The distribution and variability of heavy metals in a mountainous fir forest ecosystem in two hydrological years

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Abstract

The concentrations (for two hydrological years) of the heavy metals As, Cd, Cr, Ni and Pb as well as the isotopic $^{206}Pb/^{207}Pb$ composition in soil layers were studied in a remote mountainous Bulgarian fir (*Abies borisii regis*) forest in central Greece. The concentrations of metals in the hydrological cycle (bulk deposition and throughfall) were lower than the respective ones in the past in Europe, especially for Pb. In the litterfall consisting of mosses, lichens, insect frass and pollen the concentrations of all heavy metals were found far higher than those in the needle litterfall. An evidence of dry deposition was that all metals had higher concentrations in older needles than the young ones with the exception of Ni. Ni also had the highest concentration in the trunk wood, whereas all the other metals had a very low one. It is highly probable that Ni has an internal cycle within the fir trees. In all compartments of the standing trees the order of concentrations was Ni>Pb>Cr>Cd>As. The concentrations of metals in the ground vegetation were low with the exception of Cd, Cr and Ni in ferns. The concentrations of metals in the FH horizon and mineral soil followed the order Cr>Ni>Pb>As>Cd, whereas in the L layer Ni had the highest value. Despite the remoteness of the fir forest, the percentages of anthropogenic Pb in the soil reached an appreciable 41% in the FH horizon and lowered to 2% in the 20-40 cm layers.

Keywords: heavy metals-Bulgarian fir-concentrations-Pb isotopes

1. Introduction

Environmental pollution by heavy metals results from many sources including industrial activities, power generation, transport (especially in urban environment) and agriculture (Tipping *et al*., 2006). Forest vegetation close to such sources of pollution can be heavily affected. It is quite logical that a lot of studies have focused on the effects of heavy metals on forests located nearby of such sources (Pietrzykowski *et al*. 2014; Bache *et al*. 1992; Michopoulos *et al*. 2005; Martin *et al*., 1982; Mingorance

et al., 2007; Kapusta *et al*., 2003). However, to a lesser extent, even remote forests can also be affected. This is due to the atmosphere, which serves as a transport means in long distances (Steiness and Friedland, 2005). Moreover, heavy metals tend to accumulate in soils in time. Therefore, even in small quantities they can have adverse effects on forest functions after a long time. This is the reason that research has been carried out in remote forests. Conifers have a particular capacity to trap the dry deposition of heavy metals due to their vast foliage surface. Foliage drops as litterfall which decomposes, and heavy metals end up in soils or taken up by plants just like nutrients. One of the first studies was that of Heinrichs and Mayer (1977). The authors dealt with the distribution and cycling of Mn, Co, Cr, Cu, Ni, Pb, Sb, Sr, V, Zn, Bi, Cd, Hg and Ti in beech and spruce forests in central Germany. Van Hook *et al.,* (1977) worked with the distribution of Cd, Pb and Zn in a mixed deciduous forest in eastern Tennessee (USA). Zöttl (1985) reviewed the distribution and cycling of Mn, Cu, Cd, Pb and Be in the Black Forest in Norway spruce stands in Germany. Huang *et al*. (2011) studied the cycling of Cd, Zn, Cu and Cr in forest stands of Norway spruce in German mountains. Due to the widespread emission of Pb in the past, many studies focused on this element. Friedland and Johnson (1985) studied the distribution of Pb in forests of red spruce, balsam fir and white birch in in the southeastern USA, Turner *et al*. (1985) in forests of pitch pines and white and black oaks in New Jersey, Watmough and Dillon (2007) in forests of white pine, eastern hemlock, red oak and red maple in central Ontario, USA.

The aim of this work was to investigate the concentrations of As, Cd, Cr, Ni and Pb in a remote mountainous Bulgarian fir (*Abies borisii regis* Mattf.) forest in central Greece in as many forest compartments as possible. In addition, the isotopic Pb composition in the soil profiles was assessed to find the percentage of the anthropogenic contribution to Pb stocks in the soil. The Bulgarian fir, a hybrid between silver fir (*Abies alba* Mill) and Greek fir (*Abies cephalonica* Loudon) occupies the fir zone of central and northern Greece. These three species are hard

