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Vertical Distribution of Mercury in Forest Soils and Its Transfer to Edible Mushrooms in Relation to Tree Species

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Abstract: Soil pollution by mercury (Hg) is a global problem that poses risks to natural ecosystems and to human health. Forests represent an important recipient of Hg deposition, however, so far, very little is known about the tree species identity effects on the distribution of Hg in forest soils and its accumulation in edible mushrooms. To clarify the effect on the two main Central-European commercial forest tree species, soil samples were collected from organic F+H horizons and from mineral soil depths of 0–2, 2–10, 10–20, and 20–30 cm in mature Norway spruce (*Picea abies* (L.) H. Karst.) and European beech (*Fagus sylvatica* L.) dominated stands. Mushroom samples of the *Boletaceae* family were also collected at each sampling site. The highest Hg contents were found in the F+H layer and were significantly higher in spruce- (mean 0.46 ± 0.03 mg/kg) than in beech- (mean 0.29 ± 0.10 mg/kg) dominated stands. The variation in Hg contents in F+H was best predicted by pH, the overall lower soil pH in strongly acidic spruce stands might induce Hg immobilization in the F+H layer to cause a decrease in the bioavailability of Hg for *Xerocomellus chrysenteron* (Bull.) Šutara. In mineral soil, the Hg contents did not differ significantly between the spruce- and beech-dominated stands. The Hg content strongly correlated with the S, N, and C contents only in mineral soil; at the depths of 2–10, 10–20, and 20–30 cm, significantly also with the silt vs. sand, Al_o, and Fe_o contents. Studied mushroom species were not Hg-contaminated and, therefore, their consumption does not pose serious health risks regardless of the forest type. The results suggest that species-related soil chemistry and mineral associations, rather than different atmospheric Hg interception by spruce vs. beech, drive the vertical distribution and accumulation of Hg in forest soils.

Keywords: soil acidity; *Picea abies*; *Fagus sylvatica*; organic matter; pollution; *Xerocomellus chrysenteron*; health risk

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1. Introduction

Mercury (Hg) is a highly toxic nonessential metal, occurring in many species with different chemical properties [1–3], out of which the organic Hg compounds (e.g., dimethylmercury, methylmercury) are particularly toxic [4]. Mercury is toxic to all living organisms [5]. People receive Hg mainly through the food chain [6], which can lead to kidney dysfunction, insomnia, neurological problems, changes in nerve responses, heart problems, and impaired reproductive function [2,3]. This led to the increased attention of studies on Hg behavior in the environment since the 1980s [1].

Mercury is easily transportable in the atmosphere up to several hundred kilometres from its source, which makes it a global pollutant with the potential to contaminate even seemingly pristine areas [1,3,4]. Lamborg et al. [7] state that the anthropogenic deposition of Hg has increased by 3–5 times since the beginning of the Industrial Revolution compared to the preindustrial levels. Current global anthropogenic emissions of Hg into the atmosphere are estimated at around 2500 Mg/year [8]. The largest anthropogenic sources of Hg pollution in Europe include fossil fuel combustion and the non-ferrous metal industry [8]. The Hg compounds emitted into the atmosphere are transported by air currents and gradually return to the Earth's surface in the form of wet or dry deposition. More than 90% of this deposition enters terrestrial ecosystems, with soil being the largest recipient [1]. Vegetation plays an important role in Hg transfer from the atmosphere to the biosphere; Hg deposition in forests is typically 3–4 times higher compared to open areas due to canopy interception [9,10]. Mercury is deposited onto leaf surfaces or absorbed through stomata into the tissues, and subsequently transferred into the soil through litterfall or throughfall [9,11]. From the soil, thereafter, Hg is received by other environmental components and thus enters the wider food chain [2,5,6]. The majority of Hg enters terrestrial ecosystems in the form of inorganic compounds; however, soil organisms (sulfate-reducing bacteria and earthworms in particular) are able to produce organic Hg compounds, thereby increasing its toxicity [4].

Due to the strong potential of edible mushrooms to accumulate Hg [12,13], the consumption of their fruiting bodies can pose serious health risks. The process of metal absorption from the soil by the mushroom depends on many environmental variables, such as soil pH, soil organic matter (SOM) contents, and the particle-size distribution [4,8,14]. The major role of SOM is due to its strong potential to form complexes with Hg, which significantly affects Hg mobility [15]. Furthermore, Hg has a high ability to form strong bonds with the sulfur (S) in SOM [16] and to form insoluble HgS [15]. Bonding of Hg with clay particles [17] or with Fe and Al oxyhydroxides [18] are also considered important mechanisms of Hg stabilization in soils.

