Total and bioavailable heavy metals in the soils of two adjacent forests

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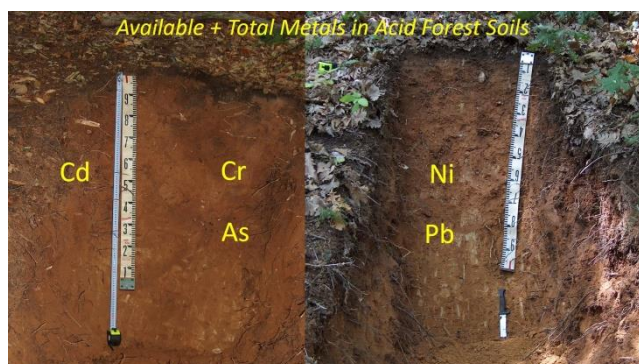[3]General Chemical Lab of Research and Analysis, Lamia, Postal Code 35131

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Graphical abstract

Keywords: Metals, soil, DTPA extraction, beech, oak

1. Introduction

Cadmium, Cr, Ni and Pb are among metals together with As, a metalloid, of great interest in bioavailability studies (due to their toxicity), as listed by the U.S. Environmental Protection Agency (EPA) (McKinney and Ron, 1992). Kim *et al.* (2015) defined three terms of metal availability. Environmental availability, which includes the total amount of heavy metal in the soil, (2) environmental bioavailability, i.e. the amount of dissolved fraction in which can be taken up by plant roots or other soil organisms, and (3) toxicological bioavailability, i.e. the amount of heavy metal which can physiologically induce bioaccumulation or other effect within the plant. Soil scientist are mainly interested in the environmental bioavailability. A great deal of experiments concerns the concentrations of heavy metals extracted with a single extractant. For instance, neutral salts are supposed to displace the readily soluble metal fraction from the exchangeable sites into soil solution. In contrast, organic chelating agents such as DTPA and EDTA are thought to behave as the organic exudates produced by plants, capable of removing metals from various soil fractions such as organic matter, carbonates and metal oxides (Fang *et al.*, 2007). DTPA can be used in both alkaline and acid soils and for this reason many researchers have used it to predict bioavailability of heavy metals and trace elements in a variety of soils (Chavda, 2018; Evanylo and Sukkariyah, 2006; Nunes *et al.*, 2014).

Environmental pollution by heavy metals is the result from many sources such as industrial activities, power generation, transport (especially in urban environments) and agriculture (Tipping *et al.,* 2006). Forests are sinks of heavy metals due to the high capacity of their soils (especially of the forest floor) to retain metals (Brumelis *et al.,* 2002; Suchara and Sucharová, 2002). It is quite logical that many studies have focused on the effects of heavy metals on forests located nearby of such sources (Pietrzykowski *et al.,* 2014; Michopoulos *et al.,* 2005).

Abstract

The concentrations and comparisons of total and available metals Cd, Cr, Ni, Pb and the metalloid As were examined in two adjacent acid forest soils in Greece under oak and beech together with the dependency of their availability. It was found that the soil in the beech plot had higher concentrations of total elements with the exception of the litter layer (L) where most metals did not differ. It is probable that the parent material of the beech soil contained some metamorphic mafic material. The surface soils for both stands were moderately enriched with Pb, Cd and As, whereas for Cr and Ni the enrichment was minimal. The concentrations of available elements (extracted with DTPA) were higher in the beech soil. The availability of most metals was affected by the pH, the organic C, the ratio of C/N and the total concentration of the metals. Through a Principal Component Analysis (PCA) analysis, it was found that 63-75% of the concentrations variance of the available metals was explained. The percentages of available metals with regard to their total concentrations in soils were higher in the beech plot in the FH layer but in the mineral layers, they did not differ apart from Pb. The concentrations of the metals in the leaves of both species in three consecutive years did not differ with the exception of Cd, the concentration of which was higher in the beech leaves.

However, to a lesser extent, even remote forests can be affected as well. This is due to the atmosphere, which serves as a transport means for metals in long distances (Steiness and Friedland, 2005).

The aim of this work was to compare the concentrations of total and available heavy metals Cr, Ni, Cd and Pb as well as the metalloid As in two acid forest soils under oak and beech and assess the dependency of their availability on certain soil properties. Moreover, it was decided to find the heavy metal concentrations in the leaves of the two main species. These forests are remote from industrial activities and can serve as a baseline for future comparisons with other forests. So far, there has been only one work (Michopoulos *et al.*, 2018) concerning heavy metals concentrations in a remote fir forest in Greece. It is the first time that a work deals with acid forest soils in which metals are expected to be more mobile. In this respect, the findings of the present work will add to our knowledge.

