

It is likely that the affinity for an alkali metal depends on the nutritional status of the organism. The mycorrhizal species *Sarcodon imbricatus* is efficient in accumulating K, Rb, and Cs: mean elements CR for fungal sporocarps of this species are 102, 676, and 259 respectively (Vinichuk et al. 2010b). Likewise, Tyler (1982), reports a mean CR for Rb in litter decomposing fungus *Collybia peronata* of 41, and a mean CR for Rb of over 100 in *Amanita rubescens*, which is mycorrhizal with beech (*Fagus sylvatica* L.). However, lower  $^{40}\text{K}$  content for mycorrhizal species is reported by Römmelt et al. (1990), which means mycorrhizal species do not necessarily accumulate alkali metals more efficiently than saprotrophic ones.

Fungal accumulation of stable and radioactive cesium appears to be species-dependent, although it is affected by local environmental conditions. The

variation in concentrations of stable and radioactive cesium in fungi of the same species is generally larger than the variation between different species (de Meijer et al. 1988) and the variation in  $^{137}\text{Cs}$  levels within the same genet of *S. varegatus* is as large as within non-genet populations of the species (Dahlberg et al. 1997). This suggests both interspecific and intrapopulation variation in the uptake of K, Rb, stable  $^{133}\text{Cs}$ , and  $^{137}\text{Cs}$ , and that their relationships can be explained by factors other than genotype identity (Vinichuk et al. 2011a). There is about two orders of magnitude variation in Cs uptake, with the highest CR value in e.g. *S. imbricatus* (256) and the lowest in *Lactarius deterrimus* (2.6) (Vinichuk et al. 2010b).

## **Cs ( $^{137}\text{Cs}$ and $^{133}\text{Cs}$ ), K and Rb in *Sphagnum* Plants**

Peatlands are areas where remains of plant litter have accumulated under waterlogged and generally nutrient-poor habitats, particularly temperate and boreal bogs in the northern hemisphere. Bogs are ombrotrophic, i.e. all water and nutrient supply to the vegetation is from aerial dust and precipitation; this results in an extremely nutrient-poor ecosystem that is often dominated by peat mosses (*Sphagnum*). *Sphagnum*-dominated peatlands with some groundwater inflow (i.e. weakly minerotrophic 'poor fens') are almost as nutrient poor and acid as true bogs. *Sphagnum* plants grown on such bogs absorb and retain substantial amounts of fallout-derived radiocesium. The radioactive cesium isotope  $^{137}\text{Cs}$  is transferred within raised bogs there is relatively high  $^{137}\text{Cs}$  bioavailability to bog vegetation and mosses (Bunzl & Kracke, 1989; Ros n et al. 2009).

The transfer of  $^{137}\text{Cs}$  within a peatland ecosystem is different from transfer in forests. In nutrient-poor but organic-matter-rich forest soils, the vertical migration rate of  $^{137}\text{Cs}$  is low, but bioavailability is often high, particularly for

mycorrhizal fungi (Olsen et al. 1990; Vinichuk & Johansson, 2003; Vinichuk et al. 2004; 2005). This is partly due to extensive fungal mycelium counteracting the downward transport of  $^{137}\text{Cs}$  (Rafferty et al. 2000), which results in a slow net downward transport of  $^{137}\text{Cs}$  in the soil profile.

In *Sphagnum*-dominated peatlands, *Sphagnum* peat is virtually clay mineral free organic matter, which also lacks fungal mycelium. The downward migration of  $^{137}\text{Cs}$  in *Sphagnum* peat is expected to be faster than in forest soil; however, Cs is continuously translocated towards the growing apex of the *Sphagnum* shoots, where it accumulates.

The chemical behavior of radiocesium in raised bog is assumed to be similar to that of stable  $^{133}\text{Cs}$  and other alkali metals, i.e. K, Rb, which have similar physicochemical properties (Ros n & Vinichuk, 2009; Vinichuk et al. 2010a). In *Sphagnum*, the relationships between K, Rb, and Cs and whether Cs follows the same pathways as K are not clearly understood.

The influence of alkali metals (K, Rb,  $^{133}\text{Cs}$ ) on  $^{137}\text{Cs}$  distribution and cycling processes in peatlands has not been well studied. Plant species growing on peat have varying capacities for influencing uptake and binding of radionuclides, but there is a lack of systematic study covering all the dominant species of *Sphagnum* peatlands and their competition for radionuclides and nutrients. The important role of *Sphagnum* mosses in mineral nutrient turnover in nutrient-poor ecosystems, in particular their role in  $^{137}\text{Cs}$  uptake and binding, necessitates clear understanding of the mechanisms involved.

Therefore, the  $^{137}\text{Cs}$  activity concentration and mass concentration of K, Rb, and  $^{133}\text{Cs}$  were analyzed within individual *Sphagnum* plants (down to 20 cm depth) and the results were published in collaboration with Professor H. Rydin (Vinichuk et al. 2010a). The distribution of Cs ( $^{133}\text{Cs}$  and  $^{137}\text{Cs}$ ), K, and Rb in

the uppermost capitulum and subapical segments of *Sphagnum* mosses were compared to determine the possible mechanisms involved in radiocesium uptake and retention within *Sphagnum* plants.

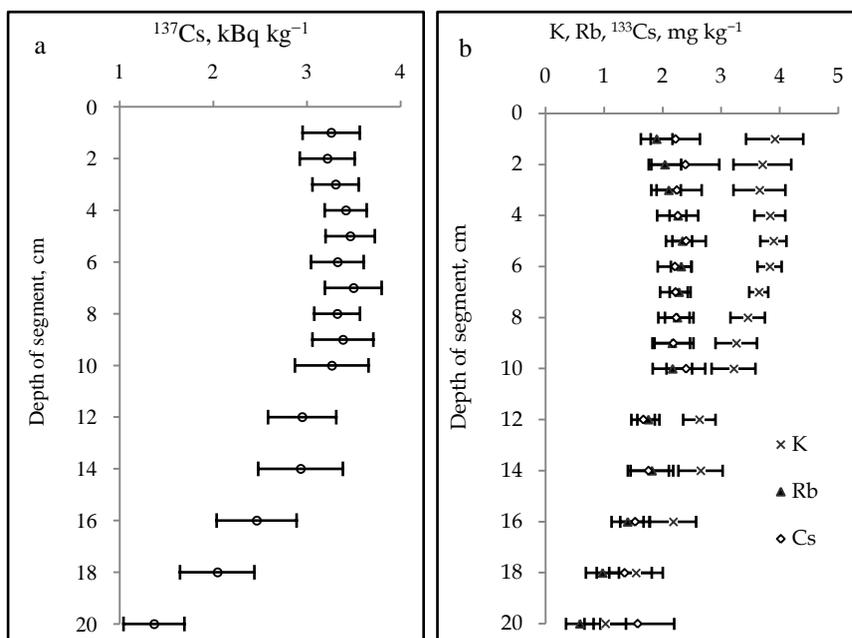
The *Sphagnum* plants were growing in a small peatland (Palsjö mossen) within a coniferous forest in eastern central Sweden, about 35 km NW of Uppsala (60°03'40"N, 17°07'47"E): the peatland area sampled was open and *Sphagnum*-dominated. A weak minerotrophic influence was indicated by the dominance of *Sphagnum papillosum*, and the presence of *Carex rostrata*, *Carex pauciflora*, and *Menyanthes trifoliata* (fen indicators in the region). The area mostly built by *Sphagnum fuscum* was dominated by dwarf-shrubs such as *Andromeda polifolia*, *Calluna vulgaris*, *Empetrum nigrum* and *Vaccinium oxycoccos*. Sampling was within a 25 m<sup>2</sup> low, flat 'lawn community' (Rydin & Jeglum, 2006) totally covered by *S. papillosum*, *S. angustifolium* and *S. magellanicum* with an abundant cover of *Eriophorum vaginatum*. The water table was generally less than 15 cm below the surface: surface water had a pH of 3.9-4.4 (June 2009).

Samples of individual *Sphagnum* shoots that held together down to 20 cm were randomly collected in 2007 (May and September) and 2008 (July, August and September). Thirteen samples of *Sphagnum* plants were collected and analyzed; three sets in 2007 and 10 sets in 2008. Each sample consisted of approximately 20-60 individual *Sphagnum* plants (mostly *S. papillosum*, in a few cases *S. angustifolium* or *S. magellanicum*). In the laboratory, the fresh, individual, erect, and tightly interwoven *Sphagnum* plants were sectioned into 1 cm (0-10) or 2 cm (10-20 cm) long segments down to 20 cm from the growing apex. The  $^{137}\text{Cs}$  activity concentrations were measured in fresh *Sphagnum* segments. Thereafter, the samples were dried at 40 °C to constant weight and analyzed for K, Rb, and  $^{133}\text{Cs}$ .

The activity concentration ( $\text{Bq kg}^{-1}$ ) of  $^{137}\text{Cs}$  in plant samples is determined by calibrated HP Ge detectors. The analysis of *Sphagnum* segments for K, Rb, and Cs is performed by a combination of ICP-AES (K concentration) and ICP-SFMS ( $^{133}\text{Cs}$  and Rb concentration) techniques at ALS Scandinavia AB, Luleå, Sweden. The detection limits were  $200 \text{ mg kg}^{-1}$  for K,  $0.04 \text{ mg kg}^{-1}$  for  $^{133}\text{Cs}$ , and  $0.008 \text{ mg kg}^{-1}$  for Rb. Relationships between K, Rb, and  $^{133}\text{Cs}$  concentrations in different *Sphagnum* segments were determined by Pearson correlation coefficients. All statistical analyses were with Minitab (© 2007 Minitab Inc.) software.

### **Distribution of Cs ( $^{137}\text{Cs}$ and $^{133}\text{Cs}$ ), K, and Rb within *Sphagnum* Plants**

The concentration of Cs ( $^{137}\text{Cs}$  and  $^{133}\text{Cs}$ ) and alkali metals K and Rb in different segments of *Sphagnum* plants provide information on differences in their uptake, distribution, and relationships. The averaged  $^{137}\text{Cs}$  activity concentrations in *Sphagnum* segments are presented in Figure 2.4. Within the upper 10 cm from the capitulum,  $^{137}\text{Cs}$  activity concentration in *Sphagnum* plants was about  $3350 \text{ Bq kg}^{-1}$ , with relatively small variations. Below 10-12 cm, the activity gradually declined with depth and in the lowest segments of *Sphagnum*,  $^{137}\text{Cs}$  activity concentration was about  $1370 \text{ Bq kg}^{-1}$ .



**Figure 2.4**  $^{137}\text{Cs}$  and alkali metals in *Sphagnum*: (a) average  $^{137}\text{Cs}$  activity concentration ( $\text{kBq kg}^{-1}$ ) in *Sphagnum* segments ( $\pm$  SE,  $n=13$ ); (b) average concentrations of K (scale values should be multiplied by  $10^3$ ), Rb ( $\times 10^1$ ), and  $^{133}\text{Cs}$  ( $\times 10^{-1}$ ) ( $\text{mg kg}^{-1}$ ) in *Sphagnum* segments ( $\pm$  SE,  $n=4$ ). Adapted from Vinichuk et al. 2010a).

