

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

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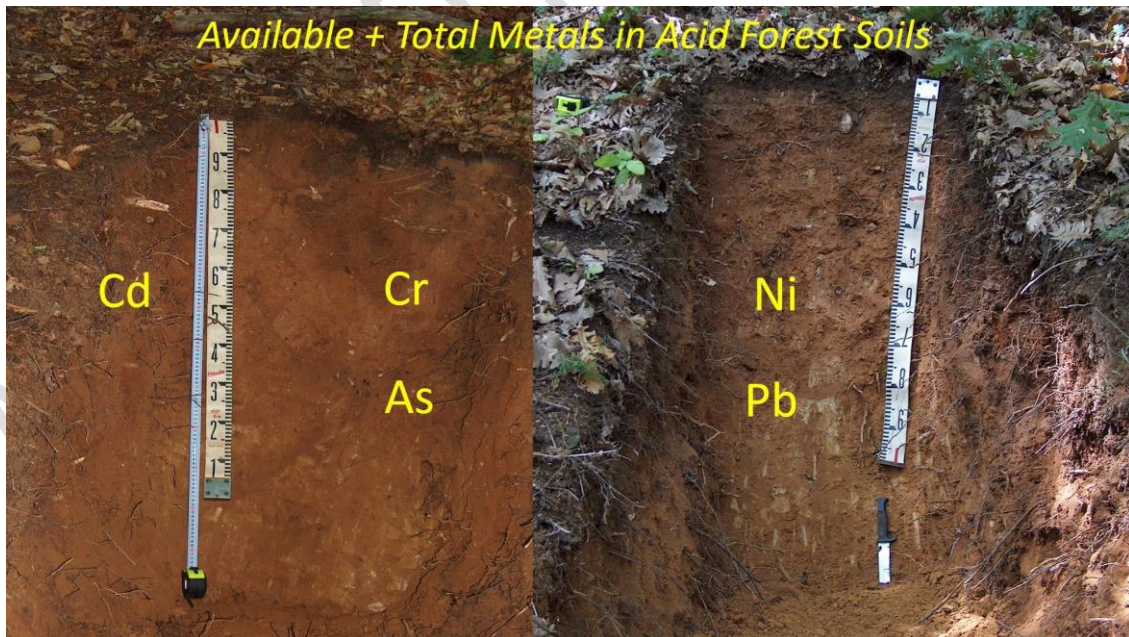
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19 GRAPHICAL ABSTRACT

20



25 **Abstract**

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

41 **Keywords**

42 Metals, soil, DTPA extraction, beech, oak,

43

44 **1. Introduction**

45 Cadmium, Cr, Ni and Pb are among metals together with As, a metalloid, of great interest in
46 bioavailability studies (due to their toxicity), as listed by the U.S. Environmental Protection Agency
47 (EPA) (McKinney and Ron, 1992). Kim *et al.* (2015) defined three terms of metal availability.
48 Environmental availability, which includes the total amount of heavy metal in the soil, (2)

49 environmental bioavailability, i.e. the amount of dissolved fraction in which can be taken up by
50 plant roots or other soil organisms, and (3) toxicological bioavailability, i.e. the amount of heavy
51 metal which can physiologically induce bioaccumulation or other effect within the plant. Soil
52 scientist are mainly interested in the environmental bioavailability. A great deal of experiments
53 concerns the concentrations of heavy metals extracted with a single extractant. For instance, neutral
54 salts are supposed to displace the readily soluble metal fraction from the exchangeable sites into soil
55 solution. In contrast, organic chelating agents such as DTPA and EDTA are thought to behave as
56 the organic exudates produced by plants, capable of removing metals from various soil fractions
57 such as organic matter, carbonates and metal oxides (Fang *et al.*, 2007). DTPA can be used in both
58 alkaline and acid soils and for this reason many researchers have used it to predict bioavailability of
59 heavy metals and trace elements in a variety of soils (Chavda, 2018; Evanylo and Sukkariyah, 2006;
60 Nunes *et al.*, 2014).

61 Environmental pollution by heavy metals is the result from many sources such as industrial
62 activities, power generation, transport (especially in urban environments) and agriculture (Tipping
63 *et al.*, 2006). Forests are sinks of heavy metals due to the high capacity of their soils (especially of
64 the forest floor) to retain metals (Brumelis *et al.*, 2002; Suchara and Sucharová, 2002). It is quite
65 logical that many studies have focused on the effects of heavy metals on forests located nearby of
66 such sources (Pietrzykowski *et al.*, 2014; Michopoulos *et al.*, 2005). However, to a lesser extent,
67 even remote forests can be affected as well. This is due to the atmosphere, which serves as a
68 transport means for metals in long distances (Steiness and Friedland, 2005).