2.   Materials and Methods

2.1. Site description

The experimental forested plots of under consideration are found in a mountainous watershed, 260 ha in size, located in central eastern Greece (Figure 1) at an elevation range of 740–1420 m. The main forest species in the oak plot is the Hungarian oak (*Quercus frainetto* Ten.) 40-70 years old, whereas in the beech plot is the *Fagus sylvatica* L. (110-130 years old). The parent material of both plots is mica schist. Detailed information on the sites of the two plots are found in Michopoulos *et al.* (2020).

2.2. Soil samples collection

The soil samples collection from both plots was done in the summer of 2007 by systematic sampling. Along three lines, distancing 25 m from each other six soil pits (5 m away from each other) were excavated. The samples collected were the L and the FH horizons and mineral soil layers from the depths 0-10 cm, 10-20 cm, 20-40 cm and 40-80 cm. There was pooling of six samples of equal volume per layer and soil depth (random selection) to have three pooled samples per layer and depth. In total, there were 18 soil samples per forest type (six layers times three replicates per layer). The samples of the FH horizon and mineral layers were air dried and passed through a 2 mm sieve stored for analysis to determine texture, pH, cation exchangeable cations, cation exchange capacity (C.E.C.) and available heavy metals. The samples of the L horizon at their initial conditions and subsamples of the FH horizon and mineral layers after sieving were pulverized in a ball mill for the aim of total analysis for organic C, total N and total heavy metals. Like the plant tissues in litterfall, soil samples were dried at 80 °C for 48 hours.

2.3. Collection of leaves

Leaves samples of both species were collected every two years in summer 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 leaves samples were dried at 80 °C for 48 h and then ground in a special mill for analysis. The data covers three collection periods, i.e. 2015, 2017 and 2019. In total, there were three pooled samples per leaf type.

2.4. Chemical analysis

2.4.1. Soil

The soil pH was determined by a glass electrode in a mixture of soil and 0.01 M $CaCl_2$ at a ratio of 1:5 (v:v). The soil texture was measured by the pipette method. The organic C and total N were determined by a CN analyzer through dry combustion.

Exchangeable cations in the FH and mineral soil layers were extracted with a 0.1 M unbuffered $BaCl_2$ solution and their concentrations were determined with an ICP-MS instrument (Thermo iCAP Qc). Cation exchange capacity (C.E.C.) was found by adding the calculated exchangeable cations.

Available heavy metals and As in soils were extracted with DTPA (Lindsay and Norvell, 1978) and their concentrations was determined with the ICP instrument.

For the total concentrations of heavy metals in the FH layer and mineral soils 0.20 g of ground soil was digested in a microwave oven with 1 mL aqua regia and 5 mL concentrated HF acid at a temperature range of 160-170 ^{0}C for a period of 20 min. The digests were diluted to 50 mL with deionized water and the concentrations of heavy metals were measured with the above-mentioned ICP instrument.

The total concentration of Al in the 0-10 cm and 40-80 cm soil layers was measured by X-ray Fluorescence Spectroscopy (XRF) model XEPOS by SPECTRO.

The limits of quantifications (LOQs) for the total Cd, Cr, Ni, As and Pb were 8.3, 251, 274, 21.6 and 48.5 µg kg^{-1}, whereas for the DTPA extractable ones the LOQs were 0.38, 2.6, 8.0, 1.2 and 5.6 µg kg^{-1}, respectively.

2.4.2. Leaves

Ground material of leaves samples were digested in a mixture of HNO_3-$HClO_4$ in a proportion of 2:1 (v:v) and the metal concentrations were determined with the ICP instrument.

All results (plant tissues and soils) were expressed in oven dry weights (105 °C for 48 hours).

2.5. Calculations and statistics

In all tables containing the soil properties of the two plots, the total heavy metal content, the concentrations of available metals and the percentages of the available metals the average concentrations and the coefficients of variability were used. The percentages of available metals and As were calculated as the percentage (%) of the DTPA extracted metal over the total metal concentration. The coefficients of variability were expressed as the percentage (%) of the standard deviation over the average values. For the statistical comparisons for the above parameters between the two plots, the Kruskal-Wallis test for non-parametric statistics was used.

The crustal enrichment factors (EF) of elements were calculated using the the equation: EFc =(CxC$_{Al}$)/sample/(Cx/C$_{Al}$) continental crust. Dantu (2009) used this equation taking into account continental crust values derived from Taylor and Mclennen's (1995). However, the lowest soil layers can also be used as reference values (Blaser *et al.,* 2000). In our work, we used the 40-80 cm one. The layer examined for enrichment was the first mineral one, i.e., 0-10 cm.