Forest trees can modify their habitat and physical environment, including soil [19–22]. Thus, the species composition of forests can both directly and indirectly affect the mobility of Hg in the soil profile and its distribution within the food chain. Recent studies suggest that the accumulation of Hg in soils and its soil profile transfer may significantly differ between coniferous and deciduous forests [21,23,24], which may be particularly important in the context of climate change and the expected species composition shift in European forests [25]. However, observational studies performed on larger scales (e.g., Gruba et al. [21]) may partly distort the relationships to some site-dependent covariates. As stated by Richardson et al. [24], further research on the effects of vegetation type on Hg contents in soil and its mobility in the environment is needed.

Norway spruce (*Picea abies* (L.) H. Karst.) and European beech (*Fagus sylvatica* L.) are two of the most abundant and economically important tree species in Central Europe [26]. These species, however, differ significantly in their influence on soil properties [19,27] and in their potential to intercept atmospheric pollutants, especially in the form of dry deposition [28]. Moreover, the highest differences in species interception can be expected in areas with increased air pollution [29]. Rothe et al. [28] found that throughfall depositions of nitrogen (N) and S compounds were about two-fold higher in spruce stands compared to beech stands. Nevertheless, the effect of beech and spruce on the Hg distribution in the soil in connection with its transfer to edible mushrooms is hitherto an understudied topic.

The aims of this study, situated in an area affected by Hg deposition, were as follows: (1) to compare the effects of beech vs. spruce on the vertical distribution of Hg in forest soils; (2) to assess the species impact on Hg bioavailability to the selected common and culinarily utilized species of edible mushrooms; and (3) to evaluate the levels of health risk represented by contaminated mushroom consumption. The hypotheses are as follows: (I) Higher forest-floor Hg contents will be observed in spruce-dominated compared to beech-dominated stands; and (II) The bioavailability of Hg will be higher in spruce-dominated stands, which will be reflected in the greater Hg contents in mushrooms.

2. Materials and Methods

2.1. Study Area

The study was carried out in the Jeseníky Mountains, the Czech Republic (Figure 1), in an area with contamination potential originating from atmospheric deposition, mostly from the Silesian industrial areas [30]. The studied area is located at an altitude of 587–990 m, with an average annual precipitation of 810 mm and an average annual temperature of 7.8 °C (considered for the period of 1989–2019; [31]). Forest stands in the area are typically dominated by Norway spruce and European beech, mostly planted in the form of even-aged monocultures.

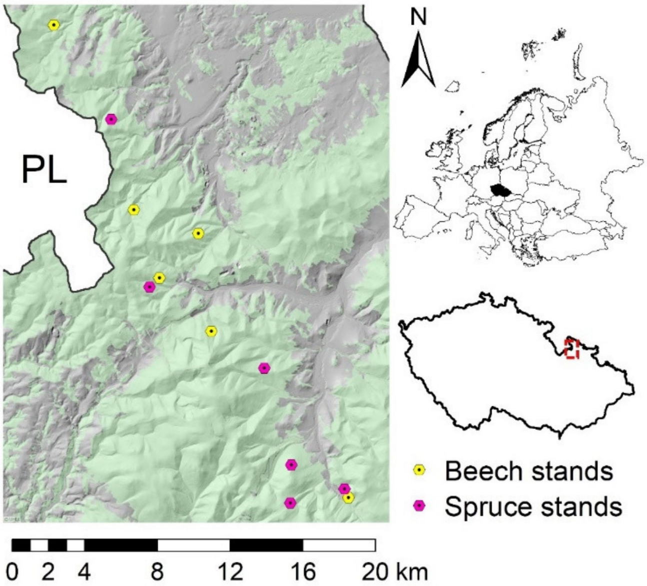

Figure 1. Study area with the sampling sites at the background layer derived from the regional forest development plans (forest areas are green); the digital model of the Czech Republic was used for shading; PL = Poland.

2.2. Soil and Mushroom Sampling

Six spruce- (*Picea abies* (L.) H. Karst.) and six beech- (*Fagus sylvatica* L.) dominated stands (representation >80%), each of the area >1 ha and age 80–100 years were selected for soil and mushroom sampling (Figure 1). The study sites were selected to have a comparable geology, with the bedrock consisting of acid metamorphic rocks (mostly phyllites and gneiss). Based on the field survey, any sites located near a potential source of pollution (e.g., frequented roads, mines, or built-up areas) were excluded from the selection, as well as forest stands with significant signs of surface soil erosion. Each forest stand (site) was interspersed with an internal hexagonal grid of 7 sampling plots laid out in 50-m distances to the plot center with the minimal 25-m buffer zone to the forest stand edge. At each plot, composite samples from the F+H organic layer and soil mineral layers at the depths of 0–2, 2–10, 10–20, and 20–30 cm were collected from three soil pits spaced 4–8 m from each other, so the sampling was never done twice under the canopy projection of a single tree individual. A total of 420 composite samples were collected into polyethylene bags, each containing about 0.5–1.0 kg of fresh sample. Mixed mushroom samples of the *Boletaceae* family were collected at each site for the determination of bioavailability and bioaccumulation of Hg, depending on the current abundance of species at individual sites. The fruiting mushroom bodies were sorted into paper bags as a mixed sample for each mushroom species. All mushroom samples were collected only under the assessed tree species, to minimize the bias due to other tree species present at the site.

