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Abstract

The range of metals used for industrial purposes - electrical engineering, solar panels, batteries - has increased substantially over the last twenty years. Some of these emerging metals are the subject of geopolitical conflict and are considered critical as their unique properties make them irreplaceable. Many of these elements are poorly studied and their biogeochemical cycles still raise many questions. Aim of this study is to analyse the soil-to-plant transfer of some of these chemical elements and to shed light on their uptake pathways.

For this purpose, the geological site of Jas Roux (France) was chosen as this alpine site is naturally rich in critical and potentially toxic elements such as As, Sb, Ba and Tl, but nevertheless is host to a high diversity of plants. Elemental concentrations were analysed in the topsoil and in 12 selected alpine plant species sampled in situ. Statistical tools were used to detect species dependent characteristics in elemental uptake.

Our analyses revealed accumulation of rare earth elements by *Saxifraga paniculata*, selective oxyanion absorption by *Hippocrepis comosa*, accumulation of Tl by *Biscutella laevigata* and *Galium corrudifolium* and an exclusion strategy in *Juniperus communis*. These findings advance our understanding of the environmental behaviour of critical metals and metalloids such as V, As, Y, Sb, Ce, Ba and Tl and might bare valuable information for phytoremediation applications.

Uptake patterns of critical metals in alpine plant species growing in an unimpaired natural site

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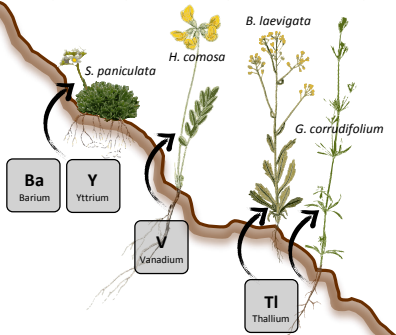
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Graphical Abstract *Alpine plant species exposed to a cocktail of critical metals*



Diverse species-related strategies

- *Saxifraga paniculata*
 - selective cation absorption
 - possible link between Y, Ba and Ca uptake
- *Hippocrepis comosa*
 - selective oxyanion absorption
 - possible link between V and Mo, Fe uptake
- *Galium corrudifolium* & *Biscutella laevigata*
 - Tl accumulation

Highlights:

- Critical metals: a need to understand their biogeochemical cycles
- Field-study of a plant community exposed to a natural cocktail of metals(oids)
- Multivariate analysis of elemental concentrations revealed species related uptake
- *S. paniculata* and *H. comosa* accumulating a different set of critical metals
- Observation of possible links in the soil/plant transfer of Mo/V/Fe and Y/Ca

1 **1 Introduction**

2 All over the world, emerging technologies require an increasingly diverse spectrum of chemical
3 elements with highly specific (photo-optical, catalytic, magnetic, etc.) properties (Celis et al.,
4 2020). Metals that have practically never been used before are now indispensable for many
5 industrial sectors and are thus the subject of soaring global demand. Due to their economic
6 importance and their supply risk, many of these metals are now classified as "critical" for high
7 tech applications (European Commission, 2020), examples being Sb in lead-acid batteries and
8 semiconductors, As in cell phones and solar cells and rare earth elements (REEs) in sensors,
9 displays and electric motors (Fortier et al., 2018; Mudd et al., 2018).

10 Despite their increased use, the biogeochemical cycles of many of these critical metals are not
11 yet fully understood and predicting either their environmental behaviour or their ecotoxicity is
12 consequently impossible (Espejo et al., 2018). Aim of this study is to analyse the soil-to-plant
13 transfer of some of these metals and to shed light on their uptake pathways. Our findings might
14 bare valuable information for phytoremediation and promote plant-based remediation
15 techniques of metal-contaminated sites. Phytoremediation techniques are relying on the
16 capacity of certain plant species to limit the uptake of the toxic elements or to accumulate them
17 in designated compartments (e.g. phytostabilisation, phytoextraction) (Favas et al., 2014).

18 Exclusion and accumulation are the two main strategies of plants to maintain homeostasis in
19 metal-rich habitats. Both involve a series of adaptations such as modified membrane transport,
20 chelation and complexation, or rhizosphere interactions for example (Furini, 2012).

21 The ability of plants to accumulate metals in their aerial parts has received considerable
22 attention and many combinations of specific plant species tolerating or accumulating a
23 particular set of elements have been discovered (Baker et al., 2010; Pollard et al., 2014).

24 However, most studies have focused on "traditional" metals such as Zn, Ni, Cd and Pb, whereas
25 the uptake pathways and metabolic impact of many elements, particularly emerging critical
26 metals, still raise many questions (Macnair, 2003; van der Ent et al., 2013).

27 Elemental uptake is influenced by chemical, physical, and biological factors. Plant species, root
28 system, root exudates, edaphic interactions, and transport mechanisms all play important roles
29 (Tangahu et al., 2011; Trakal et al., 2015; Yadav, 2010). Despite highly specialised proteins,
30 including transporters, pumps and channels, elemental selectivity remains challenging for
31 plants and each uptake mechanism is likely to let pass a range of ions due to chemical
32 similarities between the different ions (Tangahu et al., 2011). The huge number of factors and
33 complex interactions are impossible to reproduce in the laboratory, thus, to understand the

34 uptake of poorly studied elements, such as critical metals, performing in-situ studies in a natural
35 environment is advantageous.

36 The geological site of Jas Roux, located in the Ecrins National Park (Hautes-Alpes, France),
37 was chosen for the study. The distinctive reddish colour of the Jas Roux outcrops ('Roux'
38 means red) originates from a sulphosalt-forming hydrothermal mineralisation (Johan and
39 Mantiene, 2000). These sulphosalts are one of the reasons for the characteristic metal
40 concentrations of Jas Roux as they are rich in elements with a strong affinity for S, such as Pb,
41 Sn, Cu, Tl and As (Dittrich et al., 2009; Pierrot and Roger, 2018). Many of the elements found
42 in Jas Roux are used in high-tech applications and are considered to be critical for leading
43 technologies (Dittrich et al., 2009; Graedel et al., 2015). This site is thus a good proxy of chronic
44 contamination by a cocktail of metals and metalloids. As the site has never been disturbed by
45 any human activities and is located in the heart of the Ecrins National Parc, it offers ideal
46 conditions to study an established ecosystem with a plant community that has not undergone
47 human selection. The plant community can be seen as natural response to the local mineralogy
48 and may contain candidates for phytoremediation.

49 To identify such plant candidates and to study the environmental behaviour of critical metals,
50 we were guided by the following three questions:

51 To what extent are critical metals absorbed by plants?

52 Are there any species-related differences in elemental uptake?

53 Do correlations exist between critical metals and other elements in different plant parts?

54 The approach of this study was to conduct a large-scale screening of 12 plant species to identify
55 individual uptake patterns using chemical analysis, soil element extraction and statistical tools.

56 The large number of species analysed provided insights into the actual elemental
57 phytoavailability of a wide range of critical metals.

58 **2 Material and Methods**

59 **2.1 Sampling Site**

60 The geological site of Jas Roux, located in the Ecrins National Park in the district of La
61 Chapelle-en-Valgaudemar (Hautes-Alpes, France), is situated at 2170 m a.s.l. (44°48' 45" N, 6°
62 19' 18" E; WGS84) on the right-hand side of the Chabournéou stream (Johan and Mantiennne,
63 2000). Jas Roux is a hydrothermal stratiform deposit in dolomite limestone and has been
64 characterised as a sulphosalt site (Boulliard et al. 2010). The hydrothermal system was possibly
65 generated by volcanic activity involving relatively low temperatures (around 100 °C) and high
66 pressure (Johan and Mantiennne, 2000). The particular Tl-rich composition of Jas Roux has
67 attracted many mineralogists and geologists, who have identified Zn, Sb, As, Tl, Ag, Hg, Pb
68 sulphides and sulphosalts including many new sulphosalt mineral species (Boulliard et al.,
69 2010; Guillemin et al., 1970; Mantiennne, 1974; Topa et al. 2017, 2014).

70 The study zone is located below one of the reddish coloured outcrops identified by Mantiennne
71 (**Figure SM 1**) (Mantiennne, 1974). The metal-rich mineralisation is best developed in this
72 outcrop and forms a 3.8 m thick barite-rich zone (Johan and Mantiennne, 2000). The study zone
73 measured 20 m x 5 m with a slope of approximately 45° (100%) (**Figure SM 1**).

74 Soil and plant samples were collected during two field trips, one in September 2017 and one in
75 June 2018. The steep slope, variable soil depth and the presence of large rocks impeded a
76 systematic sampling approach. Nevertheless, the samples were collected as evenly distributed
77 as possible. The soil samples were collected from 10 sampling points in the study zone and
78 consisted of 1 kg of topsoil each (< 15 cm). Among the 65 plant species identified in the study
79 zone, 12 were selected, taking into account occurrence, spatial distribution and family
80 affiliation (**Table 1**). For improved readability we attributed acronyms to each of the 12 species
81 throughout the paper (**Table 1**). Depending on their abundance, between 2 and 7 replicates of
82 each species were sampled, giving a total of 55 individuals (**Table 1**). All the samples were
83 stored at 4 °C in the dark.

84

85 **2.2 Sample preparation**

86 Roots and aerial parts were separated at the collar and cleaned with 18.2 MΩ·cm ultrapure
87 water. The plant roots were additionally brushed and vortexed in ultrapure water to remove
88 adhering soil particles. The leaves of *Sax* were also brushed as they were partially covered with
89 a calcite crust. Plant samples were dried at 40 °C for 48 h. Soil samples were dried at 40 °C for
90 72 h and sieved at 2mm before analysis.

91 All the soil and plant samples were ground at 20hz in a Retsch® Mixer Mill MM 400 for 2 min
92 to obtain a fine powder. Each sample (100mg) was solubilised in acid using a Milestone
93 UltraWAVE® microwave to complete mineralisation. The acids used for the digestion of the
94 plant samples were 4mL of HNO₃ and 2mL of H₂O₂. For the soil samples, 3mL of HNO₃, 3mL
95 of HCl and 0.5 mL of HF were used to measure the total metal concentrations. Soil pH and
96 conductivity were determined in a suspension of 1g of sieved soil (< 2mm) and 5mL of
97 ultrapure water according to the French standard X31-117 (*NF X31-117*, 1994). After 5 min of
98 agitation at 80 rpm and 2 h of rest, measurements were taken using a Hanna HI-2550 bench
99 meter. To estimate the mobile and phytoavailable elemental fractions in the soil, CaCl₂
100 (0.001mol/L) and NH₄NO₃ (1mol/L) extractions have been conducted, according to standards
101 ISO 21268-2 and ISO 19730 (*ISO 19730:2008*, 2009; *NF X31-607-2*, 2004). For the CaCl₂
102 extraction, a liquid-solid ratio of 10:1 (20mL of ultrapure water and 2g of soil) was applied.
103 After 24 h of agitation at 10 rpm, the samples were centrifuged at 2500xG for 20 min, filtered
104 at 0.45 µm and analysed in ICP-MS. For the NH₄NO₃ extraction, 4g of soil were mixed with
105 10mL of ultrapure water, agitated for 2 h at 30 rpm, centrifuged at 1000xG for 15 min, filtered
106 at 0.45 µm and analysed in ICP-MS.

107 Soil mineralogy was analysed by X-ray diffraction using the X'Pert Pro MPD (XRD) equipped
108 with a Cobalt x-ray tube (= 1.79Å) running at 40 kV and 40 mA.

109 A mixture of three bulk samples of topsoil was sent to an outside laboratory (CIRAD – US49
110 Laboratoire d'analyse de Montpellier, France) for the determination of soil parameters. The soil
111 type was determined using 5 granulometric fractions according to the French standard X31-107
112 (*NF X31-107*, 2003), organic matter by combustion analysis (Dumas), labile P content using a
113 colorimetric method (Olsen) and cation exchange capacity by quantifying the dosing adsorption
114 agent [Co(NH₃)₆]Cl₃ with inductively coupled plasma atomic emission spectroscopy according
115 to the French standard X31-130 (*NF X31-130*, 1999).