For individual samples, K concentrations ranged between 508 and 4970  $\text{mg kg}^{-1}$  (mean 3096); Rb ranged between 2.4 and 31.4  $\text{mg kg}^{-1}$  (mean 18.9), and  $^{133}\text{Cs}$  ranged between 0.046 and 0.363  $\text{mg kg}^{-1}$  (mean 0.204): averaged concentrations of K, Rb, and  $^{133}\text{Cs}$  in *Sphagnum* segments are presented in Figure 2.4b. Concentrations of Rb and  $^{133}\text{Cs}$  were constant in the upper 0-10 cm segments of *Sphagnum* moss and gradually declined in the lower parts of the plant length; whereas, the concentration of K decreased with increasing depth below 5 cm. Generally, the distribution of all three alkali metals was similar to  $^{137}\text{Cs}$ , but with a weaker increase of Rb towards the surface. The  $^{137}\text{Cs}$  activity concentrations had the highest coefficient of variation (standard deviation divided

by the mean) in *Sphagnum* (43%). The coefficients of variation were 35% for K, 35% for Rb, and 37% for  $^{133}\text{Cs}$  concentrations.

Two important features affect the distributions of K, Rb,  $^{133}\text{Cs}$ , and  $^{137}\text{Cs}$  in a *Sphagnum*-dominated peatland. Firstly, this type of peatland is extremely nutrient-poor, and only a few plant and fungal species producing small fruit bodies can grow and no mycorrhiza, except ericoid mycorrhiza, exist. Secondly, the upper part of the stratigraphy is composed of living *Sphagnum* cells that selectively absorb mineral ions from the surrounding water, and the binding of K, Rb, and  $^{133}\text{Cs}$  can be at exchange sites, both outside and inside the cell.

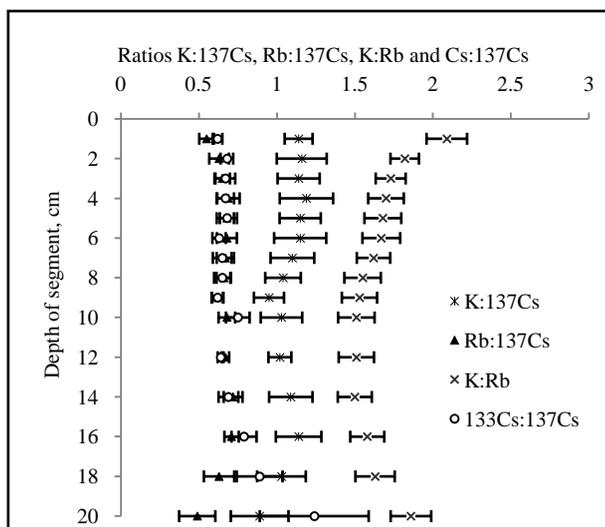
The distribution of  $^{137}\text{Cs}$  within *Sphagnum* plants is similar to stable K, Rb, and  $^{133}\text{Cs}$ . The  $^{137}\text{Cs}$  activity concentrations and concentrations of K, Rb, and  $^{133}\text{Cs}$  are highest in the uppermost 0-10 cm segments of *Sphagnum* (in the capitula and the subapical segments) and gradually decrease in older parts of the plant. Such distribution can be interpreted as dependent on the living cells of capitula and living green segments in the upper part of *Sphagnum*. Similar patterns of K distribution within *Sphagnum* plants are reported (Hájek, 2008). The  $^{137}\text{Cs}$  appears to be taken up and relocated by *Sphagnum* plants in similar ways to the stable alkali metals, as concentrations of K, Rb, and  $^{137}\text{Cs}$  activity concentrations in *Sphagnum* segments (Figure 2.4.) are similar to the depth of about 16 cm, and display a slightly different pattern in the lower part of the plant.

### **Mass Concentration and Isotopic (Atom) Ratios between $^{133}\text{Cs}$ , K, Rb, and $^{133}\text{Cs}$ , in Segments of *Sphagnum* Plants**

Ratios between mass concentrations of all three alkali metals and  $^{137}\text{Cs}$  activity concentrations, i.e.  $^{133}\text{Cs}:^{137}\text{Cs}$ , K:  $^{137}\text{Cs}$ , Rb:  $^{137}\text{Cs}$ , and  $^{133}\text{Cs}:^{137}\text{Cs}$  are constant through the upper part (0-16 cm) of *Sphagnum* plants (Figure 2.5). The

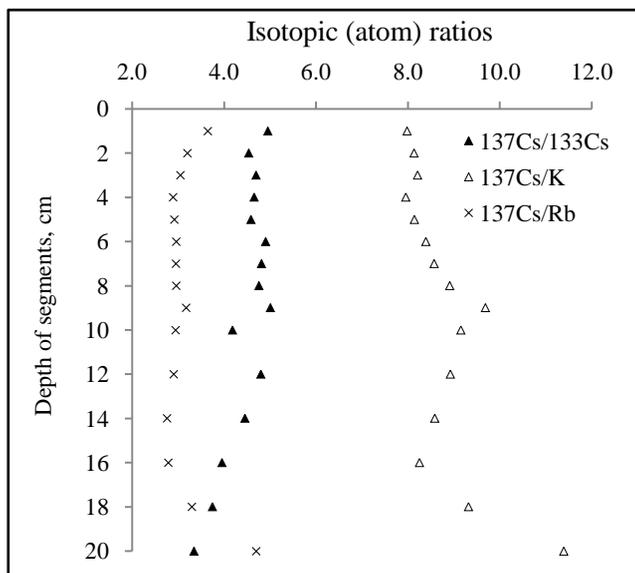
K:Rb ratio is higher in the uppermost (0-2 cm) and the lowest (18-20 cm) parts of the plant (Figure 2.5).

However, the isotopic (atom) ratios between  $^{137}\text{Cs}$  activity concentrations and mass concentrations of alkali metals, i.e.  $^{137}\text{Cs}/\text{K}$ ,  $^{137}\text{Cs}/\text{Rb}$ , and  $^{137}\text{Cs}/^{133}\text{Cs}$  have a different pattern of distribution through the upper part (0-20 cm) of *Sphagnum* plants (Figure 2.6).



**Figure 2.5** Ratios between  $\text{K}:^{137}\text{Cs}$ ,  $\text{Rb}:^{137}\text{Cs}$  (scale values should be multiplied by  $10^{-2}$ ),  $\text{K}:\text{Rb}$  ( $\times 10^2$ ), and  $^{133}\text{Cs}:^{137}\text{Cs}$  ( $\times 10^{-4}$ ) in *Sphagnum* segments. Calculations based on concentrations in  $\text{mg kg}^{-1}$  for stable isotopes and  $\text{Bq kg}^{-1}$  for  $^{137}\text{Cs}$  ( $\pm$  SE,  $n=13$  for  $^{137}\text{Cs}$ ;  $n=4$  for each of K, Rb, and  $^{133}\text{Cs}$ ) (Vinichuk et al. 2010a).

The  $^{137}\text{Cs}/\text{K}$  ratio is relatively narrow through the upper part (0-16 cm) of *Sphagnum* plants and wider with increasing depth, whereas, the  $^{137}\text{Cs}/^{133}\text{Cs}$  ratio is constant throughout the upper part (0-12 cm) of *Sphagnum* plants and becomes narrower in the lower (14-20 cm) parts. The  $^{137}\text{Cs}/\text{Rb}$  ratio is constant through the middle part (4-16 cm) of *Sphagnum* plants and somewhat narrower in the uppermost (0-4 cm) and lowest (16-20 cm) parts (Figure 2.6).



**Figure 2.6** Isotopic (atom) ratios  $^{137}\text{Cs}/\text{K}$  (scale values should be multiplied by  $10^{-12}$ ),  $^{137}\text{Cs}/\text{Rb}$  ( $\times 10^{-09}$ ), and  $^{137}\text{Cs}/^{133}\text{Cs}$  ( $\times 10^{-07}$ ) in *Sphagnum* segments. Calculations based on  $^{137}\text{Cs}$  activity concentrations and mass concentrations of K, Rb,  $^{133}\text{Cs}$  (Eq. 2) (mean values,  $n=4$  for each of  $^{137}\text{Cs}$ , K, Rb, and  $^{133}\text{Cs}$ ) (Vinichuk et al. 2010a).

The distribution of the isotopic (atom) ratios between  $^{137}\text{Cs}$  activity concentrations and mass concentrations of alkali metals K and Rb through the upper part (0-20 cm) of *Sphagnum* plants are probably conditioned by at least three processes. These include physical decay of  $^{137}\text{Cs}$  atoms with time; attainment of equilibrium between stable  $^{133}\text{Cs}$  and  $^{137}\text{Cs}$  in the bioavailable fraction of peat soil; and, the relation between cesium ( $^{133}\text{Cs}$  and  $^{137}\text{Cs}$ ), K, and Rb when taken up by the *Sphagnum* plant.

### Relationships between $^{133}\text{Cs}$ , K, Rb, and $^{137}\text{Cs}$ in Segments of *Sphagnum* Plants

The relationships between  $^{133}\text{Cs}$ , K, Rb, and  $^{137}\text{Cs}$  in separate segments of *Sphagnum* plants provide a tool for future investigation of the uptake

mechanism. There are close positive correlations between K, Rb, and  $^{133}\text{Cs}$  mass concentrations and  $^{137}\text{Cs}$  activity concentrations in *Sphagnum* segments (Table 2.9). The highest correlation is between  $^{137}\text{Cs}$  activity concentrations and Rb mass concentrations ( $r=0.950$ ;  $p=0.001$ ) and correlation between K and Rb mass concentrations ( $r=0.952$ ;  $p=0.001$ ) in 10-20 cm length of *Sphagnum* plants; however,  $^{137}\text{Cs}$  and K have a weaker correlation only when the upper 0-10 cm part of *Sphagnum* plants are analyzed ( $r=0.562$ ;  $p=0.001$ ). There is no, or negative correlation between  $^{137}\text{Cs}/^{133}\text{Cs}$  isotope (atom) ratios and mass concentrations of alkali metals (K, Rb, and  $^{133}\text{Cs}$ ) (Table 2.9).

**Table 2.9** Correlation coefficients between concentrations of potassium, rubidium, and cesium ( $^{133}\text{Cs}$  and  $^{137}\text{Cs}$ ) in *Sphagnum* segments (\*\*\*)  $p=0.001$  (Vinichuk et al. 2010a).

	$^{137}\text{Cs}$	K	Rb	$^{133}\text{Cs}$
0-10 cm length				
K	0.562***			
Rb	0.893***	0.632***		
$^{133}\text{Cs}$	0.840***	0.792***	0.802***	
$^{137}\text{Cs}/^{133}\text{Cs}$	–	–0.262	0.270	–0.157
10-20 cm length				
K	0.856***			
Rb	0.950***	0.952***		
$^{133}\text{Cs}$	0.645***	0.651***	0.664***	
$^{137}\text{Cs}/^{133}\text{Cs}$	–	0.122	0.219	–0.401

The marked decrease in  $^{137}\text{Cs}$  activity concentration below 14 cm (Figure 2.4a) raises the question as to what depth the 1986 Chernobyl horizon was when the sampling was done. A peat core was sampled in May 2003 at Åkerlänna Rösse, an open bog about 14 km SW of P åsjö mossen, by van der Linden et al. (2008). Detailed dating by  $^{14}\text{C}$  wiggle-matching indicates the Chernobyl horizon was then at a depth of 17 cm. Depth-age data estimates a linear annual peat increment of  $1.3 \text{ cm yr}^{-1}$  over the last decade ( $R^2=0.998$ ), indicating the Chernobyl horizon

would be at about 23 cm deep when the  $^{137}\text{Cs}$  sampling was done in 2007/2008. Even if there are uncertainties in applying data from different peatlands, the Chernobyl horizon should be at, or below, the lowest segments sampled. Thus,  $^{137}\text{Cs}$  has migrated upwards, although no downward migration could be tested (Vinichuk 2010a).