2.3. Sample Preparation and Analysis

Substrate samples were dried at room temperature, homogenized, and sieved through a 2 mm mesh for further analysis. For carbon (C), N and S content determination, the samples were pulverized using an agate mill.

pH was determined in mineral and organic soil-water suspension (1:5 ratio). The total C, N, and S contents were determined with the VarioMAX CNS elemental analyzer (Elementar Analysensysteme GmbH, Germany). The elemental C, N, and S analysis was performed by dry sample combustion in the presence of an oxidation catalyst (WO_3) in oxygenated airflow at the temperature of 1140 °C, where the resulting mixture of gaseous products is reduced by Cu to N_2 and SO_2 and oxidized by CuO to CO_2. The measured components (N_2, CO_2, SO_2) were separated by adsorption columns and sequentially quantified by a thermal conductivity detector (TCD).

In mineral soil samples, soil texture determination of the main grain fractions was carried out for soil particles <2 mm according to the USDA standards by dry sieving and pipette analysis [32]. The contents of oxalate-extracted Al (Al_o) and Fe (Fe_o) were determined after 0.2M ammonium oxalate (w/v ratio 0.25:10) extraction at pH 3 [33]; the concentrations of the elements in liquid extracts were measured using an atomic absorption spectrophotometer (GBS SavantAA).

Fruiting bodies of the collected mushrooms were manually cleaned of coarse debris (parts of plants, forest floor, or mineral particles) with a ceramic knife to eliminate surface contamination. The samples were sliced and dried at room temperature for several weeks in a dust-free environment. After drying, the mushroom samples were homogenized by grinding to powder in a stainless steel mill for subsequent Hg content analysis.

A single-purpose atomic absorption spectrophotometer AMA-254 (Advanced Mercury Analyzer, Altec, Czech Republic) was used for direct determination of total Hg content without sample pretreatment or pre-concentration [34]. The soil and mushroom samples were weighed onto a combustion boat, which was inserted into a combustion chamber. Each sample was dried at 120 °C for 60 s and then burned in an oxygenated atmosphere at 650 °C for 150 s. Each combustion cycle followed a 45 s system cleaning process. All samples were measured in three replicates with a relative standard deviation smaller than 10% and with a 0.004 mg/kg detection limit. Metranal 1 reference material (Analytika, Czech Republic) was used to verify the accuracy of the measurements.

2.4. Mercury Accumulation in Mushrooms and Health Risk Assessment

Bioconcentration factor (BCF) was used to assess the potential of spruce and beech to influence the transfer of Hg to edible mushrooms. BCF evaluates the relationship between mushroom and soil metal content [35], representing a simple method to quantitatively characterize the transfer of available Hg from the soil to the mushroom (accumulation ability). BCF was calculated as the mushroom-to-soil Hg content ratio. Mushrooms with a BCF value <1 belong to Hg excluders and with the BCF value >1 to Hg accumulators [12,13]. BCF was calculated for the F+H and 0–2 cm mineral soil layers, from which mushrooms primarily take up nutrients [36,37].

The health risk index (HRI) was calculated to assess the potential health risk of mushroom consumption. The index is expressed as the ratio of daily Hg intake (DI) from edible mushrooms to the oral reference dose (RfDo) [38], where HRI values >1 represent a potential health risk; RfDo value used for Hg was 0.0003 mg/kg/day [39,40]. DI was calculated using the following Equation (1) [38]:

$$\mathrm{DI} = \frac{\mathrm{C}_{mushroom} \times \mathrm{D}_{IM}}{\mathrm{BW}} \quad (1)$$

where $C_{mushroom}$ represents mushroom Hg content (mg/kg, based on dry weight), DIM represents the daily intake of mushrooms (0.03 kg, [38,41]), and BW represents an average bodyweight of the consumer (74.6, weight average of men and women in the Czech Republic [42]).

2.5. Spatial and Statistical Analysis

Map documentation was created using ArcGis 10.4; the basemap layer consisted of the 5th generation digital terrain model of the Czech Republic (Grayscale Hillshade; [43]), overlapped by the map layer of the Regional Plans of Forest Development [44].

