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Toxic elements and energy accumulation in topsoil and plants of spruce ecosystems

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ABSTRACT

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The objective of this research was to evaluate trends and relationships of energy and toxic elements accumulation in A-horizon (the depth of 0–5 cm) of soils and in selected plants of the hemioligotrophic (Dystric Cambisols) and oligotrophic (Skeletic-Rustic Podzols) spruce ecosystems situated along transect (750–1110 m a.s.l.) in the NP Slovenský raj (Eastern Slovakia). The results showed that EU limit values of risk elements in agricultural soils were exceeded for Cu and Cd at the altitude of 750–760 m a.s.l., and in case of Cd also above 1000 m a.s.l. Relationship between energy and toxic elements in soils revealed that with an increasing amount of energy, contents of Zn and Cu significantly declined with altitude ($r > -0.5$ or $r > -0.9$). The background value of Cu was exceeded in all plants, that of Zn for *Dryopteris filix-mas* and *Rubus idaeus*. Furthermore, excessive accumulation of Cd was revealed by all plants. Cu contents in soils were dominant in determining Cu uptake for *Vaccinium myrtillus* ($r > 0.5$); Zn and Cd for *V. myrtillus* ($r > 0.6$), *D. filix-mas* ($r > 0.5$ or $r > 0.8$) and *Fagus sylvatica* ($r > -0.8$ or $r > -0.5$); Zn also for *R. idaeus* species ($r > 0.4$). The soil-plant transfer coefficients higher than 1 hinted *R. idaeus* on the plots at the altitude of 960 m a.s.l. (Cd 1.1, Cu 1.2, Zn 3.1), which appears as an excellent native indicator of forest ecosystem contamination.

Keywords: forest stands; phytomass; toxicity; microelement; calorific value

Plants in the countryside are confronted with numerous stress factors due to their ability to accumulate chemical elements and energy depending on the environmental conditions (Parzych and Jonczak 2014, Yu et al. 2016). Stress factors through their influence on the amount and quality of forest litter-fall and formation of surface humus may affect nutrient cycling and nutritional status of forest ecosystems (Merino et al. 2008). In the plants growing under conditions of abiotic stressors, the content of energy-rich substances in biomass of all plant organs usually decreases compared to non-stressed plants (Hnilička et al. 2015). Also environmental factors are changed with varying altitudes and they can reflect the relationship between plant production and environment (Zhong et al. 2014). It is, therefore, necessary in all kind of research to take into account the influence of altitude.

This work is focused primarily on Cd, Cu and Zn, which belong to the elements with a high potential threat. The Spiš region (Eastern Slovakia) has been subjected to a long-term negative influence of mining, metallurgy, engineering and woodworking industry. Source of soil contamination

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by toxic elements (Cu, As, Zn, Cd, Hg) is mainly waste from the production of iron and copper ores, metal scrap. Some previous studies from this area indicated impact of soil contamination with mercury (Kuklová et al. 2010) and exceeding of limit nickel value in the surface humus horizons (Kuklová et al. 2016).

Zn toxicity in crops is less widespread than Zn deficiency (Broadley et al. 2007). In general, plant growth and quality are reduced owing to Zn inadequacy in soil. Where zinc is deficient, the physiological functions are impaired and the productivity of plants is adversely affected (Hänsch and Mendel 2009). Mousavi (2011) notes, that Zn is playing principal metabolical role in plants and is effective in energy production and Krebs cycle. The risk elements also include biologically irreplaceable microelement Cu and a non-essential Cd. Risk of these elements is in ecotoxicity and accumulation in biotic and abiotic components of the environment. Also accumulation of energy in soils and plants is associated with changes in actual amount of nutrient and risk elements in the environment and plant organs.

Therefore, the main objectives of this paper were: (1) to determine the concentration levels of potentially toxic elements (Cd, Cu, Zn) in topsoil and assimilatory organs of plants (*Fagus sylvatica*, *Dryopteris filix-mas*, *Rubus idaeus*, *Vaccinium myrtillus*) (2) to evaluate soil-plant translocation of these metals (3) to specify the trends in accumulation of energy in soils and selected plants in relation to toxic elements, taking advantage of the elevation differences of spruce monocultures.

