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ORIGINAL ARTICLE

Biogenic and contaminant heavy metal pollution in Estonian coniferous forests

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Abstract Estonia is the only country in Europe that is actively mining oil shale. Oil-shale-burning power plants have been and still are the main sources of heavy metals in Estonia. In order to establish how coniferous ecosystems are affected by Zn, Cu, Cr, Ni, Cd and Pb, heavy metal content in current-year and older needles, in litterfall needles and litterfall miscellaneous fraction, in fine roots and in soil organic horizons was analysed at six coniferous stands of ICP Forests and ICP Integrated Monitoring networks. Root uptake, translocation and accumulation indexes were calculated for each heavy metal. The highest concentrations of most of the heavy metals were found in the soil organic horizons, with the exception of Zn and Cu, the highest concentrations of which were found in fine roots. The results showed that concentrations of the rest of the heavy metals (Cr, Ni, Cd and Pb) were also higher in fine roots compared to other plant material. Significant correlations between the concentrations in soil organic horizons and fine roots indicated that heavy metals had accumulated in the soil organic horizon over time and, in some cases, they may have been transported to above-ground living biomass of coniferous trees.

Keywords Heavy metal · Fine roots · Soil organic horizon · Needles · Oil shale · ICP programmes

Introduction

Oil shale is the most important mineral resource in Estonia. It is a sedimentary rock containing kerogen that is used to produce shale oil and energy. Estonia has been at the forefront of the oil shale industry since the 1960s, making the Baltic region unique at a European level (Raukas 2010). Environmental effects of industrial oil shale pollution were most significant to Baltic and Nordic regions at the end of the 1980s. Political change, followed by drastic economic changes during the 1990s resulted in an approximate 50% decrease in energy production from oil shale and a similar decline in emitted solid particle pollution. The mineral part of oil shale contains heavy metals (e.g. chromium, nickel, lead and zinc) and therefore local ecosystems have been subjected to long-term heavy metal exposure that has resulted in accumulation (Liiv and Kaasik 2004). More recently, the majority (96%) of heavy metal emissions consist of solid particle emissions from NE Estonia's oil shale-fired electrical power producing plants and other industries (Kohv et al. 2009). The Estonian government is committed to reducing heavy metal pollution in accordance with the requirements of the Åarhus Protocol on Heavy Metals (1998) under the Convention on Long-Range Transboundary Air Pollution (CLTRAP).

Since 1990, heavy metal emissions have decreased significantly across Europe. Official data of the European

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Environmental Agency (EEA) show the most significant decline in emissions—approximately 90% since the 1990s—has occurred with lead (EEA 2015). Cadmium—the second most declined contaminant—emissions have dropped by about 75% during the same period (EEA 2015).

The EEA has defined critical loads for different elements, including heavy metals, whereby deposition above the critical load value is likely to have harmful effects on the environment (EEA 2013). Currently, critical loads of contaminants Pb and Cd are typically not exceeded in the EU. However, some EU ecosystems remain at risk due to long-term exposure to different heavy metals and possible bioaccumulation of heavy metals in plant material (EEA 2014). Therefore, continuous monitoring of heavy metals in natural ecosystems is a priority of several international programmes, e.g. the International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests (ICP Forests) and the International Cooperative Programme on Integrated Monitoring of Air Pollution Effects on Ecosystems (ICP IM).

Further reductions in air-borne pollution from the oil shale power production has been achieved via introducing new firing technology and replacing old electrostatic precipitators at thermal power plants with new ones (Hotta et al. 2005). During the period 1990–2013, Pb and Cd emissions in Estonia dropped to a similar degree as the European Union (EU) averages, i.e. in Estonia by 81 and 79%, respectively. There has also been a significant decrease in emissions of other heavy metals: Ni = 76%, Cu = 50%, Cr = 42% and Zn = 42% (Kohv et al. 2015). According to a report by Hettelingh et al. 2007, total heavy metal emissions in Estonia have decreased at a higher level than the EU average. Current emissions in Estonia are within the same range as neighbouring countries, e.g. Finland and Latvia. Recent bulk deposition of heavy metals in Estonia was measured to be approximately: Cd = 0.5 g ha^{-1} $year^{-1}$, Pb = 5.2 g ha^{-1} $year^{-1}$, Zn = 387 g ha^{-1} $year^{-1}$ and Cu = 27 g ha^{-1} $year^{-1}$ (Napa et al. 2015).

Within the framework of the monitoring programmes ICP IM and ICP Forests, heavy metal concentrations of different kinds of plant material and soil organic horizons were analysed in Estonia at four Scots pine (*Pinus sylvestris*) and two Norway spruce (*Picea abies*) natural stands, as these are the most typical coniferous forests in the region. The monitoring data of heavy metal concentrations showed very high variability at both stand and tree level. Many studies have found higher metal enrichment of topsoil organic layers compared to deeper mineral layers (e.g. Alriksson and Eriksson 2001; Ukonmaanaho et al. 2001; Bringmark et al. 2013), which has been interpreted as an effect of deposition, litter decomposition and biological uptake or a more passive uptake of heavy metals by roots (Bringmark et al. 2013; Kraepiel et al. 2015). According to the ICP Forests and ICP IM Manuals of soil sampling and analyses, all roots and large materials (e.g. branches, stones etc.) need to be removed from soil samples before chemical analyses. In environmental remediation articles, accumulation of heavy metals in fine roots has been shown by several authors (e.g. Gordon and Jackson 2000; Prapagdee et al. 2014). Given the relatively short life-span of the fine roots of Scots pine and Norway spruce, the amount of carbon and nutrients returned to the soil from turnover of fine roots may be equal or even exceed that from leaf litter (Joslin and Henderson 1987; Raich and Nadelhoffer 1989; Gordon and Jackson 2000). We were interested in the role of fine roots in terms of retention of heavy metals in forest topsoil organic layers. Our main hypotheses was that heavy metals are easily taken-up by fine roots, which means metals get into the internal cycle of stands and thus prolongs their retention in stands, thereby preventing soil purification of heavy metals.