69 The aim of this work was to compare the concentrations of total and available heavy metals Cr, Ni,
70 Cd and Pb as well as the metalloid As in two acid forest soils under oak and beech and assess the
71 dependency of their availability on certain soil properties. Moreover, it was decided to find the
72 heavy metal concentrations in the leaves of the two main species. These forests are remote from
73 industrial activities and can serve as a baseline for future comparisons with other forests. So far,

74 there has been only one work (Michopoulos *et al.*, 2018) concerning heavy metals concentrations in
75 a remote fir forest in Greece. It is the first time that a work deals with acid forest soils in which
76 metals are expected to be more mobile. In this respect, the findings of the present work will add to
77 our knowledge.

78

79 **2. Materials and Methods**

80

81 *2.1. Site description*

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

88 *2.2. Soil samples collection*

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

99 pulverized in a ball mill for the aim of total analysis for organic C, total N and total heavy metals.
100 Like the plant tissues in litterfall, soil samples were dried at 80 °C for 48 hours.

101 2.3. *Collection of leaves*

102 Leaves samples of both species were collected every two years in summer from the upper part of
103 the crown from five dominant trees and formed a pooled sample. The collection always took place
104 from the same trees. The leaves samples were dried at 80 °C for 48 h and then ground in a special
105 mill for analysis. The data covers three collection periods, i.e. 2015, 2017 and 2019. In total, there
106 were three pooled samples per leaf type.

107 2.4. *Chemical analysis*

108 2.4.1. *Soil*

109 The soil pH was determined by a glass electrode in a mixture of soil and 0.01 M CaCl₂ at a ratio of
110 1:5 (v:v). The soil texture was measured by the pipette method. The organic C and total N were
111 determined by a CN analyzer through dry combustion.

112 Exchangeable cations in the FH and mineral soil layers were extracted with a 0.1 M unbuffered
113 BaCl₂ solution and their concentrations were determined with an ICP-MS instrument (Thermo
114 iCAP Qc). Cation exchange capacity (C.E.C.) was found by adding the calculated exchangeable
115 cations.

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

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

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

125 The limits of quantifications (LOQs) for the total Cd, Cr, Ni, As and Pb were 8.3, 251, 274, 21.6
126 and 48.5 $\mu\text{g kg}^{-1}$, whereas for the DTPA extractable ones the LOQs were 0.38, 2.6, 8.0, 1.2 and 5.6
127 $\mu\text{g kg}^{-1}$, respectively.

128 2.4.2. Leaves

129 Ground material of leaves samples were digested in a mixture of $\text{HNO}_3\text{-HClO}_4$ in a proportion of
130 2:1 (v:v) and the metal concentrations were determined with the ICP instrument.

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

132

133 2.5. Calculations and statistics

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

141 The crustal enrichment factors (EF) of elements were calculated using the the equation: EF_c
142 $= (C_x/C_{Al})_{\text{sample}} / (C_x/C_{Al})_{\text{continental crust}}$. Dantu (2009) used this equation taking into account
143 continental crust values derived from Taylor and McLennan's (1995). However, the lowest soil
144 layers can also be used as reference values (Blaser *et al.*, 2000). In our work, we used the 40-80 cm
145 one. The layer examined for enrichment was the first mineral one, i.e., 0-10 cm.

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

150 **3. Results**

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

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

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

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

162 Table 4 contains the concentrations of the available metals. The concentrations were significantly
163 higher in the beech soil in all layers apart from Cd in the 0-10 cm, 10-20 cm and 20-40 cm ones.

164 The results of Figure 1 depicting the PCA axes and the parameters (as circles) are combined with
165 Table 5. The component 1 represents the horizontal axis of Figure 1, whereas the component 2 the
166 vertical one. The first principal component is the linear combination of x-variables that has
167 maximum variance (among all linear combinations). It accounts for as much variation in the data as
168 possible. As the first component explains the maximum variability, Table 5 contains only the values

169 derived from the first component's calculations. Values around 0.600 or higher are considered
170 satisfactory as they explain at least 40 % of the variability of the parameters (Comrey, 1962).

171 Therefore, from Table 5 the parameters we had the following results:

- 172 • All metals have satisfactory and positive relationship with the total heavy metal
173 content apart from Ni.
- 174 • All metals had satisfactory and positive relationship with the organic C content apart
175 from Ni.
- 176 • All metals had satisfactory and positive relationship with the ratio C/N apart from Cd
- 177 • All metals had satisfactory and negative relationship with the pH apart from Cd
- 178 • Two metals have a positive relationship with the C.E.C., Cd and Ni
- 179 • The total variance explained was higher for Pb than in any other metal.