MATERIAL AND METHODS

Study plots. The research was carried out in segments of secondary spruce ecosystems situated along altitudinal transect in National Park Slovenský Raj, Eastern Slovakia, in the years 2012–2013. Vertical transect consisted of 6 plots situated in the following altitudes: 750 m a.s.l. (20°30'43''E; 48°53'18''N); 760 m a.s.l. (20°30'42''E; 48°53'17''N); 950 m a.s.l. (20°32'07''E; 48°51'51''N); 960 m a.s.l. (20°32'12''E; 48°51'49''N); 1000 m a.s.l. (20°28'40''E; 48°52'55''N); 1110 m a.s.l. (20°28'37''E; 48°52'55''N) with considerably different stand canopy and small amount of naturally regenerated beech (5%) and fir (5%) in undergrowth. Dystric Cambisols (humus form – typical moder) were formed from grey schist (750–760 m a.s.l.) and quartz conglomerates (950–960 m a.s.l.); Skeletic-Rustic Podzols (humus form – mor) from violet-grey schist (1000 m a.s.l.) and polymict conglomerates (1110 m a.s.l.). More detailed characteristics of the sampling plots are presented in the work by Hniličková et al. (2016).

Soil analyses. In July 2013, soil samples were taken on each of six plots from the same 0–5 cm layer of organo-mineral A-horizons in triplicate. The air-dried samples were broken and fine-earth was passed through a sieve with a mesh size of 2 × 2 mm. Active soil reaction was determined by a digital pH-meter Inolab pH 720 (Weilheim, Germany), (ratio of fine earth to water 1:2.5), the content of C_{tot} and N_{tot} by NCS analyser FlashEA 1112 (Hanau, Germany). The energy value (J/g) of organo-mineral soil layers was determined using an adiabatic calorimeter IKA C-4000 (software C-402, DIN 51900, Heitersheim, Germany). Sample of weight 0.7 g to 1 g was put into the combustion bag of known caloric value (46 367 J/g) designed for combustion of samples with low specific weight. The combustion heat of the sample was calculated using the sample weight converted to dry matter. Total contents of Cd, Cu and Zn in soils were determined in *aqua regia* extract (HNO_3 + HCl 1:3) by the AAS-FAAS method using the instrument Thermo iCE 3000 (Loughborough, UK).

Plant species analyses. The samples of *Fagus sylvatica* L. leaves (from the bottom third of the tree crown), *Dryopteris filix-mas* (L.) Schott (leaves), *Vaccinium myrtillus* L. (green twigs) and *Rubus idaeus* L. species (shoots growing from creeping roots) consisting of 50 to 60 leaves/shoots were obtained by stratified random sampling on an area of 400 m^2 of each of the six studied plots in triplicate. The stand canopy varied from 40–60% to 60–100%; however, the plant samples were taken from patches with more or less similar canopy of 50–70%.

The energy value (J/g) of plant material was determined using an adiabatic calorimeter IKA C-4000 (software C-402, DIN 51900, Heitersheim, Germany). The samples weighing 0.7–1 g were pressed into a form of briquette, dried up to a constant weight at 105°C and burnt in pure oxygen under a pressure of 3.04 MPa. Total contents of Cd and Zn in plants were determined in extracts of concentrated HNO_3 by method of AAS-GTA, in case of Cu by the method of AAS-F, using a Thermo iCE 3000 (Loughborough, UK) instrument. The

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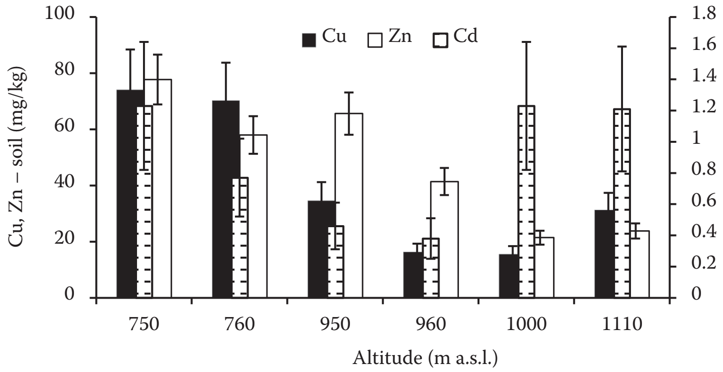

Figure 1. Content of Cu, Zn and Cd in 0–5 cm layer of organo-mineral A-horizons along altitudinal transect of forest ecosystems (arithmetic mean ± standard deviation)

total contents of toxic elements in plant shoots were determined after microwave mineralization of non-washed plant samples, because the samples were taken from relatively non-dusty areas.