Our main aim was to compare concentrations of six heavy metals—Cd, Cu, Cr, Ni, Pb and Zn—at the most typical forested ecosystem of the region, coniferous forest, in different organic material samples: living needles (separately current-year and older needles), litterfall (separately needles and miscellaneous fraction), fine roots and forest soil organic horizons. Via concentration ratios of uptake/uplift, mobility, translocation and accumulation, we aimed to follow heavy metal cycles in Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*) stands, especially to examine retention and accumulation of heavy metals in fine roots.

Materials and methods

Study sites and sampling

Data were collected at two Norway spruce (*Picea abies* (L.) Karst.) and four Scots pine (*Pinus sylvestris* (L.)) forest stands across 2009–2013 (Table 1). The sites are part of the UN ECE Convention on Long-Range Transboundary Air Pollution International Co-operative programme monitoring networks (ICP Forests and ICP IM). According to Paal (1997), forest site types vary from nutrient-poor *Cladina* to fertile *Oxalis*.

Needles

Needle samples were collected during 2009–2013 (at ICP Forests stands in 2009, 2011, 2013; at ICP IM stands in 2010, 2011, 2013). Cr and Ni for all sites was analysed in 2013 samples. All samples were analysed in accordance with the ICP Forests and ICP IM Manuals (available online at http://icp-forests.net/ and http://www.syke.fi/nature/icpim, respectively). Five trees from the main species (*Picea abies* or *Pinus sylvestris*) of the ICP Forests programme stands and three trees from the ICP IM stands were sampled per site. Needles were sampled from the upper third of the crown during the dormant period. All collected needles were sorted

Table 1 Characteristics of the six coniferous stands. Soil types were determined per the World Reference Base for Soil Resources (IUSS Working Group 2006) classification system

Stand and coordinates	Dominant tree species	Soil type	Thickness of organic layer (cm)	Average pH ($CaCl_2$) of the soil organic horizon
Saarejärve 58° 59′ 03″ N 26° 45′ 36″ E	Picea abies	Haplic podzol	OL, OF, OH (0–10)	2.8
Tõravere 58° 16′ 30″ N 26° 27′ 37″ E	Picea abies	Haplic luvisol	OL, A (0–18)	4.6
Vilsandi 58° 23′ 13″ N 21° 50′ 38″ E	Pinus sylvestris	Calcari-gleyic leptosol	OL, A (0–8)	5.2
Sagadi 59° 33′ 42″ N 26° 02′ 46″ E	Pinus sylvestris	Haplic podzol	OL, OF (0–5)	3.5
Vihula 59° 34′ 42″ N 26° 07′ 57″ E	Pinus sylvestris	Gleyic podzol	OL, OF, OH (0–10)	3.3
Saarejärve 58° 39′ 29″ N 26° 45′ 26″ E	Pinus sylvestris	Haplic podzol	OL, OF, OH (0–10)	3.0

by age, air dried and concentrations of heavy metals were determined separately for the current-year and older needles.

Litterfall

Litterfall samples were collected per the ICP Forests and ICP IM Manuals (http://icp-forests.net/ and http://www.syke.fi/nature/icpim, respectively) using funnel-shaped traps. The number of traps per stand varied from four to ten, but the total collection surface (2.5 m^2) was the same for all sites. Samples were collected monthly during 2013 (except during the winter period—December to April—when there was snow on the ground). Litter needles were separated from the remaining fine litter. Annual compound samples of both litterfall fractions were analysed in parallel.

Fine roots

During the autumns of 2012–2014, fine root (<2 mm in diameter) samples were collected from the 30-cm topsoil layer using a soil core (∅ 40 mm). Only fine roots from the soil organic layer (OL, OF and OH), which varied from 5 to 18-cm depth (Table 1), were used. However, as at two sites (Tõravere and Vilsandi), the organic layer was partly missing or less than 1-cm thick; roots from the organic-rich A layer were analysed instead. Fine roots were separated from the soil by washing, sorted into living and dead roots based on colour, elasticity and toughness (Persson 1983), and dead roots were excluded from analysis. Once cleaned of soil particles, the root samples were dried at 65 °C for 48 h and weighed. Stand specific fine root proportion was calculated based on the ratio of fine roots in the soil organic layer or upper 10-cm layer for A horizons. Fine root proportion in soil organic layers varied from 41% (Vilsandi) to 62% (Vihula and Saarejärve) in the pine stands, and from 77% (Saarejärve) to 78% (Tõravere) in the spruce stands.