The statistical dependence of available heavy metals was examined for a variety of soil parameters in the mineral soil layers by means of the Principal Component Analysis (PCA). The predictors chosen were the total metal concentrations, the pH, the percentage of clay, the concentrations of organic C and the C.E.C. values.

3. Results

Comparisons with the Kruskal-Wallis test in soils between the two plots

The soils in the beech plots had significantly lower pH values in all layers (Table 1). Apart from the L horizon, all the concentrations of organic C were higher in the beech plot. The ratio of C/N in soils was significantly higher in the beech plot for the layers 0-10 cm, 10-20 cm and 20-40 cm. The clay content was also significantly higher in the beech plot for all the mineral horizons. The CEC did not follow the same pattern with the organic C and clay. It was significantly higher in the soils of the oak plot for the 0-10 cm and the 40-80 cm layers.

Table 1 Values of some soil properties in the layers of soil profiles in the two plots. Organic C is expressed in mg g^{-1}, CEC in cmoles$_c$ kg^{-1} and clay in percentages (%). Different letters per soil property and layer denote significant difference for at least 0.05 probability level

		pH	Org. C	C/N	CEC	Clay
L	Oak		459 a (3.1)*	36.4 a (7.8)		
	Beech		473 a (0.6)	33.2 a (4.2)		
FH	Oak	6.20 a (1.6)	201 a (9.5)	20.9 a (1.3)	44.4 a (5.9)	
	Beech	5.80 b (4.9)	269 b (3.5)	19.8 a (3.1)	41.2 a (12)	
0-10 cm	Oak	5.35 a (0.7)	34.6 a (13.8)	17.5 a (2.3)	9.30 a (13)	16.2 a (3.9)
	Beech	4.83 b (2.4)	43.2 b (4.5)	20.8 b (3.8)	6.89 b (2.0)	19.7 b 5.5
10-20 cm	Oak	5.40 a (1.5)	20.9 a (15.6)	17.1 a (2.7)	5.10 a (17)	16.6 a (2.3)
	Beech	5.10 b (2.1)	29.5 b (8.7)	19.2 b (4.1)	4.70 a (5.2)	21.8 b (2.1)
20-40 cm	Oak	5.46 a (1.2)	11.8 a (3.7)	16.3 a (2.0)	3.74 a (8.1)	16.1 a (2.5)
	Beech	5.24 b (0.6)	20.9 b (12)	17.4 b (4.1)	3.58 a (9.3)	21.5 b (0.3)
40-80 cm	Oak	5.42 a (0.7)	5.66 a (16.6)	15.0 a (6.7)	3.31 a (5.2)	13.6 a (4.2)
	Beech	5.27 b (1.1)	9.08 b (3.2)	15.7 a (3.9)	2.65 b (8.5)	15.0 b (4.1)

*Coefficient of variation (%).

Table 2 Total heavy metals and As concentrations (mg kg^{-1}) in the layers of the soil profiles of the two plots. Different letters per metal and layer denote significant difference for at least 0.05 probability level

		Cd	Cr	Ni	As	Pb
L	Oak	0.165 a (44)	5.82 a (7.3)	6.08 a (9.5)	0.675 a (8.5)	4.23 a (12)
	Beech	0.229 a (2.0)	4.64 a (29)	5.16 a (9.2)	0.457 b (13)	3.76 a (15)
FH	Oak	0.665 a (1.3)	62.9 a (0.3)	31.4 a (0.3)	6.78 a (5.5)	41.1 a (5.1)
	Beech	1.07 b (3.3)	88.2 b (3.6)	38.3 b (3.9)	9.06 b (1.5)	87.3 b (0.1)
0-10 cm	Oak	0.290 a (2.0)	102 a (1.4)	43.3 a (0.6)	8.53 a (11)	26.6 a (13)
	Beech	0.266 a (11)	180 b (4.3)	56.3 b (1.0)	12.2 b (2.1)	48.7 b (5.1)
10-20 cm	Oak	0.226 a (5.0)	103 a (1.0)	43.8 a (1.6)	7.08 a (6.7)	18.8 a (12)
	Beech	0.202 a (25)	193 b (6.3)	59.4 b (5.3)	10.7 b (4.4)	29.0 b (38)
20-40 cm	Oak	0.157 a (16)	108 a (3.6)	47.5 a (4.6)	5.49 a (5.1)	13.2 a (9.3)
	Beech	0.155 a(12)	200 b (7.0)	68.0 b(6.3)	8.95 b5.3	27.9 b (4.3)
40-80 cm	Oak	0.149 a (19)	109 a (5.0)	46.2 a (3.2)	4.46 a(3.8)	7.23 a (10)
	Beech	0.178 a (14)	221 b (9.0)	66.7 b (6.1)	6.83 b (3.0)	19.2 (23) b

*Coefficient of variation (%).