Data analysis. Data were analysed with the use of a programme Statistica 9 (Tulsa, USA). The variability of measured characteristics between monitoring plots was tested by the Bartlett's test, one-way ANOVA and post-hoc Fisher's *LSD* (least significant difference) test. The Pearson's correlation coefficients were used to check the relationships between energy and element concentrations in the soils and plants. In order to determine the pollution levels of the potential trophic chains the soil-plant transfer coefficients (TC) were used. TC was calculated as a ratio of element concentration in plant species to the concentration of the same element in the soil.

RESULTS AND DISCUSSION

Content of heavy metals in soils and plants. The mean Cu contents in topsoil decreased up to the altitude of 1000 m a.s.l. and upward they slightly increased (Figure 1). The lowering of Cu contents with altitude can be apparently associated with an extreme soil acidification (Figure 2), since the lowering of the pH value of the soil is associated with an increase of the mobility of copper ions (Reddy et al. 1995). The upper limit value (70 mg/kg) of Cu in agricultural soil stated in the Act No. 220/2004 was slightly exceeded on less acidified plots (at the altitude of 750–760 m a.s.l.).

Absolutely highest Cu contents in plants were observed in *D. filix-mas* and *R. idaeus* at altitudes of 760 m a.s.l. and 960 m a.s.l. and they were significantly different from Cu contents found on other plots (Table 1). On the other hand, in case of beech leaves, the highest Cu content (15.3 mg/kg) was not significantly different from others. Similarly insignificant were also differences between Cu contents found in *V. myrtillus*. The background value of Cu in plants (10 mg/kg) stated by Markert (1995) was exceeded for *R. idaeus* and *F. sylvatica* species on all plots. In *D. filix-mas*, limit value was exceeded at the altitude of 760 m a.s.l. and for *V. myrtillus* at 1110 m a.s.l. The limit value of Cu in plants (> 12 mg/kg) referred by Hrdlička

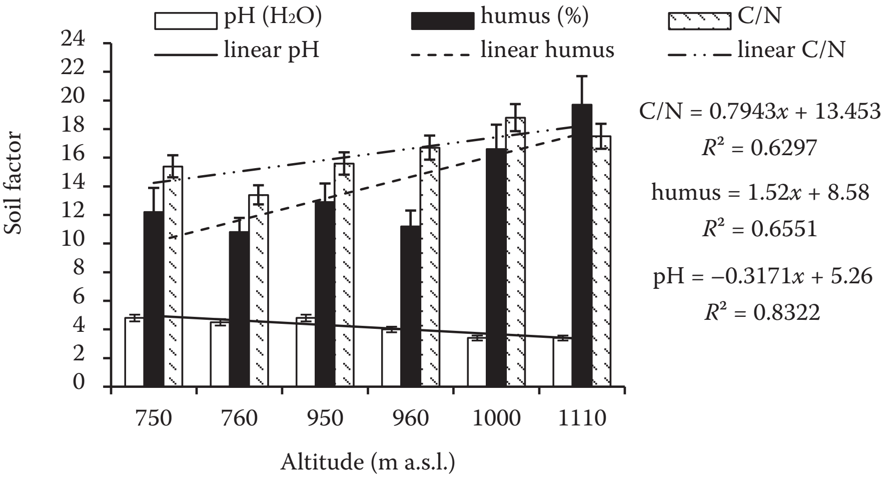

Figure 2. Values of ecological factors along altitudinal transect of forest ecosystems (arithmetic mean ± standard deviation). The correlations are significant at the 0.05 level

Table 1. Contents of toxic elements (mg/kg) in aboveground parts of plant species along altitudinal transect of forest ecosystems (arithmetic mean ± standard deviation, n = 3, nr – no record)