The relatively unchanged  $^{137}\text{Cs}/\text{K}$ ,  $^{137}\text{Cs}/\text{Rb}$ , and  $^{137}\text{Cs}/^{133}\text{Cs}$  isotopic (atom) ratios in the upper 0-14 cm part of *Sphagnum* plant and the noticeable widening below 14-16 cm supports this assumption. Upward migration of  $^{137}\text{Cs}$  has been previously reported (Ros  et al. 2009). Similarly, the majority of  $^{137}\text{Cs}$  from nuclear bomb testing in 1963 was retained in the top few centimeters of *Sphagnum* peat 20 years later, but with a lower peak at the level where the 1963 peat was laid down (Clymo, 1983).

### **Mechanisms of $^{137}\text{Cs}$ and Alkali Metals Uptake by *Sphagnum* Plants**

Presumably,  $^{137}\text{Cs}$  is bound within capitula, living green segments, and dead brown segments of *Sphagnum* plants. According to Gstoettner and Fisher (1997), the uptake of some metals (Cd, Cr, and Zn) in *Sphagnum papillosum* is a passive process as the living and dead moss accumulate metal equally. For a wide range of bryophytes, Dragović et al. (2004) found  $^{137}\text{Cs}$  is primarily bound by cation exchange, with only a few percent occurring in biomolecules. *Sphagnum* mosses have remarkably high cation exchange capacity (Clymo, 1963), and according to Russell (1988), a high surface activity of *Sphagnum* is related to its high cation exchange capacity, which ranges between 90-140 meq/100 g. In a water saturated peat moss layer, water washes (1 L de-ionized water added to a column of with a volume of 1.4 L) removed about 60% of K from *Sphagnum* (Porter B. Orr, 1975), indicating this element was held on cation exchange sites. In turn, the desiccation of living moss usually causes cation leakage from cell cytoplasm, during which

most of the effused  $\text{K}^+$  is retained on the exchange sites and reutilized during recovery after rewetting (Bates, 1997).

However, this is not necessarily the case for  $^{137}\text{Cs}$ , as  $^{137}\text{Cs}$  has a weaker correlation with K, especially in the uppermost parts of the plant, which means  $^{137}\text{Cs}$  uptake can be somewhat different from that of K. Even within the same segments of the plant,  $^{137}\text{Cs}$  activity concentrations have higher variation than K concentration. An even stronger decoupling between  $^{137}\text{Cs}$  and K is observed in the forest moss *Pleurozium schreberi*, in which  $^{137}\text{Cs}$  is retained more in senescent parts (Mattsson & Lidén, 1975). However, the close correlations found between Rb and  $^{137}\text{Cs}$  suggest similarities in their uptake and relocation: similar observations are reported for fungi (Vinichuk et al. 2010b; 2011a; 2011b).

Although some lower parts of *Sphagnum* plants are still alive and able to create new shoots, as they are still connected to the capitulum (Högström, 1997), much of lower stem is dead. Thus, the decrease in  $^{137}\text{Cs}$  activity concentration in plant segments below 10 cm indicates a release of the radionuclide from the dying lower part of *Sphagnum* and internal translocation to the capitulum.

The mechanism of radiocesium and alkali metal relocation within *Sphagnum* is probably the same active translocation as described for metabolites by Rydin & Clymo (1989). Although external buoyancy-driven transport (Rappoldt et al. 2003) could redistribute  $^{137}\text{Cs}$ , field evidence suggests buoyancy creates a downward migration of K (Adema et al. 2006); thus, this mechanism appears unlikely. Likewise, a passive downwash and upwash (Clymo & Mackay, 1987) cannot explain accumulation towards the surface.

**Alkali Earth  
Metals Ca and Sr**

**3**



Calcium (Ca) is the most common natural alkaline earth metal. In plants, calcium is a nutrient element; however, unlike in animals, its functions in plants are limited: it is mobile and necessary for cell division, cell wall, and membrane functions. The calcium content of the earth crust (2.96%) exceeds that of strontium (Sr) by several orders of magnitude ( $3.4 \times 10^{-2}\%$ ), and may discriminate strontium uptake by plants, including its radioactive isotopes. Plant roots take up Ca as divalent ions, uptake is passive, and  $\text{Ca}^{2+}$  is taken up by facilitated diffusion via a specific Ca channel. Calcium is restricted to the apoplast and an efflux mechanism is important, as Ca easily forms complexes with phosphate, which may affect the energy mechanism (Greger, 2004). Uptake of Sr appears to occur equally by either metabolic or passive processes. Strontium is considered an approximate chemical and physiological analogue of Ca and is often associated with Ca. This chemical analogy implies competition between these two ions. Due to discrimination by the chemical analogue of calcium, the transfer coefficient for Sr is low. The sorption of  $^{90}\text{Sr}$  in soils is enhanced by the increased concentration of  $\text{CO}_3^{2-}$ ,  $\text{PO}_4^{3-}$  and  $\text{SO}_4^{2-}$  anions and the by the co-precipitation of Sr compounds with low solubility and low assimilability. Therefore, Sr has low bioavailability in soils with a high content of exchangeable forms of phosphorus and sulfur.

Concentrations of Ca and Sr in soil fractions: bulk soil, rhizosphere, soil-root interface, fungal mycelium and fruit bodies of fungi are relatively high (Table 3.1).

The concentration of Ca is found to be slightly higher in the rhizosphere fraction and mycelium than in the bulk soil and soil-root interface fractions. Strontium concentration is higher in the rhizosphere. Fungal sporocarps

contained noticeably less calcium and strontium than mycelium. Despite a very high level of Ca in bulk soil, the mechanism of Ca exclusion appears efficient.

**Table 3.1** Concentrations of alkaline earth metals ( $\text{mg kg}^{-1}$  DW) and bioconcentration ratios (BCR: defined as concentration of the element ( $\text{mg kg}^{-1}$  DW) in the specific fraction divided by concentration of the element ( $\text{mg kg}^{-1}$  DW) in bulk soil), in soil fractions and fungi, mean values  $\pm$  standard deviation (Vinichuk et al. 2010b)<sup>1</sup>.

Element	Bulk soil	Rhizo-sphere	Soil-root-interface	Fungal mycelium	Fruit bodies
Concentrations					
Ca	11785 $\pm$ 11 335 <sup>a</sup>	16042 $\pm$ 9 513 <sup>b</sup>	10514 $\pm$ 7 122 <sup>a</sup>	15 780 $\pm$ 9 992 <sup>b</sup>	377.2 $\pm$ 293.2
Sr	17.14 $\pm$ 10.6 <sup>a</sup>	22.5 $\pm$ 7.77 <sup>b</sup>	18.7 $\pm$ 7.85 <sup>a</sup>	17.9 $\pm$ 6.77 <sup>a</sup>	0.873 $\pm$ 0.749
Bioconcentration ratios					
Ca	-	1.27 $\pm$ 0.51	0.80 $\pm$ 0.24	2.03 $\pm$ 1.56	0.06 $\pm$ 0.06
Sr	-	1.32 $\pm$ 0.53	1.18 $\pm$ 0.56	1.31 $\pm$ 0.66	0.10 $\pm$ 0.18

<sup>1</sup>Means within rows with different letters (a or b) are significantly different ( $p < 0.01$ ).

**Table 3.2** Element bioconcentration ratios (BCR:  $\text{mg kg}^{-1}$  DW in fungi) / ( $\text{mg kg}^{-1}$  DW in bulk soil) for fungal sporocarps.

Sampling plots according to Vinichuk et al. (2010b)	Species	Ca	Sr
4	<i>Boletus edulis</i>	0.02	0.01
6	<i>Cantharellus tubaeformis</i>	0.04	0.03
10	<i>Collybia peronata</i> <sup>a</sup>	0.09	0.05
7	<i>Cortinarius armeniacus</i>	0.13	0.04
5	<i>C. odorifer</i>	0.01	0.04
8	<i>C. spp.</i>	0.21	0.14
8-10	<i>Hypholoma capnoides</i> <sup>a</sup>	0.05	0.66
1	<i>Lactarius deterrimus</i>	-	0.03
3	<i>L. scrobiculatus</i>	0.01	0.03
6	<i>L. trivialis</i>	0.05	0.03
5-7	<i>Sarcodon imbricatus</i>	0.04	0.02
2	<i>Suillus granulatus</i>	0.03	0.01
8-10	<i>Tricholoma equestre</i>	0.02	0.09

<sup>a</sup>Saprophyte.

Fungal sporocarps varied in their ability to accumulate alkaline earth metals, although the concentration of calcium within fruit bodies of different species was several orders of magnitude higher than the concentration of strontium (Table 3.2).

Although the alkaline earth metal radium (Ra) was also included in this study, the concentrations were below the detection limit ( $0.005 \text{ mg kg}^{-1} \text{ d.w.}$ ) and were excluded.

Calcium uptake correlates fairly well with Sr uptake in fungi: the Pearson correlation of Sr and Ca in fruit bodies of fungi is 0.904 (P-value = 0.000).



**Transition Metals**  
**Cr, Co, Ni, Cu, Zn,**  
**Cd, Hg, and Pb**

**4**



Mycorrhizal fungi acquire essential macronutrients, such as phosphorus (Burgess et al. 1993), and are efficient at taking up and accumulating microelements (Smith and Read, 1997). However, this ability includes the accumulation of non-essential elements and trace metals, such as the heavy metal cadmium (Cd), which is an element of concern for food quality, essential zinc (Zn), and the trace element copper (Cu) (Falandysz et al. 2008). All Cu in the organic surface of coniferous forest soils layer may be accumulated in ectomycorrhizas (Berthelsen et al. 1995). The accumulation of these elements can have important consequences for their retention, mobility, and availability in forest ecosystems.

Most heavy metals are not chemically degraded (Kirpichtchikova et al. 2006) but they are accumulated in surface layers of soils (Basta et al. 2005). Thus, these elements tend to bioaccumulate, and sporocarps mainly of ectomycorrhizal macrofungi can contain extremely high levels of heavy metals. Cadmium is intensively accumulated by fungi (Collin-Hansen, 2002, Rudawska & Leski, 2005), and relatively high Cu and Zn concentrations (bioaccumulation values higher than 1) in certain species of wild growing fungi are reported (Blanuša et al. 2001; Elekes et al. 2010).

Soil fungi, especially fungal sporocarps, are involved in the recycling of heavy metals in forest ecosystems through bioaccumulation (Lepp et al. 1987), and fungal sporocarps have been suggested as a useful object for monitoring environmental pollution (Byrne et al. 1976). Furthermore, the capacity for retaining metal among species of fungi is wide (Pérez et al. 2007) and the capability of fungi to accumulate elements differs (Campos et al. 2009). Essential nutrients, such as cobalt (Co) and nickel (Ni), and non-essential lead (Pb) appear to be excluded from fungi, as they do not accumulate (Berthelsen et al. 1995;

Holan and Volesky, 1995). Similarly, mushroom fruit bodies do not accumulate chromium (Cr) (Berthelsen et al. 1995) or mercury (Hg) (Falandysz et al. 2012).

Although fungal sporocarps only comprise a few percentage of the total fungal biomass, i.e. biomass of mycelia in soil constitutes a major part of the total biomass of fungi (Horton and Bruns 2001), the majority of fungal biomass in soil is located in the upper soil horizons below the soil surface (Bååth, 1980). If Cd levels in sporocarps of ectomycorrhizal fungi are similar to those in soil mycelium (Berthelsen et al. 1995), a noticeable amount of soil metal located within soil fungi will be expected.