A pilot study was conducted at the Saarejärve pine stand to assess the uptake and accumulation of heavy metals in ectomycorrhizal mycelia. Samples of mycelia were collected from root ingrowth nets installed in the soil during 2010 to study annual production of fine roots. In 2011, after one growth season, 15 nets were harvested. Root-associated mycelia that had grown through the net were collected and cleaned of soil particles.

Soil samples

Samples of soil organic and mineral horizons from the ICP Forests Level II plots were collected during 2008–2010 per the ICP Forests Manual of Soil Sampling and Analyses (2004). Soil samples from the ICP IM stands were collected during 2010 according to the ICP IM Manual (2004). Mineral layers were collected, as the soil sampling was part of a larger study. As concentrations of heavy metals were significantly lower in the mineral soil horizons, in this study data of only the organic horizons were used (OL, OF and OH layers). When OF and OH layers were missing, the uppermost organic-rich soil layer—A horizon—was used in order to include all stands. OL, OF and OH layers were sampled with a 40x25x6 cm frame placed on the forest floor, after which all the green parts and mosses were removed. The sub horizons OL, OF and OH were cut-out separately along the frame using a sharp knife, and the thickness of each layer measured. However, in the framework of this study, these layers were dealt as a single soil organic horizon. The A horizon was sampled from depths of 0–20 cm with a soil auger or spade. Per layer one composite sample was made from five subsamples collected from different pits. All roots and large materials (e.g. branches, stones) were removed; samples were air dried and sieved through a 2-mm mesh before chemical analyses. Before chemical analyses, the soil organic layer samples were also milled.

Chemical analyses

All samples used in this study were chemically analysed by the laboratory of Estonian Environment Research Centre. The laboratory holds a certificate of the ICP Programmes to conduct chemical analysis and is accredited by the Estonian Accreditation Centre. Soil and plant material heavy metal content analysis parameters can be found in the official documents of the Estonian Accreditation Centre (available at www.eak.ee).

Chemical analyses of Cd, Cr, Cu, Ni, Pb and Zn concentrations in needles, litterfall, fine roots and soil organic horizons were performed using the ICP-AES method (inductively via couples plasma spectroscopy, ISO 11885 STJnr.M/U91 for Cu and Zn; STJnr.M/U for Cd, Ni, Pb, SFS 5074, certification verified by Eesti Akrediteerimiskeskus 2013); pre-treatment was done using a microwave oven with *Aqua regia* for soil organic horizon samples and with HNO_3 for the other plant material samples.

Indexes

Based on the data of heavy metal concentrations in fine roots, soil organic horizons, litterfall and living needles, three different indexes were calculated to characterise the movement and retention of heavy metals in the pine and spruce stands.

Root uptake factor (RUF)

$$\mathrm{RUF} = C_{\mathrm{FR}} \Big/ C_{\mathrm{SO}}$$

The root uptake factor is the ratio of heavy metal concentration in fine roots (C_{FR}) to the soil organic horizon (or to A horizon) (C_{SO}). A similar bioconcentration factor has been described by Prapagdee et al. (2014). The RUF shows the ability of roots to obtain and or retain certain heavy metals from the soil organic horizon. An RUF >1 indicates high demand and or mobility of a certain heavy metal. An RUF <1 equates to low mobility of a heavy metal and or its strong attachment to soil organic horizons.

Translocation factor (TF)

$$\mathrm{TF} = C_{\mathrm{N\_}Current} \Big/ C_{\mathrm{FR}}$$

The translocation factor has been used by Yoon et al. (2006) and Prapagdee et al. (2014) and is the ratio of heavy metal concentration in shoots to that in fine roots, thus describing heavy metal translocation inside trees. A higher TF value represents efficient transportation of heavy metals from fine roots to needles. Using the data available from the ICP programmes, TF in our study represents the ratio of heavy metal concentrations in current-year needles ($C_{\mathrm{N\_Current}}$) to fine roots (C_{FR}).

Accumulation index (AI)

$$\mathrm{AI} = C_{\mathrm{LF\_}Needles} \Big/ C_{\mathrm{N\_}current}$$

The accumulation index is the ratio of heavy metal concentrations in the litterfall needle fraction ($C_{\mathrm{LF\_Needles}}$) to current-year living needles ($C_{\mathrm{N\_current}}$). AI represents heavy metal accumulation in older needles.

Statistical analyses

Statistical analyses were performed using STATISTICA 7.0 software, MS Excel 2010 and Canoco (Version 4.5) (Ter Braak and Šmilauer 1998).

To detect any significant correlations between different samples and heavy metals, Spearman's rank order correlation for non-parametric data was used. Only significant ($p < 0.05$) results are presented (starting from correlation 0.8).

Redundancy analysis (RDA) of logarithmically transposed data was used to illustrate and detect connections between plant materials and soil organic layer samples, sites and heavy metals. The Monte Carlo permutation test available in Canoco evaluates the significance of constrained ordination models (Lepš and Šmilauer 2003) and was used to verify the significance of each variable and ordination axis.