Altitude (m a.s.l.)	*Fagus sylvatica*			*Dryopteris filix-mas*			*Rubus idaeus*			*Vaccinium myrtillus*		
	Cu	Zn	Cd	Cu	Zn	Cd	Cu	Zn	Cd	Cu	Zn	Cd
750	14.6 ± 5.1^{A}	36.2 ± 5.1^{A}	0.27 ± 0.09^{A}	7.9 ± 2.8^{A}	34.8 ± 4.9^{A}	0.08 ± 0.03^{A}	11.1 ± 3.9^{A}	40.6 ± 5.7^{A}	0.55 ± 0.18^{A}	nr	nr	nr
760	14.6 ± 5.1^{A}	30.1 ± 4.2^{B}	0.36 ± 0.12^{B}	19.1 ± 6.7^{B}	48.2 ± 6.8^{B}	0.18 ± 0.06^{A}	12.1 ± 4.2^{A}	42.0 ± 5.9^{A}	0.67 ± 0.22^{A}	nr	nr	nr
950	10.3 ± 3.6^{A}	31.3 ± 4.4^{B}	0.29 ± 0.10^{A}	8.6 ± 3.0^{A}	32.9 ± 4.6^{A}	0.13 ± 0.04^{A}	11.4 ± 4.0^{A}	45.7 ± 6.4^{A}	0.22 ± 0.07^{A}	11.5 ± 4.0^{A}	27.7 ± 3.9^{A}	0.13 ± 0.04^{A}
960	12.9 ± 4.5^{A}	30.2 ± 4.2^{B}	0.27 ± 0.09^{A}	10.9 ± 3.8^{A}	31.4 ± 4.4^{A}	0.08 ± 0.03^{A}	20.0 ± 7.0^{B}	29.8 ± 4.2^{A}	0.40 ± 0.13^{A}	9.6 ± 3.4^{A}	25.6 ± 3.6^{A}	0.15 ± 0.05^{A}
1000	15.3 ± 5.4^{A}	29.9 ± 4.2^{B}	0.15 ± 0.05^{C}	9.3 ± 3.3^{A}	47.7 ± 6.7^{B}	0.52 ± 0.17^{B}	11.3 ± 4.0^{A}	67 ± 9.4^{B}	1.07 ± 0.35^{B}	10.6 ± 3.7^{A}	24.8 ± 3.5^{A}	0.08 ± 0.03^{A}
1110	12.1 ± 4.2^{A}	26.4 ± 3.7^{B}	0.26 ± 0.09^{A}	9.7 ± 3.4^{A}	53.3 ± 7.5^{B}	1.14 ± 0.37^{C}	13.3 ± 4.7^{A}	50.8 ± 7.1^{C}	0.44 ± 0.14^{C}	13.5 ± 4.7^{A}	40 ± 5.6^{B}	0.35 ± 0.11^{B}

The letters indicate differences in elements contents at the significance level α = 0.05 (one-way ANOVA, Fisher-*LSD* test)

and Kula (1998) is only slightly higher than that stated by Markert (1995). The growth depression of sensitive plants was observed at 15–20 mg/kg Cu in tissues (Kloke et al. 1984), and 10% yield decrease is most likely at Cu concentration within the range of 10–30 mg/kg (MacNicol and Beckett 1985). Cu concentrations in plants were mostly lower compared to the surface soil layers. Significant was only correlation between Cu contents in *V. myrtillus* and soil (Table 2). Cu was significantly cumulated only in *R. idaeus* (TC = 1.2) growing on the plots situated at the altitude of 960 m a.s.l. (Figure 3). This fact indicates better bioaccumulation ability of the species compared to other plants and according to Skorbiłowicz (2015) it can prove an increased bioavailability of the given substance for a particular organism.

The mean Zn contents found in surface soil layers decreased more or less up to the altitude of 1000 m a.s.l. and upward only slightly increased (Figure 1). The upper limit value of Zn in agricultural soil (200 mg/kg) stated in the Act No. 220/2004 was not exceeded on any plot of transect. The lowering of Zn contents towards higher altitudes (Figure 1) can be associated with higher soil acidity (Figure 2), since the increasing solubility of Zn is associated with a decrease in acidity of soils (Reddy et al. 1995). This fact is obviously related to climatic conditions, where the lack of soil Zn manifests in cold and wet conditions (Mousavi 2011). According to Kabata-Pendias (2011) acid leaching is a crucial factor in Zn mobilization and might lead to losses of this metal from soil horizons, particularly of podzols.