116

117 **2.3 Chemical analyses**

118 The samples were analysed by inductively coupled plasma mass spectrometry (ICP-MS, Perkin
119 Elmer NexIon 300X). For the elements Mg, Al, P, K, Ca and S, the results of inductively
120 coupled plasma optical emission spectroscopy (ICP-OES) were occasionally used to reach
121 higher accuracy (Perkin Elmer 4300 DV). The calibration curves were established using multi-
122 solution standards CCS4, CCS5, CCS6 from Inorganic Ventures (New Jersey USA) diluted in
123 2% HNO₃ solution. The internal standards were Rh103 for ICP-MS and Y for ICP-OES. Oak
124 leaves (V464) provided by the French National Institute for Agriculture Food and Environment

125 (INRAE) were used as the external reference for plant samples. For the soil, a rendzina sample
126 (ISA 979) provided by Wageningen Evaluating Programs for Analytical Laboratories
127 (WEPAL) was used. EnviroMAT drinking water EP-L-3 and subterranean water ES-H-2 (SCP
128 Sciences) were used as internal references and for quality control.

129 ICP analyses aimed to quantify critical elements (As, V, Y, Sb, Ba, Ce, Tl) and major soil
130 elements and nutrients (Mg, Al, P, S, K, Ca, Mn, Fe, Ni, Cu, Zn, Mo) present in Jas Roux. The
131 critical elements were chosen based on the study on the EU's list of critical raw materials and a
132 review of critical minerals and will be called critical metals throughout the manuscript
133 (European Commission, 2020; Hayes and McCullough, 2018). The detection limit for each
134 element was determined using handling blanks analysed at each ICP analysis (**Table SM 1**).
135 The coefficients of variances for all certified elements were calculated for the internal and
136 external standards (**Table SM 1**).

137 The translocation factor (TF) was calculated to identify the transfer strategies of the different
138 plant species. The TF is obtained by dividing the elemental concentrations in the aerial parts by
139 the concentrations in the roots (Radziemska et al., 2017). The TF was calculated for each plant
140 sample and averaged according to the respective plant species. A heatmap with adjusted
141 colormap breaks was used to illustrate the TF.

142

143 **2.4 Statistical analysis**

144 All statistical analyses were performed with the open-source software R studio. The complete
145 dataset for the plants contains 110 observations (aerial parts + roots) and 19 variables (chemical
146 elements). In the first step, a principal component analysis (PCA) was conducted on the aerial
147 parts and the roots separately to capture the essence of the datasets and to identify the features
148 that explain most of the variation in chemical composition. For this purpose, the packages
149 ‘FactomineR’ and ‘factoextra’ were used (Kassambara, 2017a; Kassambara and Mundt, 2020;
150 Lê et al., 2008). Missing values were implemented using the package ‘missMDA’, which
151 reduced their influence on the PCA results to a minimum (Josse and Husson, 2016).

152 In the second step, an unsupervised clustering analysis was performed. The clustering tendency
153 of the two datasets (aerial parts + roots) was tested using the Hopkins test from the package
154 ‘clustertend’ before performing portioning clustering (K-means) (YiLan and RuTong, 2015).
155 K-means is a clustering algorithm that returns the natural grouping of data points based on their
156 similarity (elemental concentrations), not on their identity (plant species). The determination of
157 the number of clusters as well as the clustering itself and the visualisation were performed using
158 the package ‘factoextra’ (Kassambara, 2017b; Kassambara and Mundt, 2020). Next, a

159 PERMANOVA was performed on the complete dataset, using the package ‘vegan’ (Oksanen
160 et al., 2020) to examine to what degree the factors "plant species" and "plant parts" influenced
161 the elemental concentrations in the plants. Statistical significance between species was assessed
162 using a Welch one-way ANOVA with a post hoc “Games-Howell” test (Games and Howell,
163 1976), which is part of the package ‘userfriendlyscience’ (Peters, 2019). The paired
164 comparisons were visualised using the algorithm for a letter-based comparison embedded in
165 the package ‘multcompView’ (Graves et al., 2019; Piepho, 2004). For statistical analyses, a p-
166 value < 0.05 was used as the cutoff for significance. Statistical difference is shown by a
167 sequence of letters within which the absence of matches indicates significant difference. All the
168 figures were created using a colour-blind-friendly colormap (Chang, 2013).

169

170 **3 Results**

171 **3.1 Properties, parameters and elemental composition of the Jas Roux soil**

172 The soil pH of the study zone was 6.8 ± 0.3 (n=10) and conductivity was 58.4 ± 12.6 $\mu\text{S}/\text{cm}$.
173 Analysis of the soil parameters identified the soil as a poorly carbonated (0.51%) sandy loam
174 containing 2.08% organic matter (**Table SM 2**). The cation exchange capacity was 6.1
175 $\text{mmol}_c/100$ g (**Table SM 2**). The mineralogy of the topsoil was dominated by barite, calcite,
176 jarosite, kaolinite, muscovite, quartz and K-feldspar (**Figure SM 2**). **Table 2** lists the mean
177 total concentrations in the soil (n=10) and standard deviations (SD) for the 19 selected elements
178 compared to average soil concentrations, as well as the results of the CaCl_2 and the NH_4NO_3
179 extractions. Except for Mg, Al, P, K, Mn, Fe, Ba and Tl, the phytoavailable (NH_4NO_3) fraction
180 was inferior to 1 mg/kg DW.

181

182 **3.2 Elemental uptake of plants**

183 3.2.1 Elemental concentrations in the roots and aerial plant parts

184 **Figure 1** shows the range of elemental concentrations in the aerial parts and the roots of all
185 sampled individuals compared to total concentrations in the soil. With the exception of Ca, P
186 and Mg, the soil concentrations were higher than in the plant parts. The range of concentrations
187 of macronutrients (Mg, P, S, K, Ca) was similar in the roots and the aerial parts, whereas the
188 concentrations of trace elements were lower in the aerial parts than in the roots (**Figure 1**). The
189 variability was very strong among the 53 plant samples compared to variability in the soil.

190

191 3.2.2 PCA based examination of correlations between critical metals and other elements in
192 different plant parts

193 Plant responses to high metal concentrations are species dependent and can even vary from one
194 population to another (Gonneau et al., 2014; Pošćić et al., 2013; Rajabpoor et al., 2019). In this
195 study, data analysis was performed at species and plant part levels to identify the different
196 strategies. In the first step, a PCA was performed to capture the essence of the datasets.

197 The PCA biplot (**Figure 2**) merges the score plot of the individuals, represented by species
198 dependant symbols, and the loading plot of the variables (elemental concentrations) represented
199 by arrows, for aerial plant parts (**a**) and roots (**c**). In the aerial parts, the two first dimensions
200 (Dim1 and Dim2) explained 61.2% of total variance (**Figure 2a**). A first group of positively
201 correlated variables, composed of Mg, Ca, Mn, Ni, Zn, Y, Ba and Ce concentrations, formed
202 Dim1 (33.3% of total variance) (**Figure SM 3**). A second group of positively correlated
203 variables, including Al, V, Fe, As, Mo and Sb concentrations, contributed to Dim2 (27.9% of
204 total variance) (**Figure SM 3**). The score plot shows that all *Sax* individuals clustered together,
205 mainly contributed to Dim 1 and were associated with high concentrations of the first group of
206 elements. A cluster of *Hip* together with some single *Glo*, *Dia* and *Gal* individuals influenced
207 Dim2 (27.9% of total variance) and was characterised by higher concentrations of the second
208 group of elements.

209 In roots, 59.3% of total variance was explained by the first two dimensions (**Figure 2c**). Three
210 main groups of elements with strong positive intra-group correlations, were distinguished. The
211 first group was composed of Al, Ca, V, Mn, Ni, Zn, Y and Ce and contributed to Dim1 (42.8%
212 of total variance) (**Figure SM 3**). The second group was composed of P, S and Tl and mainly
213 contributed to Dim2 (16.5% of total variance). The third group comprised Fe, As, Sb and Ba
214 and influenced both Dim 1 and Dim2 (**Figure SM 3**).

215 All seven individuals of *Sax* and one individual of *Gal* were associated with the first group
216 while the second group of variables was connected by individuals of *Eup* and one individual of
217 *Gal*.

218 Isolated individuals of *Dac* and *Eup* were associated with the third group of variables.

219 Overall, the PCA shows that specific groups of chemical elements were decisive in explaining
220 the variance in roots and aerial plant parts driven by the individuals of *Sax* and *Hip* and by a
221 single individual each of *Dia*, *Glo*, *Gal*.

222 Cluster analysis confirmed the tendencies revealed by the PCA. Three clusters of individuals
223 were distinguished in the aerial plant parts based on their similar metal absorption patterns
224 (**Figure 2b**). The first group contained all seven individuals of *Sax* while the second group

225 contained all three individuals of *Hip* as well as a single individual each of *Glo*, *Gal* and *Dia*.
226 The third group contained all other individuals showing proximity among *Pil* and *Eup*
227 individuals.

228 Only two clusters were distinguished in the roots (**Figure 2d**). *Sax* and *Hip* individuals were
229 part of the same cluster whereas all individuals of *Pil*, *Bis*, *Dia*, *Ver* and *Jun* formed the second
230 cluster. Individuals of *Hip*, *Dac*, *Gal* and *Eup* were part of both clusters.

231

232 3.2.3 Species related differences in uptake of critical metals

233 According to the PERMANOVA, the factor “plant species” explained 65.65% of the variance
234 in elemental concentrations while only 4.4% was explained by the factor "plant part" (**Table**
235 **SM 3**).

236 A Welch one-way ANOVA test combined with a Games-Howell post hoc test (**Table 3**, **Table**
237 **4**) showed that three species, *Jun*, *Sax* and *Hip*, stood out from the others.

238 *Jun* generally had lower elemental concentrations in its aerial parts than the other species.
239 Indeed, *Jun* contained the lowest mean concentrations of Mg, Al, S, K, V, Fe, Ni, Cu, Zn, As,
240 Y, Sb, Ce and Tl. The concentrations of all these elements were significantly lower than in at
241 least one other species (**Table 3**). However, this tendency was not observed in the roots (**Table**
242 **4**).

243 In turn, *Sax* had the highest concentrations of many of the elements analysed. In the aerial parts,
244 significant differences in the concentrations of Mg, Ca, Mn, Ni, Y and Ce were found. The
245 concentrations of Ca, Mn, Ni and Y were significantly higher than in all the other species. In
246 the roots, the highly concentrated elements were very similar to those in the aerial parts. The
247 concentrations of Al, Ca, Mn, Ni, As and Ce were significantly higher than in at least three of
248 the other species.

249 The third species with characteristic elemental concentrations was *Hip*. The elements
250 accumulated in the aerial parts of *Hip* were Al, V, Fe, As, Sb and Mo. Differences in Al, V, Fe
251 and As were significantly higher than in the other species. Differences in the concentrations of
252 Sb and Mo were not significant. It should be noted that average concentrations of Mo in *Hip*
253 were about 5 times higher in roots (up to 26.9 mg/kg DW) and 30 times higher in the aerial
254 parts (up to 53.7 mg/kg DW), than in all the other species.

255 The significance between groups (species) depends on both the variability within each group
256 and between the groups. Species with highly variable concentrations are therefore not identified
257 as significantly differing from one another. For example, *Bis* and *Gal* had locally high
258 concentrations of Tl in their aerial parts, up to 541 mg/kg DW in *Bis* and up to 1091 mg/kg DW

259 in *Gal*. Despite the fact that no individual of another species exceeded 100 mg/kg DW, the
260 differences were not significant. The concentrations of Ba in *Sax* are another example of non-
261 significant differences due to high variability. The maximum concentration measured in the
262 aerial parts was 5007 mg/kg, while in the other species 1445 mg/kg was the maximum
263 concentration measured.

264

265 3.2.4 Identification of candidates for phytoremediation applications

266 The translocation-factor (TF) is a useful tool to identify the transfer strategy of a plant. The
267 higher the factor, the larger the share of the element translocated to the aerial parts (Macnair,
268 2003).

269 **Figure 3** is a heatmap of the mean TFs for the plant species and chemical elements investigated
270 with a colour spectrum ranging from pale yellow to dark red. The darker the colour of a box,
271 the higher the TF. The TF was low or highly variable in the majority of the twelve plant species.
272 *Bis* had the highest TF for many of the critical metals analysed (Mg, V, As, Y, Sb, Ce, Tl). In
273 particular, the translocation of Tl was very high (TF=19.4±17.2). A second species with a high
274 TF for Tl was *Gal* (TF=2.0±0.5). *Dia* also translocated many critical metals to the aerial parts
275 as shown by the high TF for Mg, V, As, Ce and Tl. *Hip*, on the other hand, had the highest TF
276 for Mo (TF=3.8±4.4). In *Sax*, the TF was low for most of the critical metals (As, Y, Ce, Sb, Tl)
277 and the only elements that were preferably stored in the leaves were Mg (TF=1.3±0.2) and Ca
278 (TF=2.1±0.8). *Jun* only had low TFs.