Results

Comparison of heavy metal concentrations in fine roots, needles, litterfall and soil organic horizons

A comparison between the different types of samples revealed highest concentrations of heavy metals in either fine roots or soil organic horizons. On average, Zn had significantly higher concentrations on average of all analysed heavy metals. Zn and Cu—necessary micronutrients for plant growth (Tangahu et al. 2011)—concentrations were on average highest in fine roots (109 mg kg^{-1} and 10 mg kg^{-1}, respectively) compared to organic soil horizons and the other plant sample types (Fig. 1). Of the other studied heavy metals (Pb, Cd, Cr and Ni), highest average concentrations were found in soil organic horizons, i.e. 24 mg kg^{-1} of Pb, 6 mg kg^{-1} of Cr, 4 mg kg^{-1} of Ni and 0.6 mg kg^{-1} of Cd. Furthermore, average concentrations of Zn were higher in litterfall (56 mg kg^{-1} for the needle fraction and 52 mg kg^{-1} for the misc. fraction) and in older needles

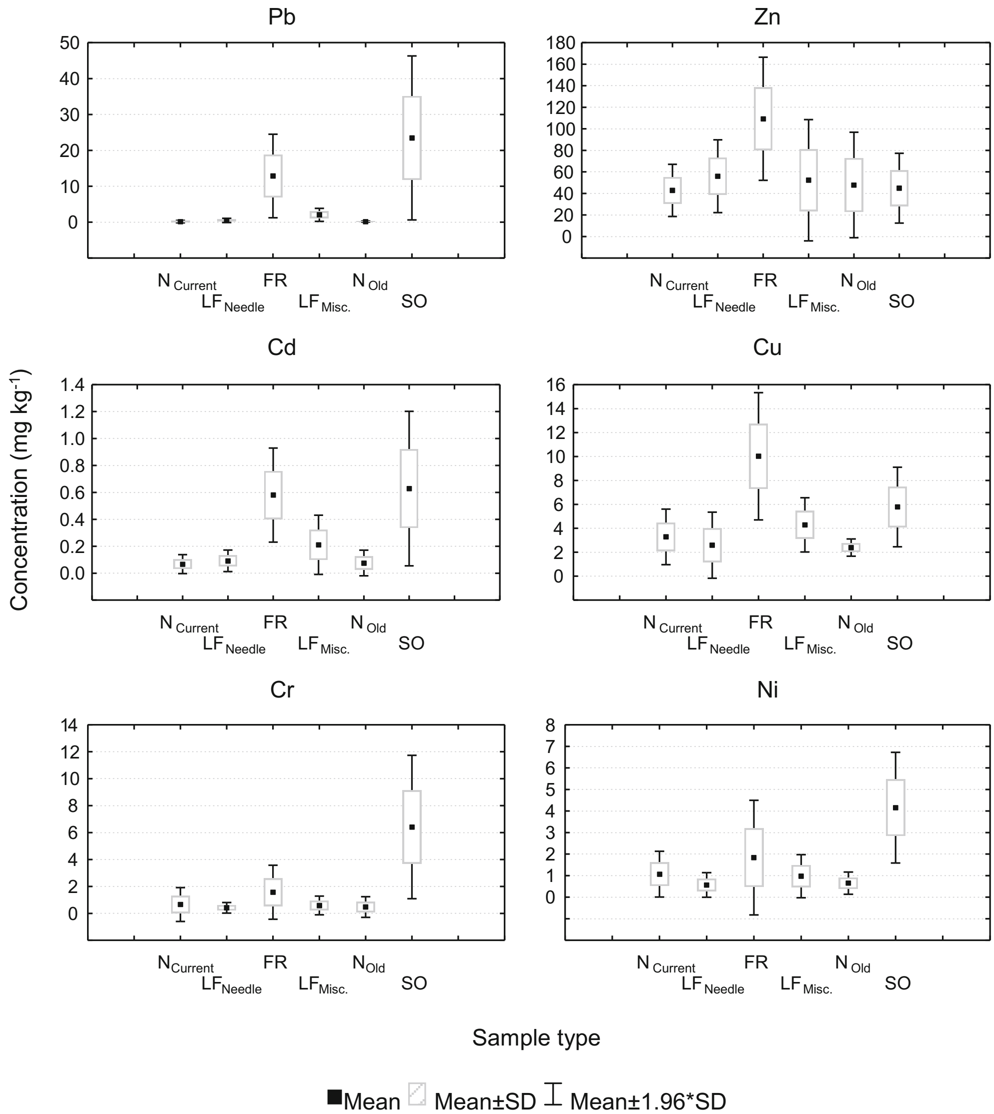

Fig. 1 Mean concentration (mg kg^{-1}) of Zn, Cu, Cd, Cr, Pb and Ni in: $N_{Current}$ current-year living needles, LF_{Needle} litterfall needle fraction, *FR* fine roots, $LF_{Misc.}$ litterfall miscellaneous fraction, N_{Old} older living needles and *SO* soil organic horizons. *Boxes* indicate ± standard deviation and *whiskers* indicate mean ± 1.96*standard deviation

(48 mg kg^{-1}) than in soil organic horizons (45 mg kg^{-1}). Average Pb, Cr, Ni and Cd concentrations in fine roots were higher compared to average concentrations in litterfall or needles (Fig. 1).

To complement the data in Fig. 1, a pilot study at Saarejärve pine stand was carried out to collect ectomycorrhizal mycelia. Chemical analysis revealed that concentrations of heavy metals in ectomycorrhizal mycelia were the following (Table 2.): average Cu = 29 mg kg^{-1}, Zn = 560 mg kg^{-1}, Pb = 37 mg kg^{-1}, Cd = 0.4 mg kg^{-1}, Cr = 14 mg kg^{-1} and Ni = 9 mg kg^{-1}.