180

181 The percentages (%) of the available metals (Figures 2-6) for Pb were higher in all layers (starting
182 from the FH one) in the beech plot. For Ni and As the concentrations were also higher in the beech
183 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
184 different behavior. In the FH layer, the concentrations were lower in the oak plot but higher in the
185 0-10 cm in the same plot. Finally, in the 40-80 cm it was higher in the beech plot.

186

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

190

191

192

193 4. Discussion

194 4.1. Total concentrations of metals in soils

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

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

214 The baseline concentrations of As in soils are generally of the order of 5–10 mg kg⁻¹ (Smedley and
215 Kinniburgh, 2002). However, higher values have been found in mineral uncontaminated soils (10-

216 24 mg kg⁻¹) of forests (Huang and Matzner, 2007). In our work the As concentration ranged from
217 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.

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

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

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

235 4.2. Availability

236 4.2.1. General comments

237 The PCA disclosed some relations of available metals with the other soil properties (Figure 1 and
238 Table 5), which help explain the comparisons of available metal concentrations in Table 3 between
239 the two plots. First, the percentages that the parameters chosen to enter the PCA explained a

240 satisfactory percentage for all metals especially for Pb. Nearly all the DTPA extracted metals had a
241 negative relation with pH and a positive one with organic C and clay content. Under the aerobic and
242 acidic to near-neutral conditions typical of many forest environments, heavy metals are strongly
243 adsorbed by oxide minerals. The H protons can dissolve some oxides thus releasing metals in the
244 soil solution. The lower the pH the more active the H protons are. The organic matter played a
245 significant role. In the large majority of cases, the concentrations of DTPA extracted metals
246 followed the order of organic matter (Table 1) and consequently their concentrations were
247 significant higher in the soil of the beech plot. A point worth mentioning is that the C/N ratio
248 affected positively (apart from Cd). This means that decomposition rate was not as important for
249 metals in terms of availability. This is reflected in the finding that the beech soil had higher
250 concentrations of available metals although the C/N ratio was higher than that in the oak plot.
251 Another finding was that the concentrations of available metals (apart from Ni) were positively
252 related to the total metal concentration. That means that weathering is important to the enrichment
253 of the available metals. The positive relation of clays with metals (apart from Cd) indicates the
254 adsorption of metals to hydrous oxides, which are abundant in clay minerals.

255 4.2.2. *Specific comments*

256 Cd

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

264 Cr

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

274 As

275 Arsenic forms anionic species in soils and many sequential extraction protocols applied to the
276 arsenic fractionation are based on the fractionation of phosphorus, making use of the fact of a
277 chemical similarity between the two elements (Dybowska *et al.* 2005). For this reason, Cai *et al.*
278 (2002) suggested the use of potassium phosphate (KH_2PO_4) to extract the available As fraction. In
279 any case, the DTPA solution is still in use for As (Karak *et al.*, 2011; Marin *et al.*, 2001; Sadiq,
280 1986). In our work As was negatively correlated with pH. One would expect the opposite as As is
281 mobilized by high pH values (Moreno-Jiménez, 2012). However, these pH values should be
282 approximately 8.0 to bring about these changes. Smedley and Kinniburgh (2002) showed that as pH
283 increases, especially above pH 8.5, As desorbs from the Fe and Mn oxide surfaces, thereby
284 increasing the concentration of As in solution. The pH values in the soils of the two plots in our
285 work are far lower.

286 Ni

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

292 Pb

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

297 *4.2.3 Percentages of available metals*

298 In all the FH layers, the percentages of all metals were significantly higher in the beech plot
299 (Figures 2-6). Rékási and Filem (2015) also found that the percentages for Cd, Ni and Pb were
300 higher in acidic soils collected from the top mineral soils (0-20 cm), either arable or forested in
301 Hungary. For Pb the percentages were significantly higher for all soil layers in the beech plot. This
302 is probably due to two factors: the high affinity of Pb for the organic matter and the consistently
303 higher concentrations of organic C in all layers of the beech plot. However, in our work the
304 consistent difference mentioned above did not appear in all mineral layers for the rest of the metal
305 percentages. It can be concluded that additional factors (apart from pH) play an important role. The
306 concentrations of metals bound to hydrous oxides and the magnitude of C.E.C. can also be
307 influential. The CEC was higher in the 0-10 cm and 40-80 cm layers in the soil under oak so, to a
308 certain degree, it can counterbalance the effects of pH.