Mycelium, particularly sporocarps, accumulates Cd and fungal sporocarps may contain much higher Cd content than most plants (Byrne et al. 1976), up to ten times higher cadmium concentrations than in the soil in which they grow (Lepp et al. 1987). Consequently, the consumption of sporocarps of edible fungi with high Cd contents represents an important pathway by which Cd enters the human food system (Zurera-Cosano et al. 1988).

Thus, fungal mycelium appears important in the uptake of cadmium, copper, and zinc from soil, and their accumulation by soil fungi may be related to their ability to solubilize minerals of those metals, converting them into oxalates that are precipitated by the fungus in the local environment and in association with the mycelium (Fomina et al. 2005). When cadmium, copper, and zinc are taken up from the soil, other interactions may occur. Cadmium and zinc are in the same size and have similar chemical properties and zinc ions may act as antagonists of cadmium and copper. Even though ectomycorrhizal fungi profoundly affect forest ecosystems through mediating nutrient uptake and maintaining forest food webs, there are few studies on the ability of fungal mycelium to accumulate cadmium, copper, and zinc in mycelia (Thomet et al. 1999). The accumulation of metals in

mycorrhizal biomass in field conditions is studied to a much lesser extent and the rhizosphere appears to be important for understanding the transfer of metals from soil to fungi (Berthelsen et al. 2000). However, the fungi/soil concentration ratio for metals is not well studied under field conditions; neither is the difference between the uptake of metals from soil to fungi (mycelium/soil ratio) and transport within fungal thalli (sporocarps/mycelium ratio).

The concentration of chromium in soil-root interface fraction and fruiting bodies of fungi is 4-7 times lower than in the bulk soil (Table 4.1; Vinichuk, 2012a).

**Table 4.1** Mean concentrations of transition metals ( $\text{mg kg}^{-1}$  DW) in soil fractions and fungi, mean values  $\pm$  standard deviation<sup>1</sup>. (Vinichuk, 2012a, 2012b, 2013).

Element	Bulk soil	Rhizo sphere	Soil-root-interface	Fungal mycelium	Fruit bodies
Cr	2.79 $\pm$ 0.65 <sup>a</sup>	4.31 $\pm$ 1.4 <sup>a</sup>	1.05 $\pm$ 0.33 <sup>b</sup>	2.65 $\pm$ 0.94 <sup>a</sup>	0.19 $\pm$ 0.09
Co	0.79 $\pm$ 0.44 <sup>a</sup>	1.06 $\pm$ 0.6 <sup>a</sup>	0.59 $\pm$ 0.29 <sup>a</sup>	0.98 $\pm$ 0.65 <sup>a</sup>	0.11 $\pm$ 0.13
Ni	3.45 $\pm$ 2.1 <sup>a</sup>	4.62 $\pm$ 2.1 <sup>a</sup>	2.01 $\pm$ 1.0 <sup>a</sup>	3.13 $\pm$ 1.9 <sup>a</sup>	0.55 $\pm$ 0.34
Cu	10.4 $\pm$ 7.8 <sup>a</sup>	13.1 $\pm$ 5.5 <sup>a</sup>	11.9 $\pm$ 4.5 <sup>a</sup>	15.8 $\pm$ 5.7 <sup>a</sup>	28.8 $\pm$ 17.3
Zn	38.5 $\pm$ 24.7 <sup>a</sup>	31.9 $\pm$ 9.6 <sup>a</sup>	44.2 $\pm$ 5.4 <sup>a</sup>	69.7 $\pm$ 22.1 <sup>b</sup>	120.2 $\pm$ 5.7
Cd	0.29 $\pm$ 0.13 <sup>a</sup>	0.35 $\pm$ 0.11 <sup>a</sup>	0.81 $\pm$ 0.39 <sup>b</sup>	1.5 $\pm$ 1.6 <sup>b</sup>	3.1 $\pm$ 3.1
Hg	0.18 $\pm$ 0.1 <sup>a</sup>	0.16 $\pm$ 0.04 <sup>b</sup>	0.10 $\pm$ 0.03 <sup>b</sup>	0.24 $\pm$ 0.11 <sup>b</sup>	0.70 $\pm$ 0.96
Pb	18.4 $\pm$ 8.6 <sup>a</sup>	16.5 $\pm$ 6.2 <sup>a</sup>	7.7 $\pm$ 2.4 <sup>b</sup>	12.6 $\pm$ 4.7 <sup>a</sup>	0.25 $\pm$ 0.17

<sup>1</sup>Means within rows with different letters (a or b) are significantly different ( $p < 0.01$ ).

The concentration of Cr in fungal mycelium is not differing from the concentration in bulk soil and also Cr does not accumulate in either mycelium or in fruit bodies: bioconcentration ratios of this element do not exceed 1 (Table 4.2).

The concentration of Co and Ni in sporocarps is lower than the concentration in bulk soil and mycelium. Among other species, *Cortinarius armeniacus* and *Cortinarius* sp. have the highest content of all metals studied (Table 4.3).

Species of the genus *Cortinarius* absorb Cr more intensely (two orders of magnitude) than other studied species (Vinichuk, 2012a).

**Table 4.2** Element bioconcentration ratios (BCR: mg kg<sup>-1</sup> DW in specific fraction) / (mg kg<sup>-1</sup> DW in bulk soil), mean values ± standard deviation).  
Adapted from Vinichuk, 2012a, 2012b, 2013).

Element	Rhizo-sphere	Soil-root-interface	Fungal mycelium	Fruit bodies
Cr	1.09±0.22	0.27±0.24	0.78±0.12	0.13±0.09
Co	1.07±0.45	0.62±0.29	1.34±0.76	0.22±0.34
Ni	1.18±0.40	0.49±0.14	0.98±0.38	0.21±0.14
Cu	1.3±0.5	1.2±0.6	1.9±0.8	3.4±2.2
Zn	1.5±0.6	2.2±0.9	2.4±1.4	3.3±2.1
Cd	1.6±0.7	4.0±2.8	5.7±4.6	8.8±9.2
Hg	1.4±0.7	0.87±0.46	1.74±1.0	2.7±1.1
Pb	1.18±0.50	0.56±0.26	0.87±0.60	0.013±0.008

**Table 4.3** Element bioconcentration ratios (BCR: mg kg<sup>-1</sup> DW in fungi) / (mg kg<sup>-1</sup> DW in bulk soil) for fungal sporocarps.

Sampling plots according to Vinichuk et al. (2010b)	Species	Cr	Co	Ni	Cu	Zn	Cd	Hg	Pb
4	<i>Boletus edulis</i>	0.01	0.013	0.28	1.22	1.55	3.37	1.93	0.004
6	<i>Cantharellus tubaeformis</i>	0.04	0.048	0.12	4.39	2.23	1.09	0.89	0.011
10	<i>Collybia peronata</i> <sup>a</sup>	0.13	0.316	0.22	7.24	0.46	18.78	11.69	0.015
7	<i>Cortinarius armeniacus</i>	0.03	0.795	0.48	3.98	3.60	33.23	5.89	0.013
5	<i>C. odorifer</i>	0.02	0.034	0.05	0.42	3.85	7.19	0.97	0.016
8	<i>C. spp.</i>	0.99	1.084	0.45	6.74	5.14	17.23	2.34	0.030
8-10	<i>Hypholoma capnoides</i> <sup>a</sup>	0.50	0.045	0.18	3.02	0.52	2.27	1.09	0.005
1	<i>Lactarius deterrimus</i>	0.01	0.004	0.07	0.65	3.60	3.89	1.42	0.009
3	<i>L. scrobiculatus</i>	0.01	0.198	0.16	3.28	2.48	6.92	1.05	0.016
6	<i>L. trivialis</i>	0.02	0.113	0.31	3.33	2.93	4.41	1.23	0.024
5-7	<i>Sarcodon imbricatus</i>	0.02	0.237	0.21	2.09	8.37	9.87	9.17	0.019
2	<i>Suillus granulatus</i>	0.01	0.005	0.01	1.93	2.82	0.91	0.84	0.008
8-10	<i>Tricholoma equestre</i>	0.25	0.024	0.22	5.54	5.06	5.80	0.38	0.004

<sup>a</sup>Saprophyte.

Fungi vary in their ability to take up chromium: particularly high concentrations of this element are found in fungal species with wood as a substrate, such as *Rhizina undulata* Fr. (Jonnalagadd et al. 2006). In addition, hexavalent chromium, Cr (VI) is taken up by fungi in quantities that are one order of magnitude higher than the total uptake of chromium (Figueiredo et al. 2007).

Metabolic processes in fungi occur most intensively in their fruiting bodies, but data indicate concentrations of both nickel and chromium in mycelium of fungi is 6-14 times higher than the concentration in fruit bodies (Vinichuk, 2012a). Differences between the concentrations of elements in the fruit bodies and mycelium of fungi suggest both elements are absorbed by the fungal mycelium, whereas, only a small part is transferred to fruiting bodies.

The concentration of Cr in fruiting bodies of fungi does not depend on metal content in the soil. According to bioconcentration ratios, values of chromium and nickel in species of fungi and forest soil fractions can be placed in the decreasing order: rhizosphere>bulk soil > mycelia> soil-root interface > fruiting bodies (Vinichuk, 2012a).

In bulk soil, the concentrations of Cu and Cd are not different from the concentrations in the rhizosphere, although the values for both elements are slightly higher in the rhizosphere fraction (Table 4.1). In the rhizosphere fraction, Zn and Pb concentrations are lower than in bulk soil. The soil-root interface fraction has a higher Cd concentration than bulk soil, but the concentrations of Cu and Zn in the soil-root interface fraction are similar to the concentrations in the bulk soil and rhizosphere fractions. Zinc and cadmium concentrations are higher in fungal mycelium fractions than in the bulk soil and rhizosphere fractions.

Although, the fungal mycelium fraction is more concentrated with Cu, this is not statistically significant. In the soil-root interface fraction, the increase in Cd was 4 times larger than in bulk soil, and in mycelium, the increase in Cd was 5.7 times larger than in bulk soil. Zinc concentration increased by a factor of 2.2 in the soil-root interface fraction and 2.4 in mycelium, compared with bulk soil. Copper concentrations vary widely among analyzed fractions. However, no significant difference in Cu concentrations among different soil fractions is found (Table 4.1).

BCR defined as the concentration of the element ( $\text{mg kg}^{-1}$  DW) in the specific fraction divided by the concentration of the element ( $\text{mg kg}^{-1}$  DW) in bulk soil for the 0-10 cm soil layer had a similar pattern, but the enrichment of Cu, Zn, and Cd in fungal material was more evident, particularly in the sporocarps (Table 4.2). The bioconcentration ratios for mentioned elements vary among the species: the sporocarp: bulk soil bioconcentration ratios are presented in Table 4.3. Most species of fungi, except *Lactarius deterrimus* and *Cortinarius odorifer*, accumulate Cu, and most fungal species, except saprotrophic fungi *Hypholoma capnoides* and *Collybia peronata*, accumulate Zn. All fungal sporocarps, except *Suillus granulatus*, accumulate Cd. The bioconcentration ratio values for Cd are high, for example, the BCR for *Cortinarius armeniacus* is above 30. Alonso et al. (2003) also find differences in the concentrations of metals (Cd, Cu, and Zn) and their bioconcentration potential.

Fungal sporocarps accumulate larger amounts of Cu, Zn, and Cd than mycelium. For example, in fungal sporocarps collected from the same plots where soil samples and mycelium are extracted, Cu concentrations are about 1.8 times higher than Cu concentrations in mycelium. The concentration of Zn in fungal sporocarps is about 1.4-fold higher than in the corresponding fungal mycelium, and the concentration of Cd is about 1.5-fold higher (Tables 4.2 and 4.3).