Statistical analyses were carried out in the R software, version 4.0.5 [45]. Linear mixed-effects models (LMEM) fitted by residual maximum likelihood were used for the analysis of dependent soil data [46], whereby the site factors were always used as random effects to control for pseudoreplication, while the forest type, sampling depth, or the soil covariables (depending on the analysis) were typically used as fixed effects. Fixed effects were tested for significance by default using the analysis of variance (ANOVA) with the Satterthwaite's method in the "lme4" package [46], as well as by the likelihood ratio test (based on the comparison to the reduced model, after refitting by maximum likelihood). Competing fixed effects were compared by the F-value statistic to select among the best models. The distributions of model residuals were evaluated using quantile–quantile plots. Marginal and conditional R^2 were calculated for the selected model as the measures of variance explained by the fixed effects and by the whole model, respectively. For comparability of regression coefficients, soil data were centered (mean = 0) and standardized (standard deviation = 1) prior to analysis, so the models had a null intercept. In LMEM applied for the summary statistics, however, unscaled data were used instead.

3. Results

3.1. Effect of Soil Properties on Soil Profile Hg Distribution

The distribution of Hg in the soil profile was regulated by different soil properties (Tables 1 and 2) in individual soil layers. While pH was the main covariate of Hg content in the F+H layer, explaining more than 68% of the variability, strong positive associations with S, N, and C prevailed in the mineral soil (Table 2). Significant relationships between the Hg and C, N, and S contents, however, were observed in all soil layers (Table 1). In the mineral soil at the depths of 2–10, 10–20, and 20–30 cm, Hg contents were also positively correlated with the silt, Al_o, and Fe_o, while negatively correlated with the sand contents. In contrast, C:N and clay contents showed no significant relationship with the Hg contents in any of the studied soil layers.

Table 1. Fixed effect coefficients in mixed-effects models of Hg contents at different soil depths, significant at $p < 0.001$ based on ANOVA using Satterthwaite's method; n.s., no significant effect; NA, not applicable. The site factor was used as random effect in the model.

Layer/Depth (cm)	C	N	S	C:N	pH	Sand	Silt	Clay	Al_o	Fe_o
F+H	0.240	0.243	0.327	n.s.	−0.801	NA	NA	NA	NA	NA
0–2	0.574	0.643	0.618	n.s.	−0.392	n.s.	n.s.	n.s.	n.s.	n.s.
2–10	0.706	0.833	0.649	n.s.	n.s.	−0.512	0.516	n.s.	0.616	0.561
10–20	0.785	0.705	0.614	n.s.	n.s.	−0.289	0.362	n.s.	0.625	0.644
20–30	0.762	0.670	0.715	n.s.	−0.294	−0.200	0.238	n.s.	0.671	0.487

3.2. Tree-Species Effects on Hg Distribution in the Soil Profile

Significant differences were found in soil Hg contents between the spruce- and beech-dominated stands only in the organic F+H layer (Figure 2 and Table A1). In beech-dominated stands, the mean Hg contents in the F+H layer were 0.29 ± 0.10 mg/kg, while in spruce stands, they were 0.46 ± 0.03 mg/kg (Table A1), with the tree species factor explaining more than 50% of the variability (data not shown). In contrast, no significant differences between tree species were found in the mineral soil layers (Figure 2).

Table 2. The best mixed effects models of Hg contents with selected independent soil variables (covariates) as fixed effects; all fixed effects were significant at $p < 0.001$ based on the likelihood-ratio test. Random effects were represented by the factor site. In the "full model", the fixed effects were represented by soil covariate; in the "reduced model", the fixed effects were replaced by a constant. For each soil layer, fixed effect coefficients, the variance corresponding to fixed and random effects, and the proportion of variance explained by fixed effects (marginal R^2) and by the whole model (conditional R^2) are provided.

Layer/Depth	**Full Model**								**Reduced Model**		
	Covariate	**Coefficient**		**Variance**			**R^2**		**Variance**		**R^2**
(cm)	**(Name)**	**Estimate**	**SE**	**Fixed**	**Random**	**Residual**	**Marginal**	**Conditional**	**Random**	**Residual**	**Conditional**
F+H	pH	−0.801	0.075	0.641	0.041	0.258	0.682	0.726	0.695	0.293	0.704
0–2	S	0.618	0.075	0.382	0.172	0.338	0428	0.621	0.405	0.583	0.410
2–10	N	0.833	0.074	0.694	0.117	0.266	0.645	0.753	0.321	0.664	0.326
10–20	C	0.785	0.066	0.616	0.027	0.289	0.661	0.690	0.467	0.521	0.473
20–30	C	0.762	0.068	0.581	0.063	0.224	0.669	0.742	0.596	0.393	0.603