309 *4.2.4. Uptake of metals by beech and oak trees*

310 Cadmium was the only metal that had significantly higher concentrations in the beech and oak
311 leaves despite the significant differences in the available amounts of the rest metals in the two plots.
312 It seems that plants have defensive mechanisms that do not allow metals to concentrate in leaves
313 tissues. Different strategies developed by plants against metal toxicity include chelation of with
314 ligands, and/or compartmentation of metals in the vacuoles (Marschner, 1989). Consequently, the
315 metal uptake does not always agree with availability indices derived from soil analysis. Another
316 point is that the concentrations of extracted metals with a chelating agent are far higher than those
317 in the actual soil solution. For example, the percentages of available Pb in the beech soil in our
318 work ranged from 6.3 to 12.8%. In contrast, Davies (1995) reported a range of 0.02 to 0.13% in soil
319 solution in a variety of soils. The soil analysis is necessary, however, because it shows where
320 problems may occur in future times. In any case, the Cd concentration in beech leaves in our work
321 is low. It is within the range that Bezlova et al. (2016) found for Cd in beech leaves in the Central
322 Balkan National Park, in Bulgaria.

323

324 **5. Conclusions**

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

329

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337

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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 cmol_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	11.8 a	16.3 a	3.74 a	16.1 a

		(1.2)	(3.7)	(2.0)	(8.1)	(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 (%)

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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	193 b	59.4 b	10.7 b	29.0 b

		(25)	(6.3)	(5.3)	(4.4)	(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 b (5.3)	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 (%)

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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 (%)

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Table 4 Concentrations ($\mu\text{g kg}^{-1}$) 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 (%)

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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

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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 (%)

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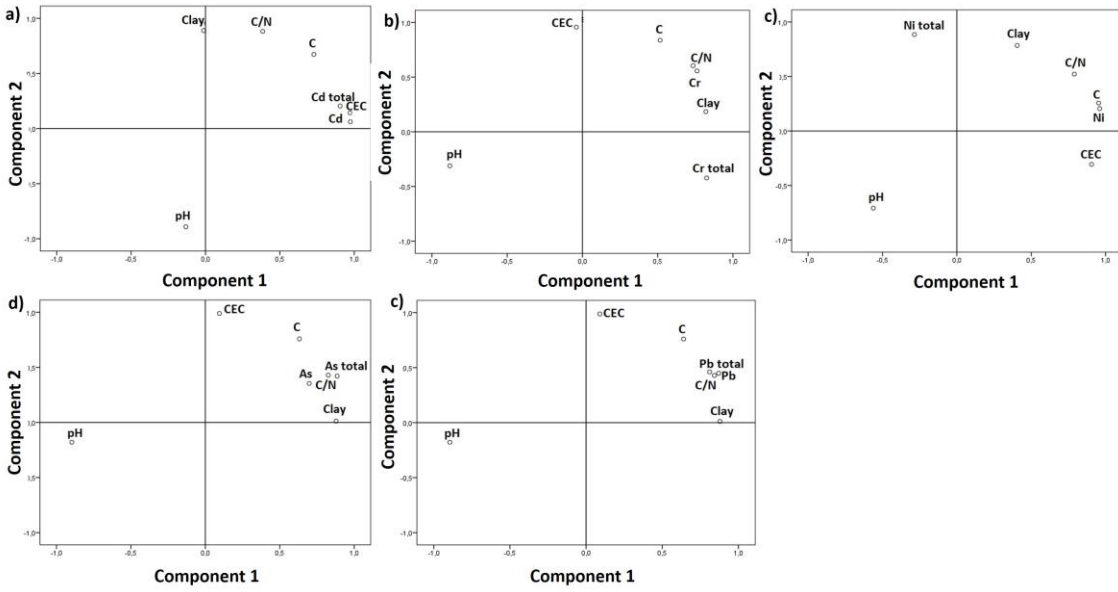
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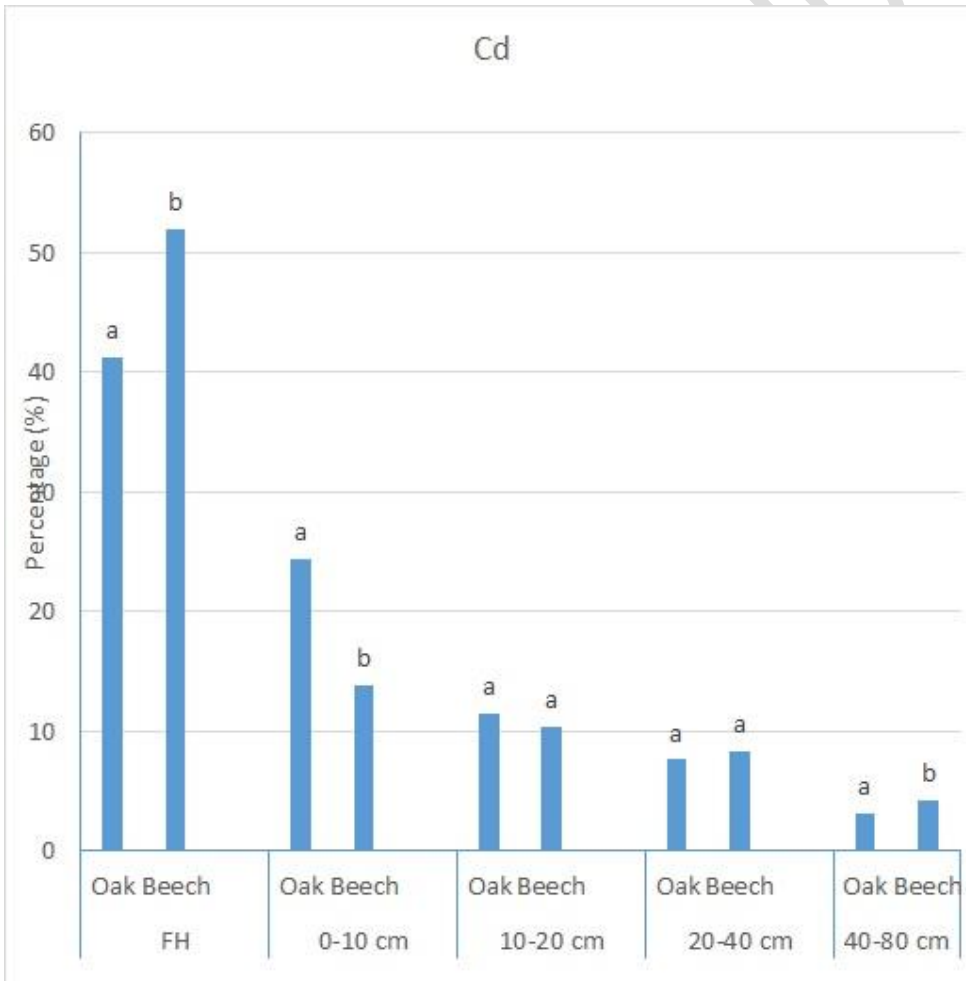
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Figure Captions