Table 3 Crustal enrichment factors for the mineral layer 0-10 cm with heavy metals and As

	Cd	Cr	Ni	As	Pb
Oak	2.20 (23)	1.03 (9.8)	1.03 (8.8)	2.11 (15)	4.05(15)
Beech	1.62(12)	0.88(12)	0.91 a(13)	1.92 (8.5)	2.89 (36)

*Coefficient of variation (%).

With regard to the total amounts of metals in the L layer only As had higher concentrations in the oak plot (Table 2). Further, down the soil profiles the beech plot had higher concentrations in all metals apart from Cd that had higher concentration in the beech plot in the FH layer.

Table 3 contains the crustal enrichment factors for the mineral layer 0-10 cm.

Table 4 contains the concentrations of the available metals. The concentrations were significantly higher in the beech soil in all layers apart from Cd in the 0-10 cm, 10-20 cm and 20-40 cm ones.
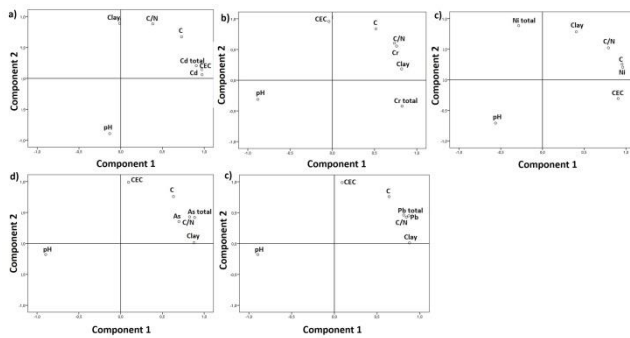
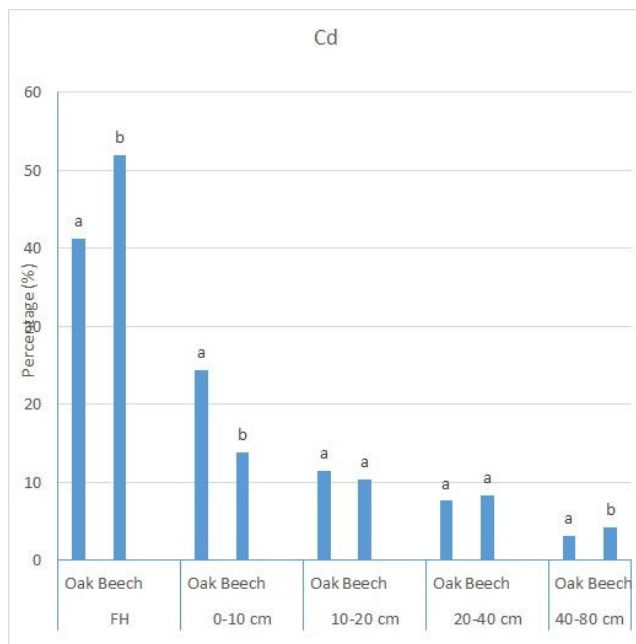**Figure 1.** PCA ordination diagram for heavy metals and As.

Figure 2. Percentage (%) of available Cd in soil layers. Bars with different letters denote significant difference for at least 0.05 probability level.

The results of Figure 1 depicting the PCA axes and the parameters (as circles) are combined with Table 5. The component 1 represents the horizontal axis of Figure 1, whereas the component 2 the vertical one. The first principal component is the linear combination of x-variables that has maximum variance (among all linear combinations). It accounts for as much variation in the data as possible. As the first component explains the maximum variability, Table 5 contains only the values derived from the first component's calculations. Values around 0.600 or higher are considered satisfactory as they explain at least 40 % of the variability of the parameters (Comrey, 1962). Therefore, from Table 5 the parameters we had the following results:

- All metals have satisfactory and positive relationship with the total heavy metal content apart from Ni.

- All metals had satisfactory and positive relationship with the organic C content apart from Ni.

- All metals had satisfactory and positive relationship with the ratio C/N apart from Cd.

- All metals had satisfactory and negative relationship with the pH apart from Cd.

- Two metals have a positive relationship with the C.E.C., Cd and Ni

- The total variance explained was higher for Pb than in any other metal.

The percentages (%) of the available metals (Figures 2-6) for Pb were higher in all layers (starting from the FH one) in the beech plot. For Ni and As the concentrations were also higher in the beech plot only for the FH layer and for Cr in the FH and 0-10 cm were higher in the beech plot. Cd had a different behavior. In the FH layer, the concentrations were lower in the oak plot but higher in the 0-10 cm in the same plot. Finally, in the 40-80 cm it was higher in the beech plot.