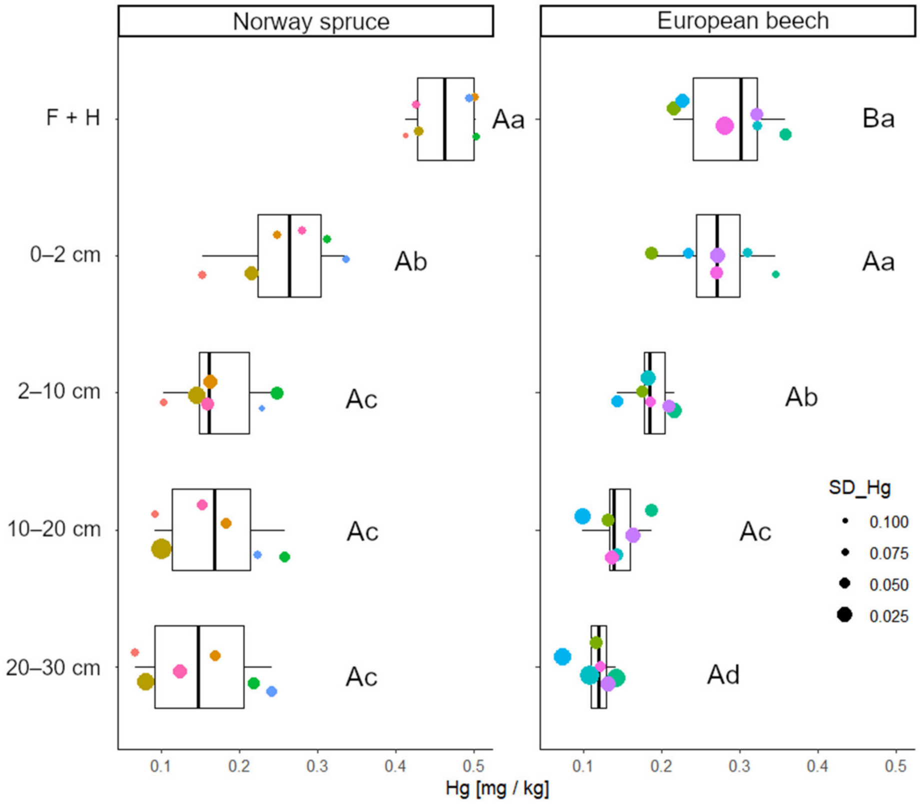

Figure 2. Vertical distribution of mercury (Hg) in forest soils in relation to the dominant tree species; different uppercase letters indicate significant differences between forest types (likelihood ratio test); different lowercase letters indicate significant differences between sampling depths within a particular forest type (Tukey's test); boxes represent quartiles; points represent mean values at a particular site (differentiated by colors); the within-site variation is indicated by standard deviation (SD) that is inversely related to point size.

Significant decreases in the Hg contents along the gradient of sampling depth have been observed in both the spruce-and beech-dominated stands (Figure 2), however, the trajectory of this effect differed between the species. In spruce-dominated stands, the highest Hg accumulation was observed in the F+H layer and its contents decreased rapidly towards the 2–10-cm depth. Below the 2–10-cm depth, the Hg contents did not change significantly, mainly due to considerable variability among individual stands. In beech-dominated stands, the most Hg-enriched layers were F+H and 0–2-cm depth, while the mineral soil layers showed a significant vertical decrease in the Hg contents down to a depth of 20–30 cm.

3.3. Effect of Tree Species on the Bioaccumulation of Hg in Mushrooms and the Related Health Risks

The mean Hg contents for individual mushroom species ranged between 0.056 and 2.50 mg/kg in the spruce-dominated stands and from 0.18 to 0.53 mg/kg in the beech-dominated stands, with no detectable differences among the forest types or mushroom species, mostly due to the low number of samples for most mushroom species (Table 3). The only mushroom species found in all forest stands was *X. chrysenteron*, whose bioaccumulation index showed a higher mean value for the F+H layer in beech- (BCF = 1.31), compared to spruce-dominated stands (BCF = 0.78) (Table 3). However, neither the BCF values for the 0–2-cm depth, nor the HRI values differed significantly between the forest types. Overall, the HRI values indicate a predominant absence of Hg-posed health risks (Table 3) even at long-term consumption of collected mushroom species; the limit value was exceeded in one sample of *I. badia* only.