to distinguish and occupy a significant area in the central and southern Europe. The *Abies* species are very sensitive to environmental stress. The silver fir was the first species to present symptoms of forest decline in the past in southern Germany (Krauss *et al.*, 1986). In the last decades, the mortality rate of these fir species was very high (Certini *et al.*, 2000; Raftoyannis et al., 2008; Markalas, 1992). So far, there have been two works on heavy metal distribution on *Abies* species by Gandois *et al.* (2010) and Gandois and Probst (2012). The authors examined the distribution of Zn, Ni, Cu Pb and Cd in a silver fir ecosystem without including litterfall. There has also been one work on the limits of decomposition rates set on silver fir litterfall by concentrations of heavy metals in south Italy (Berg *et al.*, 2003). In conclusion, the information for such valuable species as *Abies* is very limited. Furthermore, most studies on heavy metals in forests have focused on Pb and Cd. The present work has also included As, Cr and Ni. As the fir forest under consideration is situated on a good quality site, it can serve as reference to future heavy metals biogeochemical studies.

2.   Materials and Methods

2.1 Study area

The experimental plot is situated in the mountain Timfristos in central Greece, at an altitude of 1170 m. It has an area of 0.27 ha and is enclosed in a catchment of a total area of 147 ha. The average annual rain height is 1530 mm. The vegetation cover consists of an even aged Bulgarian fir (*Abies borisii regis* M.) stand in good health having an average age of 100 years approximately. The ground vegetation consists mainly of ferns (*Pteridium aquilinum* L.), shrubs (*Rubus hirtus* W. & K.), herbs (*Sanicula europaea* L., *Geranium lucidum* L., *Geranium rotundifolium* L., *Luzula forsteri* Sm.) and plants from the family *Gramineae* such as *Melica uniflora* R. and *Brachypodium sylvaticum* H.

The soil was developed on sandy flysch, it is deep and classified as Humic Alisols (FAO, 1988).

2.2 Experimental set up and sampling

2.2.1 Bulk and throughfall deposition collection

In the year 1997, bulk and throughfall deposition collectors were installed in the plot to monitor the quantity and chemistry of precipitation. Bulk deposition was collected with three collectors placed in a forest opening very close to the stand. For throughfall measurements, 30 collectors were placed randomly under the tree canopies in the plot. Bulk and throughfall deposition collectors were identical and each of them consisted of an 18 cm diameter polyethylene funnel attached through a one-meter long hose to a five litre polyethylene bottle. Litter was blocked from entering the funnels with a plug of tulle. The assembly was enclosed vertically in a PVC drainage pipe, dug firmly into the soil to keep the collecting bottle underground so as to exclude light from the water samples. The volume of bulk and throughfall deposition for each collector was measured in

the field with a graduated cylinder and a pooled sample was brought back to the laboratory for analysis.

Deposition collection was done weekly and water volume was measured immediately. Afterwards, samples were placed in plastic bottles and stored at 0 °C in a refrigerator. After each sample collection, all collectors and funnels were washed thoroughly with deionized water. From the middle of November till the beginning of April funnels and bottles were replaced with plastic bags to collect snow.

Both bulk deposition and throughfall formed a pooled sample per month by mixing the weekly samples according to their volume.

All water samples passed through a 0.45 μm filter prior to analysis.

2.2.2 Soil samples collection

The soil samples collection was carried out by means of systematic sampling. Inside the plot, along three lines distancing 25 m from each other six soil pits were excavated. Each pit was 5 m away from each other. From each soil pit, the samples collected were the L and the FH horizon by means of a frame 15 x 15 cm and mineral soil layers from the depths 0-10 cm, 10-20 cm, 20-40 cm and 40-80 cm. There was mixture of six samples of equal volume per horizon and soil depth to have three pooled samples per horizon and depth. The samples of the L horizon at their initial conditions and those of the FH horizon and mineral layers after sieving were pulverized in a ball mill for the purpose of total heavy metal analysis.

2.2.3 Litterfall collection

For litterfall collection, 10 littertraps in the form of cylindrical plastic buckets, each having a collecting area of 0.242 m^2, were placed systematically along a line in the plot, approximately 0.50 m above ground at a distance of 10 m from each other. The bottom of each bucket was perforated so that rain or snowmelt water could drain out. A plastic net was put at the bottom of the littertraps to avoid loss of small material. Collection of litterfall was done monthly or for longer periods, depending on the accessibility of the area due to snow. A composite sample was formed and transported to the laboratory for analysis. Needles, twigs, lichens, mosses, insect frass and pollen were separated and dried at 80 °C for 48 hours.

2.2.4 Sampling years

Bulk deposition, throughfall, soil and litterfall were collected for two hydrological years (2012-2013 and 2013-2014). Each hydrological year started on the 1st of October and ended on the 30th of September.