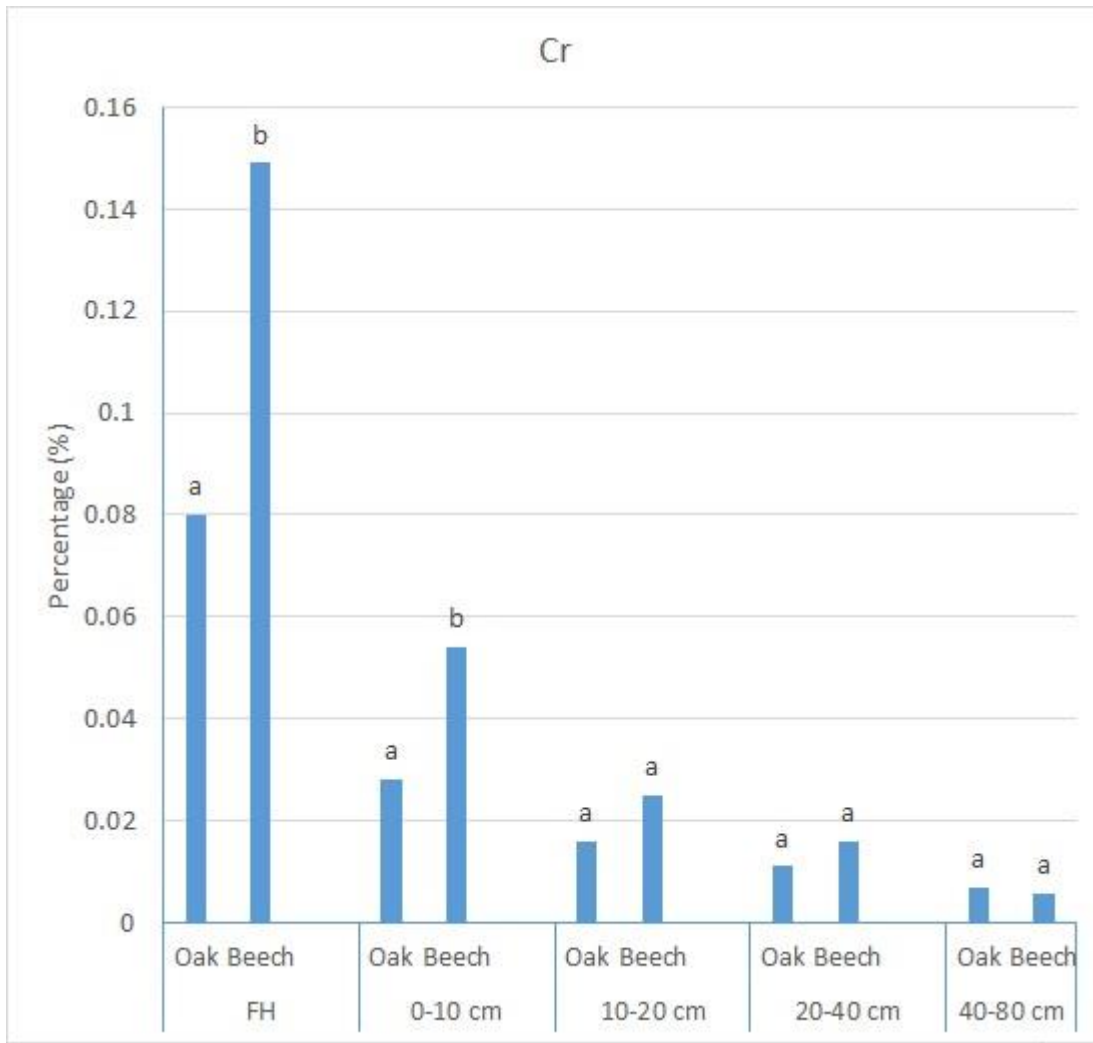
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556 Figure 1. PCA ordination diagram for heavy metals and As