For most living cells, Cu is both an essential micronutrient and a toxic heavy metal. Mycorrhizal fungi extensively accumulate copper (Blanuša et al. 2002; Falandysz et al. 2011). Results obtained from the top layers of coniferous forest soils in central Sweden (Vinichuk, 2012b) indicate that copper concentration increases in the order: bulk soil < soil-root interface < rhizosphere < fungal mycelium < fungal sporocarps. However, the statistically higher concentration of copper, compared to that in bulk soil, is only found in fungal sporocarps (Vinichuk, 2012b), as is reported in other studies (Collin-Hansen et al. 2002; Elekes et al. 2010), where the concentration of Cu is higher in fungal sporocarps than in topsoil. Copper accumulation ( $BCR > 1$ ) is found in the majority of fungal sporocarps species analyzed, and both Blanuša et al. (2002) and Elekes et al. (2010) report copper accumulation in all sporocarps, irrespective of species. Although copper concentration in mycelium is about 1.5-2-fold higher than in soil, this does not differ significantly from bulk soil.

Although mycorrhizal fungi sporocarps are more capable of accumulating copper than mycelium, the data for Cu accumulation by mycelium *in situ* are limited (Brzostowski et al. 2011). The values for mean Cu concentration in mycelium are lower than reported values for ectomycorrhiza ( $337 \pm 140 \mu\text{g g}^{-1}$  mycorrhiza) from the top organic layer of Norwegian coniferous forest soils contaminated with Cu (Berthelsen et al. 1995).

Zinc concentrates in mushroom thalli and increases in the order: bulk soil < soil-root interface < fungal mycelium < fungal sporocarps. The concentration of Zn in rhizosphere appears lower than in bulk soil, when the concentration of Zn is slightly (not significantly) higher in the soil-root interface fraction than in bulk soil. The concentration of zinc is about 2-fold higher in fungal mycelium and about 3-fold higher in sporocarps than in bulk soil (Vinichuk, 2012b). Alonso et al. (2003) and Elekes et al. (2010) also find a similar concentration of

Zn in fungal sporocarps. Data on zinc concentration in mycelium extracted from the soil in which sporocarps grow are limited. However, Berthelsen et al. (1995) report mean Zn concentrations of  $456 \pm 201 \mu\text{g g}^{-1}$  in mycorrhiza, which is higher than the values obtained by Vinichuk (2012b, 2013). The difference may be due to the area studied, as the study by Berthelsen et al. (1995) was located in a part of Norway exposed to considerable amounts of airborne deposition of heavy metals, such as Cd and Zn and concentrations of naturally occurring Zn in the biota and/or litter in the area studied by Vinichuk (2012b, 2013) are in a range of  $50\text{--}200 \text{ mg kg}^{-1} \text{ DW}$  (Brun et al. 2010).

Cadmium concentrations increase in the order: bulk soil < rhizosphere < soil-root interface < fungal mycelium < fungal sporocarps. Fungi appeared to have high preference for cadmium, as the accumulation of Cd by fungi, both mycelium and especially fungal sporocarps, is pronounced. Cadmium concentration in mycelium is about 5 times higher than in bulk soil, and cadmium concentration in sporocarps is about 2 times higher than in mycelium extracted from soil of the same plots where fungal sporocarps are sampled.

The accumulation of cadmium by fungal sporocarps appears species-dependent. Fungi have a tendency to accumulate Cd: the mean BCR is about 6:1 for mycelium and 9:1 for sporocarps (Vinichuk, 2012b, 2013). The ability of fungi to accumulate Cd is documented, and extremely high levels of cadmium in fruiting bodies of some fungi, especially the toxic *Amanita* sp., are reported by Kalač et al. (1991) and Rudawska & Leski (2005). This might explain the species-wide variation in both mycelium and sporocarps, as the standard deviation values for cadmium are as high as the mean values. However, not only fungal thallus is enriched with Cd, as the rhizosphere and soil-root interface fractions are noticeably enriched with Cd, although this is only significantly different for the soil-root interface fraction.

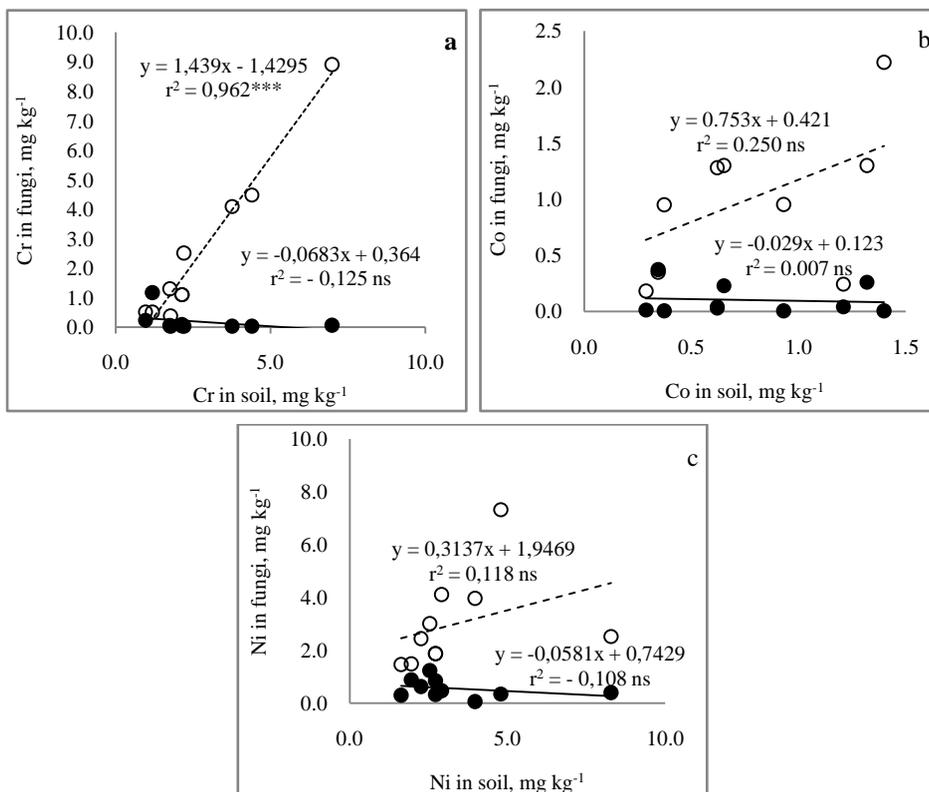
The concentrations of all three metals (Cu, Zn, and Cd) in sporocarps are about two times higher than in fungal mycelium; although Thomet et al. (1999) report similar concentrations of Cd and Zn in isolated mycelia and stems of corresponding fruiting bodies.

Fungi (mycelium and sporocarps) preferentially accumulate Cd over Zn and Cu. Thus, based on the BCR values obtained for fungal sporocarps and mycelium, (Table 4.2), the bioaccumulation of the metals can be ranked in the order:  $Cd^{2+} > Zn^{2+} > Cu^{2+}$ , with a relative ratio of 100:41:38. In terms of the amounts of metals accumulated by mycelium and sporocarps per mass unit, the metals can be ranked in the order  $Zn^{2+} > Cu^{2+} > Cd^{2+}$ , with a relative ratio of 41:13:1.

Fungi tend to accumulate mercury: the Hg content is slightly higher in rhizosphere and lower in soil-root-interface fraction than in soil, and Hg concentration is about 1.5-2.0 times higher in fungal mycelium and about 2.5-3.0 times higher in fruit bodies than in bulk soil (Tables 4.1; 4.2; and, 4.3).

## **Relationships between Transition Metals Cr, Co, Ni, Cu, Zn, Cd, Hg, and Pb in Soil and Fungi**

The total element content in the soil or soil biota provides little insight in elucidating into element availability and uptake mechanism. As the interactions between plants, nutrients (metals), and fungi are complex, the relationships between Cr, Co, Ni, Cu, Zn, Cd, Hg, and Pb concentrations in fungi (both fungal fruit bodies and mycelium) and soil can be useful. The concentrations of some metals found in fungi are related to its concentration in soil when uptake of other metals in fungi is independent on soil concentration.

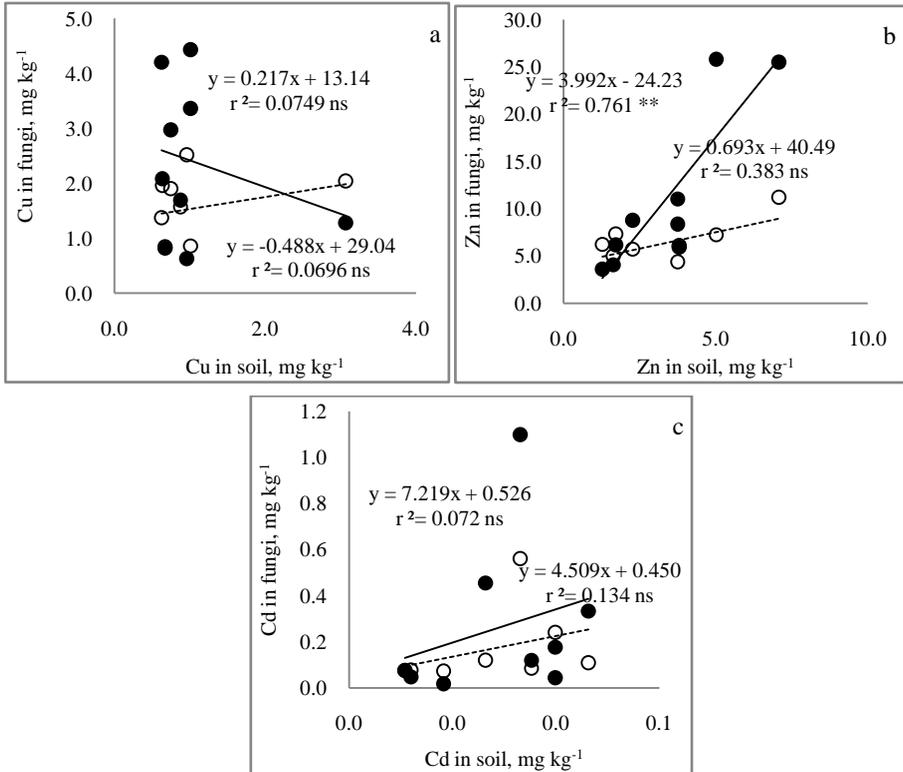


**Figure 4.1** Relationships between concentration of Cr (a), Co (b) and Ni (c) in fungal sporocarps (filled circle, solid line) and soil mycelium (empty circle, dotted line) in relation to the soil in which they are growing. \*\*\*  $p < 0.001$ . Adapted from Vinichuk, (2012a, 2012b).

Thus, the concentration of Cr and Ni in fruit bodies of fungi appear independent of the concentration of the elements in the soil (Figure 4.1). There is a close relation ( $r^2 = 0.962$ ) between the concentration of Cr in fungal mycelium and the concentration in soil, but Co and Ni concentration in the fungal mycelium only weakly relate to its soil concentration. A lack of relationship between Cr content in mushrooms (fruiting bodies) and the content in soil (total and mobile forms) is reported by Sybyrkyna (2012). There are no significant correlations

between the concentrations of Cr, Co and Ni in fungi and soil pH or between the concentrations and soil organic matter content (data are not presented).

The relationships between the concentration of Cu, Zn, and Cd in fungal sporocarps and soil mycelium in relation to the concentration in the soil they were growing are displayed in Figure 4.2.