2.2.5 Ground vegetation collection

The ground vegetation was collected in a systematic way by means of a frame having an area of 0.544 m^2. The collection took place in September of 2009. The categories collected were herbs, ferns and grasses. Each category formed a pooled sample, which included both leaves and stems.

All the categories of ground vegetation were dried at 80 °C for 48 hours.

2.2.6 Needles collection

Conifers retain needles for a few years. For this reason it is advisable, for nutrition or pollution studies, to sample needles of the last year growth (current), second and older years. In our study, needle samples of current, second year and older needles were collected every two years in winter (dormant period) from the upper part of the crown from five dominant trees and formed a pooled sample. The collection always took place from the same trees. The three fractions of needles were dried at 80 °C for 48 hours.

2.2.7 Bark and trunk wood collection

Samples of bark and wood were taken from the same trees from which foliage was collected and formed pooled samples.

2.3 Chemical analysis

The soil pH was determined by a glass electrode in a mixture of soil and water at a ratio of 1:5 per volume. The soil texture was measured by the pipette method. The organic C was determined by a C analyzer.

Exchangeable cations in soils were extracted with a 0.1 M $BaCl_2$ solution and cation exchange capacity (C.E.C.) was calculated by adding the calculated exchangeable cations.

Concentrations of metals in $BaCl_2$ extracts, deposition and soil solution were determined with an ICP-MS instrument (Thermo iCAP Qc). Soil and plant tissues (needles, litterfall, wood, bark and ground vegetation) were digested in a microwave oven with HF and aqua regia at a temperature range of 160-170 °C for 20 min. Likewise, the metal concentrations were measured with the same ICP-MS instrument.

The Pb isotope ratios were determined with an X series 2 ICP-MS (Thermo).

The quality assurance of the metal analysis was checked with the use of sediment samples (TAQC!-River Sediment, training on Analytical Control-Water Framework directive) and that of the isotope with the use of certified reference material (BCR-2 (United States Geological Survey).

2.4 Calculations

2.4.1 Statistics

The coefficients of variation (%) were calculated as the percentages of the standard deviations over the means for the heavy metal concentrations and the isotopic ratios in soils.

2.4.2 Pb isotopic ratios

The percentage of anthropogenic Pb was calculated according to the following equation (Emanuel and Erel, 2002):

Percentage of anthropogenic Pb = 100 x [($^{206}Pb/^{207}Pb$ geogenic-$^{206}Pb/^{207}Pb$ soil sample)/($^{206}Pb/^{207}Pb$ geogenic-$^{206}Pb/^{207}Pb$ anthropogenic)].

The geogenic Pb can be considered the one in the lowest soil layers (Saether et al., 2011). Accordingly, the isotopic ratio of $^{206}Pb/^{207}Pb$ in the 40-80 cm layer in the fir soil represented the ratio of the geogenic Pb.

The average value of $^{206}Pb/^{207}Pb$ (1.13) in the European atmosphere (Saether et al., 2011) was used as the isotopic ratio of the anthropogenic Pb.

3. Results and Discussion

Concentrations of heavy metals in remote ecosystems can be used to delineate the levels of pollution or those of normal concentrations. Normal concentrations are usually considered those of remote from pollution sources ecosystems although in the past there had been problems with atmospheric metal transport. For this reason, examining the past from literature and comparison with current data is another useful procedure.

Table 1. Concentrations ($\mu g\ L^{-1}$) of heavy metals in bulk and throughfall deposition in the two hydrological years

2012-2013					
Bulk					
	As	**Cd**	**Cr**	**Ni**	**Pb**
Mean	0.075	0.024	0.041	0.358	0.163
Range	(0.027-0.357)	(0.05-0.101)	(0.001-0.174)	(0.122-1.34)	(0.039-0.384)
Throughfall					
	As	**Cd**	**Cr**	**Ni**	**Pb**
Mean	0.108	0.016	0.118	0.683	0.301
Range	(0.041-1.01)	(0.09-0.069)	(0.055-0.383)	(0.228-4.58)	(0.107-1.42)
2013-2014					
Bulk					
	As	**Cd**	**Cr**	**Ni**	**Pb**
Mean	0.109	0.069	0.307	0.669	0.412
Range	(0.054-0.192)	(0.021-0.937)	(0.080-1.54)	(0.368-2.14)	(0.210-0.861)
Throughfall					
	As	**Cd**	**Cr**	**Ni**	**Pb**
Mean	0.119	0.025	1.47	0.789	0.925
Range	(0.065-0.237)	(0.001-0.063)	(0.010-5.19)	(0.430-1.34)	(0.278-3.65)