The clearly highest mean Zn contents in plants were found in *R. idaeus*, *D. filix-mas* and *V. myrtillus* growing at the altitude of 1000–1110 m a.s.l. and they were mostly significantly different from Zn contents found in plants at lower altitudes (750–960 m a.s.l.) (Table 1). In the case of *F. sylvatica*, a trend of continual lowering of Zn contents towards higher altitudes was observed. The background value of Zn in plants (50 mg/kg) stated by Markert (1995) was slightly exceeded only in *R. idaeus* and *D. filix-mas*, growing at altitudes

Table 2. Pearson's correlation coefficients between ecological characteristics of soils and plants (n = 18)

	Cu	Zn	Cd
Relationship between toxic elements in soils and plants			
Fagus sylvatica	– 0.219	– 0.854**	– 0.556*
Dryopteris filix-mas	– 0.232	0.508*	0.802**
Rubus idaeus	0.268	0.473*	– 0.148
Vaccinium myrtillus	0.546*	0.657**	0.656**
Relationship between energy and toxic elements in plants			
Fagus sylvatica	– 0.033	– 0.153	– 0.413*
Dryopteris filix-mas	0.074	0.044	– 0.200
Relationship between energy and toxic elements in soils			
A-horizon (0–5 cm)	– 0.879**	– 0.512*	– 0.274

*α = 0.05; **α = 0.01

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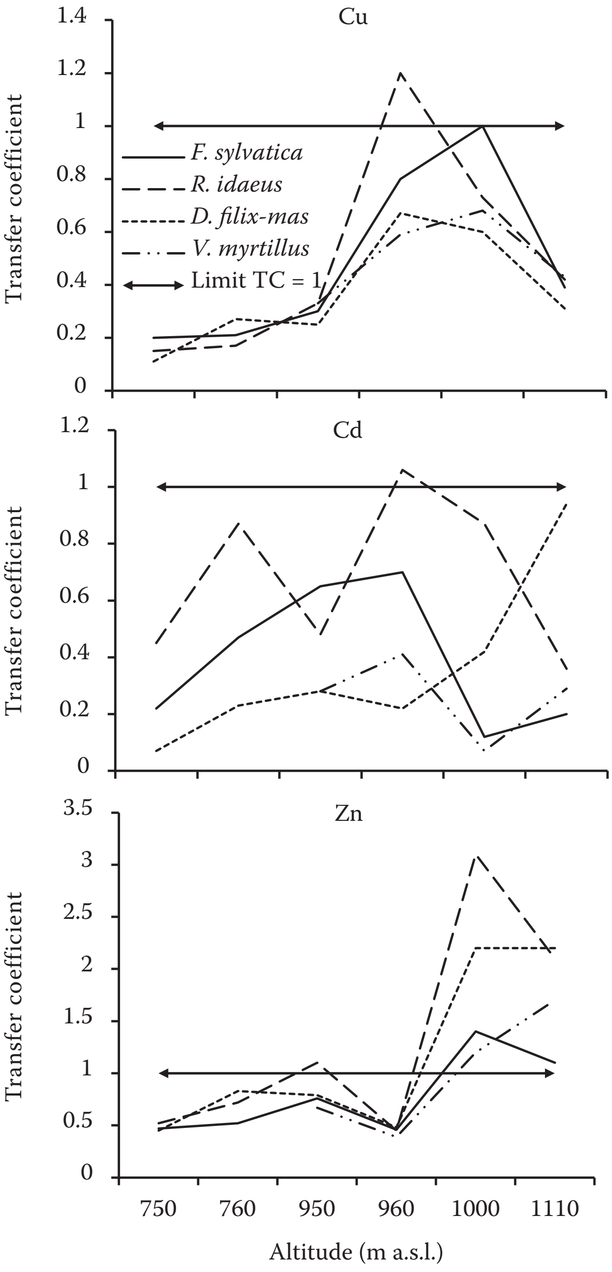

Figure 3. Soil-plant transfer coefficients of Cu, Zn and Cd along altitudinal transect of forest ecosystems (the ratios higher than 1 point out the contamination of soil, or better accumulation ability of plant species)

above 1000 m a.s.l. (Table 1). However, the reference level 100 μg/g, which could be considered as a common indicator of possible immissions according to Dmuchowski and Bytnerowicz (1995), was not exceeded. According to Marschner (1995) the toxic value for plants is a concentration > 300 μg/g. The correlations of Zn concentrations for *D. filix-mas*, *V. myrtillus*, *R. idaeus* species and soils were significantly positive, while in case of *F. sylvatica* they were negative (Table 2). A similar relationship ($r = 0.693$) was found in the study of *V. myrtillus* species by Belanovic et al. (2013). The TC higher than 1 was found in plants growing at altitudes above 1000 m a.s.l. (Figure 3), where the soil acidity is the highest (Figure 2) and Zn contents in soils (Figure 1) are the lowest. This fact points out better Zn solubility and thereby availability to plants.