279

280 4 Discussion

281 4.1 Jas Roux - A good site to study a plant community exposed to a cocktail 282 of metals and metalloids in a natural ecosystem

283 The Jas Roux outcrop has fairly been studied regarding its exceptional mineral composition
284 (Biagioni et al., 2015; Johan and Mantiene, 2000; Topa et al., 2014). However, the surrounding
285 ecosystem, including the soil and the plants has never been studied before.

286 The concentrations of Zn (602 mg/kg), As (2382 mg/kg), Mo (19.3 mg/kg), Sb (778 mg/kg),
287 Ba (27010 mg/kg) and Tl (242 mg/kg) were far above ordinary soil concentrations (**Table 2**).
288 Soil concentrations in European topsoil of Zn are usually around 68 mg/kg, of As, 11 mg/kg,
289 of Mo, 0.9 mg/kg, of Sb, 1 mg/kg, of Ba, 400 mg/kg, and of Tl, 0.8 mg/kg (**Table 2**) (Salminen
290 et al., 2005). Except for Mo, all these elements have also been identified as elements associated
291 with the primary minerals in the Jas Roux outcrops, namely barite, quartz and pyrite, as well as
292 Zn, Sb, As, Tl, Ag, Hg and Pb sulphides and sulphosalts (Boulliard et al., 2010). In terms of

293 mobility and phytoavailability Zn, As, Mo, Sb, Ba and Tl remained < 0.1% of the total
294 concentrations and only Ba exceeded 1 mg/kg with 16.0 mg/kg after CaCl₂ extraction and 199
295 mg/kg after NH₄NO₃ extraction. These concentrations are comparable with those measured in
296 soils close to an active barite mining region in China with up to 111 mg/kg of phytoavailable
297 Ba (Lu et al., 2019). The mobility and phytoavailability of As, Sb and Zn were low compared
298 to those in contaminated soils with comparable total concentrations (Pueyo et al., 2004; Qasim
299 et al., 2015). This low mobility indicates the dominance of stable minerals in Jas Roux. Unlike
300 other sulphosalt sites, Jas Roux has not been disturbed by mining activities which generally
301 increase metal mobility (Resongles et al., 2014; Wei et al., 2018).
302 Even though many of the characteristic elements of Jas Roux are known to be toxic for plants
303 (As, Tl etc.), plant diversity is that of a classical alpine ecosystem (Landolt, 2015; Michalet et
304 al., 2002). It is possible that the relatively low phytoavailability of the metals helps the plants
305 to maintain their homeostasis in the short term (**Table 2**), but long-term exposure to low doses
306 can result in a high real exposition, to which plants need to adapt.
307 Plants use different strategies to adapt to high concentrations of toxic elements in the soil. Some
308 plants are able to exclude the metals and others to accumulate the elements in specific
309 compartments (Tremel-Schaub and Feix, 2005). Among the accumulating metallophytes,
310 metals are generally accumulated in the roots, but some species promote translocation to the
311 aerial plant parts (Krämer, 2010). Different statistical approaches have been used to identify the
312 different adaption strategies and revealed characteristic uptake-patterns for the two plant
313 species *Sax* and *Hip* (**Figure 2b**).

314

315 **4.2 *Saxifraga paniculata* - A cation accumulating species with an affinity for** 316 **REEs and alkaline earth metals**

317 In terms of elemental concentration in roots and aerial parts *Sax* stood out among the 12 species
318 analysed (**Figure 2**). In the aerial parts, the PCA showed the individuals of *Sax* to be associated
319 with Mg, Ca, Mn, Ni, Zn, Y, Ba and Ce (**Figure 2**). Among these elements, the concentrations
320 of Ca, Mn, Ni, Y were significantly higher than in all the other species (**Figure 2, Table 3**). It
321 is worth noting that the majority of elements accumulated in the aerial parts of *Sax* are divalent
322 cations (Ba, Ni, Mn, Ca, Zn and Mg) while the three elements with the highest concentrations
323 (Mg, Ca and Ba) are alkaline earth elements. This set of accumulated elements could be caused
324 by overexpression of nonselective cation channels or increased activity of a single type of metal
325 cation transporter, as their selectivity is known to be rather low (Demidchik and Maathuis,
326 2007; Thomas et al., 2005).

327 The genus *Saxifraga* comprises about 400 species and some of them have already been studied
328 regarding metal tolerance (Gornall, 1987). To give one example, *Saxifraga oppositifolia*,
329 *Saxifraga cespitosa* and *Saxifraga azoides* growing in Spitzbergen contained higher
330 concentrations of Pb and Zn in their aerial parts than concentrations in the soil (Jóźwik and
331 Magierski, 1991). Further, a Cu tolerant population of *Saxifraga stellaris* growing on polluted
332 soils was identified in southern Germany, with concentrations up to 900 mg/kg DW (Adlassnig
333 et al., 2013; Ryszka et al., 2019; Sissolak, 1984).

334 *Sax* belongs to a specific taxonomic section (sect. *Ligulatae*) characterised by strong guttation
335 (Gornall, 1987). Guttation is a process to regulate root pressure and nutrient flow by excreting
336 phloem and xylem sap through openings on the leaf surface and is observable in a wide range
337 of plant species, including many Saxifragaceae (Singh, 2014, 2013). The openings are called
338 hydathodes and, in contrast to stomata, remain permanently open (Singh, 2014). The guttation
339 process is thought to help plants expel excessive nutrient salts and harmful elements (Hossain
340 et al., 2017; Neumann et al., 1997; Singh, 2013; Stahl, 1919). In some species of *Saxifraga*,
341 including *Sax*, the repeated evaporation of guttation droplets leads to the formation of a calcite
342 crust on the leaf surface (Arnott and Pautard, 1970; Schmidt, 1930; Wightman et al., 2017).
343 These deposits appear to be the result of increased Ca uptake and might be a strategy to reduce
344 the cytotoxic effects of Ca while maintaining high flow rates (Nazrul Islam and Kawasaki,
345 2015). In the present study, the accumulation of Ca by *Sax* is reflected in the significantly higher
346 Ca-concentrations measured in the roots and the aerial parts (**Table 3, Table 4**) and the high
347 translocation factor (**Figure 3**).

348 This increased Ca uptake could also explain the higher concentrations of REEs (Y, Ce) in *Sax*
349 compared to those in the other species, as REEs are probably absorbed through the same
350 pathway as Ca because of their similar ionic radius (Carpenter et al., 2015; Kastori et al., 2010;
351 Rengel, 1994; van der Ent et al., 2017). Ozaki et al. (1997) observed increased REE-
352 concentrations combined with higher Ca and Ba uptake in several REE-accumulating fern
353 species and suspected a possible link between these elements.

354 The accumulation of REEs is of particular interest for this study as REEs are crucial for modern
355 technologies. Compared to the average concentration in the soil (4.22 ± 1.04 mg/kg) the roots of
356 *Sax* individuals contained nearly twice as much Y (7.37 ± 3.24 mg/kg) (**Figure 4**). This raises
357 the question of how *Sax* is able to absorb such a high ratio of Y (**Figure SM 4**). Y soil
358 mineralogy in Jas Roux soil has not been studied so far. Y can be associated with several
359 mineral phases, but one common soil mineral based on Y is Xenotime (YPO₄) (Ramos et al.,
360 2016). Xenotime has low solubility and phosphate minerals are generally difficult for plants to

361 access (Darcy et al., 2018; Liu and Byrne, 1997; Tropper et al., 2011). However, there are some
362 exceptions - like white lupin or cassava - which can take up a fraction of soil P that is not
363 accessible to other plant species. In the case of cassava, the mechanisms allowing the increased
364 P-uptake are strong mycorrhization (Zaag et al., 1979). In white lupin, on the other hand,
365 increased root exudation is combined with a cluster root system (Braum and Helmke, 1995). In
366 the case of *Sax*, root exudates are more likely to play an important role, as the plant is able to
367 grow in very extreme habitats, in high altitudes and with little organic matter (Burga et al.,
368 2004; Neuner et al., 1999).

369

370 **4.3 *Hippocrepis comosa* - A oxyanion accumulating species with a particular** 371 **affinity for Mo**

372 The second plant species showing a characteristic pattern in elemental uptake was *Hip* (**Figure**
373 **2b**). In the aerial parts, all three individuals were positively correlated with the elements As,
374 Fe, Sb, Mo, V and Al (**Figure 2**) with a particularly high TF for Mo (TF=3.8±4.4) (**Figure 3**).
375 The fact that none of these elements accumulated in the aerial parts of *Sax* led to two easily
376 distinguishable groups, separating the two species (**Figure 2b**).

377 Among the elements accumulated by *Hip* typical elemental correlations became visible. Due to
378 their geochemical similarities, As and Sb (for example) are considered to be related elements
379 (Herath et al., 2017). A positive correlation between these two elements has already been
380 observed in plants and increased Sb uptake is presumed to be the result of damaged cell
381 membrane integrity caused by As (Feng et al., 2013; Müller et al., 2013).

382 There is also a strong link between the uptake mechanisms of Mo and Fe (Bittner, 2014). Most
383 Mo-enzymes also require Fe-containing redox groups and many studies reported increased Mo
384 uptake in the presence of Fe and vice versa (Bittner, 2014; Kannan and Ramani, 1978; Zimmer
385 and Mendel, 1999).

386 Three major hypotheses are proposed to explain the characteristic uptake pattern of *Hip*:

387 I) One hypothesis is a characteristic modification of the rhizosphere that would influence the
388 redox potential. This modification could locally increase phytoavailability of the accumulated
389 elements, as, except for Al, all are redox sensitive elements (As, Fe, Sb, Mo, and V).

390 II) The second hypothesis concerns the uptake pathway of the elements. It is known that As,
391 Sb, Mo, and V are usually absorbed as oxyanions by plants (Hossain et al., 2018; Kabata-
392 Pendas, 2011). Vanadate (VO_4^{3-}) and arsenate (AsO_4^{3-}) enter the plant through P transporters,
393 due to similarities in structure and charge (Chen et al., 2021; Meharg and Hartley-Whitaker,
394 2002, Han et al., 2016). Antimonate, on the other hand, is more likely to be absorbed through

395 less selective anion transporters, such as those for Cl^- and NO_3^- , or an incompletely sealed or
396 damaged Casparian strip (Feng et al., 2013; Tschan et al., 2009b, 2009a). However, little is
397 known about Mo uptake by roots. Because of structural and behavioural similarities between
398 molybdate (MoO_4^{2-}) and sulphate (SO_4^{2-}), it is plausible that sulphate transporters are involved
399 (Bittner, 2014; Zimmer and Mendel, 1999).

400 Overexpression or increased activity of so many different transporters, responsible for the
401 particular elemental concentrations found in *Hip*, is unlikely. Nevertheless, a very unselective
402 anion transporter, able to transport phosphate, sulphate, nitrate and chlorate, could be involved.
403 Such a transporter was identified in barley by Preuss et al. (2010), for example.

404 III) The third hypothesis is based on the N fixing property of *Hip*, as Mo and Fe are known to
405 play an essential role as cofactors during symbiotic N fixation (Hossain et al., 2018). For this
406 reason, legume plants like *Hip* have a higher demand for Mo than non-N fixing plants (Kabata-
407 Pendas, 2011). It is plausible that *Hip* is able to promote Mo absorption in order to guarantee
408 N fixation under Mo deficient conditions. However, in Mo rich soils like Jas Roux (19.9 ± 3.71
409 mg/kg), this property might lead to an increased uptake and thus explain the comparatively high
410 Mo concentrations in *Hip* (**Figure 4**). It is worth mentioning that V may also interfere during
411 N fixation by replacing Mo in the nitrogenase enzyme (Hernandez et al., 2009).

412 Individually, none of the three hypotheses explains all the elemental concentrations observed
413 in *Hip*. Nevertheless, a combination of the mechanisms could be a valid explanation. Al is the
414 only element whose accumulation by *Hip* remains difficult to explain, as this species is
415 generally found in Ca rich sites and is sensitive to high Al concentrations (Michalet et al., 2002).
416 Concentrations of Al > 1000 mg/kg, like those we measured in aerial parts of *Hip* (1336 mg/kg
417 DW), are rare in higher plants and are considered as Al accumulation (Kabata-Pendas, 2011).
418 The tolerance of toxic elements (Al, As) makes *Hip* an interesting candidate for remediation of
419 polluted soils as its ability to fix N thereby improving soil fertility could be an additional
420 advantage (Pajuelo et al 2008).