3.1 Hydrological cycle

In the second hydrological year, the concentrations of all metals in bulk and throughfall deposition (Table 1) were higher than those in the first year (with the exception of Cd, which had identical concentrations in throughfall in both years). It can be assumed that some dry deposition of metals could have taken place in the second year

In an early work, Heinrichs and Mayer (1977) determined the average concentrations of Cd, Pb, Cr and Ni in the hydrological cycle of Norway spruce (*Picea abies* L. Karst) in the Solling Mountains in central Germany. The concentrations found in bulk deposition were 1.2 µg L^{-1} for Cd, 2.1 µg L^{-1} for Cr, 38 µg L^{-1} for Pb and 1.4 µg L^{-1} for Ni. For throughfall the concentrations for the same metals were 3.3 µg L^{-1} for Cd, 99 µg L^{-1} for Pb, 2.9 µg L^{-1} for Cr and 9.0 µg L^{-1} for Ni. In a later study also in Germany in the Fichtelgebirge mountains dominated by Norway spruce forests Huang *et al*., (2011) found median values for bulk deposition 0.03 µg L^{-1} for Cd, 0.05 µg L^{-1} for Cr and 0.48 µg L^{-1} for Ni, whereas in throughfall the median values for the same metals were 0.09 µg L^{-1}, 0.24 µg L^{-1} and 1.02 µg L^{-1}, respectively. The concentrations of Pb are missing from the last work. Itoh *et al*. (2006) found Pb concentrations in three rural forests in Japan ranging from 0.66 µg L^{-1} to 1.73 µg L^{-1} in bulk deposition and 0.50 to 1.01 µg L^{-1} in throughfall. The concentrations of As was not examined. It is obvious that the metal concentrations in the study of Heinrichs and Mayer (1977) were far higher than those in our work but even in the more recent papers, the metal concentrations found were either equal or higher.

3.2 Litterfall

The rest litterfall, i.e. mosses and lichens together, insect frass and pollen had the highest concentrations with the exception of Pb and Ni, which also had high values in the twigs (Table 2) just like their respective concentrations in the twigs of standing biomass. Insect frass originated mainly from *Choristoneura murinana*, a needle eating moth from the *Tortricidae* family, which is always present in this area. Pollen production was profuse in the second hydrological year. Mosses and lichens often serve as an indication of heavy metal pollution. In a mosses survey in Germany Pesch *et al*. (2008) found much lower concentrations in all the metals of our work (with the exception of Pb, which were similar). The heavy metal concentrations in lichens vary extremely. Nimis *et al*. (2000) found great ranges in all the metals of our work in the lichen *Xanthoria parietina* in NE Italy. In this study, there was no separation of lichens and mosses so the origin of metals cannot easily be concluded. That is a stimulus for further research. The cone scales is wooden tissue and apart from Ni all the metals had low concentration. The sperms and its wings do not have a high surface and apart from Ni the metal concentration was low. That can be an evidence of internal translocation of Ni in all plant tissues. It is interesting that frass and pollen had a high metal concentration. Probably this is due to their large surface. In any case, it is a proof that metals are deposited through the atmosphere.

Table 2. Concentrations (mg kg^{-1}) of heavy metals in the various components of litterfall in the two hydrological years

2012-2013					
Needles	As	Cd	Cr	Ni	Pb
Mean	0.507	0.102	1.08	3.30	2.34
Range	(0.140-1.37)	(0.06-0.142)	(0.62-2.05)	(3.17-4.55)	(1.00-3.64)
Twigs	0.412	0.303	1.87	4.42	5.07
Frass	0.602	0.174	2.51	4.32	3.44
Cone scales	0.027	0.040	0.252	3.08	0.33
Rest	0.781	0.250	3.11	6.69	5.27
2013-2014					
Needles	As	Cd	Cr	Ni	Pb
Mean	0.200	0.100	0.902	3.328	1.84
Range	(0.154-0.287)	(0.062-0.149)	(0.668-1.24)	(2.79-3.86)	(0.97-2.72)
Twigs	0.353	0.308	1.85	3.97	7.42
Frass and dust	0.678	0.259	3.34	5.85	6.34
Cone scales	0.029	0.052	0.144	2.45	0.607
Sperms	0.135	0.098	1.00	5.21	0.817
Pollen	0.658	0.230	3.70	5.43	4.73
Rest	0.795	0.261	3.98	7.61	7.41

Nearly all studies on heavy metals in litterfall have dealt with fluxes rather than concentrations. The only works in literature so far that have met this objective, are those of Kapusta *et al*. (2003) referring to leaves litterfall in mixed forest ecosystems in Cracow, Poland and Huang et al. (2011) to litterfall of *Picea abies* in Germany. Kapusta *et al*. (2003) found higher concentrations of Pb and Cd than those in the needles of fir in our work. Only in high distances from Cracow the metal concentrations were similar to our results. Huang *et al*. (2011) found almost identical concentrations of Cd (0.10 mg kg^{-1}), and lower concentrations of Cr (0.62 mg kg^{-1}) and Ni (1.70 mg kg^{-1}) with regard to our results.