The Cd contents in soil layers decreased up to the altitude of 960 m a.s.l. and upward they increased more than 3-fold (Figure 1). The upper limit value of Cd in agricultural soil (1 mg/kg) stated in the Act No. 220/2004 was exceeded on the lowest-situated plot with less acid soil (at the altitude of 750 m a.s.l.) and also on the highest-situated plots with very acid soils (at altitudes above 1000 m a.s.l.). Since in the very acid soils a higher solubility can be expected and thereby also leaching of Cd owing to high mountain precipitation, this fact is probably associated with the influence of air pollution. The values higher than background contents reflect an anthropogenic impact on the Cd status in topsoils (Kabata-Pendias 2011).

The highest content of Cd was found in *R. idaeus* at the altitude of 1000 m a.s.l., in *D. filix-mas* and *V. myrtillus* species at the altitude of 1110 m a.s.l., however, in case of *F. sylvatica* only at the altitude of 760 m a.s.l. (Table 1). Cd contents found in the studied plants were in the range of the limit values in plants (0.1–2.4 mg/kg) reported by Bowen (1979). According to Hrdlička and Kula (1998) the excessive value of Cd in plants represents > 0.2 mg/kg. However, the background value in plants (0.05 mg/kg) reported by Markert (1995) was exceeded on all plots. Cd concentrations in plants were mostly lower compared to the soil layers, with exception of *R. idaeus* species. The correlations between Cd concentrations in *V. myrtillus*, *D. filix-mas* and soils were significantly positive, in case of *F. sylvatica* they were negative. The highest TC for Cd was found for *R. idaeus*, i.e. 1.06 (maximum at the altitude of 960 m a.s.l.) (Figure 3). Better ability to cumulate Cd showed the leaves of *Urtica dioica* L. and *Taraxacum* species (TC 2–4.3) growing along a water stream in alluvial plain (Zeidler 2005).

Content of energy in soils and plants. Significantly higher were the energy values in soils found at altitudes above 950 m a.s.l. ($F_{(5,12)} = 14.06$; $\alpha < 0.001$), probably due to a slowed decomposition of organic matter in topsoil horizons (humus content 16.6–19.7%) (Figure 4). The ash contents

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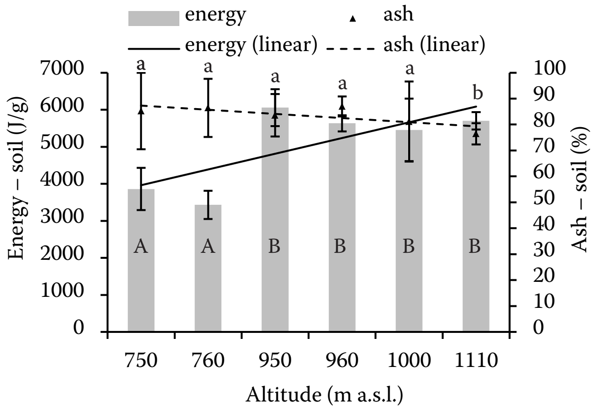

Figure 4. Energy and ash contents in 0–5 cm layer of organo-mineral A-horizons along altitudinal transect of forest ecosystems (arithmetic mean ± standard deviation). The letters indicate differences in energy and ash contents at the significance level α = 0.05 (one-way ANOVA, Fisher-*LSD* test)

found in soil layers fluctuated from 81% to 87%. Relationship between accumulated energy and toxic elements in soils revealed that correlation of the energy and Zn was moderately negative, correlation of energy and Cu was strongly negative (Table 2).