The concentrations of heavy metals and As in leaves are showed in Table 6. Only Cd was found to differ significantly between the plots. The high variability in comparison with the variability in soils is obvious.

4. Discussion

4.1. Total concentrations of metals in soils

From Table 1, it can be seen that the soil under beech had significantly lower pH values in all soil layers, higher clay content and organic C. The difference in these properties affect both total and available metal concentrations as will be seen below. As the soil in the two plots is not contaminated, the comparison of total metal concentrations (Table 2) with other soils in literature will be made also with non-contaminated ones. The average contents of Cd in soils lie between 0.06 and 1.1 mg kg^{-1} (Kabata-Pendias and Pendias, 2000). In our work, the Cd content was rather low ranging from 0.15 to 0.229 mg kg^{-1}. Shahid *et al.* (2017) quoted some ranges of total concentrations of Cr in soils, which reached up to 100 mg kg^{-1}. Blaser *et al.* (2000) found higher values of total Cr in Swiss forest soils derived from schist (127 mg kg^{-1}) and Hernandez *et al.* (2003) measured 108 mg kg^{-1} in the mineral horizons of forest soil over metamorphic rocks. In our work, we found higher concentrations than 120 mg kg^{-1} in the mineral layers of the beech plot (maximum 221 mg kg^{-1}). This finding can be ascribed not only to the higher clay content but also to some mineralogical influence. There must be some mixture of metamorphic mafic rocks with the mica schist in the parent material of the beech plot as these concentrations of Cr are met in soils derived from mafic rocks (Shahid *et al.,* 2017).

The average concentration of Ni in soils is 40 mg kg^{-1} (Uren, 1992). Close to these values are the averages of forest soil profiles in Swiss forest soils (Blaser *et al.*, 2000). In the oak plot in our work, the values are also close to the 40 mg kg^{-1}. In the beech plot, however, the values are higher reaching 68 mg kg^{-1} in the 20-40 cm layer.

The baseline concentrations of As in soils are generally of the order of 5–10 mg kg^{-1} (Smedley and Kinniburgh, 2002). However, higher values have been found in mineral uncontaminated soils (10-24 mg kg^{-1}) of forests (Huang and Matzner, 2007). In our work the As concentration

ranged from 0.46 mg kg^{-1} in the L horizon to 12.2 mg kg^{-1} in the 0-10 cm mineral layer of the beech soil.

For Pb mean values for soils range from 10 to 67 mg kg^{-1} having an average of 32 mg kg^{-1} (Kabata-Pendias and Pendias, 2000). In our work and especially in the lower soil layers, which are not subject to Pb deposition, the top concentration for Pb value was 29 mg kg^{-1} (beech plot).

Despite the supposedly common parent material for both plots, there were significant differences in concentrations even for the 40-80 cm layer apart from Cd (Table 2). In all cases, the beech soil had higher metal concentrations. Possible explanation can be the low pH values in the soil under beech, the high organic matter and clay content and possibly some mineralogical difference in the parent material of the beech soil. Low pH can accelerate weathering and release metals which subsequently are retained by clay minerals and organic matter.

The crustal enrichments factors in both plots were very low for Cr and Ni. Sutherland (2000) quoted five contamination categories based on enrichment factors values. According to those values, the Cr and Ni in both plots are listed as minimal and the rest metals as moderate. A similar result was found in forest soils in Germany, where the proportions of the variance in the Ni and Cr (extracted with aqua regia) concentrations explained by the parent rock type were 43 and 47%, respectively, whereas for Pb it was 25% (Utermann *et al.*, 2019). For the rest elements, the moderate enrichment is considered low for As, Cd and taking into account their capacity for long-range transport in the atmosphere and subsequent deposition in forests (Steinnes and Friedland, 2005).

Table 4 Concentrations (μg kg^{-1}) of DTPA extractable heavy metals and As in the layers of the soil profiles of the two plots. Different letters per metal and layer denote significant difference for at least 0.05 probability level

		Cd	Cr	Ni	As	Pb
FH	Oak	275 a (2.6)	48.1 a (7.7)	2410 a (1.6)	156 a (6.3)	5268 a (8.3)
	Beech	557 b (3.0)	132 b (5.0)	4968 b (2.4)	349 b (7.1)	12543 b (17)
0-10 cm	Oak	70.9 a (16)	28.6 a(20)	459 a(26)	45.9 a(25)	1966 a(13)
	Beech	36.7 b (4.2)	96.9 b (4.1)	638 b (12)	51.6 b (6.4)	4240 b (8.1)
10-20 cm	Oak	26.2 a (34)	17.5 a (16)	198 a (26)	29.9 a (6.4)	1060 a(21)
	Beech	20.2 a(9.6)	48.3 b (37)	364 b (14)	48.5 b(12)	3078 b(9.7)
20-40 cm	Oak	12.0 a(17)	11.8 a (22)	95.9 a (13)	26.2 a (6.3)	529 a (8.1)
	Beech	12.9 a (11)	32.3 b (31)	175 b (33)	41.2 b (2.1)	1756 b (19)
40-80 cm	Oak	4.71 a (12)	7.31 a (48)	45.2 a (20)	30.8 a(7.8)	268 a (7.8)
	Beech	7.31 b (10)	12.3 a (42)	66.4 b (12)	47.0 b (5.2)	1140 b (12)

*Coefficient of variation (%).