3.3 Standing tree

When comparing heavy metal concentrations in the various compartments of a coniferous forest it is useful to have the concentrations in new and older parts in the standing tree. Nutrients, in conifers, move from old needles to younger ones during senescence (Ranger *et al.*, 1997). Therefore, metals assuming a physiological role are expected to have lower concentrations in older needles. If, however, needles accumulate metals due to atmospheric deposition, the opposite can happen. Sometimes both processes take place, i.e. metals have an internal circulation and at the same time, metals deposited from the atmosphere accumulate in older plant parts. As a result, the outcome depends on which rate is higher. In our work, all metals had higher concentrations in older needles than the young ones with the exception of Ni (Table 3).

Table 3. Concentrations (mg kg^{-1}) of heavy metals in the various compartments of the standing fir trees

Compartment	As	Cd	Cr	Ni	Pb
Bark	<0.01	0.162	<0.01	0.501	0.189
Trunk wood	<0.01	0.055	<0.01	0.277	0.040
Current year twigs	<0.01	0.212	0.119	5.37	1.36
Last year twigs	0.066	0.411	0.300	4.25	2.49
Branches	<0.01	0.153	<0.01	0.952	0.117
Current year needles	0.026	0.033	0.120	3.81	0.612
Second year needles	0.042	0.037	0.229	2.53	1.56
Older needles	0.117	0.063	0.420	2.90	3.62

Lower concentrations of Ni in current needles than in older ones were also found by Nikonov *et al.* (2001) in Scotch pines in Russia. The same concentration pattern was observed with the twigs of current and second year. Current year and second year twigs are those on which current and second year needles are attached. It is interesting that Ni also had higher concentrations in the younger twigs than the older ones. It can be concluded that an internal circulation takes place for this metal. In all compartments of the standing trees the order of concentrations was Ni>Pb>Cr>Cd>As. With respect to the metals in our work, Gandois *et al.* (2010) found the same concentration order for Ni, Pb and Cd in standing trees needles (mixed ages) of silver fir with the exception of trunk wood and branches where Pb had a higher concentration than Ni. In terms of the metals concentration magnitude in needles, results vary in literature. Gandois *et al.* (2010) found similar concentrations with our work in needles for Cd (0.08 mg kg^{-1}), Ni (2.75 mg kg^{-1}), but lower concentrations of Pb (0.33 mg kg^{-1}). In second year needles of *Pinus masoniana*, Kuang et al. (2007) found higher concentrations of Cr (0.97 mg kg^{-1}), higher concentrations of Cd (0.26 mg kg^{-1}), similar concentrations of Pb (1.39 mg kg^{-1}) and lower concentrations of Ni (0.46 mg kg^{-1}). In mixed (in terms of age) needles of *Picea abies*, Mankovska (1997) found similar concentrations of Pb (1.73 mg kg^{-1}) and Ni (2.60 mg kg^{-1}) and higher concentrations of Cd (0.19 mg kg^{-1}), Cr (0.68 mg kg^{-1}) and As (1.41 mg kg^{-1}). Wyttenbach *et al.* (1996) found lower concentrations of As than those in our work in the needles of *Picea abies* in five age classes of remote forests in Switzerland (0.008-0.025 mg kg^{-1}). However, the same authors found higher concentrations of As in current year needles of *Picea abies* and *Abies alba* in the Black Forest in Germany (0.06-0.08 mg kg^{-1}).