The mean energy contents found in beech leaves increased up to the altitude of 950 m a.s.l. and upwards they more or less slightly fluctuated (Figure 5a). The mean energy contents in *D. filix-mas* continually decreased to the altitude of 960 m a.s.l. and upwards they have only slightly risen (Figure 5a). This species reacted sensitively by a drop of energy with increasing altitude compared to the *F. sylvatica*. Some studies have shown that energy content of plants can be affected by the absorptance to radiation of plant surface, plant shape and orientation. Besides that, Baruch (1982) hinted a tendency to increase energy content of plants in terms of environmental stress. A moderate negative relationship between accumulated energy and Cd content was revealed only by beech leaves ($r > -0.4$). The correlations between Zn-energy and Cu-energy in the studied species were insignificant. Similar results showed *D. filix-mas* species (Table 2). The beech leaves sampled at the altitude of 750–760 m a.s.l. had higher ash contents compared with higher-situated plots (Figure 5b). The ash contents in *D. filix-mas* decreased at the altitude of 950 m a.s.l. (cambisols) and again they were growing upwards (podzols). For instance, Burvall (1997) observed extreme ash variations between grass grown on high clay soils and soils with high content of humus, with ash contents of 10.1% and 2.2%, respectively.

In conclusion, the research conducted on plots situated along the altitudinal transect is an appropriate tool for identifying the impact of air pollution on the status and development of forest ecosystems. Quantification of toxic metals in topsoil indicated higher contents of Cu and Cd over the recommended limit values. Relationship between energy and toxic elements in soils revealed that with an increasing amount of energy, the content of Zn and Cu with altitude significantly declined. Significant was also a decreased content of Cd with an increasing amount of energy in beech leaves. Based on the ability to accumulate metals, studied plants across transect can be ranked into

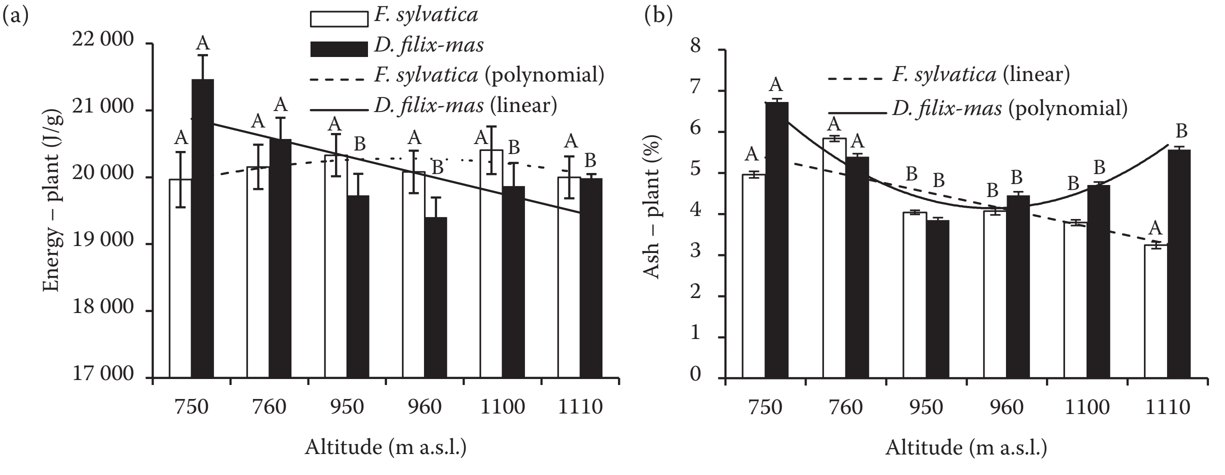

Figure 5. Content of (a) energy and (b) ash in aboveground part of plant species along altitudinal transect of forest ecosystems (arithmetic mean ± standard deviation). The letters indicate differences in energy and ash contents at the significance level α = 0.05 (one-way ANOVA, Fisher-*LSD* test)

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following series: (Cu) *F. sylvatica* > *R. idaeus* > *V. myrtillus* > *D. filix-mas*; (Zn, Cd) *R. idaeus* > *D. filix-mas* > *F. sylvatica* > *V. myrtillus*. Due to generally acid soil conditions and the impact of soil contamination, critical levels of Cd were reached in all plants. Higher heavy metal plant/soil transfer coefficients were found for plants above 960 m a.s.l. However, the highest TC for Cd, Cu and Zn showed *R. idaeus* species, which appears as an excellent native indicator of forest ecosystem contamination.

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