Table 5 Component matrix with the percentage (%) of variance explained and the relations of the available P and soil parameters derived from the PCA

	Cd	Cr	Ni	As	Pb
Variance	64.2	63.9	62.6	70.9	75.1
C/N	0.385	0.735	0.790	0.826	0.842
Org. C	0.729	0.516	0.953	0.632	0.640
pH	-0.132	-0.881	-0.562	-0.897	-0.895
Clay	-0.013	0.820	0.405	0.878	0.879
CEC	0.972	-0.041	0.906	0.095	0.091
Total Cd	0.905				
Total Cr		0.826			
Total Ni			-0.286		
Total As				0.885	
Total Pb					0.811

Table 6 Concentrations (mg kg^{-1}) of heavy metals and As in the leaves of oak and beech of the two plots. Different letters per metal and layer denote significant difference for at least 0.05 probability level

	Cd	Cr	Ni	As	Pb
Oak	0.030 a (17)	0.758 a (21)	2.25 a (6.2)	0.091 a (50)	9.20 a (19)
Beech	0.122 b (42)	0.691 a (37)	2.69 a (28)	0.109 a(62)	8.24 a (77)

*Coefficient of variation (%).

4.2. Availability

4.2.1. General comments

The PCA disclosed some relations of available metals with the other soil properties (Figure 1 and Table 5), which help explain the comparisons of available metal concentrations in Table 3 between the two plots. First, the percentages

that the parameters chosen to enter the PCA explained a satisfactory percentage for all metals especially for Pb. Nearly all the DTPA extracted metals had a negative relation with pH and a positive one with organic C and clay content. Under the aerobic and acidic to near-neutral conditions typical of many forest environments, heavy metals are strongly adsorbed by oxide minerals. The H

protons can dissolve some oxides thus releasing metals in the soil solution. The lower the pH the more active the H protons are. The organic matter played a significant role. In the large majority of cases, the concentrations of DTPA extracted metals followed the order of organic matter (Table 1) and consequently their concentrations were significant higher in the soil of the beech plot. A point worth mentioning is that the C/N ratio affected positively (apart from Cd). This means that decomposition rate was not as important for metals in terms of availability. This is reflected in the finding that the beech soil had higher concentrations of available metals although the C/N ratio was higher than that in the oak plot. Another finding was that the concentrations of available metals (apart from Ni) were positively related to the total metal concentration. That means that weathering is important to the enrichment of the available metals. The positive relation of clays with metals (apart from Cd) indicates the adsorption of metals to hydrous oxides, which are abundant in clay minerals.

4.2.2. Specific comments

Cd. Cadmium was the only metal the available concentration of which was not always higher in the soil of the beech plot (Table 4). Cd is more soluble than the rest metals and leaching might have occurred from the FH layer enriching the mineral layers. Removal of Cd due to the soil pH can be the cause. Krosshavn *et al.* (1993) found that as pH becomes lower, Cd is gradually removed from the soil organic matter and becomes more available to plants. Although it is known that Cd is negatively related to pH, in this work the relation was not significant (albeit negative as well). The positive relationship of available Cd with the CEC is a sign that the exchangeable Cd is important.

Cr. The availability of Cr depends on its chemical form. Cr has two oxidation numbers, III and VI, the last is the more toxic. The effect of soil pH on Cr sorption/desorption in soil varies with its chemical form. The desorption of Cr (III) from soil solid into solution is most important at low pH, while Cr (VI) adsorption on soil particles enhances with decrease in pH (Choppala *et al.,* 2016). The relation of Cr with organic matter is complex. Soils having high level of organic matter can create reduced condition and alter redox potential in soil via proliferation of the microorganisms. Cr (VI) is reduced to the less toxic Cr (III) (Shahid *et al.,* 2017). In our plots, this can probably happen in the FH layer of the soils where the percentage of organic C is high. Cr is readily adsorbed by hydrous oxides (Rai and Zachara, 1989) and this is the reason for the good relation with clay.