421

422 **4.4 Translocation factor reveals specific plant strategies**

423 The differentiation of *Hip* and *Sax* from the other species was mainly caused by high
424 concentrations of a particular set of elements that made both species stand out in the PCA and
425 the cluster analysis. However, this statistical approach failed to detect affinities for single
426 elements, or adaption strategies associated with particularly low concentrations. The
427 translocation factor in combination with the mean elemental concentrations, revealed further
428 adaption strategies and species-related tendencies in elemental uptake (**Figure 3**).

429 For instance, *Bis* and *Dia* did not form a distinct group in the PCA or in the cluster analysis but
430 both had TFs > 1 for a large number of critical metals (Mg, V, Mn, As, Y, Sb, Ba, Ce, Tl). In
431 particular, the TF of *Bis* for Tl was remarkably high (TF=19.4±17.2) indicating Tl accumulation
432 (256±224 mg/kg) (**Figure 4**). While no explanation was found in the literature for the high TF
433 of *Dia*, *Bis* belongs to the Brassicaceae family, which includes many hyperaccumulating
434 species (Babula et al., 2008; van der Ent et al., 2013). *Bis* itself has already been mentioned as
435 a Tl accumulating species (LaCoste et al., 1999; Pošćić et al., 2013). But *Bis* was not the only
436 Tl accumulating species, as *Gal*, which has never previously been associated with metalliferous
437 sites, also showed high concentrations of Tl in its aerial parts (254±410 mg/kg) and high
438 translocation (TF=2.0±0.5).

439 Another species that did not stand out in the statistical analysis was *Jun*. Nevertheless, *Jun* had
440 the lowest concentrations of the elements Mg, Al, S, K, V, Fe, Ni, Cu, Zn, As, Y, Sb, Ce and
441 Tl in its aerial parts (**Table 3**). This exclusion strategy seems to consist in limited translocation
442 to the aerial parts, as the TF was low for all elements (**Figure 3**). Given that *Jun* is the only
443 lignifying plant among the 12 species we analysed, its exclusion capacity might be due to
444 different plant-internal barriers or reduced flow rates due to its small leaf surface area. Even
445 though no studies have demonstrated metal exclusion in Juniperus species, metal concentrations
446 in other conifers (Pinales) have already been reported. For example, Mingorance et al. (2007)
447 found *Pinus pinea* growing in an industrial area was able to exclude Al, Ba, Cr, Fe, and Pb. In
448 light of the results of our study, *Jun* could be an interesting candidate for phytostabilisation
449 thanks to its high resistance to metal contamination, its widespread occurrence and its
450 ruggedness (Saladin, 2015).

451

452 **5 Conclusions**

453 Among 12 plant species exposed to a cocktail of metals and metalloids in an entirely natural
454 environment, two species stood out: *Hippocrepis comosa* and *Saxifraga paniculata*, each of
455 which accumulated an individual set of elements. *Saxifraga paniculata* preferably absorbed
456 divalent cations, but also the REEs Y and Ce. This uptake pattern could be explained by the Ca
457 accumulation property of *Saxifraga paniculata*. The affinity to Ca might cause an increased
458 absorption of other elements with a similar ionic radius like Y and Ce, or similar ionic charge
459 like Mn and Ba. *Hippocrepis comosa*, on the other hand, preferably absorbed oxyanions,
460 perhaps due to overexpression or increased activity of a non-selective anion transporter.
461 Furthermore, *Hippocrepis comosa* is an interesting candidate for phytoremediation of polluted
462 soils, as its ability to fix N₂ improves soil fertility and could thus facilitate the establishment of
463 other species.

464 Different tolerance strategies were identified among the studied plant species. *Biscutella*
465 *laevigata* and *Dianthus sylvestris* favoured translocation of multiple critical metals to their
466 aerial parts while, *Juniperus communis* avoided translocation and used an exclusion strategy.
467 Particularly high concentrations of Tl were found in *Biscutella laevigata* and *Galium*
468 *corrudifolium*. While hyperaccumulation of Tl has already been reported in *Biscutella*
469 *laevigata*, it is a new discovery in *Galium corrudifolium*. The exclusion capacity of *Juniperus*
470 *communis subsp. nana* might represent a potential for phytostabilisation applications. Erosion
471 of a polluted soil could be prevented in the long term with no risk of transfer into the food chain
472 because *Juniperus communis subsp. nana* prevents elemental translocation and the shrub is not
473 consumed by grazing animals.

474 These results advance our understanding of soil-to-plant transfer of poorly studied but
475 increasingly used critical metals and point to the potential of several plant species for future
476 phytoremediation techniques.

477

478

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489

490 **Abbreviations:**

491 ANOVA..... Analysis Of Variance

492 a.s.l. Above Sea Level

493 DW Dry Weight

494 ICP-MS Inductively Coupled Plasma Mass Spectrometry

495 ICP-OES Inductively Coupled Plasma Optical Emission Spectroscopy

496 PCA Principal Component Analysis

497 PERMANOVA..... Permutational Multivariate Analysis Of Variance

498 PGMs Platinum Group Metals

499 REEs Rare Earth Elements

500 SD Standard Deviation

501 subsp. Subspecies

502 TF Translocation Factor

503 XRD X-Ray Diffraction

504

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821

1 Introduction

2 All over the world, emerging technologies require an increasingly diverse spectrum of chemical
 3 elements with highly specific (photo-optical, catalytic, magnetic, etc.) properties (Celis et al.,
 4 2020). Metals that have practically never been used before are now indispensable for many
 5 industrial sectors and are thus the subject of soaring global demand. Due to their economic
 6 importance and their supply risk, many of these metals are now classified as "critical" for high
 7 tech applications (European Commission, 2020), examples being Sb in lead-acid batteries and
 8 semiconductors, As in cell phones and solar cells and rare earth elements (REEs) in sensors,
 9 displays and electric motors (Fortier et al., 2018; Mudd et al., 2018).

10 ~~Their increasing use also involves increased risk of their dispersion in the environment during~~
 11 ~~their extraction, use, and disposal. However~~Despite their increased use, the biogeochemical
 12 cycles of many of these critical metals are not yet fully understood and predicting either their
 13 environmental behaviour or their ecotoxicity is consequently impossible (Espejo et al., 2018).

14 ~~This knowledge, however,~~Aim of this study is essential to ~~choose~~analyse the ~~right remediation~~
 15 ~~techniques~~soil-to-plant transfer of some of these metals and to shed light on their uptake
 16 ~~pathways. Our findings might bare valuable information~~ for ~~polluted sites. In recent~~
 17 ~~years,~~phytoremediation and promote plant-based ~~remediation~~ techniques (~~phytoremediation~~)
 18 ~~have become a feasible and environmentally friendly solution to treat~~of metal-contaminated
 19 ~~soils (Geras'kin et al., 2011; Tangahu et al., 2011; van der Ent et al., 2017)-sites.~~

20 Phytoremediation techniques are relying on the capacity of certain plant species to limit the
 21 uptake of the toxic elements or to accumulate them in designated compartments (e.g.
 22 phytostabilisation, phytoextraction...-) (Favas et al., 2014). Exclusion and accumulation are
 23 the two main strategies of plants to maintain homeostasis in metal-rich habitats. Both involve
 24 a series of adaptations such as modified membrane transport, chelation and complexation, or
 25 rhizosphere interactions for example (Furini, 2012).

26 The ability of plants to accumulate metals in their aerial parts has received considerable
 27 attention and many combinations of specific plant species tolerating or accumulating a
 28 particular set of elements have been discovered (Baker et al., 2010; Pollard et al., 2014).
 29 However, most studies have focused on "traditional" metals such as Zn, Ni, Cd and Pb, whereas
 30 the uptake pathways and metabolic impact of many elements, particularly emerging critical
 31 metals, still raise many questions (Macnair, 2003; van der Ent et al., 2013).

32 Elemental uptake is influenced by chemical, physical, and biological factors. Plant species, root
 33 system, root exudates, edaphic interactions, and transport mechanisms all play important roles
 34 (Tangahu et al., 2011; Trakal et al., 2015; Yadav, 2010). Despite highly specialised proteins,

35 including transporters, pumps and channels, elemental selectivity remains challenging for
36 plants and each uptake mechanism is likely to let pass a range of ions due to chemical
37 similarities between the different ions (Tangahu et al., 2011). The huge number of factors and
38 complex interactions are impossible to reproduce in the laboratory, thus, to understand the
39 uptake of poorly studied elements, such as critical metals, performing in-situ studies in a natural
40 environment is advantageous.

41 The geological site of Jas Roux, located in the Ecrins National Park (Hautes-Alpes, France),
42 was chosen for the study. The distinctive reddish colour of the Jas Roux outcrops ('Roux'
43 means red) originates from a sulphosalt-forming hydrothermal mineralisation (Johan and
44 Mantiene, 2000). These sulphosalts are one of the reasons for the characteristic metal
45 concentrations of Jas Roux as they are rich in elements with a strong affinity for ~~sulphur~~S, such
46 as ~~lead, antimony, copper, thallium~~Pb, Sn, Cu, Tl and ~~arsenic~~As (Dittrich et al., 2009; Pierrot
47 and Roger, 2018). Many of the elements found in Jas Roux are used in high-tech applications
48 and are considered to be critical for leading technologies (Dittrich et al., 2009; Graedel et al.,
49 2015). This site is thus a good proxy of chronic contamination by a cocktail of metals and
50 metalloids. As the site has never been disturbed by any human activities and is located in the
51 heart of the Ecrins National Parc, it offers ideal conditions to study an established ecosystem
52 with a plant community that has not undergone human selection. The plant community can be
53 seen as natural response to the local mineralogy and may contain candidates for
54 phytoremediation.

55 To identify such plant candidates and to study the environmental behaviour of critical metals,
56 we were guided by the following three questions:

57 To what extent are critical metals absorbed by plants?

58 Are there any species-related differences in elemental uptake?

59 Do correlations exist between critical metals and other elements in different plant parts?

60 The approach of this study was to conduct a large-scale screening of 12 plant species to identify
61 individual uptake patterns using chemical analysis, soil element extraction and statistical tools.

62 The large number of species analysed provided insights into the actual elemental
63 phytoavailability of a wide range of critical metals.

64 2 Material and Methods

65 2.1 Sampling Site

66 The geological site of Jas Roux, located in the Ecrins National Park in the district of La
67 Chapelle-en-Valgaudemar (Hautes-Alpes, France), is situated at 2170 m a.s.l. (44°48' 45" N, 6°
68 19' 18" E; WGS84) on the right-hand side of the Chabournéou stream (Johan and Mantienné,
69 2000). Jas Roux is a hydrothermal stratiform deposit in dolomite limestone and has been
70 characterised as a sulphosalt site (Boulliard et al. 2010). The hydrothermal system was possibly
71 generated by volcanic activity involving relatively low temperatures (around 100 °C) and high
72 pressure (Johan and Mantienné, 2000). The particular ~~thallium~~Tl-rich composition of Jas Roux
73 has attracted many mineralogists and geologists, who have identified Zn, Sb, As, Tl, Ag, Hg,
74 Pb sulphides and sulphosalts including many new sulphosalt mineral species (Boulliard et al.,
75 2010; Guillemin et al., 1970; Mantienné, 1974; Topa et al. 2017, 2014).

76 The study zone is located below one of the reddish coloured outcrops identified by Mantienné
77 (**Figure SM 1**) (Mantienné, 1974). The metal-rich mineralisation is best developed in this
78 outcrop and forms a 3.8 m thick barite-rich zone (Johan and Mantienné, 2000). The study zone
79 measured 20 m x 5 m with a slope of approximately 45° (100%) (**Figure SM 1**).