To provide an overview of the variability of heavy metal concentrations, a variation coefficient (CV%) was calculated for all the organic material samples (Fig. 1). Heavy metal concentrations in current-year living needles exhibited the most variability, especially Pb (CV% = 102%) and Cr (CV% = 97%). Lowest variability characterised soil organic horizon samples, where CV% varied between 29 and 50%.

To find similarities in the pathways of different heavy metals in the different types of samples, Spearman's rank order correlation coefficient (r_s) was calculated. Seven different correlation variants were detected (Table 3.). In most cases, the strongest statistically significant correlation was obtained between fine roots and soil organic layers; this applied to Cu, Pb, Cd, Ni and Cr (r_s for all ~0.9). Zn had no statistically significant correlations between any types of samples.

According to RDA, the stands and fractions of plant materials and the soil organic layer described 85.7% of total variation of heavy metal accumulation. Concentrations of Ni, Cr, Pb and Cd correlated with the first axis, which was interpreted to represent accumulation in both the soil organic layer and fine roots; Axis 1 explained 69.3% of total variability. Axis 2 correlated with variation of Zn concentration in fine roots; the Tõravere stand (*Haplic Luvisol* soil type) had low Zn concentrations in fine roots and the highest Ni and Cr concentrations

Table 2 Average concentrations of heavy metals (±SE) in mg kg^{-1} in the soil organic horizon, ectomycorrhizal mycelia and fine root samples from Saarejärve Scots pine test stand. Datasets from the Saarejärve stand consisted of soil organic horizons data from 2010 and 2015, fine roots data from 2013 and 2015 and mycelia from 2010 (single measurement)

	Cu (±SE) (mg kg^{-1})	Zn (±SE) (mg kg^{-1})	Pb (±SE) (mg kg^{-1})	Cd (±SE) (mg kg^{-1})	Cr (±SE) (mg kg^{-1})	Ni (±SE) (mg kg^{-1})
Soil organic horizon	7 (2)	47 (3)	35 (4)	0.6 (0.4)	6 (1)	4 (1)
Mycelia	29	560	37	0.4	14	9
Fine roots	11 (1)	188 (48)	28 (5)	1.0 (0.3)	2 (1)	2 (<1)

in the soil organic layer. Geographical location of study sites alone was not significant (Fig. 2).

Decreasing series of heavy metal concentrations in different samples were compared to detect the same types of heavy metal cycling patterns in a stand. The concentrations of two main nutrient heavy metals (Zn and Cu) declined differently in studied samples. The highest Zn and Cu concentrations were found in fine roots, not soil organic layers, as occurred with the other heavy metals. Zn concentrations in soil organic layers were low compared to litterfall samples.

Enrichment of soil organic horizons by Ni, Cr and especially the contaminants Pb and Cd was observed; fine roots also retained high concentrations of these heavy metals. Translocation and or accumulation of Pb and Cd to/in older needles were observed; concentrations of these heavy metals were lowest in current-year living needles.

The series of similar decreasing concentrations of Ni and Cr may indicate the same type of cycling pattern of these heavy metals in the ecosystem.

Indexes: RUF, TF and AI

Three different indexes describing heavy metal cycling in coniferous stands were calculated: root uptake factor (RUF), translocation factor (TF) and accumulation index (AI) (Table 4).

Table 3 Correlations (Spearman's rank order) between litterfall needles and miscellaneous fraction (LF_{Needle} and LF_{Misc}), current-year and older living needles ($N_{Current}$ and N_{Old}), fine roots (FR) and the soil organic layer (SO). Only statistically significant ($p < 0.05$, marked with *) correlation coefficients (r_s) are shown

	Cu	Zn	Pb	Cd	Cr	Ni
FR-SO	.880*	–	.886*	.853*	.943*	.928*
FR-$N_{Current}$	–	–	–	–	.943*	–
$LF_{Needles}$-N_{Old}	–	–	–	–	.900*	–
$N_{Current}$-$LF_{Misc.}$	–	–	–	.886*	–	–
$LF_{Needles}$-$LF_{Misc.}$	–	–	–	.900*	–	–
FR-$LF_{Misc.}$	–	–	–	–	–	.928*
$N_{Current}$-N_{Old}	.900*	–	.900*	–	–	–

The micronutrients Zn and Cu had the highest RUF (RUF_{Zn} = 2.63 and RUF_{Cu} = 1.58, respectively; Table 4.). The lowest RUF was measured for Pb, Ni and Cr (RUF_{Pb} = 0.60, RUF_{Ni} = 0.40 and RUF_{Cr} = 0.23, respectively). These results indicate higher mobility of the micronutrients Zn and Cu and a strong attachment of Ni, Cr and Pb to soil organic horizons.

Pb (TF_{Pb} = 0.02) and Cd (TF_{Cd} = 0.13) had the lowest average TF compared to the biogenic elements Cu (TF_{Cu} = 0.34) and Zn (TF_{Zn} = 0.42). The heavy metal most efficiently translocated was Ni, the TF ratio for which was 0.77.