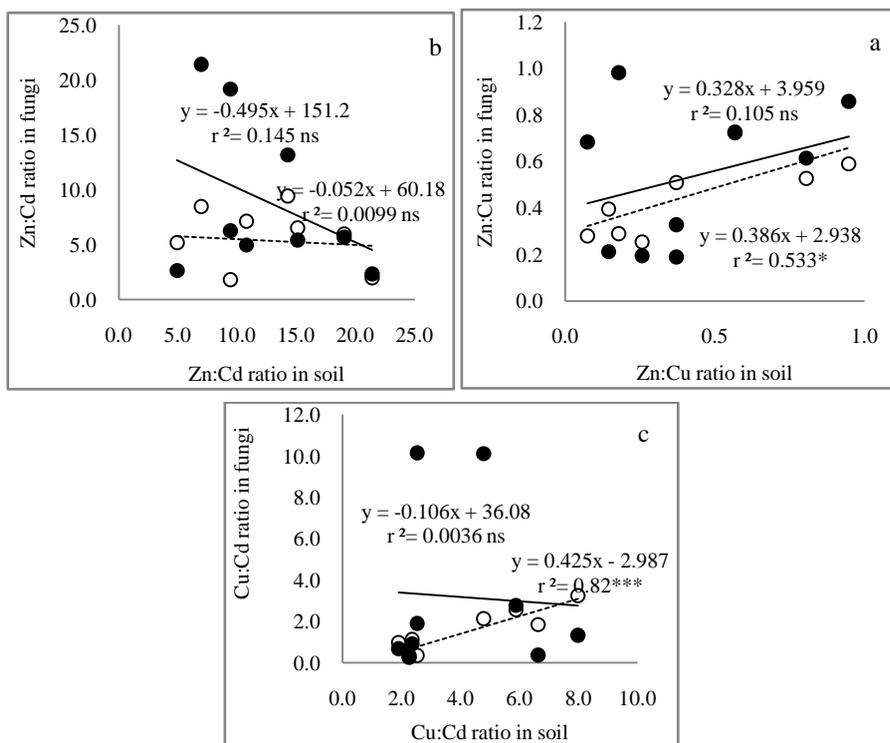


**Figure 4.2** Relationships between the concentration of Cu (a), Zn (b) and Cd (c) in fungal sporocarps (filled circle, solid line) and soil mycelium (empty circle, dotted line) in relation to the concentration in the soil they were growing. \*\*  $p < 0.01$ .

Adapted from Vinichuk, (2012a, 2012b, 2013).

Sporocarps and mycelium Cu concentrations do not depend on soil concentrations; however, Zn concentrations in soil correlate to Zn concentrations

in both sporocarps and mycelium. There were no significant correlations between Cd in soil and in either mycelium or in sporocarps (Vinichuk, 2013).



**Figure 4.3** Ratios of Zn:Cu (a), Zn:Cd (b), and Cu:Cd (c) in fungal sporocarps (filled circle, solid line) and soil mycelium (empty circle, dotted line) in relation to the ratios in the soil they were growing. \*  $p < 0.05$ ; \*\*\*  $p < 0.001$ , (Vinichuk, 2013).

Thus, the only metal concentration in soil that correlates with the concentration in sporocarps, and to a certain extent in mycelium, is essential Zn. There are no correlations between Cu in soil and in mycelium or sporocarps, or between Cd in soil and in mycelium or sporocarps. The concentrations of Cd and chemically related Zn strongly correlate in both mycelium and sporocarps (data not presented), whereas, Cu only poorly correlates with Cd in sporocarps and not correlate in mycelium (Vinichuk, 2013). Schmitt & Meisch (1985) and

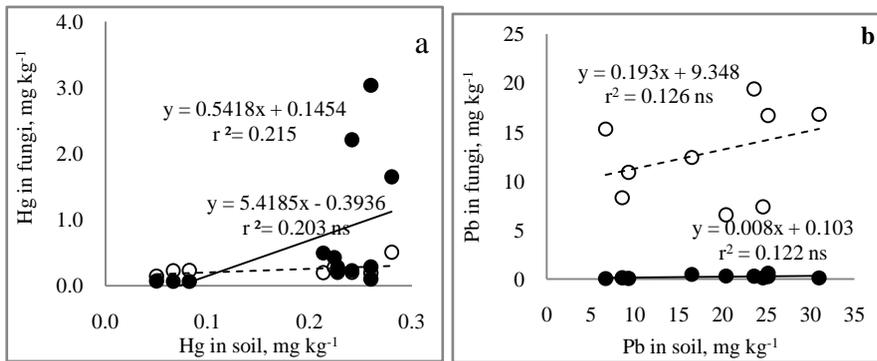
Thomet et al. (1999) also find cadmium uptake by fungi does not correlate with the uptake of zinc and copper.

The possible competition between Cu, Zn, and Cd in various transfer steps and the relationships between the concentrations of these three metals in soil, mycelia, and fungal sporocarps are estimated (Vinichuk, 2013). When comparing ratios between Cu, Zn, and Cd in soil and fungi (Figure 4.3), there is a moderate and strong positive correlation between the Zn:Cu and Cu:Cd ratios in soil and fungal mycelium (Figure 4.3a, b, c).

The relationships between Zn:Cu, Zn:Cd, and Cu:Cd ratios in fungal sporocarps and soil mycelia, with respect to the soil in which they were growing (Figure 4.3), indicate differences in the uptake of these metals by fungi. The close positive correlations between Zn:Cu ratios in fungal mycelium and in soil suggest the uptake of both metals by fungi is balanced and there are similarities in the uptake mechanism. However, metal accumulation capability of fungi is species specific and mainly depends on its accumulation mechanism (Demirbaş 2002). The opposite phenomenon is observed for Zn:Cd ratios in soil and fungi: the relationships between Zn:Cd ratios in soil mycelium, sporocarps and in soil are absent or even negative. The relationships between Cu:Cd in soil and fungi are unclear: there is a high positive correlation for mycelium, but no correlation for sporocarps. The lack of correlation in the Zn:Cd and Cu:Cd ratios in sporocarps is probably due to the variable concentration of Cd in sporocarps, indicating species dependent competition between Cu or Zn and Cd. The extent of Cd and Zn transfer from soil to mushrooms is species-specific and influenced by the availability of the two heavy metals and the age of the mushroom (Thomet et al. 1999). The variation in heavy metal content among edible mushrooms species also depends on the ability of the species to extract elements from the substrate (Radulescu et al. 2011), and there are significant

linear correlations between lead and cadmium concentrations in certain species i.e. *Boletus edulis* and *Paxillus involutus* (Kalač et al. 1991). In addition, there is a competitive interaction between Cd and Zn in the growth of fungi and on the nutrient uptake capacity of root systems (Krznaric et al. 2008).

There is a very weak correlation in Pb concentration in both sporocarps and mycelium whereas mercury content in soil appears to be correlated with the concentration of Hg in mycelium and in fruit bodies of fungi (Figure 4.4.a).



**Figure 4.4** Relationships between concentration of Hg (a) and Pb (b) in fungal sporocarps (filled circle, solid line) and soil mycelium (empty circle, dotted line) in relation to the concentration in the soil in which they were growing.

The mercury content in fungal sporocarps varies, whereas, the opposite is observed for mycelium (Vinichuk, 2013).

Correlation coefficients among the concentration of transition metals in fruit bodies of fungi are presented in Table 4.4. Uptake of Cd and Cu by fungi correlates well with Hg and uptake of Ni is related to concentration of Co, Cd, and Pb.

**Table 4.4** Correlation coefficients among the concentration of elements in fruitbodies of fungi (Vinichuk, 2013).

	Cr	Co	Ni	Cu	Zn	Cd	Hg
Co	0.51*						
Ni	0.14	0.64*					
Cu	0.21	0.40	0.37				
Zn	0.35	0.33	0.41	0.42			
Cd	0.09	0.51*	0.66*	0.41	0.41		
Hg	-0.11	0.31	0.43	0.67*	0.10	0.75**	
Pb	0.26	0.46	0.62*	0.58*	0.22	0.44	0.39

\*  $P < 0.05$ , \*\*  $P < 0.01$

The capability of fungal species to accumulate essential (Co, Ni) and non-essential (Pb) metals differs (Pérez et al. 2007; Campos et al. 2009; Vetter, 2005). The concentrations of Co, Ni, and Pb are generally evenly distributed between soil fractions and mycelium, whereas, the concentration of these metals in sporocarps is lower. Thus, elimination of the metals from sporocarps appears effective. Lead elimination from the sporocarps is more pronounced, as the concentration in sporocarps is about 70 times lower than in bulk soil and about 50 times lower than in mycelium. Similarly, Campos et al. (2009) observe only absorption of Pb, but not accumulation, in the ectomycorrhizal fungus *Cantharellus cibarius*. The differences in metal concentrations between the mycelium and the sporocarps (assuming they belong to the same species) indicate there is no further transport of Co, Ni, and Pb within sporocarps after being taken up by mycelium from soil, i.e. these metals are not actively transported from mycelium to sporocarps. In organic soils, Pb is more strongly bound to soil, whereas, e.g. Cd is only weakly bound (Villaverde et al. 2009).

The estimated values for soil mycelium biomass range from 30 to 60 mg DW of mycelium per one gram DW of soil (0-5 cm layer). Assuming the dry bulk density in the upper layers of the soil in the Forsmark area (Forsmark lies in

east-central Sweden, N 60°22'; E 18°13') is  $0.4 \text{ g cm}^{-3}$  (Lundin et al. 2004), fungal mycelium is an important constituent of the organic surface soil, comprising between 3 and 6% by volume in the upper 5-cm of the forest soil. Based on the metal concentrations in bulk soil, rhizosphere, soil-root interface fractions, and mycelium extracted from the same soil, fungal mycelium appear to accumulate relatively small fractions of soil metals. Assuming the concentration of chromium ( $2.65 \text{ mg kg}^{-1}$  dry weight) and nickel ( $3.13 \text{ mg kg}^{-1}$  dry weight) in the mycelium, mycelium of upper forest soil layer may comprise 2.9-5.8% of chromium and 2.7-5.4% of nickel from their total content in soil. Fungal mycelium can also comprise between 4.6-9.1% of the total Cu in soil, 5.4-10.9% of the total Zn in the soil, and 15.5-31.7% of the total Cd in the soil. In fungal mycelium, the accumulation is estimated to be 3.5-6.9% for cobalt, 2.0-3.9% for Pb and 4.0-8.0% for Hg. Estimates of fungal biomass in soil may be relevant: Berthelsen et al. (1995) report similar data for fungal biomass in forest soil from Norway. Although the fungal content of Cu, Zn, and Cd was higher in Norwegian forest soil, possibly due to the difference in concentration level of metals in soil, as the study area in Norway had been exposed to airborne deposition of heavy metals, thus, explaining the higher accumulation by mycelium of fungi. The fungal content of Pb (2-4%) was similar to the < 3% reported by Berthelsen et al. (1995). In the mycelium of fungi in the upper (0-5 cm) layer of forest soil can be allocated 2.9-5.8% of the total amount of chromium in soil.

**Semimetals (As)**

**5**



Arsenic (As) has been analyzed in a number of fungal sporocarps (Byrne et al. 1976; Kalač et al. 1991; Slekovec & Irgolic 1996). High arsenic concentrations are characteristic of the genus *Agaricus* (Vetter, 1994). However, little is known about the accumulation of As by fungal mycelia and its contribution to the total element content. No difference in As concentration in bulk soil and rhizosphere has been found, when soil-root interface fraction contains lower amounts of the elements (Table 5.1, unpublished data).