80 Soil and plant samples were collected during two field trips, one in September 2017 and one in
81 June 2018. The steep slope, variable soil depth and the presence of large rocks impeded a
82 systematic sampling approach. Nevertheless, the samples were collected as evenly distributed
83 as possible. The soil samples were collected from 10 ~~evenly distributed~~ sampling points in the
84 study zone and consisted of 1 kg of topsoil each (< 15 cm). Among the 65 plant species
85 identified in the study zone, 12 were selected, ~~each belonging to a different plant family~~ (**Table**
86 **1**); taking into account occurrence, spatial distribution and family affiliation (Table 1). For
87 improved readability we attributed acronyms to each of the 12 species throughout the paper
88 (Table 1). Depending on their abundance, between 2 and 7 replicates of each species were
89 sampled, giving a total of 55 individuals. (**Table 1**). All the samples were stored at 4 °C in the
90 dark.

91

92 2.2 Sample preparation

93 Roots and aerial parts were separated at the collar and cleaned with 18.2 MΩ·cm ultrapure
94 water. The plant roots were additionally brushed and vortexed in ultrapure water to remove
95 adhering soil particles. The leaves of *Saxifraga paniculata* ~~Sax~~ were also brushed as they were
96 partially covered with a calcite crust. Plant samples were dried at 40 °C for 48 h. Soil samples
97 were dried at 40 °C for 72 h and sieved at 2mm before analysis.

98 All the soil and plant samples were ground at 20hz in a Retsch® Mixer Mill MM 400 for 2 min
99 to obtain a fine powder. Each sample (100mg) was solubilised in acid using a Milestone
100 UltraWAVE® microwave to complete mineralisation. The acids used for the digestion of the
101 plant samples were 4mL of HNO₃ and 2mL of H₂O₂. For the soil samples, 3mL of HNO₃, 3mL
102 of HCl and 0.5 mL of HF were used to measure the total metal concentrations. Soil pH and
103 conductivity were determined in a suspension of 1g of sieved soil (< 2mm) and 5mL of
104 ultrapure water according to the French standard X31-117 (*NF X31-117*, 1994). After 5 min of
105 agitation at 80 rpm and 2 h of rest, measurements were taken using a Hanna HI-2550 bench
106 meter. To estimate the mobile and phytoavailable elemental fractions in the soil, CaCl₂
107 (0.001mol/L) and NH₄NO₃ (1mol/L) extractions have been conducted, according to standards
108 ISO 21268-2 and ISO 19730 (*ISO 19730:2008*, 2009; *NF X31-607-2*, 2004). For the CaCl₂
109 extraction, a liquid-solid ratio of 10:1 (20mL of ultrapure water and 2g of soil) was applied.
110 After 24 h of agitation at 10 rpm, the samples were centrifuged at 2500xG for 20 min, filtered
111 at ~~45µm~~0.45 µm and analysed in ICP-MS. For the NH₄NO₃ extraction, 4g of soil were mixed
112 with 10mL of ultrapure water, agitated for 2 h at 30 rpm, centrifuged at 1000xG for 15 min,
113 filtered at 0.45 µm and analysed in ICP-MS.

114 Soil mineralogy was analysed by X-ray diffraction using the X'Pert Pro MPD (XRD) equipped
115 with a Cobalt x-ray tube (= 1.79Å) running at 40 kV and 40 mA.

116 A mixture of three bulk samples of topsoil was sent to an outside laboratory (CIRAD – US49
117 Laboratoire d'analyse de Montpellier, France) for the determination of soil parameters. The soil
118 type was determined using 5 granulometric fractions according to the French standard X31-107
119 (*NF X31-107*, 2003), organic matter by combustion analysis (Dumas), labile ~~phosphorous~~P
120 content using a colorimetric method (Olsen) and cation exchange capacity by quantifying the
121 dosing adsorption agent [Co(NH₃)₆]Cl₃ with inductively coupled plasma atomic emission
122 spectroscopy according to the French standard X31-130 (*NF X31-130*, 1999).

123

124 **2.3 Chemical analyses**

125 The samples were analysed by inductively coupled plasma mass spectrometry (ICP-MS, Perkin
126 Elmer NexIon 300X). For the elements Mg, Al, P, K, Ca and S, the results of inductively
127 coupled plasma optical emission spectroscopy (ICP-OES) were occasionally used to reach
128 higher accuracy (Perkin Elmer 4300 DV). The calibration curves were established using multi-
129 solution standards CCS4, CCS5, CCS6 from Inorganic Ventures (New Jersey USA) diluted in
130 2% HNO₃ solution. The internal standards were Rh103 for ICP-MS and Y for ICP-OES. Oak
131 leaves (V464) provided by the French National Institute for Agriculture Food and Environment

132 (INRAE) were used as the external reference for plant samples. For the soil, a rendzina sample
133 (ISA 979) provided by Wageningen Evaluating Programs for Analytical Laboratories
134 (WEPAL) was used. EnviroMAT drinking water EP-L-3 and subterranean water ES-H-2 (SCP
135 Sciences) were used as internal references and for quality control.

136 ICP analyses aimed to quantify critical metalelements (As, V, Y, Sb, Ba, Ce, Tl) and major
137 soil elements and nutrients (Mg, Al, P, S, K, Ca, Mn, Fe, Ni, Cu, Zn, Mo) present in Jas Roux.

138 The critical elements were chosen based on the study on the EU's list of critical raw materials
139 and a review of critical minerals and will be called critical metals throughout the manuscript
140 (European Commission, 2020; Hayes and McCullough, 2018). The detection limit for each
141 element was determined using handling blanks analysed at each ICP analysis- (Table SM 1).

142 The coefficients of variances for all certified elements were calculated for the internal and
143 external standards (**Table SM 1**).

144 The translocation factor (TF) was calculated to identify the transfer strategies of the different
145 plant species. The TF is obtained by dividing the elemental concentrations in the aerial parts by
146 the concentrations in the roots (Radziemska et al., 2017). The TF was calculated for each plant
147 sample and averaged according to the respective plant species. A heatmap with adjusted
148 colormap breaks was used to illustrate the TF.

149

150 **2.4 Statistical analysis**

151 All statistical analyses were performed with the open-source software R studio. The complete
152 dataset for the plants contains 110 observations (aerial parts + roots) and 19 variables (chemical
153 elements). In the first step, a principal component analysis (PCA) was conducted on the aerial
154 parts and the roots separately to capture the essence of the datasets and to identify the features
155 that explain most of the variation in chemical composition. For this purpose, the packages
156 ‘FactomineR’ and ‘factoextra’ were used (Kassambara, 2017a; Kassambara and Mundt, 2020;
157 Lê et al., 2008). Missing values were implemented using the package ‘missMDA’, which
158 reduced their influence on the PCA results to a minimum (Josse and Husson, 2016).

159 In the second step, an unsupervised clustering analysis was performed. The clustering tendency
160 of the two datasets (aerial parts + roots) was tested using the Hopkins test from the package
161 ‘clustertend’ before performing portioning clustering (K-means) (YiLan and RuTong, 2015).
162 K-means is a clustering algorithm that returns the natural grouping of data points based on their
163 similarity (elemental concentrations), not on their identity (plant species). The determination of
164 the number of clusters as well as the clustering itself and the visualisation were performed using
165 the package ‘factoextra’ (Kassambara, 2017b; Kassambara and Mundt, 2020). Next, a

166 PERMANOVA was performed on the complete dataset, using the package ‘vegan’ (Oksanen
167 et al., 2020) to examine to what degree the factors "plant species" and "plant parts" influenced
168 the elemental concentrations in the plants. Statistical significance between species was assessed
169 using a Welch one-way ANOVA with a post hoc “Games-Howell” test (Games and Howell,
170 1976), which is part of the package ‘userfriendlyscience’ (Peters, 2019). The paired
171 comparisons were visualised using the algorithm for a letter-based comparison embedded in
172 the package ‘multcompView’ (Graves et al., 2019; Piepho, 2004). For statistical analyses, a p-
173 value < 0.05 was used as the cutoff for significance. Statistical difference is shown by a
174 sequence of letters within which the absence of matches indicates significant difference. All the
175 figures were created using a colour-blind-friendly colormap (Chang, 2013).

176

177 **3 Results**

178 **3.1 Properties, parameters and elemental composition of the Jas Roux soil**

179 The soil pH of the study zone was 6.8 ± 0.3 (n=10) and conductivity was 58.4 ± 12.6 $\mu\text{S}/\text{cm}$.
180 Analysis of the soil parameters identified the soil as a poorly carbonated (0.51%) sandy loam
181 containing 2.08% organic matter (**Table SM 2**). The cation exchange capacity was 6.1
182 $\text{mmol}_c/100$ g (**Table SM 2**). The mineralogy of the topsoil was dominated by barite, calcite,
183 jarosite, kaolinite, muscovite, quartz and K-feldspar (**Figure SM 2**). **Table 2** lists the mean
184 total concentrations in the soil (n=10) and standard deviations (SD) for the 19 selected elements
185 compared to average soil concentrations, as well as the results of the CaCl_2 and the NH_4NO_3
186 extractions. Except for Mg, Al, P, K, Mn, Fe, Ba and Tl, the phytoavailable (NH_4NO_3) fraction
187 was inferior to 1 mg/kg DW.

188

189 **3.2 Elemental uptake of plants**

190 3.2.1 Elemental concentrations in the roots and aerial plant parts

191 **Figure 1** shows the range of elemental concentrations in the aerial parts and the roots of all
192 sampled individuals compared to total concentrations in the soil. With the exception of Ca, P
193 and Mg, the soil concentrations were higher than in the plant parts. The range of concentrations
194 of macronutrients (Mg, P, S, K, Ca) was similar in the roots and the aerial parts, whereas the
195 concentrations of trace elements were lower in the aerial parts than in the roots (**Figure 1**). The
196 variability was very strong among the 53 plant samples compared to variability in the soil.

197

198 3.2.2 PCA based examination of correlations between critical metals and other elements in
199 different plant parts

200 Plant responses to high metal concentrations are species dependent and can even vary from one
201 population to another (Gonneau et al., 2014; Pošćić et al., 2013; Rajabpoor et al., 2019). In this
202 study, data analysis was performed at species and plant part levels to identify the different
203 strategies. In the first step, a PCA was performed to capture the essence of the datasets.

204 The PCA biplot (**Figure 2**) merges the score plot of the individuals, represented by species
205 dependant symbols, and the loading plot of the variables (elemental concentrations) represented
206 by arrows, for aerial plant parts (**a**) and roots (**c**). In the aerial parts, the two first dimensions
207 (Dim1 and Dim2) explained 61.2% of total variance (**Figure 2a**). A first group of positively
208 correlated variables, composed of Mg, Ca, Mn, Ni, Zn, Y, Ba and Ce concentrations, formed
209 Dim1 (33.3% of total variance) (**Figure SM 3**). A second group of positively correlated
210 variables, including Al, V, Fe, As, Mo and Sb concentrations, contributed to Dim2 (27.9% of
211 total variance) (**Figure SM 3**). The score plot shows that all *Sax* individuals clustered together,
212 mainly contributed to Dim 1 and were associated with high concentrations of the first group of
213 elements. A cluster of *Hip* together with some single *Glo*, *Dia* and *Gal* individuals influenced
214 Dim2 (27.9% of total variance) and was characterised by higher concentrations of the second
215 group of elements.

216 In roots, 59.3% of total variance was explained by the first two dimensions (**Figure 2c**). Three
217 main groups of elements with strong positive intra-group correlations, were distinguished. The
218 first group was composed of Al, Ca, V, Mn, Ni, Zn, Y and Ce and contributed to Dim1 (42.8%
219 of total variance) (**Figure SM 3**). The second group was composed of P, S and Tl and mainly
220 contributed to Dim2 (16.5% of total variance). The third group comprised Fe, As, Sb and Ba
221 and influenced both Dim 1 and Dim2 (**Figure SM 3**).

222 All seven individuals of *Sax* and one individual of *Gal* were associated with the first group
223 while the second group of variables was connected by individuals of *Eup* and one individual of
224 *Gal*.

225 Isolated individuals of *Dac* and *Eup* were associated with the third group of variables.

226 Overall, the PCA shows that specific groups of chemical elements were decisive in explaining
227 the variance in roots and aerial plant parts driven by the individuals of *Sax* and *Hip* and by a
228 single individual each of *Dia*, *Glo*, *Gal*.

229 Cluster analysis confirmed the tendencies revealed by the PCA. Three clusters of individuals
230 were distinguished in the aerial plant parts based on their similar metal absorption patterns
231 (**Figure 2b**). The first group contained all seven individuals of *Sax* while the second group

232 contained all three individuals of *Hip* as well as a single individual each of *Glo*, *Gal* and *Dia*.
233 The third group contained all other individuals showing proximity among *Pil* and *Eup*
234 individuals.