As. Arsenic forms anionic species in soils and many sequential extraction protocols applied to the arsenic fractionation are based on the fractionation of phosphorus, making use of the fact of a chemical similarity between the two elements (Dybowska *et al.,* 2005). For this reason, Cai *et al.* (2002) suggested the use of potassium phosphate (KH_2PO_4) to extract the available As fraction. In any case, the DTPA solution is still in use for As (Karak *et al.,* 2011; Marin *et al.,* 2001; Sadiq, 1986). In

our work As was negatively correlated with pH. One would expect the opposite as As is mobilized by high pH values (Moreno-Jiménez, 2012). However, these pH values should be approximately 8.0 to bring about these changes. Smedley and Kinniburgh (2002) showed that as pH increases, especially above pH 8.5, As desorbs from the Fe and Mn oxide surfaces, thereby increasing the concentration of As in solution. The pH values in the soils of the two plots in our work are far lower.

Ni. A characteristic of Ni in soils is its rapid removal of Ni from solution at low concentrations. This is due to sorption by any of the solid phases of soil: amorphous layer silicates; organic matter; hydrous oxides and carbonates (Uren, 1992). This is probably the explanation of the high correlation with the organic C and C.E.C. in our work. The lack of relation with the total Ni is probably due to its slow release by weathering.

Pb. A clear relationship between the available Pb and pH has not been found by all researchers. This element is more related to organic matter than the other soil properties (Davies, 1995). Nevertheless, Michopoulos (1999) found a significant and negative relationship between Pb concentrations extracted with EDTA and pH in a variety of forest soils under beech.
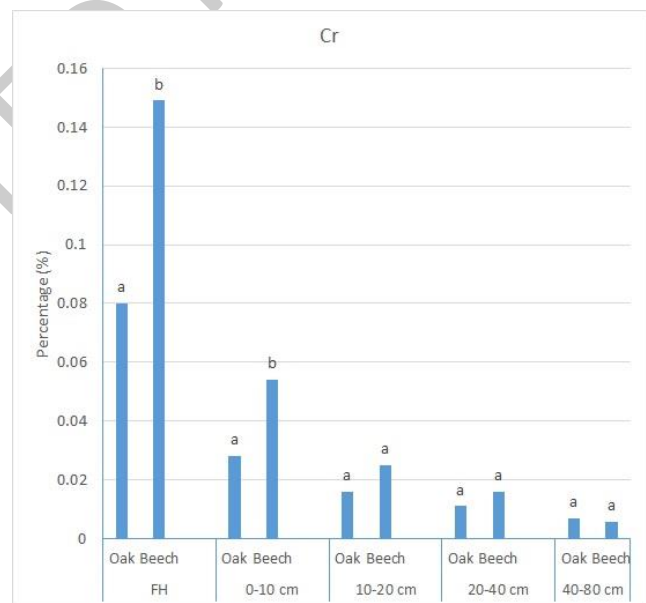

Figure 3. Percentages (%) of available Cr in soil layers. Bars with different letters denote significant difference for at least 0.05 probability level.

4.2.3. Percentages of available metals

In all the FH layers, the percentages of all metals were significantly higher in the beech plot (Figures 2-6). Rékási and Filem (2015) also found that the percentages for Cd, Ni and Pb were higher in acidic soils collected from the top mineral soils (0-20 cm), either arable or forested in Hungary. For Pb the percentages were significantly higher for all soil layers in the beech plot. This is probably due to two factors: the high affinity of Pb for the organic matter and the consistently higher concentrations of organic C in all layers of the beech plot. However, in our work the consistent difference mentioned above did not appear in