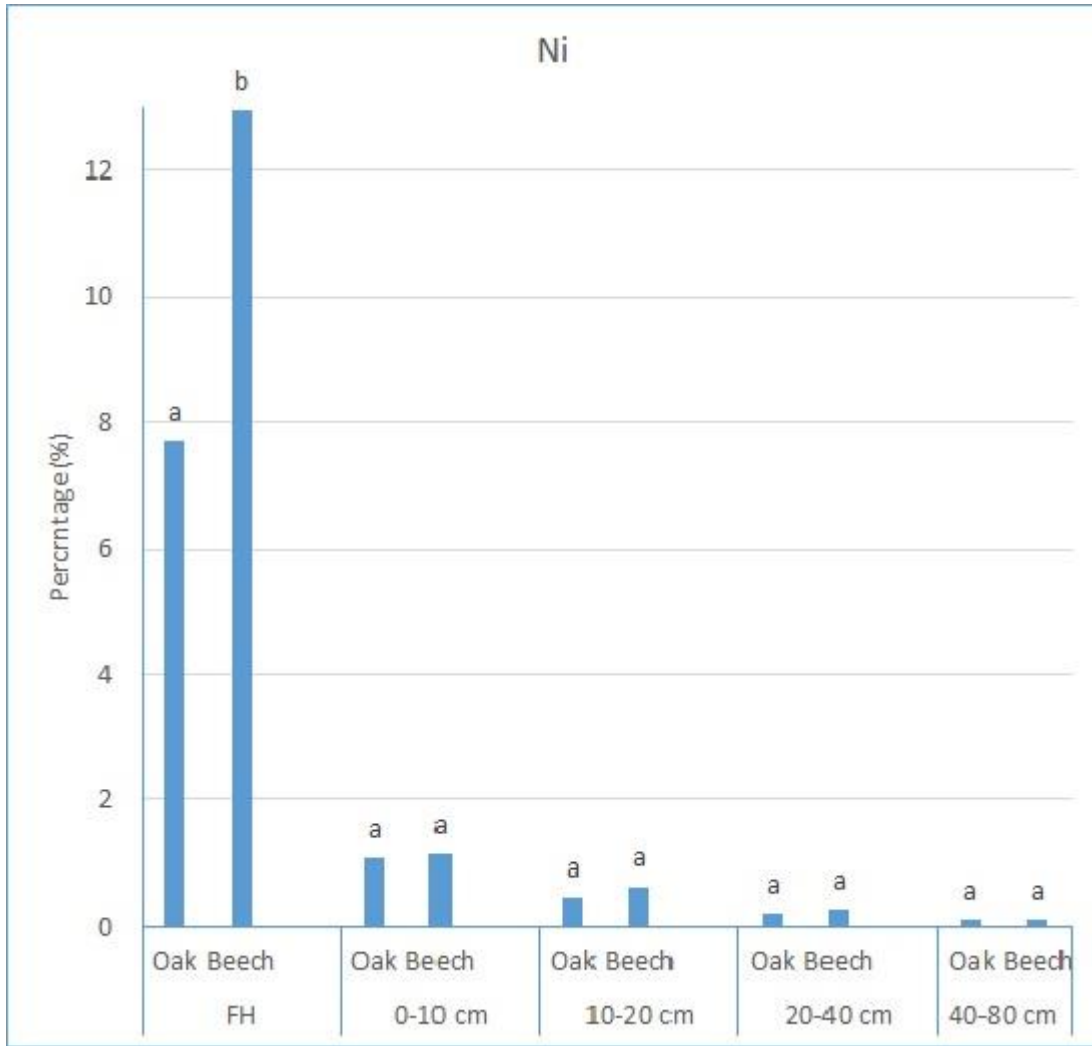
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558 Figure 2. Percentage (%) of available Cd in soil layers. Bars with different letters denote significant
559 difference for at least 0.05 probability level