235 Only two clusters were distinguished in the roots (**Figure 2d**). *Sax* and *Hip* individuals were
236 part of the same cluster whereas all individuals of *Pil*, *Bis*, *Dia*, *Ver* and *Jun* formed the second
237 cluster. Individuals of *Hip*, *Dac*, *Gal* and *Eup* were part of both clusters.

238

239 3.2.3 Species related differences in uptake of critical metals

240 According to the PERMANOVA, the factor “plant species” explained 65.65% of the variance
241 in elemental concentrations while only 4.4% was explained by the factor "plant part" (**Table**
242 **SM 3**).

243 A Welch one-way ANOVA test combined with a Games-Howell post hoc test (**Table 3**, **Table**
244 **4**) showed that three species, *Jun*, *Sax* and *Hip*, stood out from the others.

245 *Juniperus communis* (*Jun*) generally had lower elemental concentrations in its aerial parts than
246 the other species. Indeed, *Jun* contained the lowest mean concentrations of Mg, Al, S, K, V, Fe,
247 Ni, Cu, Zn, As, Y, Sb, Ce and Tl. The concentrations of all these elements were significantly
248 lower than in at least one other species (**Table 3**). However, this tendency was not observed in
249 the roots (**Table 4**).

250 In turn, *Saxifraga paniculata* (*Sax*) had the highest concentrations of many of the elements
251 analysed. In the aerial parts, significant differences in the concentrations of Mg, Ca, Mn, Ni, Y
252 and Ce were found. The concentrations of Ca, Mn, Ni and Y were significantly higher than in
253 all the other species. In the roots, the highly concentrated elements were very similar to those
254 in the aerial parts. The concentrations of Al, Ca, Mn, Ni, As and Ce were significantly higher
255 than in at least three of the other species.

256 The third species with characteristic elemental concentrations was *Hippocrepis comosa* (*Hip*).
257 The elements accumulated in the aerial parts of *Hip* were Al, V, Fe, As, Sb and Mo. Differences
258 in Al, V, Fe and As were significantly higher than in the other species. Differences in the
259 concentrations of Sb and Mo were not significant. It should be noted that average concentrations
260 of Mo in *Hip* were about 5 times higher in roots (up to 26.9 mg/kg DW) and 30 times higher in
261 the aerial parts (up to 53.7 mg/kg DW), than in all the other species.

262 The significance between groups (species) depends on both the variability within each group
263 and between the groups. Species with highly variable concentrations are therefore not identified
264 as significantly differing from one another. For example, *Bis* and *Gal* had locally high
265 concentrations of Tl in their aerial parts, up to 541 mg/kg DW in *Bis* and up to 1091 mg/kg DW

266 in *Gal*. Despite the fact that no individual of another species exceeded 100 mg/kg DW, the
267 differences were not significant. The concentrations of Ba in *Sax* are another example of non-
268 significant differences due to high variability. The maximum concentration measured in the
269 aerial parts was 5007 mg/kg, while in the other species 1445 mg/kg was the maximum
270 concentration measured.

271

272 3.2.4 Identification of candidates for phytoremediation applications

273 The translocation-factor (TF) is a useful tool to identify the transfer strategy of a plant. The
274 higher the factor, the larger the share of the element translocated to the aerial parts (Macnair,
275 2003).

276 **Figure 3** is a heatmap of the mean TFs for the plant species and chemical elements investigated
277 with a colour spectrum ranging from pale yellow to dark red. The darker the colour of a box,
278 the higher the TF. The TF was low or highly variable in the majority of the twelve plant species.
279 *Bis* had the highest TF for many of the critical metals analysed (Mg, V, As, Y, Sb, Ce, Tl). In
280 particular, the translocation of Tl was very high (TF=19.4±17.2). A second species with a high
281 TF for Tl was *Gal* (TF=2.0±0.5). *Dia* also translocated many critical metals to the aerial parts
282 as shown by the high TF for Mg, V, As, Ce and Tl. *Hip*, on the other hand, had the highest TF
283 for Mo (TF=3.8±4.4). In *Sax*, the TF was low for most of the critical metals (As, Y, Ce, Sb, Tl)
284 and the only elements that were preferably stored in the leaves were Mg (TF=1.3±0.2) and Ca
285 (TF=2.1±0.8). *Jun* only had low TFs.

286

287 4 Discussion

288 4.1 Jas Roux - A good site to study a plant community exposed to a cocktail 289 of metals and metalloids in a natural ecosystem

290 The Jas Roux outcrop has fairly been studied regarding its exceptional mineral composition
291 (Biagioni et al., 2015; Johan and Mantiene, 2000; Topa et al., 2014). However, the surrounding
292 ecosystem, including the soil and the plants has never been studied before.

293 The concentrations of Zn (602 mg/kg), As (2382 mg/kg), Mo (19.3 mg/kg), Sb (778 mg/kg),
294 Ba (27010 mg/kg) and Tl (242 mg/kg) were far above ordinary soil concentrations (**Table 2**).
295 Soil concentrations [in European topsoil](#) of Zn are usually around 68 mg/kg, of As, 11 mg/kg,
296 of Mo, 0.9 mg/kg, of Sb, 1 mg/kg, of Ba, 400 mg/kg, and of Tl, 0.8 mg/kg (**Table 2**) (Salminen
297 et al., 2005). Except for Mo, all these elements have also been identified as elements associated
298 with the primary minerals in the Jas Roux outcrops, namely barite, quartz and pyrite, as well as
299 Zn, Sb, As, Tl, Ag, Hg and Pb sulphides and sulphosalts (Boulliard et al., 2010). In terms of

300 mobility and phytoavailability Zn, As, Mo, Sb, Ba and Tl remained < 0.1% of the total
301 concentrations and only Ba exceeded 1 mg/kg with 16.0 mg/kg after CaCl₂ extraction and 199
302 mg/kg after NH₄NO₃ extraction. These concentrations are comparable with those measured in
303 soils close to an active barite mining region in China with up to 111 mg/kg of phytoavailable
304 Ba (Lu et al., 2019). The mobility and phytoavailability of As, Sb and Zn were low compared
305 to those in contaminated soils with comparable total concentrations (Pueyo et al., 2004; Qasim
306 et al., 2015). This low mobility ~~is due to~~ indicates the ~~fact that~~ dominance of stable minerals in
307 ~~Jas Roux is a natural ecosystem that.~~ Unlike other sulphosalt sites, Jas Roux has not been
308 disturbed by mining activities ~~that~~ which generally increase metal mobility. ~~(Resongles et al.,~~
309 2014; Wei et al., 2018).
310 ~~The particularity of Jas Roux is its location in the heart of the Ecrins National Parc and the fact~~
311 ~~that, unlike other sulphosalt sites, it has never been disturbed by mining activities (Ilmen et al.,~~
312 2016; Klemm et al., 2004). ~~The plant community in Jas Roux has had millennia to adapt to an~~
313 ~~environment with no human intervention.~~

314 Even though many of the characteristic elements of Jas Roux are known to be toxic for plants
315 (As, Tl etc.), plant diversity is that of a classical alpine ecosystem (Landolt, 2015; Michalet et
316 al., 2002). It is possible that the relatively low phytoavailability of the metals helps the plants
317 to maintain their homeostasis in the short term (**Table 2**), but long-term exposure to low doses
318 can result in a high real exposition, to which plants need to adapt.

319 Plants use different strategies to adapt to high concentrations of toxic elements in the soil. Some
320 plants are able to exclude the metals and others to accumulate the elements in specific
321 compartments (Tremel-Schaub and Feix, 2005). Among the accumulating metallophytes,
322 metals are generally accumulated in the roots, but some species promote translocation to the
323 aerial plant parts (Krämer, 2010). Different statistical approaches have been used to identify the
324 different adaption strategies and revealed characteristic uptake-patterns for the two plant
325 species ~~*Saxifraga paniculata* and *Hippocrepis comosa*~~ *Sax* and *Hip* (**Figure 2b**).

326

327 **4.2 *Saxifraga paniculata* - A cation accumulating species with an affinity for** 328 **REEs and alkaline earth metals**

329 In terms of elemental concentration in roots and aerial parts ~~*Saxifraga paniculata*~~ *Sax* stood out
330 among the 12 species analysed (**Figure 2**). In the aerial parts, the PCA showed the individuals
331 of *Sax* to be associated with Mg, Ca, Mn, Ni, Zn, Y, Ba and Ce (**Figure 2**). Among these
332 elements, the concentrations of Ca, Mn, Ni, Y were significantly higher than in all the other
333 species (**Figure 2, Table 3**). It is worth noting that the majority of elements accumulated in the

334 aerial parts of *Saxifraga paniculata* Sax are divalent cations (Ba, Ni, Mn, Ca, Zn and Mg) while
335 the three elements with the highest concentrations (Mg, Ca and Ba) are alkaline earth elements.
336 This set of accumulated elements could be caused by overexpression of nonselective cation
337 channels or increased activity of a single type of metal cation transporter, as their selectivity is
338 known to be rather low (Demidchik and Maathuis, 2007; Thomas et al., 2005).

339 The genus *Saxifraga* comprises about 400 species and some of them have already been studied
340 regarding metal tolerance (Gornall, 1987). To give one example, *Saxifraga oppositifolia*,
341 *Saxifraga cespitosa* and *Saxifraga azoides* growing in Spitzbergen contained higher
342 concentrations of Pb and Zn in their aerial parts than concentrations in the soil (Jóźwik and
343 Magierski, 1991). Further, a Cu tolerant population of *Saxifraga stellaris* growing on polluted
344 soils was identified in southern Germany, with concentrations up to 900 mg/kg DW (Adlassnig
345 et al., 2013; Ryszka et al., 2019; Sissolak, 1984).

346 ~~*Saxifraga paniculata*, the species analysed in this study,~~ Sax belongs to a specific taxonomic
347 section (sect. *Ligulatae*) characterised by strong guttation (Gornall, 1987). Guttation is a
348 process to regulate root pressure and nutrient flow by excreting phloem and xylem sap through
349 openings on the leaf surface and is observable in a wide range of plant species, including many
350 Saxifragaceae (Singh, 2014, 2013). The openings are called hydathodes and, in contrast to
351 stomata, remain permanently open (Singh, 2014). The guttation process is thought to help plants
352 expel excessive nutrient salts and harmful elements (Hossain et al., 2017; Neumann et al., 1997;
353 Singh, 2013; Stahl, 1919). In some species of *Saxifraga*, including *Saxifraga paniculata* Sax,
354 the repeated evaporation of guttation droplets leads to the formation of a calcite ~~crust of calcium~~
355 ~~carbonate~~ on the leaf surface (Arnott and Pautard, 1970; Schmidt, 1930; Wightman et al., 2017).
356 These deposits appear to be the result of increased Ca uptake and might be a strategy to reduce
357 the cytotoxic effects of Ca while maintaining high flow rates (Nazrul Islam and Kawasaki,
358 2015). In the present study, the accumulation of Ca by *Saxifraga paniculata* Sax is reflected in
359 the significantly higher Ca-concentrations measured in the roots and the aerial parts (**Table 3**,
360 **Table 4**) and the high translocation factor (**Figure 3**).

361 This increased Ca uptake could also explain the higher concentrations of REEs (Y, Ce) in
362 *Saxifraga paniculata* Sax compared to those in the other species, as REEs are probably absorbed
363 through the same pathway as Ca because of their similar ionic radius (Carpenter et al., 2015;
364 Kastori et al., 2010; Rengel, 1994; van der Ent et al., 2017). Ozaki et al. (1997) observed
365 increased REE-concentrations combined with higher Ca and Ba uptake in several REE-
366 accumulating fern species and suspected a possible link between these elements.