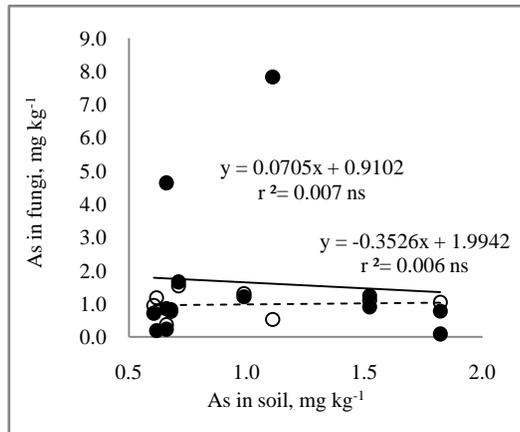
**Table 5.1** Mean concentration of arsenic ( $\text{mg kg}^{-1}$  DW) in soil fractions and fungi and bioconcentration ratios (BCR), mean values  $\pm$  standard deviation.

Bulk soil	Rhizosphere	Soil-root-interface	Fungal mycelium	Fruit bodies
Element concentration <sup>1</sup>				
0.97 $\pm$ 0.44 <sup>a</sup>	1.0 $\pm$ 0.34 <sup>a</sup>	0.66 $\pm$ 0.29 <sup>b</sup>	0.98 $\pm$ 0.37 <sup>a</sup>	1.63 $\pm$ 2.19
Bioconcentration ratios				
-	1.19 $\pm$ 0.51	0.70 $\pm$ 0.18	1.16 $\pm$ 0.63	2.01 $\pm$ 2.40

<sup>1</sup>Means within rows with different letters (a or b) are significantly different ( $p < 0.01$ ). BCR defined as concentration of the element ( $\text{mg kg}^{-1}$  DW) in the specific fraction divided by concentration of the element ( $\text{mg kg}^{-1}$  DW) in bulk soil).

The concentration of arsenic in mycelium was almost the same as concentration in the soil and about 1.5 times higher in fruit bodies than in mycelium. Thus, arsenic does not appear to be accumulated by mycelium and only moderately by fruit bodies of fungi (CR = 1.6). *Cortinarius* spp. tend to accumulate higher amounts of As than other species.

There was no relationship between the concentration of As in fungal sporocarps or soil mycelium and concentration in the soil in which they are growing (Figure 5.1).



**Figure 5.1** Relationships between concentration of As in fungal sporocarps (filled circle, solid line) and soil mycelium (non-filled circle, dotted line) in relation to the soil in which they are growing.

The values for soil mycelium biomass are space between 30 and 60 mg DW of mycelium per one gram DW of soil. With the assumption, the dry bulk density in the upper layers of the soil in the Forsmark study area is  $0.4 \text{ g cm}^{-3}$  (Lundin et al. 2004), and the As concentration in bulk soil and mycelium (Table 5.1.), it appears fungal mycelium accumulate between 3.0 and 6.1% of the total As in soil.

**Actinides Th and U**

**6**



Some fungi have the ability to take up thorium and uranium, but this capability differs depending on the species (Campos et al. 2009; Borovička et al. 2011, Turtiainen et al. 2013). Although thorium and uranium concentrations in fungi are usually measurable, in exceptionally low amounts (Mietelski et al, 2002; Rufyikiri et al, 2002), there have been attempts to analyze the content of these trace elements in mushroom mycelium growing *in vivo* (Thomet et al. 1999; Berthelsen et al. 1995). It is not known how natural isotopes coming from soil are taken up by mycelium and into fruiting bodies and through the food chain. The role of soil fractions such as rhizosphere and soil-root interface appears important for the understanding of elemental uptake by fungi.

Thorium and uranium contents in mushrooms seem depend on the acidity of the soil. Results of our previous study (Vinichuk, 2012c) indicate a positive correlation exists between pH and uranium ( $r=0.91$  for mushroom mycelium;  $r=0.50$  for fruiting bodies) and between pH and thorium content ( $r=0.33$  mushroom mycelium;  $r=0.52$  for fruiting bodies). Although, there are currently no available data on soil acidity effects on the uptake of thorium and uranium by ectomycorrhizal fungi, the biomass of fungi in the genus *Penicillium* absorbs most uranium at pH 3-7.5 (Galun et al. 1983). There is an inversely proportional relationship for thorium ( $r=-0.66$ ) and uranium ( $r=-0.51$ ) between the content of organic matter in the soils studied and concentrations of elements in the mushroom mycelium.

Thorium content in the rhizosphere fraction is slightly higher than its content in soil (Table 6.1.).

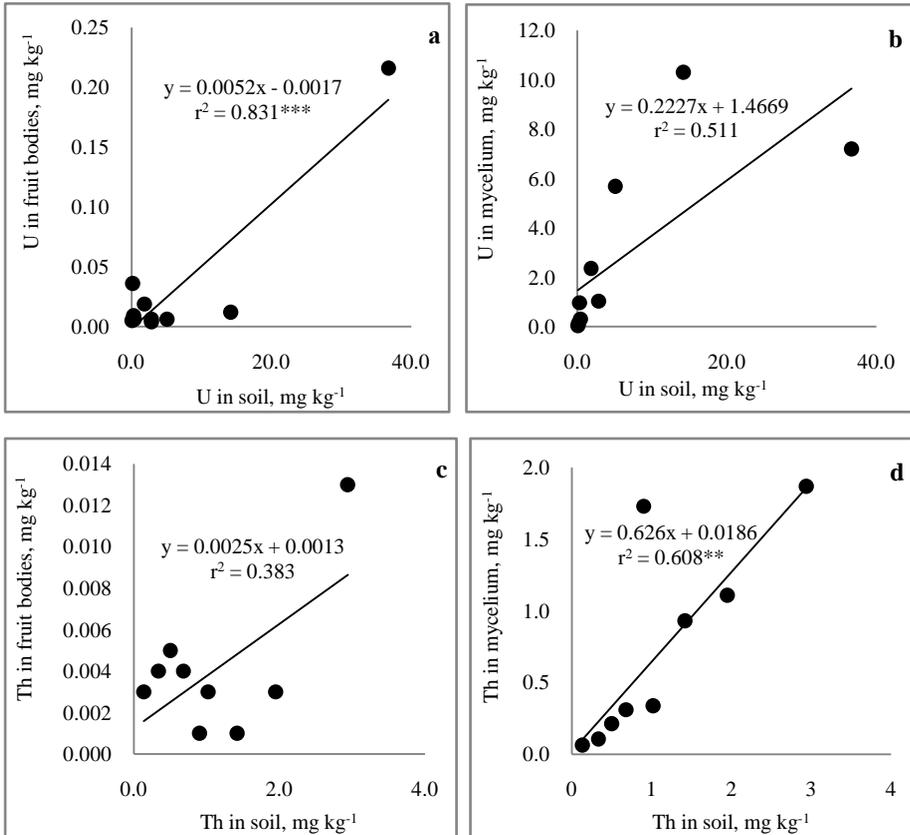
**Table 6.1** Mean concentrations of actinides (mg kg<sup>-1</sup> DW) in soil fractions and fungi and bioconcentration ratios (BCR), mean values ± standard deviation. Adapted from Vinichuk, 2012c).

Element	Bulk soil	Rhizosphere	Soil-root interface	Fungal mycelium	Fruit bodies
Element concentrations					
Th	1.10 ±0.30 <sup>a</sup>	1.45 ±0.5 <sup>a</sup>	0.28 ±0.096	0.74 ±0.23 <sup>a</sup>	0.0041 ±0.0009
U	6.85 ±4.02 <sup>a</sup>	9.36 ±3.99 <sup>a</sup>	5.79 ±2.81 <sup>a</sup>	3.11 ±1.24 <sup>a</sup>	0.026 ±0.016
Bioconcentration ratios					
Th	-	0.85 ±0.24	0.18 ±0.04	0.64 ±0.16	0.006 ±0.002
U	-	1.05 ±0.19	0.52 ±0.18	0.99 ±0.29	0.035 ±0.021

The means within rows with different letters (a, b) represent a significant difference ( $p < 0.01$ ). BCR defined as concentration of the element (mg kg<sup>-1</sup> DW) in the specific fraction divided by concentration of the element (mg kg<sup>-1</sup> DW) in bulk soil).

The concentration of thorium in mushroom mycelium is slightly lower than its content in the soil, whereas, the content in fruit bodies is two orders of magnitude lower than in the soil. As the soil-root interface fraction is essentially the finest plant roots with adhered soil particles, it could be argued the root system of plants (trees) takes up but does not accumulate neither uranium nor thorium. The concentration of thorium in the soil-root fraction is lower than the Th content in the bulk soil. Thus, the content of these elements in plant tissue can be assumed as lower: after being absorbed, metals accumulate in the roots. Uranium and thorium concentrations in mycelium are generally lower than in bulk soil, whereas, metal concentration in fruit bodies is orders of magnitude lower than in bulk soil. Borovička et al. (2011) also report similar results for thorium and uranium content in fruit bodies of mushrooms. Uranium appears to have similar behavior to thorium. The median concentration of this element is higher in bulk soil (6.85 mg kg<sup>-1</sup>) than in mycelium (3.11 mg kg<sup>-1</sup>). In fruit bodies, uranium is only found in trace amounts (0.026 mg kg<sup>-1</sup>), indicating a low uptake rate of this element by fruit bodies and efficient exclusion of U from fungi (Table 6.1).

The content of uranium and thorium in mushrooms appears to depend on the concentration of these isotopes in the soil and there are significant correlations between uranium concentration in bulk soil and mycelium and in bulk soil and in fruit bodies (Figure 6.1).



**Figure 6.1** Relationships between concentration of U (a, b) and Th (b, c) in fungal sporocarps and soil mycelium in relation to the soil in which they are growing. Adapted from Vinichuk, (2012c).

Uptake of uranium by mycelium correlates with calcium ( $r=0.86$ ) and strontium uptake ( $r=0.79$ ,  $P<0.05$ ). Uranium concentration in fruit bodies of fungi correlates with Ca concentration ( $r=0.62$ ,  $P<0.01$ ). Mietelski et al. (2002)

reported uranium in fruit bodies of mushrooms in measurable amounts; however, there was no uranium accumulation in mushroom species examined. Uranium uptake and translocation by the arbuscular mycorrhizal fungus *Glomus intraradices* under root-organ culture conditions are reported by Rufyikiri et al. (2002), although no accumulation of U was observed.

Thus, both uranium and thorium enter the fruiting bodies of fungi but they do not be accumulated. Bioconcentration ratios of uranium and thorium in the fruit bodies of fungi species studied in Sweden have a wide range of variation, but significantly less than 1 (Table 6.2).

**Table 6.2** Element bioconcentration ratios (BCR: mg kg<sup>-1</sup> DW in fungi) / (mg kg<sup>-1</sup> DW in bulk soil) for fungal sporocarps. Adapted from Vinichuk, (2012c).

Sampling plots according to Vinichuk et al. (2010b)	Species	Th	U
4	<i>Boletus edulis</i>	0.0011	0.0296
6	<i>Cantharellus tubaeformis</i>	0.0029	0.0014
10	<i>Collybia peronata</i> <sup>a</sup>	0.0746	0.0857
7	<i>Cortinarius armeniacus</i>	0.0059	0.0149
5	<i>C. odorifer</i>	0.0100	0.0059
8	<i>C. spp.</i>	0.0119	0.2105
8-10	<i>Hypholoma capnoides</i> <sup>a</sup>	0.0149	0.0429
1	<i>Lactarius deterrimus</i>	0.0044	0.0012
3	<i>L. scrobiculatus</i>	0.0015	0.0103
6	<i>L. trivialis</i>	0.0029	0.0021
5-7	<i>Sarcodon imbricatus</i>	0.0020	0.0049
2	<i>Suillus granulatus</i>	0.0007	0.0008
8-10	<i>Tricholoma equestre</i>	0.0224	0.0714

<sup>a</sup>Saprophyte.