Ni also had the highest concentration in the trunk wood, whereas all the other metals had very low ones (less than 5000 fold with regard to Ni). With the hypothesis that trunk wood accumulates elements mainly from the soil and its metal concentration does not reflect the atmospheric pollution (Rossini Oliva and Mingorance, 2006), it can be inferred there is a substantial Ni take up from the soil. Nygård *et al.* (2012) argued that anthropogenic input is less important for Ni and Cr than Pb and As. However, in our work the concentration of Cr in the older needles was higher than that in the new ones. Therefore, some deposition input of Cr had taken place in the fir forest. The second metal in concentration in wood tissue was Cd. Although Cd has no known biological role, Cd uptake by living cells via essential cation routes is possible (Clemens, 2006). In the stem wood of a sugar maple (*Acer sacharum*) forest, Morrison and Hogan (1986) also found higher concentrations of Ni (0.5 mg kg^{-1}) with regard to Cd (0.19 mg kg^{-1}) and Pb (0.3 mg kg^{-1}). Some researchers have used the ratio of the metal concentrations in tree bark over that in wood. If this ratio is over one, there is indication of atmospheric pollution (Rossini Oliva and Mingorance, 2006). In the fir stand Cd, Ni and Pb had a ratio over one, whereas As and Cr had no detectable concentrations in both trunk and wood. These results, however, have to be interpreted with caution. Gandois *et al.* (2010) argue that in remote and unpolluted forests the increased metal concentrations in tree barks might be due to metal leaching from canopy and not directly from the atmosphere. The Ni concentrations in the second and older year's needles were lower than those in the first year. Assuming that the comparison of different year plant parts is the best method to assess metal accumulation, the bark over wood tissue method is questionable.

3.4 Ground vegetation

The concentrations of metals in the ground vegetation were low (Table 4). These plants are annual and their metal concentration is mainly associated with uptake and deposition in one year. An exception was Cd, Cr and Ni concentrations in ferns, which were rather high in comparison with the concentrations in the standing trees. In comparison with herbs and grasses, ferns have an extensive rooting system and the most probable cause is the uptake through soil. So far, there has been only one work by Martin *et al.* (1982) concerning heavy metal concentrations in the ground vegetation in forests.

Although the forest was contaminated deciduous woodland, the authors found high concentrations of Cd in ground vegetation and especially in ferns in comparison with Pb (the other metals cited in our work were not examined). Maybe ferns can serve as an index for Cd uptake.

Table 4. Concentrations (mg kg-1) of heavy metals in the various plant groups of ground vegetation

	As	Cd	Cr	Ni	Pb
Herbs	0.049	0.076	1.33	3.00	0.651
Ferns	0.001	0.262	1.23	7.26	0.122
Grasses	0.001	0.004	0.010	0.364	0.019

3.5 Soils

The coefficient of variation was highest in the L horizon for all metals (Table 5). This can be attributed to the variation of absorbing capacity of the L layer, as decomposition differs in space, variation in throughfall deposition and plant (including ground vegetation) take up of metals. The heavy metal content of the soil depends on several factors. The parent material, the pH, the organic matter, the clay content and cation exchange capacity. The parent material of the soil in our work is flysch, which is a sedimentary marine formation. This type of flysch alternates sandy and clay content. In general, the clayey sediments have higher concentrations of heavy metals than sandstones (Chimidorzhieva *et al.*, 2012). The pH of the soil is slightly acidic as depth increases (Table 6) and the organic matter becomes lower with depth. These two factors contribute to lower metal concentrations with depth, but the relatively high clay content counteracts this trend. Another important factor when comparing heavy metal concentrations of various soils is the method of digestion. The use of HF acid brings about complete dissolution of the silicate minerals, whereas the other concentrated acids do not have that ability. Consequently, the rest of the acids will result in lower concentrations. Nasseem (1980) found that total concentrations of Cu and Zn in alluvial and calcareous soils using the HF digests were approximately 20% higher than the corresponding ones estimated using aqua regia digests. The use of X-ray fluorescence and fusion with alkali carbonates or metaborate salts are also supposed to give total concentrations. In any case, the method must be mentioned.

Table 5. Concentrations (mg kg^{-1}) of heavy metals in soil layers of the fir plot

Layer	As	Cd	Cr	Ni	Pb
L	0.639	0.077	3.00	7.32	4.60
	(113)*	(57)	(36)	(91)	(115)
FH	5.58	0.591	78.1	43.9	38.6
	(12)	(15)	(12.2)	(10.4)	(7.4)
0-10 cm	8.02	0.313	125	67.6	22.3
	(3.4)	(4.5)	(3.2)	(4.2)	(6.7)
10-20 cm	8.55	0.264	126	65.1	20.6
	(1.7)	(12)	(2.5)	(6.4)	(2.9)
20-40 cm	9.13	0.201	128	72.1	16.5
	(4.0)	(11)	(0.97)	(5.0)	(13)
40-80 cm	9.47	0.176	135	73.4	14.5
	(5.2)	(18)	(1.6)	(8.0)	(12)