367 The accumulation of REEs is of particular interest for this study as REEs are crucial for modern
368 technologies. Compared to the average concentration in the soil (4.22 ± 1.04 mg/kg) the roots of
369 *Saxifraga paniculata*Sax individuals contained nearly twice as much Y (7.37 ± 3.24 mg/kg)
370 (Figure 4). This raises the question of how *Saxifraga paniculata*Sax is able to absorb such a
371 high ratio of Y (Figure SM 4). ~~The most Y soil mineralogy in Jas Roux soil has not been studied~~
372 ~~so far. Y can be associated with several mineral phases, but one~~ common soil mineral based on
373 Y is Xenotime (YPO_4) ~~which~~(Ramos et al., 2016). Xenotime has low solubility (Liu and Byrne,
374 1997; Ramos et al., 2016; Tropper et al., 2011). ~~However, and phosphate chemistry in iron-rich~~
375 ~~environments like Jas Roux is highly complex and the formation of mixed amorphous phases~~
376 ~~is possible. Phosphate~~ minerals are generally difficult for plants to access (Darcy et al., 2018);
377 Liu and Byrne, 1997; Tropper et al., 2011). However, there are some exceptions - like white
378 lupin or cassava - which can take up a fraction of soil P that is not accessible to other plant
379 species. In the case of cassava, the mechanisms allowing the increased P-uptake are strong
380 mycorrhization (Zaag et al., 1979). In white lupin, on the other hand, increased root exudation
381 is combined with a cluster root system (Braum and Helmke, 1995). In the case of *Saxifraga*
382 *paniculata*Sax, root exudates are more likely to play an important role, as the plant is able to
383 grow in very extreme habitats, in high altitudes and with little organic matter (Burga et al.,
384 2004; Neuner et al., 1999).

385

386 **4.3 *Hippocrepis comosa* - A oxyanion accumulating species with a particular** 387 **affinity for Mo**

388 The second plant species showing a characteristic pattern in elemental uptake was *Hippocrepis*
389 *comosa*Hip (Figure 2b). In the aerial parts, all three individuals were positively correlated with
390 the elements As, Fe, Sb, Mo, V and Al (Figure 2) with a particularly high TF for Mo
391 ($TF = 3.8 \pm 4.4$) (Figure 3). The fact that none of these elements accumulated in the aerial parts
392 of *Saxifraga paniculata*Sax led to two easily distinguishable groups, separating the two species
393 (Figure 2b).

394 Among the elements accumulated by *Hippocrepis comosa*Hip typical elemental correlations
395 became visible. Due to their geochemical similarities, As and Sb (for example) are considered
396 to be related elements (Herath et al., 2017). A positive correlation between these two elements
397 has already been observed in plants and increased Sb uptake is presumed to be the result of
398 damaged cell membrane integrity caused by As (Feng et al., 2013; Müller et al., 2013).

399 There is also a strong link between the uptake mechanisms of Mo and Fe (Bittner, 2014). Most
400 Mo-enzymes also require Fe-containing redox groups and many studies reported increased Mo

401 uptake in the presence of Fe and vice versa (Bittner, 2014; Kannan and Ramani, 1978; Zimmer
402 and Mendel, 1999).

403 Three major hypotheses are proposed to explain the characteristic uptake pattern of *Hippocrepis*
404 *comosa*: Hip:

405 I) One hypothesis is a characteristic modification of the rhizosphere that would influence the
406 redox potential. This modification could locally increase phytoavailability of the accumulated
407 elements, as, except for Al, all are redox sensitive elements (As, Fe, Sb, Mo, and V).

408 II) The second hypothesis concerns the uptake pathway of the elements. It is known that As,
409 Sb, Mo, and V are usually absorbed as oxyanions by plants (Hossain et al., 2018; Kabata-
410 Pendias, 2011). Vanadate ($\text{H}_2\text{VO}_4\text{VO}_4^{3-}$) and arsenate ($\text{H}_2\text{AsO}_4\text{AsO}_4^{3-}$) enter the plant through
411 phosphorousP transporters, due to similarities in structure and charge (Chen et al., 2021;
412 Meharg and Hartley-Whitaker, 2002); Han et al., 2016). Antimonate, on the other hand, is more
413 likely to be absorbed through less selective anion transporters, such as those for Cl^- and NO_3^- ,
414 or an incompletely sealed or damaged Casparian strip (Feng et al., 2013; Tschan et al., 2009b,
415 2009a). However, little is known about molybdenumMo uptake by roots. Because of structural
416 and behavioural similarities between molybdate ($\text{HMoO}_4\text{MoO}_4^{2-}$) and sulphate ($\text{HSO}_4\text{SO}_4^{2-}$), it
417 is plausible that sulphate transporters are involved (Bittner, 2014; Zimmer and Mendel, 1999).
418 Overexpression or increased activity of so many different transporters, responsible for the
419 particular elemental concentrations found in *Hippocrepis comosa* Hip, is unlikely. Nevertheless,
420 a very unselective anion transporter, able to transport phosphate, sulphate, nitrate and chlorate,
421 could be involved. Such a transporter was identified in barley by Preuss et al. (2010), for
422 example.

423 III) The third hypothesis is based on the nitrogenN fixing property of *Hippocrepis comosa* Hip,
424 as Mo and Fe are known to play an essential role as cofactors during symbiotic nitrogenN
425 fixation (Hossain et al., 2018). For this reason, legume plants like *Hippocrepis comosa* Hip have
426 a higher demand for Mo than non-nitrogenN fixing plants (Kabata-Pendias, 2011). It is
427 plausible that *Hippocrepis comosa* Hip is able to promote Mo absorption in order to guarantee
428 nitrogenN fixation under Mo deficient conditions. However, in Mo rich soils like Jas Roux
429 (19.9 ± 3.71 mg/kg), this property might lead to an increased uptake and thus explain the
430 comparatively high Mo concentrations in *Hippocrepis comosa* Hip (**Figure 4**). It is worth
431 mentioning that vanadiumV may also interfere during nitrogenN fixation by replacing Mo in
432 the nitrogenase enzyme (Hernandez et al., 2009).

433 Individually, none of the three hypotheses explains all the elemental concentrations observed
434 in *Hippocrepis comosa* Hip. Nevertheless, a combination of the mechanisms could be a valid

435 explanation. AluminiumAl is the only element whose accumulation by Hippocrepis comosaHip
436 remains difficult to explain, as this species is generally found in Ca rich sites and is sensitive to
437 high Al concentrations (Michalet et al., 2002). Concentrations of Al > 1000 mg/kg, like those
438 we measured in aerial parts of Hippocrepis comosaHip (1336 mg/kg DW), are rare in higher
439 plants and are considered as Al accumulation (Kabata-Pendias, 2011). The tolerance of toxic
440 elements (Al, As) makes Hippocrepis comosaHip an interesting candidate for remediation of
441 polluted soils as its ability to fix nitrogenN thereby improving soil fertility could be an
442 additional advantage (Pajuelo et al 2008).

444 **4.4 Translocation factor reveals specific plant strategies**

445 The differentiation of Hippocrepis comosa and Saxifraga paniculataHip and Sax from the other
446 species was mainly caused by high concentrations of a particular set of elements that made both
447 species stand out in the PCA and the cluster analysis. However, this statistical approach failed
448 to detect affinities for single elements, or adaption strategies associated with particularly low
449 concentrations. The translocation factor in combination with the mean elemental
450 concentrations, revealed further adaption strategies and species-related tendencies in elemental
451 uptake (Figure 3).

452 ~~Biological factors are a useful tool to identify adaption strategies. Together with the mean~~
453 ~~elemental concentrations, the translocation factor indeed revealed further species related~~
454 ~~tendencies in elemental uptake (Figure 3).~~

455 For instance, Biscutella laevigataBis and Dianthus sylvestrisDia did not form a distinct group
456 in the PCA or in the cluster analysis but both had TFs > 1 for a large number of critical metals
457 (Mg, V, Mn, As, Y, Sb, Ba, Ce, Tl). In particular, the TF of Biscutella laevigataBis for Tl was
458 remarkably high (TF=19.4±17.2) indicating Tl accumulation (256±224 mg/kg) (**Figure 4**).
459 While no explanation was found in the literature for the high TF of Dianthus sylvestris,
460 Biscutella laevigataDia, Bis belongs to the Brassicaceae family, which includes many
461 hyperaccumulating species (Babula et al., 2008; van der Ent et al., 2013). Biscutella
462 laevigataBis itself has already been mentioned as a Tl accumulating species (LaCoste et al.,
463 1999; Pošćić et al., 2013). But Biscutella laevigataBis was not the only Tl accumulating species,
464 as Galium corrudifoliumGal, which has never previously been associated with metalliferous
465 sites, also showed high concentrations of Tl in its aerial parts (254±410 mg/kg) and high
466 translocation (TF=2.0±0.5).

467 Another species that did not stand out in the statistical analysis was Juniperus communis subsp.
468 nanaJun. Nevertheless, Juniperus communis subsp. nanaJun had the lowest concentrations of

469 the elements Mg, Al, S, K, V, Fe, Ni, Cu, Zn, As, Y, Sb, Ce and Tl in its aerial parts (**Table 3**).
470 This exclusion strategy seems to consist in limited translocation to the aerial parts, as the TF
471 was low for all elements (**Figure 3**). Given that *Juniperus communis subsp. nana* Jun is the only
472 lignifying plant among the 12 species we analysed, its exclusion capacity might be due to
473 different plant-internal barriers or reduced flow rates due to its small leaf surface area. Even
474 though no studies have demonstrated metal exclusion in Juniperus species, metal concentrations
475 in other conifers (Pinales) have already been reported. For example, Mingorance et al. (2007)
476 found *Pinus pinea* growing in an industrial area was able to exclude Al, Ba, Cr, Fe, and Pb. In
477 light of the results of our study, *Juniperus communis subsp. nana* Jun could be an interesting
478 candidate for phytostabilisation thanks to its high resistance to metal contamination, its
479 widespread occurrence and its ruggedness (Saladin, 2015).

480

481 5 Conclusions

482 ~~Jas Roux is a unique site to understand the soil-to-Among 12~~ plant transfer of critical metals in
483 ~~an ecosystemspecies~~ exposed to a cocktail of metals and metalloids in an entirely natural
484 environment ~~that has never been affected by human activities. The statistical approach used in~~
485 ~~the present study revealed species related uptake patterns in the aerial plant parts. The~~ two
486 species ~~that stood out-were:~~ *Hippocrepis comosa* and *Saxifraga paniculata*, each of which
487 accumulated an individual set of elements.

488 ~~Different hypotheses for these particular uptake patterns are proposed. Saxifraga paniculata~~
489 preferably absorbed divalent cations, but also the REEs Y and Ce. ~~The ionic radius of both~~
490 ~~elements resembles that of calcium, which was strongly accumulated~~This uptake pattern could
491 be explained by the Ca accumulation property of Saxifraga paniculata. The affinity to Ca might
492 cause an increased absorption of other elements with a similar ionic radius like Y and Ce, or
493 similar ionic charge like Mn and Ba. *Hippocrepis comosa*, on the other hand, preferably
494 absorbed oxyanions, perhaps due to overexpression or increased activity of a non-selective
495 anion transporter. ~~In turn, this anion affinity is probably linked to the nitrogen fixation activity~~
496 ~~of Hippocrepis comosa, which increases its demand for the anionic cofactor Mo (Kabata-~~
497 ~~Pendias, 2011; Zimmer and Mendel, 1999).~~Furthermore, Hippocrepis comosa is an interesting
498 candidate for phytoremediation of polluted soils, as its ability to fix N₂ improves soil fertility
499 and could thus facilitate the establishment of other species.

500 ~~Further, different~~Different tolerance strategies were identified among the ~~12~~studied plant
501 species. ~~On the one hand,~~ *Biscutella laevigata* and *Dianthus sylvestris* favoured translocation
502 of multiple critical metals to their aerial parts while, ~~on the other hand,~~ *Juniperus communis*
503 avoided translocation and used an exclusion strategy. Particularly high concentrations of Tl
504 were found in *Biscutella laevigata* and *Galium corrudifolium*. While hyperaccumulation of Tl
505 has already been reported in *Biscutella laevigata*, it is a new discovery in *Galium corrudifolium*.

506 ~~Likewise, the~~The exclusion capacity of *Juniperus communis subsp. nana* ~~has never previously~~
507 ~~been documented and~~ might represent a potential for phytostabilisation applications. Erosion
508 of a polluted soil could be prevented in the long term with no risk of transfer into the food chain
509 because *Juniperus communis subsp. nana* prevents elemental translocation and the shrub is not
510 consumed by grazing animals.

511 ~~To conclude, several promising species among the 12 candidates were identified in the course~~
512 ~~of this study.~~ These results advance our understanding of soil-to-plant transfer of poorly studied
513 but increasingly used critical metals and point to the potential of several plant species for future
514 phytoremediation ~~and phytoextraction~~ techniques.