For most species of fungi, BCR for Th  $\leq 0.01$ , whereas, the BCR for U are almost an order of magnitude higher but do not exceed 0.2. The coefficient of variation of BCR for U in fruit bodies of fungi is almost 3 times higher than for Th. Similar BCR values are reported by Baeza & Guillén (2006) in the fruiting

bodies of fungi in forest ecosystems in Spain (Th=0.030-0.62; U=0.043-0.49). Species of the genus *Cortinarius* absorb U at one or two orders of magnitude more intensively than other species.

The saprotroph species *Collybia peronata* has the highest BCR for Th. Mycorrhizal species, especially members of Cortinariaceae (*Gymnopilus hybridus* (Fr.) Maire) are characterized by elevated levels of both of these naturally radioactive elements (Campos et al. 2009). According to Baeza & Guillén research (2006), the highest rates of thorium and uranium uptake are in a representative of Cortinariaceae, e.g. ectomycorrhizal species *Hebeloma cylindrosporum* Romagn, which is often found on sandy forest soils low in organic matter. Due to its exceptional ability to absorb radioactive elements, this species is used as bioindicator of soil contamination.

Although metabolism in fungi occurs most intensely within their fruit bodies, the concentration of thorium and uranium in fungal mycelium is more than two orders of magnitude higher than the concentration in fruit bodies, indicating a minor role of both elements in spore formation within sporocarps.

Estimates based on uranium and thorium concentrations in bulk soil and mycelium and mycelium content in typical forest soils in Sweden indicate that the mycelium in upper forest soil layers may comprise 2.0-5.0% of the total thorium content of soil and 1.4-2.7% of the total uranium content of soil. However, the direct method used for estimating mycelium biomass may not provide complete removal of soil mycelium; especially for the upper enriched organic matter soil layers where hyphae mycelium closely intertwine with semi-decomposed organic remains. Therefore, the data on thorium and uranium contents in mushroom mycelium may be underestimated rather than overestimated.

The uptake of uranium and thorium in fungal fruit bodies do not relate:  
Pearson correlation of U and Th in fruit bodies of fungi is 0.050 ( $p=0.870$ ).

## Conclusions

### Cesium ( $^{137}\text{Cs}$ and $^{133}\text{Cs}$ ) and Alkali Metals

The concentrations of the three stable alkali elements K, Rb, and  $^{133}\text{Cs}$  and the activity concentration of  $^{137}\text{Cs}$  have been determined in various components of Swedish forest – bulk soil, rhizosphere, soil-root interface fraction, fungal mycelium, and fungal sporocarps (Vinichuk et al. 2010b). The soil-root interface fraction is distinctly enriched with K and Rb, compared with bulk soil. Potassium concentration increases in the order bulk soil < rhizosphere < fungal mycelium < soil-root interface < fungal sporocarps, whereas, Rb concentration increases in the order bulk soil < rhizosphere < soil-root interface < fungal mycelium < fungal sporocarps. Cesium is generally evenly distributed within bulk soil, rhizosphere, and soil-root interface fractions, indicating no  $^{133}\text{Cs}$  enrichment in these forest compartments.

The uptake of K, Rb, and  $^{133}\text{Cs}$  during the entire transfer process between soil and sporocarps occurs against a concentration gradient. For all three alkali metals, the levels of K, Rb, and  $^{133}\text{Cs}$  are at least one order of magnitude higher in sporocarps than in fungal mycelium. Potassium uptake appears to be regulated by fungal nutritional demands for this element and fungi have a high preference for the uptake of Rb and K than for Cs. According to the efficiency of uptaking by fungi, the three elements can be ranked in the order  $\text{Rb}^+ > \text{K}^+ > \text{Cs}^+$ , with a relative ratio of 100:57:32.

Although the mechanism of Cs uptake by fungi may be similar to that of Rb, the uptake mechanism for K appears to be different. The variability in isotopic (atom) ratios of  $^{137}\text{Cs}/\text{K}$ ,  $^{137}\text{Cs}/\text{Rb}$ , and  $^{137}\text{Cs}/^{133}\text{Cs}$  in the fungal sporocarps

suggests they are not species specific. The relationships between the concentration ratio  $^{137}\text{Cs}/^{133}\text{Cs}$  and K, Rb, and  $^{133}\text{Cs}$  in fungal sporocarps have wide and inconsistent variation, and the concentration of K, Rb, and  $^{133}\text{Cs}$  in sporocarps appears to be independent of the  $^{137}\text{Cs}/^{133}\text{Cs}$  isotopic ratio.

The study of *S. variegatus* sporocarps (Vinichuk et al. 2011a) sampled within 1 km<sup>2</sup> forest area with high  $^{137}\text{Cs}$  fallout from the Chernobyl accident confirms  $^{133}\text{Cs}$  and  $^{137}\text{Cs}$  uptake is not correlated with uptake of K; whereas, the uptake of Rb is closely related to the uptake of  $^{133}\text{Cs}$ . Furthermore, the variability in  $^{137}\text{Cs}$  and alkali metals (K, Rb, and  $^{133}\text{Cs}$ ) among genotypes in local populations of *S. variegatus* is high and the variation appears to be in the same range as found in species collected at different localities. The variations in concentrations of K, Rb, and  $^{133}\text{Cs}$  and  $^{137}\text{Cs}$  activity concentration in sporocarps of *S. variegatus* appear to be influenced more by local environmental factors than by genetic differences among fungal genotypes.

### **Cs ( $^{137}\text{Cs}$ and $^{133}\text{Cs}$ ), K and Rb in *Sphagnum* Plants**

The distribution of  $^{137}\text{Cs}$  within *Sphagnum* plants (down to 20 cm depth) (Vinichuk et al. 2010a) is similar to stable K, Rb, and  $^{133}\text{Cs}$ . The  $^{137}\text{Cs}$  activity concentrations and concentrations of K, Rb, and  $^{133}\text{Cs}$  are highest in the uppermost 0-10 cm segments of *Sphagnum* (in the capitula and the subapical segments) and gradually decrease in older parts of the plant. Such distribution can be interpreted as dependent on the living cells of capitula and living green segments in the upper part of *Sphagnum*. The  $^{137}\text{Cs}$  appears to be taken up and relocated by *Sphagnum* plants in similar ways to the stable alkali metals, as concentrations of K, Rb, and  $^{137}\text{Cs}$  activity concentrations in *Sphagnum* segments are similar to the depth of about 16 cm, and display a slightly different pattern in the lower part of the plant.

## **Alkali Earth Metals Ca and Sr**

Concentrations of Ca and Sr in soil fractions: bulk soil, rhizosphere, soil-root interface, fungal mycelium and fruit bodies of fungi are relatively high. The concentration of Ca and Sr is found to be higher in the rhizosphere fraction. Fungal sporocarps contained noticeably less calcium and strontium than mycelium and the mechanism of Ca and Sr exclusion appears efficient.

Fungal sporocarps varied in their ability to accumulate alkaline earth metals, although the concentration of calcium within fruit bodies of different species was several orders of magnitude higher than the concentration of strontium.

Calcium uptake correlates fairly well with Sr uptake in fungi: the Pearson correlation of Sr and Ca in fruit bodies of fungi is 0.904 (P-value = 0.000).

## **Transition Metals Cr, Co, Ni, Cu, Zn, Cd, Hg, and Pb**

The capability of mycorrhizal fungi sporocarps to accumulate copper is higher than in mycelium: fungal sporocarps have statistically higher concentrations of Cu than the bulk soil (Vinichuk, 2012b, 2013). Zinc concentration in fungal mycelium is 2-fold higher than in bulk soil and 3-fold higher in sporocarps. The accumulation of cadmium is pronounced in both mycelium and fungal sporocarps: Cd concentration in mycelium is about 5 times higher than in bulk soil and about 2 times higher in sporocarps than in mycelium extracted from soil in the same plots where fungal sporocarps were sampled. Accumulation of Cd by fungal sporocarps is apparently species dependent.

Zinc concentration in sporocarps, and to a lesser extent in mycelium, depends on the concentration of Zn in the soil, whereas, the uptake of Cu and Cd by both sporocarps and mycelium does not correlate with their concentration in soil.

Thus, the uptake of Zn and Cu by fungi is balanced, implying similarities in the uptake mechanism.

The uptake of Cu, Zn, and Cd during the entire transfer process between soil and sporocarps occurs against a concentration gradient. For all three metals, the levels of Cu, Zn, and Cd in sporocarps is about two times higher than in fungal mycelium. Thus, fungi (mycelium and sporocarps) preferentially bioaccumulate Cd over Zn and Cu, and the bioconcentration values can be ranked in the order:  $Cd^{2+} > Zn^{2+} > Cu^{2+}$ , with a relative ratio of 100:41:38.

The concentration of Co and Ni in mycelium (Vinichuk, 2012a) is similar to the concentrations in other soil fractions (bulk soil, rhizosphere, soil-root interface); whereas, the concentrations in sporocarps are about 5-9 times lower than in mycelium. The concentration of Pb in mycelium is about 1.5 times lower than in other soil fractions (bulk soil, rhizosphere, soil-root interface) and about 50 times lower than in sporocarps. The mycorrhizal fungi (mycelium and sporocarps) only absorb Co, Ni, and Pb but do not accumulate these metals in tissue.

Fungal mycelium of upper forest soil layer may comprise 2.9-5.8% of chromium and 2.7-5.4% of nickel from their total content in soil. Fungal mycelium can also comprise between 4.6-9.1% of the total Cu in soil, 5.4-10.9% of the total Zn in the soil, and 15.5-31.7% of the total Cd in the soil. In fungal mycelium, the accumulation is estimated to be 3.5-6.9% for cobalt, 2.0-3.9% for Pb and 4.0-8.0% for Hg. In the mycelium of fungi in the upper (0-5 cm) layer of forest soil can be allocated 2.9-5.8% of the total amount of chromium in soil.

## **Semimetals (As)**

No difference in As concentration in bulk soil and rhizosphere has been found, when soil-root interface fraction contains lower amounts of the elements (Unpublished data). The concentration of arsenic in mycelium was about the same as concentration in the soil and about 1.5 times higher in fruit bodies than in mycelium. Thus, arsenic does not appear to be accumulated by mycelium and only moderately by fruit bodies of fungi. Fungal mycelium accumulates between 3.0 and 6.1% of the total As in soil.

## **Actinides Th and U**

Thorium concentration varies from of 0.0041 mg kg<sup>-1</sup> dry weight in fruit bodies of fungi to 1.45 mg kg<sup>-1</sup> dry weight fraction in the rhizosphere (Vinichuk, 2012c). Uranium concentration varies from 0.026 mg kg<sup>-1</sup> dry weight in fruiting bodies of fungi to 9.36 mg kg<sup>-1</sup> dry weight fraction in the rhizosphere. Both natural isotopes do not accumulate in either mushroom mycelium or their fruit bodies: bioconcentration ratios in fruit bodies are on average 0.006 for Th and 0.035 for U. Concentrations of Th and U isotopes in fruit bodies of fungi are about 270 times lower than in the bulk soil. The content of Th and U in mushroom mycelium and fruiting bodies depends on the concentration of these elements in the soil: as the concentrations of these elements in the soil increases, their content in mushrooms also increases. Mycelia of upper (0-5 cm) layers of the forest soil can comprise up to 5.0% of total thorium and 3.0% of total uranium content in soil.



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