Table 3. Mushroom Hg content (mg/kg), bioconcentration factor (BCF), and Health Risk Index (HRI) in relation to tree species; different lowercase letters indicate significant differences between the forest types at $p < 0.05$ (ANOVA).

Mushroom Species		**Norway Spruce**				**European Beech**			
		Hg	**BCF**		**HRI**	**Hg**	**BCF**		**HRI**
			F+H	**0–2 cm**			**F+H**	**0–2 cm**	
Xerocomellus chrysenteron (Bull.) Šutara	Average	0.36a	0.78b	1.47a	0.47a	0.35a	1.31a	1.41a	0.46a
(*n* = 6 + 6)	S.D.	0.07	0.17	0.52	0.09	0.06	0.40	0.48	0.07
Imleria badia (Fr.) Vizzini	Average	0.54	1.12	1.54	0.71	0.29	0.97	1.03	0.38
(*n* = 6 + 4)	S.D.	0.88	1.76	2.22	1.15	0.14	0.45	0.54	0.18
Xerocomellus pruinatus (Fr. & Hök) Šutara	Average	0.38	0.79	1.68	0.50	0.35	1.39	1.44	0.46
(*n* = 3 + 2)	S.D.	0.08	0.20	0.78	0.11	0.02	0.12	0.05	0.02

4. Discussion

4.1. Effect of Soil Properties on Soil Profile Hg Distribution

Given the literature, it could be assumed that the main mechanism of Hg retention in the F+H layer is the binding of Hg with S and SOM [15]. However, the decisive significance of these properties in the F+H layer was not evidenced, just as it was not significant for the differentiation between beech and spruce stands in Gruba et al. [21]. In contrast, the main variable affecting Hg contents in the F+H layer was pH (Table 2), which was not considered at all in the above-mentioned study. Given that soil pH is significantly affected by the tree species identity [19], which is also confirmed by this study (Table A1), this result can be largely attributed to the effect of tree species, as revealed by the mixed model (see Section 3.2).

The strongly acidic reaction of the organic F+H layer under spruce (Table A1) with pH of around three may be associated with the low dissolution of Hg [14]. In such conditions, possible co-precipitation of Hg and SOM occurs due to their strong affinity [47]. Mercury particularly well binds with humic acids [5,17,48,49]. Humic acids are insoluble under strongly acidic conditions [14,49], which significantly reduces their precipitation-driven leaching from the F+H layer, which would result in decreased Hg mobility [48]. In contrast, fulvic acid-bound Hg is more mobile [17], which could be the main mechanism of Hg migration to deeper layers in the studied soils. However, fulvic acids of upland forest soils contain only small amounts of Hg [49]. Moreover, Hg-fulvic acid association can enhance Hg adsorption on minerals, particularly at low pH [50]. Weaker association of C, N, and S to Hg contents in the F+H layer compared to the mineral horizons may also indicate the importance of another process that causes Hg retention but is not directly related to organic matter, such as Hg adsorption on hydrous manganese oxides [51,52]. This assumption is supported by the fact that spruce litter tends to be Mn-richer compared to most other tree species [20], including beech [27]. Clarifying this hypothesis will, however, require further research.

It can be expected that at least one of the above-mentioned processes does initiate the increased Hg retention and accumulation in the spruce F+H layer, which indirectly leads to limited Hg migration into deeper soil layers. Mercury immobilization in the spruce F+H layer and thus the limitation of its bioavailability was also indicated by lower BFC values for *X. chrysenteron* in spruce- as compared to beech-dominated stands (Table 3). Mercury retention in the organic F+H layer in spruce-dominated stands may also be one of the reasons for the distinct vertical distribution of Hg in the soil profile (Figure 2). While only a limited amount of Hg can migrate from the spruce F+H layer to different mineral soil depths, in beech stands, the vertical distribution of Hg is more gradual, probably due to less pronounced Hg binding to Al complexes, while a stronger association to soil texture in the deeper mineral soil layers (Supplementary Tables S1 and S2).

The results further suggest that the principal process controlling the distribution of Hg in the mineral layers (0–30 cm depth) were the Hg-SOM and Hg-S associations (Tables 1 and 2). Strong binding of Hg with SOM and S has already been documented [5,15,16,53]. Furthermore, although a continuing litter-related negative association of Hg to soil pH can still be observed in the 0–2 cm layer, at deeper soil layers, the associations to soil minerals (sand and silt fractions, Al_o and Fe_o contents) are significant (Table 1), which may signal a stronger control of mineral bonding and pedogenic processes such as podsolization. Richardson et al. [24] draw similar conclusions, stating that Hg accumulation in mineral soils is primarily controlled by soil properties. However, the somewhat weak subsoil Hg accumulation indicated that the vertical Hg mobility associated with dissolved organic matter and its stabilization by Al_o and Fe_o (as described by Gómez-Armesto et al. [18]) occurs only to a limited extent. The association with soil texture suggests that Hg contents decreased with increasing particle size, which is in accordance with early observations [14,21].