*Coefficient of variation

The highest concentrations for Pb and Cd were found in the FH layer (Table 5), whereas for the other metals the mineral layers had the highest concentrations. Pb forms covalent bonds with organic matter and Cd is mainly bound in the exchangeable form. The high magnitude of both organic C and C.E.C. in the FH layer is an explanation for that. Cd is not enriched in the mineral layers as after the FH horizon its concentrations decrease (Table 5). The proportion of Cd transported in soils is larger than the other metals (Bergvist, 1987). It is probable that the pH values in our soils were not low enough to promote Cd migration, as it is known that low pH significantly increases Cd solubility (Christensen, 1984). The highest concentration in the FH layers and the mineral layers was that of Cr followed by Ni. The metal concentrations in the mineral soil followed the order Cr>Ni>Pb>As>Cd. The same order was found by Blaser et al. (2000) for Swiss forest soils using X-ray fluorescence analysis (the authors did not include Cd in their study). In addition, the ranges of metal concentrations in soil layers found by Blaser *et al*. (2000) were quite similar with those found in the present work. Lower concentrations for Cd, Ni and Pd were found by Gandoi *et al*. (2010) who used HF to digest samples of forest soils in northeastern France. However, those soils had a sandy texture. Hernandez *et al*. (2003) using HF acid to digest various forest soils in France found the concentration order Cr>Pb>Ni>Cd apart from some soils where the Ni concentrations were higher than Pb. They also found (like in our work) increasing concentrations with depth of Cr and Ni and ascribed it to weathering and/or leaching within the soil profile. Vanemechen *et al*. (1997) quoted the concentrations and ranges of some heavy metals in European forest soils determined by digestion with aqua regia. The metals Cr, Ni, Pb Cd were included. The concentration ranges found in the soils, in mg kg^{-1} , were for Cr 50-100, for Ni 30-60 for Cd 0.8-1.5 and for Pb 50-100. Nevertheless, the concentrations were lower than the values found in this work with the

exception of Cr. Hernandez *et al*., (2003) also found high concentrations of Cr (similar to our work) in forest soils (using HF acid).

Table 6. Selected properties of the soil in the fir stand. Organic C and clay are expressed in percentages (%) and C.E.C. in cmols$_c$ per kg of soil.

Layer	pH	Org. C	C.E.C.	Clay
FH	6.48	23.0	57.8	
	(0.24)*	(13)	(9.5)	
0-10 cm	6.07	5.12	37.3	27.2
	(1.99)	(20.0)	(14.8)	(2.02)
10-20 cm	5.77	3.36	23.6	31.2
	(3.15)	(10.3)	(12.9)	(2.1)
20-40 cm	5.54	2.75	17.2	33.0
	(1.18)	(3.51)	(5.3)	(6.7)
40-80 cm	5.32	1.53	11.3	34.9
	(1.49)	(19.3)	(5.3)	(14.1)

Coefficient of variation

As is probably the least studied metal in forest soils despite its toxicity. Hansen *et al*., (2001) using hot HNO$_3$ to digest soil samples from Chinese forests found slightly lower values than those in our work with the exception of some extreme high values in one soil type although the authors claimed that the soils were in unpolluted areas. Nygård *et al*. (2012) also used hot HNO$_3$ to digest soil organic horizons from Norwegian forests and found a lower average value of As concentration than that in our work although there was a large range of values in their results.

The heavy metal concentrations in the lowest soil layers can be used to represent the natural geological background. This approach was used by Blaser *et al*., (2000). If the depth 40-80 cm reflects the lithogenic concentrations, there was enrichment only for Pb and Cd (Table 5). Therefore, it can be assumed that both of those metals in the other soil layers were derived from anthropogenic origin. However, as mentioned, As and Cr concentrations were enriched in older fir needles. It can be concluded that higher metal concentrations in deeper soil layers cannot be the only criterion to assess anthropogenic impact.

3.6 Percentage of anthropogenic Pb in soils

The method of determination the percentages of anthropogenic Pb in soils is based on the stable isotopic ratio that Pb had in petrol (related to its mineral origin) and its relation to that of geogenic origin which also is stable depending of course on the rock type (Sturgies and Barrie, 1987). Among all isotopes, the ^{206}Pb/^{207}Pb ratio is used widely. Not all the quantity of anthropogenic Pb found in the environment comes from petrol used in the past decades. Shotyk *et al*. (2004) found that 50-75% of anthropogenic Pb was preindustrial Pb. At a local scale, the introduction of Pb was done about 1000 years ago when the Cu mining became widespread (Mighall *et al*., 2009). There has been no information on the preindustrial Pb use in the area of research. Therefore, the use of ^{206}Pb/^{207}Pb ratio was taken equal to the average value, i.e. 1.13 found in the European atmosphere (Saether *et al*., 2011).