Pb had the highest accumulation index (AI_{Pb} = 4.16), with a ratio several times higher than any other heavy metal. Relatively high accumulation was characteristic of Cd, Cr and Zn (AI >1). Ni and Cu had the lowest AI ratios, with a high correlation between younger needles and older needles suggesting low accumulation. Stands where Scots pine was the dominant species had higher AI_{Cr} compared to Norway spruce plots. Spruce dominated plots had much lower AIs than the overall averages ($AI_{Cr\ pine}$ = 1.64, $AI_{Cr\ spruce}$ = 0.50 and $AI_{Cr\ AVG}$ = 1.26) (Table 4).

Discussion

Ecosystems in Estonia have been subjected to long-term heavy metal exposure due to intense use of oil shale during the previous decades. Levels of current and historical emissions and heavy metal deposition can be found in different plant materials and soil samples of natural coniferous stands. Heavy metal concentrations are affected by various biological, anthropogenic and site specific factors; therefore, high variability is characteristic of heavy metal data collected from natural coniferous stands (Fig. 1).

High concentrations of Zn and low concentrations of Cd characterised all the studied samples. Micro quantities of Zn, Cu and Ni are necessary plant nutrients (Tangahu et al. 2011); however, in higher amounts, they are toxic (Kamal et al. 2004). Our data revealed a distinct difference between concentrations of biogenic heavy metals (Zn and Cu) compared to contaminant heavy metals (Pb, Cd and Cr). In the plant

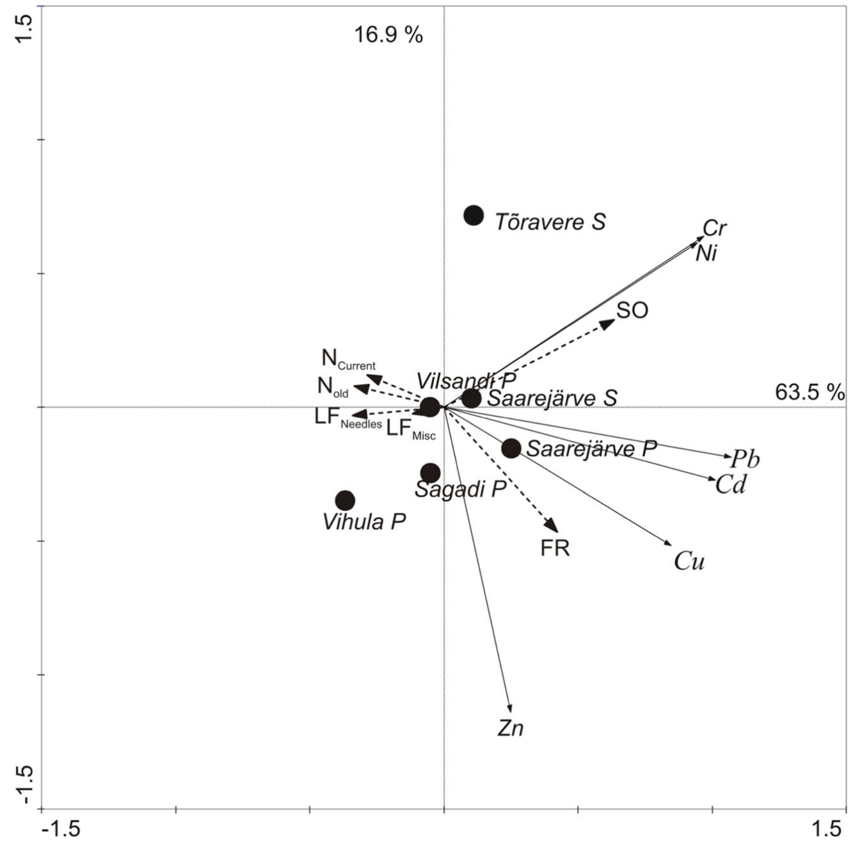

Fig. 2 RDA ordination biplot of heavy metal concentration (*solid arrows*) in litterfall needles and miscellaneous fraction (LF_{Needle} and $LF_{Misc.}$), current-year and older living needles ($N_{Current}$ and N_{Old}), fine roots (*FR*) and in soil organic (*SO*) layer (*dashed arrows*) across various coniferous stands (*dots* with stand name, *P* indicates pine and *S* spruce stands). The samples of plant materials, soil organic layer and stands accounted for 85.7% of total variability. Axis 1 describes 63.5% and Axis 2 16.9% of the total variation in heavy metal concentrations (at *p* value 0.001)

material samples, especially fine roots, both biogenic elements were always present in higher concentrations than the other heavy metals, which probably indicate a biological demand for Zn and Cu by pine and spruce trees. The highest concentrations of Cd, Pb and Cr were found in soil organic horizons (Fig. 1). Our previous study of heavy metals in forest soils revealed Zn cycling by pine and spruce from the soil litter layer, as evidenced by concentrations of Zn in the litter layer being higher than in the other soil organic layers, which was not the case for the other heavy metals (Napa et al. 2015).