all mineral layers for the rest of the metal percentages. It can be concluded that additional factors (apart from pH) play an important role. The concentrations of metals bound to hydrous oxides and the magnitude of C.E.C. can also be influential. The CEC was higher in the 0-10 cm and 40-80 cm layers in the soil under oak so, to a certain degree, it can counterbalance the effects of pH.
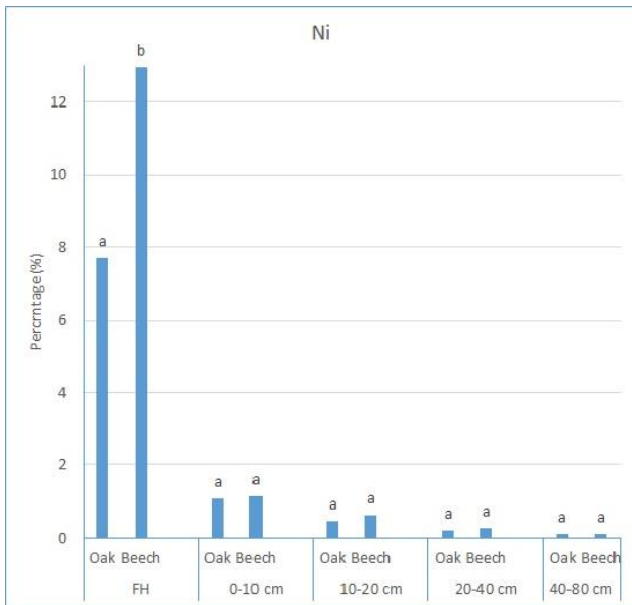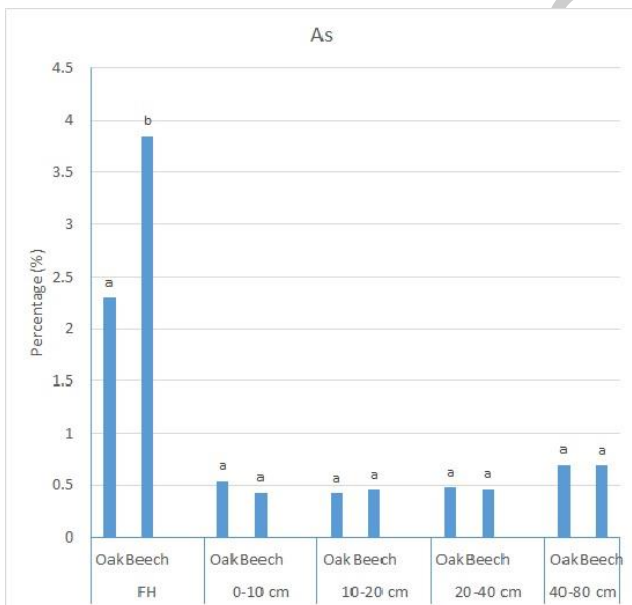

Figure 4. Percentages (%) of available Ni in soil layers. Bars with different letters denote significant difference for at least 0.05 probability level.

Figure 5. Percentages (%) of available As in soil layers. Bars with different letters denote significant difference for at least 0.05 probability level.

4.2.4. Uptake of metals by beech and oak trees

Cadmium was the only metal that had significantly higher concentrations in the beech and oak leaves despite the significant differences in the available amounts of the rest metals in the two plots. It seems that plants have defensive mechanisms that do not allow metals to

concentrate in leaves tissues. Different strategies developed by plants against metal toxicity include chelation of with ligands, and/or compartmentation of metals in the vacuoles (Marschner, 1989). Consequently, the metal uptake does not always agree with availability indices derived from soil analysis. Another point is that the concentrations of extracted metals with a chelating agent are far higher than those in the actual soil solution. For example, the percentages of available Pb in the beech soil in our work ranged from 6.3 to 12.8%. In contrast, Davies (1995) reported a range of 0.02 to 0.13% in soil solution in a variety of soils. The soil analysis is necessary, however, because it shows where problems may occur in future times. In any case, the Cd concentration in beech leaves in our work is low. It is within the range that Bezlova *et al.* (2016) found for Cd in beech leaves in the Central Balkan National Park, in Bulgaria.

5. Conclusions

The parameters selected explained a satisfactory percentage for all available metals in the soils of the two forest plots. The pH and organic matter in the beech plot played an important role in accumulating higher metal content than the oak plot. The availability indices cannot always explain the differences in metal uptake by trees but they are useful to predict future environmental changes.
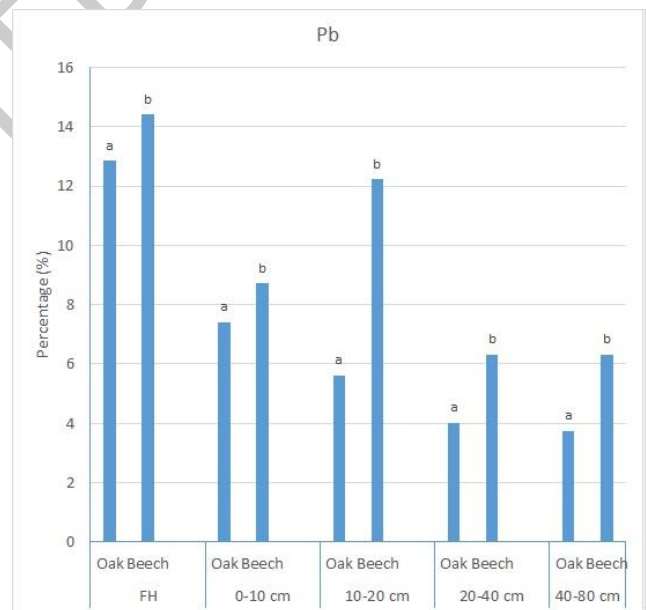

Figure 6. Percentages (%) of available Pb in soil layers. Bars with different letters denote significant difference for at least 0.05 probability level.

invaluable practical assistance with all the problems encountered.

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