4.2. Effect of Tree Species on the Soil Profile Hg Distribution

The results confirm the first hypothesis that spruce has a higher potential for Hg accumulation in the organic F+H layer (Figure 2). Richardson et al. [24] also observed higher Hg accumulation in organic horizons under coniferous stands. Ballabio et al. [8] considered coniferous stands larger Hg pools than deciduous stands. In contrast, Gruba et al. [21] found no significant difference between the soils underneath beech and spruce. This inconsistency can be attributed to the large geographical extent of the study and the potential interference with other environmental factors, which could blur their results.

Spruce has a higher potential than beech to intercept atmospheric pollutants [28] and, for example, Ballabio et al. [8] expect the deposition fluxes to be the main cause for higher Hg contents underneath the coniferous stands. This may be mainly associated with the finding that throughfall dominates Hg fluxes into the coniferous forest soil, while the litterfall is the main Hg flux to the soil in deciduous forests [23]. This study's results, however, suggest that it was rather the effect of tree species on soil pH than the differences between tree species in the potential to intercept the pollutants that caused higher Hg contents in the F+H layer of spruce stands. At the same time, although, it is necessary to consider the potentially higher acid deposition loads in spruce stands that contribute to soil acidification. These factors can therefore overlap and are difficult to separate.

The absence of between-species differences in the Hg content at a mineral soil depth of 0–30 cm, however, suggests that mineral soil Hg inputs are comparable despite the higher Hg immobilization in the spruce F+H layer on one side, and higher atmospheric Hg interception in coniferous stands on the other [8,24,28]. This supports the assumption that tree species only affect forest floor Hg retention, and that mineral soil comprises an equal Hg sink regardless of the tree species [21] in managed forests. Nevertheless, both pH and C:N were significantly differentiated throughout the soil profile of the forest types (Table A1), including Hg relations with soil properties (Supplementary Tables S1 and S2), which demonstrates the species effect on soil chemistry down to the 30-cm depth.

4.3. Effect of Tree Species on the Hg Transfer to Mushrooms and Related Health Risks

The species-specific Hg accumulation in the studied mushrooms (Table 3) was not confirmed in contrast to other studies [13,37,41]. The results, however, indicate potential tree species effect on Hg bioaccumulation in mushrooms. Although the differences in the Hg content in mushrooms were negligible between the beech- and spruce-dominated stands, the BCF suggested a more efficient Hg accumulation from the F+H layer in the beech-dominated stands or rather limited Hg uptake from the F+H layer in the spruce-dominated stands. Thus, hypothesis II was not confirmed, most probably due to Hg immobilization in the organic F+H layer in the spruce-dominated stands.

Observed Hg contents in mushrooms (Table 3) showed low levels compared to the contamination limit (<0.5–5 mg/kg) [41], which indicates low Hg contamination in the study area. Similarly, the potential health risk assessment associated with mushroom consumption was considered negligible (Table 3), since only one sample of *I. badia* (3.27) could pose a potential risk. Potential factors to have caused this single extreme value are the age of the mushroom or the influence of microhabitat [35]. However, tree species effect implications on Hg bioaccumulation and the associated health risks posed by mushroom consumption with Hg require further research due to the limited sample size and the low number of studied species.

5. Conclusions

Although the characteristics related to SOM content had a significant influence on Hg contents in all studied soil layers, the results showed a closer relationship with pH, particularly in the F+H layer, which is often not considered at all in the published studies. With increasing soil depth, the typical associations of Hg contents with S, N, and C were also supplemented with a positive association to the silt, Al_o, and Fe_o contents, which is consistent with similarly focused studies.

The highest Hg contents were observed in the F+H layer. Significantly higher Hg contents in the F+H layer in spruce stands were most likely due to the strongly acidic pH, which probably led to Hg precipitation followed by retention and accumulation. Hg immobilization was also reflected in reduced bioavailability for *X. chrysenteron* uptake. The results of this study suggest that rather than different spruce and beech potential to intercept Hg deposition, it was their effect on soil chemistry that is important for Hg distribution in the soil. However, potentially higher acid deposition loading in spruce stands, caused by superior coniferous tree capacity to intercept pollution, can also lead to more intensive soil acidification. These factors can therefore overlap and are difficult to separate.