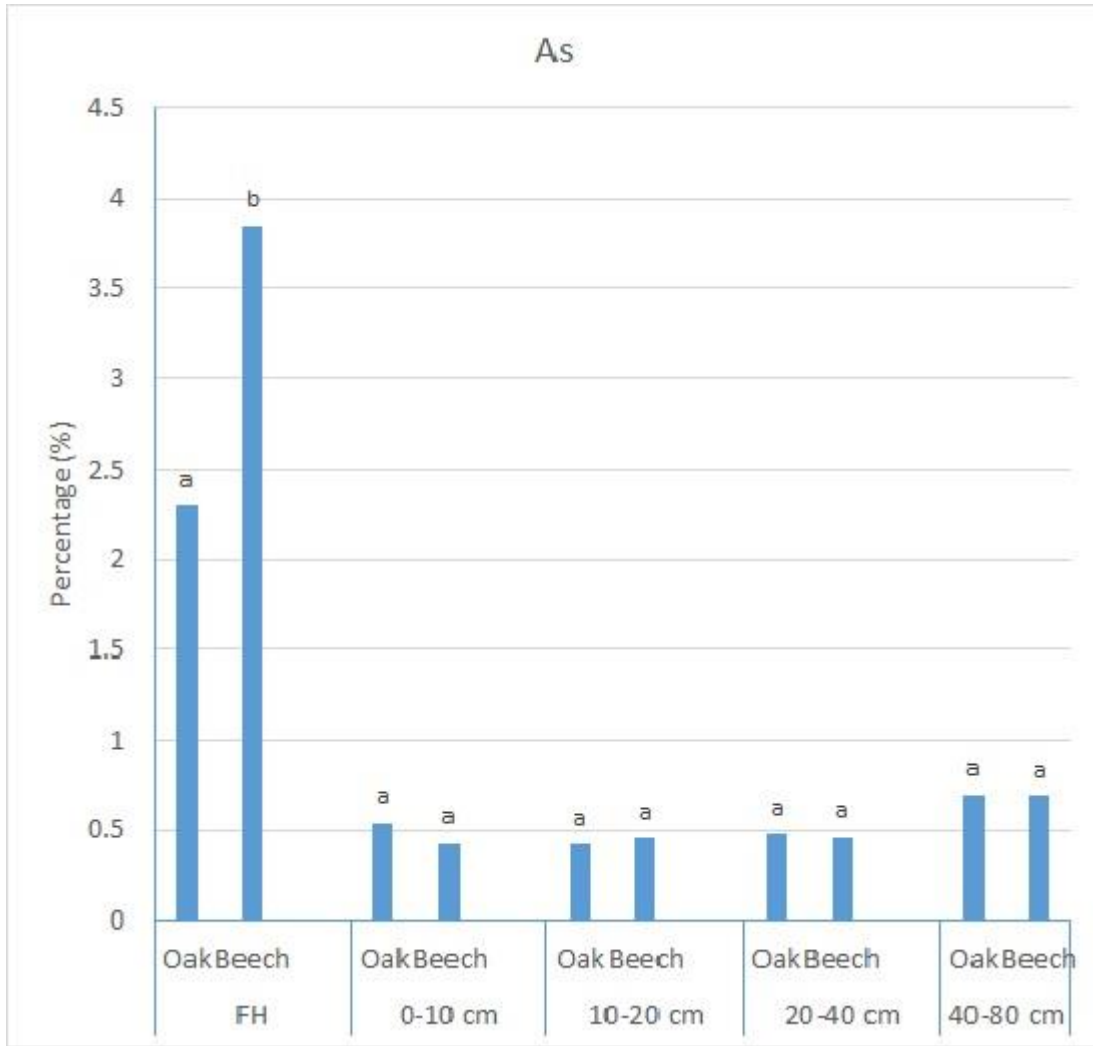
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561 Figure 3. Percentages (%) of available Cr in soil layers. Bars with different letters denote significant
 562 difference for at least 0.05 probability level