According to the RDA ordination, the Tõravere stand differed from other stands. Tõravere had higher Ni and Cr concentrations in the soil organic horizons and fine root samples compared to the other studied stands. The Saarejärve spruce and pine stands differed to the Tõravere and Vilsandi stands and Vihula and Sagadi stands, by having three times and ~50% higher Pb concentrations in soil organic layer, respectively. Although Cd concentrations were very low at all the studied stands, the Saarejärve stands had Cd concentrations in the soil organic horizons that were ~50% higher than at the

Table 4 Indexes (± SE) describing heavy metals cycling in coniferous stands: root uptake factor (RUF) is the ratio of heavy metal concentrations in fine roots compared to soil organic horizons (or to A horizons); translocation factor (TF) is the ratio of heavy metal concentrations in current-year needles compared to fine roots; accumulation index (AI) is the ratio of heavy metal concentrations in the litterfall needle fraction compared to current-year living needles

	Index	Cu (± SE)	Zn (± SE)	Pb (± SE)	Cd (± SE)	Cr (± SE)	Ni (± SE)
Scots pine	RUF	1.67 (0.22)	2.65 (0.53)	0.55 (0.06)	0.98 (0.05)	0.21 (0.03)	0.31 (0.07)
	TF	0.40 (0.06)	0.50 (0.11)	0.03 (0.02)	0.17 (0.04)	0.28 (0.10)	0.96 (0.27)
	AI	0.81 (0.68)	1.29 (0.17)	4.31 (1.60)	1.46 (0.18)	1.64 (0.36)	0.67 (0.35)
Norway spruce	RUF	1.39 (0.33)	2.58 (0.58)	0.71 (0.35)	1.03 (0.41)	0.28 (0.03)	0.58 (0.06)
	TF	0.24 (0.04)	0.27 (0.05)	0.01 (<0.01)	0.05 (<0.01)	0.51 (0.01)	0.39 (0.01)
	AI	0.70 (0.11)	1.41 (0.38)	3.85 (1.74)	1.52 (0.81)	0.50 (0.35)	0.71 (0.05)
Average	RUF	1.58 (0.17)	2.63 (0.37)	0.60 (0.10)	1.00 (0.11)	0.23 (0.03)	0.40 (0.07)
	TF	0.34 (0.05)	0.42 (0.08)	0.02 (0.01)	0.13 (0.04)	0.36 (0.08)	0.77 (0.21)
	AI	0.77 (0.07)	1.33 (0.15)	4.16 (1.11)	1.48 (0.24)	1.26 (0.34)	0.68 (0.22)

other stands. The most likely cause for the contrast between Tõravere and the other stands was the effect of soil type. Tõravere had a thin mull organic layer and humus-rich A layer. The effect of location and distance of stands from industries using oil shale (located mainly in NE Estonia) was not assessed in the current study, although the heavy metal stores at stands are most likely connected to the consequences of pollution from intense oil shale use over the past decades. Today, the importance of stand location is rather insignificant compared to oil shale usage-related air pollution per se and the amount of heavy metals deposited in the past; air pollution from oil shale has decreased significantly since the 1980s, which is a very positive achievement.

The accumulation index (AI) showed heavy metal concentrations were higher in litterfall needles than current-year living needles (Table 4). Accumulation of heavy metals in older parts of trees (e.g. older needles and bark) has been well described in previous studies (Alriksson and Eriksson 2001; Ukonmaanaho et al. 2001; Asi et al. 2009). In the current study, the AI suggests that contaminant heavy metals, especially Cd and Pb, accumulate in older needles that are then withdrawn from the tree via litterfall. When investigating differences between tree species, it must be considered that according to the ICP IM and ICP Forests data, the maximum age of older needles varies from 3 to 4 years for Scots pine and 6 to 9 years for Norway spruce. Therefore, it was rather surprising that in spruce dominated stands the accumulation index for some heavy metals (Ni, Cr and Cu) was especially low compared to pine dominated stands, indicating lower retention and favourable translocation of these heavy metals from older spruce needles.

Despite the highest concentrations of Cr, Ni, Cd and Pb occurring in soil organic horizons, concentrations of these heavy metals were high in fine roots, especially compared to needles and litterfall. For example, mean concentrations of Pb in fine roots were approximately 99% higher than in current-year living needles and 84% higher than in older living needles (Fig. 1). This kind of passive uptake of heavy metals by fine roots was also reflected in the correlation analysis, which showed that the most frequent and strongest correlation occurred between fine roots and soil organic horizons for Cu, Pb, Cd, Ni and Cr (Table 3). Again, the only exception was Zn, for which no statistically significant correlations were detected. Hence, there is reason to suggest that Zn is a growth element in high demand by conifers. That heavy metal concentrations in soil organic layers and fine roots were especially high compared to recent average loads of heavy metal deposition (Napa et al. 2015) indicates previous high deposition levels and retention of the pollutants in the soil organic horizons of coniferous forests.

Fine roots and current-year living needles are short-lived in the context of coniferous ecosystems; therefore, heavy metals contained in these materials return to the cycle relatively quickly. Roots regulate the biological availability of heavy metals mainly through phytostabilisation or risofiltration (Tangahu et al. 2011). Differences in decomposition rates, for example of fine roots and needle litter, can also affect and enhance the retention of heavy metals in soil organic layers, especially in terms of fine roots litter (McClaugherty et al. 1984; Kriiska et al. 2015). Even in areas with relatively low heavy metal deposition, e.g. Scandinavia or Estonia, the retention of heavy metals could slow the decomposition process (Lomander and Johansson 2001), inducing the further accumulation of heavy metals in soil organic horizons where heavy metals can stay bound for decades (Froberg et al. 2011).