No significant differences in the Hg content between spruce- and beech-dominated stands were found in mineral soil layers (0–30 cm depth). However, there are significant differences in pH and C:N between the forest types throughout the entire soil profile, which demonstrates the species effect on soil chemistry down to the 30-cm depth. The secondary effects on pedogenic processes are probably the reason behind the different Hg distribution patterns in the mineral soil. The tree species effects on the pedogenetic processes associated with the Hg translocation (mobility) within the soil should be given more attention in the future.

Studied mushroom species were not Hg-contaminated and do not pose serious health risks by Hg intoxication to their consumers. BCF results indicate a more efficient Hg accumulation in beech stands, or rather limited accumulation of Hg in spruce stands. Potentially different Hg accumulation efficiency in mushrooms in relation to tree species and soil contents highlights the need for further research.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/f12050539/s1, Table S1: Coefficients of the fixed effects in mixed-effects models of Hg contents in different soil depths, calculated separately for the Norway spruce stands; the parameters are significant at $p < 0.05$ in ANOVA using the Satterthwaite's method; n.s., no significant effect; NA, not applicable. Table S2: Coefficients of the fixed effects in mixed-effects models of Hg contents in different soil depths, separately for European beech stands; the parameters are significant at $p < 0.05$ in ANOVA using the Satterthwaite's method; n.s., no significant effect; NA, not applicable.

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Appendix A

Table A1. Soil characteristics at different sampling depths depending on the tree species; mean estimates of the mixed model (lower and upper limits of the mean 95% confidence interval), with forest type and site as fixed and random effects, respectively; were not significant, the fixed effects were replaced by a constant (without considering the forest type); NS = Norway spruce; EB = European beech; n.a. not applicable.

Variable	Species	F+H				0–2 cm				2–10 cm				10–20 cm				20–30 cm			
		Lower	Mean	Upper	a	Lower	Mean	Upper	a	Lower	Mean	Upper	a	Lower	Mean	Upper	a	Lower	Mean	Upper	a
Hg (mg/kg)	NS	0.427	0.461	0.496	***	0.230	0.264	0.298		0.157	0.180	0.204		0.127	0.156	0.185		0.102	0.133	0.164	
	EB	0.188	0.287	0.387																	
C (g/kg)	NS	357	376	396	**	109	120	131		57.3	64.9	72.5		39.2	47.9	56.6		30.1	39.8	49.6	
	EB	266	316	366																	
N (g/kg)	NS	16.3	16.9	17.6		5.16	6.12	7.09	*	3.38	3.90	4.43		2.32	2.82	3.31		1.73	2.26	2.78	
	EB					5.30	7.67	10.0													
S (g/kg)	NS	2.05	2.17	2.28	**	0.624	0.692	0.760		0.417	0.486	0.555		0.291	0.362	0.432		0.240	0.306	0.372	
	EB	1.43	1.78	2.13																	
C:N	NS	20.9	21.7	22.5	***	17.9	19.3	20.7	**	16.4	19.0	21.5	*	16.6	19.6	22.6	*	17.6	20.7	23.9	*
	EB	16.9	19.0	21.1		12.8	16.0	19.1		9.4	15.1	20.8		8.5	15.2	21.9		8.3	15.3	22.4	
pH	NS	3.64	3.77	3.90	***	3.76	3.89	4.01	*	4.03	4.11	4.20	**	4.28	4.39	4.51	**	4.49	4.59	4.70	*
	EB	4.06	4.51	4.95		3.83	4.23	4.62		4.16	4.46	4.76		4.39	4.72	5.04		4.58	4.90	5.22	
Sand (%)	NS	n.a.	n.a.	n.a.		34.6	38.7	42.8		36.2	40.8	45.5		37.8	42.6	47.4		41.1	45.2	49.4	
	EB																				
Silt (%)	NS	n.a.	n.a.	n.a.		50.1	54.2	58.3		48.1	53.2	58.2		46.8	52.0	57.2		45.3	49.7	54.2	
	EB																				
Clay (%)	NS	n.a.	n.a.	n.a.		6.23	7.09	7.95		5.16	6.05	6.93		4.38	5.40	6.42		4.00	5.02	6.03	
	EB																				
Al$_{O}$ (g/kg)	NS	n.a.	n.a.	n.a.		1.67	2.60	3.53	**	2.22	3.81	5.40	·	4.86	6.57	8.28		5.56	7.67	9.78	
	EB					2.36	4.79	7.21		2.00	5.97	9.94									
Fe$_{O}$ (g/kg)	NS	n.a.	n.a.	n.a.		5.11	6.42	7.74		5.80	7.90	10.01		5.61	7.87	10.14		4.84	6.79	8.75	
	EB																				

[a] Significance levels for the fixed effects: '***' $p < 0.001$; '**' $p < 0.01$; '*' $p < 0.05$, '·' < 0.1.

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