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

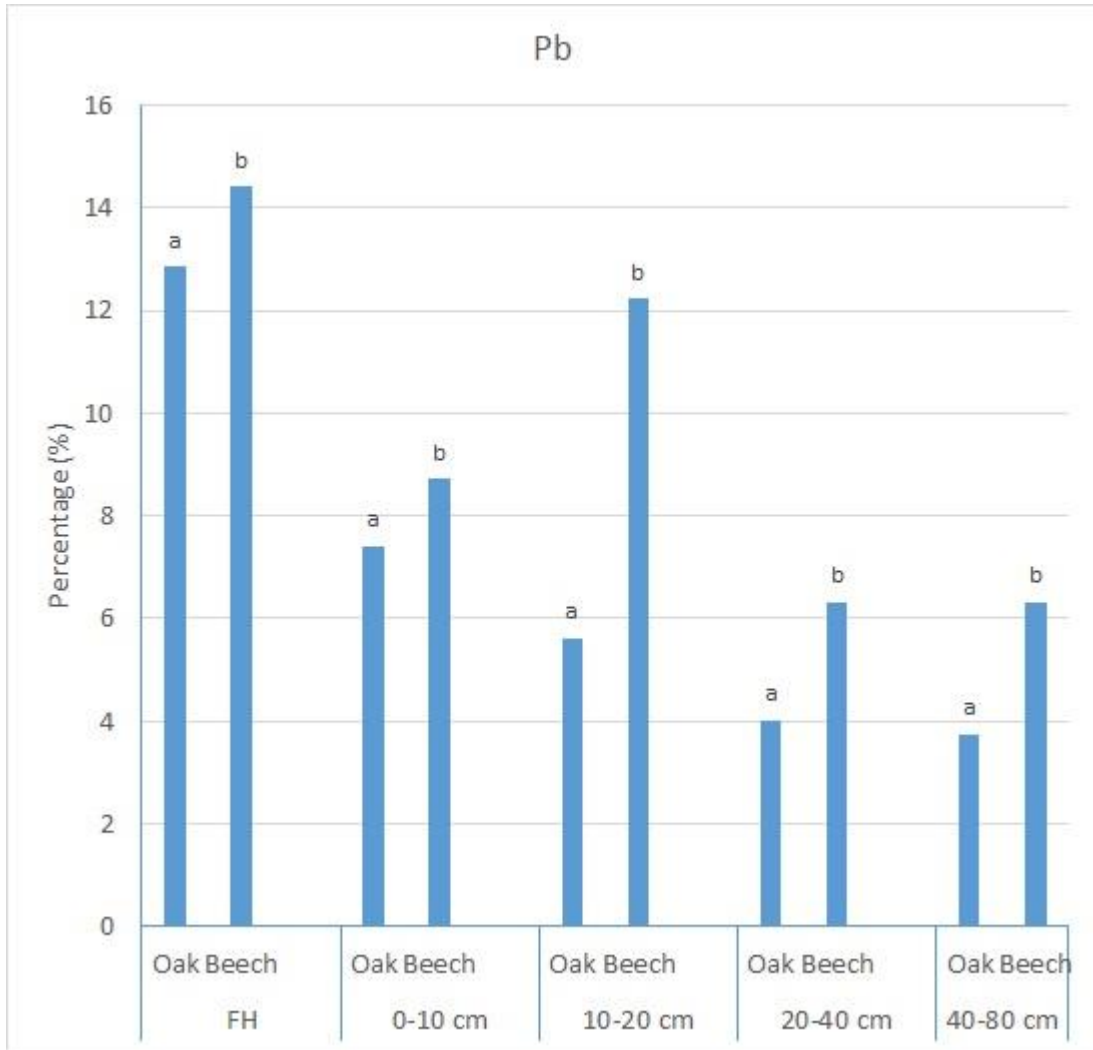


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Figure 5. Percentages (%) of available As in soil layers. Bars with different letters denote significant difference for at least 0.05 probability level



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

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