The root-soil complex affects both heavy metal concentrations in soil layers and roots (Jobbagy and Jackson 2004). For example, the role of mycelia in the heavy metal concentrations of the fine roots of conifers is rather poorly documented in the literature. In the current study, chemical analyses of the root-associated mycelia at one test site—the Saarejärve pine stand—revealed that Ni, Cr, Cu and Zn concentrations in mycelia were several times higher than in litterfall, living needles, fine roots and soil organic horizon samples (Table 2). Higher concentrations of these heavy metals in mycelia may be connected to their higher absorption area in comparison to e.g. fine roots (Clarholm and Skyllberg 2013). Nevertheless, the highest concentrations of the contaminants Cd and Pb occurred in the soil organic horizons.

The higher concentrations of heavy metals in mycelia might be connected to root exudates or the symbiotic relationship between mycelia and roots, whereby the former obtains necessary heavy metals (Tangahu et al. 2011). The study by Tangahu et al. (2011) supports the theory that one role of mycelia might be to increase the availability of necessary micronutrients to plants in cooperation with soil microorganisms.

Some heavy metals, e.g. Zn and Cu, are more mobile in ecosystems than others (Bergkvist et al. 1989). In the current study, the root uptake factor showed higher mobility of Zn and Cu, but also Cd. The mobility of heavy metals depends on several soil-related factors, such as pH, level of dissolved organic carbon content and the presence of organic ligands in leachate (Bergkvist et al. 1989). PH <5.5–6.0 increases heavy metal leaching and availability; hence a soil pH >6 decreases the availability of some heavy metals, e.g. Pb (Kabata-Pendias 2011; Hale et al. 2012). Per our data, low pH of the upper organic topsoil layers was characteristic of the studied podzol soils (Table 1). A continuous statistically significant downward trend in the pH of soil water at the Saarejärve pine stand has been recorded over the past decades (Frey and Frey 2015), indicating run-off of alkaline ions and thereby acidification of the upper soil organic layers. The significant decrease in oil shale usage-related alkaline particle air pollution since the beginning of the 1990s is almost certainly one reason for the acidification of the studied conifer stands, which has resulted in the mobility of previously retained heavy metals in the upper soil organic layers. When heavy metals are taken-up by rather passive below-ground uplift processes, the subsequent translocation process from roots to needles—described by the translocation factor TF—is slow. Consequently, the studied species—Scots pine and Norway spruce—rather modestly accumulate heavy metals in needles (Table 4). However, it should be noted that our study did not cover the whole uptake process, for example a significant proportion of uplifted heavy metals are accumulated in stem wood, branches and especially bark (Alriksson and Eriksson 2001; Opydo et al. 2005; Danesino 2009). Translocation of heavy metals from soil to plant and inside trees depends on both the metal and tree species (Kabata-Pendias 2011). Norway spruce tends to translocate some heavy metals (e.g. Ni) more efficiently than Scots pine (Table 4). Simultaneous antagonistic or synergistic behaviour of heavy metals may affect the uptake of heavy metals. Antagonistic/synergistic interactions may occur inside the plant and around and or on the roots (Kabata-Pendias 2011). Cu, Zn and Cr have been found to have the most antagonistic interactions (Kabata-Pendias 2011).

The translocation factor (TF) of Cd and Pb was especially low for all studied stands, indicating that despite the high concentrations of these heavy metals in fine roots, Pb and Cd were inefficiently transported from the fine roots to above-ground living biomass of the studied trees. Another interesting observation was that the TF for Ni was as high as or higher than the TF for Cu and Zn, suggesting that Ni is a necessary plant micronutrient and easily translocated to needles. The mobility of different heavy metals is dissimilar within different plants (Alloway 1995). According to Chaney and Giordano (1977), Zn and Cd are easily translocated within plants, Ni and Cu are translocated to some extent and Cr and Pb are not prone to translocation. This would explain the high TF for Cu, Zn and Ni and low TF for Pb in trees found during the current study (Table 4). The low TF for the otherwise easily mobile Cd might be due to antagonism between Zn and Cd (Kabata-Pendias 2011).

Conclusions

High concentrations of heavy metals in fine roots, and significant correlations between heavy metal concentrations in soil organic horizons and in fine roots, indicate that heavy metals accumulated during the peak oil-shale-usage period have left their mark on soil organic layers, and that in some cases they may still be transported to above-ground living biomass of coniferous trees. However, a distinction has to be made between biogenic heavy metals (Zn and Cu) and contaminant heavy metals (Pb, Cd, Cr and Ni) as not all heavy metals were accumulating in the same parts of the ecosystem to an equal extent. Our data showed a distinct difference between concentrations of the main biogenic heavy metals compared to the contaminant heavy metals in litterfall, living needles, fine roots and soil organic horizon samples. Concentrations of both of the biogenic elements were higher than those of the contaminant elements in plant material samples, which is likely to indicate a biological demand for Zn and Cu for growth. The accumulation of Zn and Cu in fine roots and particularly in root-associated mycelia should be emphasised as this indicates active root uptake of these microelements from soil organic layers. Therefore, it is evident that fine roots prolong the stay of heavy metals in coniferous stands and slow down the natural purification process of coniferous ecosystems.

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