As mentioned for total dissolution of soil minerals by strong acids with regard to heavy meal concentrations, the same remark has to be made for the Pb isotopic ratios. The total dissolution involving HF gives higher values of ^{206}Pb/^{207}Pb ratios. The reason is the existence of resistant minerals to weak acids like zirconium, which tend increase the ^{206}Pb/^{207}Pb ratio (Erel *et al*., 2004).

The values of ^{206}Pb/^{207}Pb in the various mineral soil layers (Table 7) had very low coefficients of variations, far lower than those in the Pb concentrations (Table 6). That means that the anthropogenic Pb migrated more or less uniformly down the soil profiles. All values increased with depth. Similar distributions of ^{206}Pb/^{207}Pb with depth was found by Wilke *et al*. (2001) and Steinnes *et al*. (2005) in soils under Scotch pine in forest soils in Slovakia and Norway, respectively.

Table 7. Values of the ^{206}Pb/^{207}Pb ratios in soil layers of the fir plot and percentages of anthropogenic Pb

Layer	Ratios	Percentage
FH	1.168 (0.27)*	41.1
0-10 cm	1.185 (0.10)	13.5
10-20 cm	1.150 (0.22)	6.25
20-40 cm	1.193 (0.27)	2.08
40-80 cm	1.194 (0.17)	-

Coefficient of variation

The values of ^{206}Pb/^{207}Pb in the present work ranged from 1.168 in the FH horizon to 1.194 in the 40-80 cm layer. In forest soils in Sweden the isotopic ratio range was found 1.3->2 in the C horizons, whereas in surface soils the range was 1.14-1.18 (Bindler, 2011). With regard to the percentages of anthropogenic Pb, the values varied from 41% in the FH horizon to 2% in the 20-40 cm layers (Table 7). In comparison with values found in other forest soils,

the percentages are rather low. Watmouth and Hutchinson (2004) found that approximately 60% of Pb in the top soil under conifers in Ontario of Canada was anthropogenic. Itoh *et al.*, (2007) found 55% of anthropogenic Pb in the forest floor under Japanese cedar 120 km from Tokyo. Even in pristine areas of northern Norway more than 80% of the humus layer was found to be of anthropogenic origin (Steinnes *et al.*, 2005).

In conclusion, the soil under consideration was found to have lower percentages of anthropogenic Pb than the forests of industrial countries in Europe. Nevertheless, the percentage of anthropogenic Pb reached a value of 41% in the FH horizon.

4. Conclusions

- In comparison with the past, the metal concentrations in bulk and throughfall deposition in the fir stand were found lower. Nevertheless, the metal concentrations in the second hydrological year were higher than the first one indicating atmospheric deposition.

- In litterfall, insect frass and pollen had the highest metal concentrations among all fractions. This was probably a combination of their large surface area and metal dry deposition. With regard to past information from conifers, litterfall needles had lower concentrations of Pb and Cd but higher ones of Ni and Cr.

- The high concentrations of metals (with the exception of Ni) in older tree parts such as needles and twigs is a strong evidence of dry deposition.

- Ni appears to have an internal cycle in trees more intensive than the other metals. This conclusion was derived from the finding that Ni had the highest concentration in the trunk wood under the hypothesis of course, that trunk accumulates elements mainly from soil. Nevertheless, its high concentrations in pollen and insect frass litterfall testifies to the existence of dry deposition of this metal too.

- In the ground vegetation, ferns had higher concentrations of Cd, Cr and Ni with regard to the respective concentrations in the standing trees. High concentrations of Cd in ferns have been reported by other authors. Probably, ferns could serve as an index for Cd uptake from soils.

- In soils the highest concentrations for Pb and Cd were found in the FH layer but the highest concentrations (in absolute magnitude) for all metals were those of Cr and Ni. In the mineral layers the order of concentrations was Cr>Ni>Pb>As>Cd. The metal enrichment in deep soil layers cannot be the only criterion to assess anthropogenic impact. The comparison of new and old plant tissues metal concentrations can also reveal information.

- The isotopic ratios of $^{206}Pb/^{207}Pb$ showed that although the soil in the fir stand had lower percentages of anthropogenic Pb than the forests of industrial countries in Europe, still an appreciable percentage (41%) of anthropogenic Pb was found in the FH soil horizon.

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