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Abbreviations:

ANOVA.....	Analysis Of Variance
a.s.l.	Above Sea Level
DW	Dry Weight
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
PCA	Principal Component Analysis
PERMANOVA.....	Permutational Multivariate Analysis Of Variance
PGMs	Platinum Group Metals
REEs	Rare Earth Elements
SD	Standard Deviation
subsp.	Subspecies
TF	Translocation Factor
XRD	X-Ray Diffraction

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Response to reviewers

We would like to thank both reviewers for their efforts reviewing our revised manuscript. Thanks to their comments we were able to improve consistency and straightforwardness of the paper.

Reviewer #1: The authors have addressed most issues raised by the reviewers. I miss a deeper discussion on the control of mineralogy on the metal bioavailability for these plants, and comparison of metal phytoextraction with the same plants but in cleaner environment.

We fully agree that it would be valuable to perform more soil characterization to have a better knowledge on soil mineralogy. However, the majority of the observed variability in metal concentrations could be explained by the plant species. Therefore, we focused our discussion on species related particularities. We identified interesting couples of plant/critical element in this study, for which we will perform further studies, including advanced mineralogy characterization and a plantation of Jas Roux species on an artificially doped soil. Despite that, I consider that the manuscript could be published after minor changes below.

Some minor comments:

I found the Graphical Abstract low informative

We revised the graphical abstract to include additional information and hope the new version is an improvement.

Line 19: avoid the use of ellipsis

We replaced the ellipsis by e.g. in L17: "(e.g. phytostabilisation, phytoextraction)"

Line 60: be consistent with section numbering, Introduction should be the point 1

Thank you for this comment, the oversight has been corrected accordingly

Line 77: the difficulties commented by the authors in the response to reviewers letter should be included in the manuscript.

We agree with the reviewers' comment and added an additional sentence L75-78: "The steep slope, variable soil depth and the presence of large rocks impeded a systematic sampling approach. Nevertheless, the samples were collected as evenly distributed as possible. The soil samples were collected from 10 sampling points in the study zone and consisted of 1 kg of topsoil each (< 15 cm)."

Line 102: 0.45 μ m ??

The mistake has been corrected for CaCl₂ and NH₄NO₃ extractions

Line 283: which soils? European, worldwide? I am afraid that these values range notably according to background geology. I guess that the authors refer to European average values

Table 2 indicates average European topsoil concentrations according to Salminen et al., 2005. The information has been added to the text according to the reviewers' proposition L288-290: "Soil concentrations in European topsoil of Zn are usually around 68 mg/kg, of As, 11 mg/kg, of Mo, 0.9 mg/kg, of Sb, 1 mg/kg, of Ba, 400 mg/kg, and of Tl, 0.8 mg/kg (Table 2)"

Lines 293-295: also, the mineralogy, with predominance of stable minerals, plays an important role in the low metal mobility

We agree with the reviewer that this information was missing in this paragraph. We modified the paragraph accordingly. L299-301: This low mobility indicates the dominance of stable

minerals in Jas Roux. Unlike other sulphosalt sites, Jas Roux has not been disturbed by mining activities which generally increase metal mobility (Resongles et al., 2014; Wei et al., 2018).

Lines 357-361: other minerals can host significant concentrations of Y, so I find this part too speculative without a deeper mineralogical study of the substrate by SEM-EDS or similar techniques.

We agree with the reviewers' comment and reduced the speculative part of this paragraph. L357-361: "Y soil mineralogy in Jas Roux soil has not been studied so far. Y can be associated with several mineral phases, but one common soil mineral based on Y is Xenotime (YPO₄) (Ramos et al., 2016). Xenotime has low solubility and phosphate minerals are generally difficult for plants to access (Darcy et al., 2018; Liu and Byrne, 1997; Tropper et al., 2011)."

Line 399: this is not sulphate but bisulphate. Please clear it up

We changed the chemical formula in L392 + L398 to the unprotonated forms to be more consistent.

Line 413: please be consistent, use the chemical symbol or the name

We changed all element names to the chemical symbol.

Line 422: accumulators??

This sentence seems right as it is. The subject of the sentence is "concentrations". L416-417: "Concentrations of Al > 1000 mg/kg, like those we measured in aerial parts of Hippocrepis comosa (1336 mg/kg DW), are rare in higher plants and are considered as Al accumulation (Kabata-Pendias, 2011)."

Line 437: As is a metalloid not a metal. Neither is critical

Indeed, this is an important comment. We used the term "critical metals" instead of "critical elements" as it is a standing term in literature and includes metalloids like As and Sb (<https://doi.org/10.1016/j.resconrec.2019.104669>). We added a sentence to explain our choice of critical elements and justify the presence of As which is considered critical in many studies summarized by Hayes and McCullough - 2018. L130-133: "The critical elements were chosen based on the study on the EU's list of critical raw materials and a review of critical minerals and will be called critical metals throughout the manuscript (European Commission, 2020; Hayes and McCullough, 2018)."

Reviewer #3: Metal accumulation in plants have been widely studied in the past few decades. As stated by Fehlaue et al., however, the case of rare metals accumulation in plants is limited. Totally, the work present here is very important and interesting, but I think the manuscript should be revised again according to the following suggestions or comments.

We thank reviewer #3 for the kind words. Thanks to the comments and suggestions we were able to further improve our manuscript.

Line 10: "Their increasing use also involves increased risk of their dispersion", what is the main focus of your study? You introduced rare element application in industry, its risks to humans and how to remove them by phytoremediation. I recommend the authors to highlight the most important goal of the study throughout the manuscript.

Indeed, the main goal of the study was mentioned too late in the introduction. Thanks to the reviewers' comments we modified our second paragraph to improve the straightforwardness of the introduction. L12-15: "Aim of this study is to analyse the soil-to-plant transfer of some

of these metals and to shed light on their uptake pathways. Our findings might bare valuable information for phytoremediation and promote plant-based remediation techniques of metal-contaminated sites."

Furthermore, the main goal of the study is specified again in the introduction, the abstract and the conclusion.

L32-35: "The huge number of factors and complex interactions are impossible to reproduce in the laboratory, thus, to understand the uptake of poorly studied elements, such as critical metals, performing in-situ studies in a natural environment is advantageous."

L474-476: "These results advance our understanding of soil-to-plant transfer of poorly studied but increasingly used critical metals and point to the potential of several plant species for future phytoremediation techniques."

Line 43: "lead, antimony, copper, thallium and arsenic". You used the abbreviated names of some heavy metals before.

We increased consistency by using exclusively the atomic symbol instead of the names.

Line 79: "12 were selected", If two or more plants belonging to a certain species, how did you make the choice?

We chose the sampled species according to occurrence, spatial distribution and family. The family was different for each of the 12 species, except for *Dactylis glomerata* and *Festuca laevigata* that both belong to the family of Poaceae. We modified the sentence to improve clarity. L78-80: "Among the 65 plant species identified in the study zone, 12 were selected, taking into account occurrence, spatial distribution and family affiliation (Table 1)."

Lines 201-225: Pls add the figure captions of Hip, Glo, Dia, etc., following the figure 2.

The acronyms of each species are introduced in Table 1. Additionally, we added the information to the caption of each figure as proposed by the reviewer.

Figure 2: Another question, did the PCA show statistical significance?

No, a PCA does not allow to draw any conclusions regarding statistical significance. It is more of a visualization of the dataset. The clustering however gives statistical information about clusters within the dataset. Statistical differences between species regarding the elemental concentrations have been tested using a Games Howel test and are illustrated in Table 3 and 4.

Section 4.4: Pls reference to previous article <http://dx.doi.org/10.1016/j.jhazmat.2016.08.079>

We added the reference to the citation in L 392-394: "Vanadate (VO₄³⁻) and arsenate (AsO₄³⁻) enter the plant through phosphorous transporters, due to similarities in structure and charge (Chen et al., 2021; Meharg and Hartley-Whitaker, 2002, Han et al., 2016)."

Lines 227-258: Jun, Sax, Hip, etc., pls add the information of the abbreviated names in the first use. See Q4 and check the mistakes throughout the manuscript. You can use the full names and give the abbreviated information in the first use and use the abbreviated names in other parts.

We followed the reviewers' advice and replaced all full names by the acronyms. We kept the full names only in the conclusion and the abstract as these parts need to be understandable without reading the full paper. In order to introduce the acronyms, we added a sentence in material and methods referring to Table 1. L80-81: "For improved readability we attributed acronyms to each of the 12 species throughout the paper (Table 1)". Additionally, all acronyms are explained in the figure captions.

Lines 260-273: TF is one of the indexes to isolate the potential plants for phytoremediation. Why didn't you focus on other indexes, e.g., BF?

We also calculated the bioconcentration factor ($C_{\text{roots}}/C_{\text{soil}}$) and the migration ratio ($C_{\text{leaves}}/C_{\text{soil}}$) using the total soil concentrations. Both factors can give additional information about elemental uptake by plants. In our case however the translocation factor ($C_{\text{leaves}}/C_{\text{roots}}$) was sufficient to illustrate the main results.

Line 433: Biological factors? You only showed TF in this study.

We improved the sentence according to the reviewers' comment. L426-428: "The translocation factor in combination with the mean elemental concentrations, revealed further adaption strategies and species-related tendencies in elemental uptake (Figure 3)."

Conclusions: Its too long, pls cut down this part. Conclusion is not a summary for each part but a concise conclusion of your findings.

We fully agree with the reviewers' comment we cut down the conclusion significantly (291 words instead of 352 words). We hope that this improved overall readability and precision.

Table 1: Selected plant species, n = number of replicates

Species	Family	Acronym	n
<i>Biscutella laevigata</i>	Brassicaceae	Bis	4
<i>Dactylis glomerata</i>	Poaceae	Dac	3
<i>Dianthus sylvestris</i>	Caryophyllaceae	Dia	3
<i>Euphorbia cyparissias</i>	Euphorbiaceae	Eup	7
<i>Festuca laevigata</i>	Poaceae	Fes	7
<i>Galium corrudifolium</i>	Rubiaceae	Gal	6
<i>Globularia cordifolia</i>	Globulariaceae	Glo	3
<i>Hippocrepis comosa</i>	Fabaceae	Hip	3
<i>Juniperus communis subsp. nana</i>	Cupressaceae	Jun	3
<i>Pilosella peleteriana</i>	Asteraceae	Pil	7
<i>Saxifraga paniculata</i>	Saxifragaceae	Sax	7
<i>Veronica fruticans</i>	Plantaginaceae	Ver	2

Table 2: Average total, mobile (0.001 mol/L CaCl₂) and phytoavailable (1 mol/L NH₄NO₃) concentrations and standard deviations (SD) in mg/kg DW of topsoil from Jas Roux (n=10), as well as average concentrations in European topsoil (n≥840) from Salminen et al. 2005. The concentrations were measured by ICP-MS except for total concentrations of Al, K, Ca and S that were measured by ICP-OES.

	Mg	Al	P	S	K	Ca	V	Mn	Fe	Ni	Cu	Zn	As	Y	Mo	Sb	Ba	Ce	Tl
Total	1679	12172	678	11258	21153	3890	110	3401	58930	59.9	44.6	602	2382	4.22	19.3	778	27182	6.61	242
SD	457	1467	112	4892	2134	3651	11.1	802	11219	8.12	3.96	161	975	1.04	3.71	287	6157	2.61	145
CaCl₂	55.53	1.65	0.595	NA	79.8	NA	0.004	1.62	5.95	0.049	0.043	0.272	0.649	0.003	0.016	0.446	16.0	0.006	0.778
SD	19.6	0.986	0.350	NA	26.0	NA	0.002	1.18	4.85	0.027	0.013	0.146	0.560	0.002	0.012	0.219	6.22	0.003	0.720
NH₄NO₃	106	0.237	1.52	NA	112	NA	0.003	2.61	0.718	0.048	0.121	0.350	0.103	0.006	0.006	0.113	199	0.001	7.60
SD	34.9	0.176	0.794	NA	27.9	NA	0.001	3.25	0.610	0.024	0.044	0.166	0.096	0.003	0.004	0.054	93.3	0.001	6.78
European topsoil							68.1			37.3	17.3	68.1	11.6	22.7	0.943	1.04	400	52.2	0.821
SD							47.0			136	19.0	141	20.1	15.7	1.31	2.04	213	31.3	1.02

Table 3: Average concentrations (mg/kg DW) and standard deviation (SD) in the aerial parts of each plant species analysed. The *highest* and *lowest* concentrations of each element are highlighted. The statistical difference between the species ($\alpha=0.05$), is shown for each element by a sequence of letters within which the absence of matches indicates significant difference (NA=not available). Except for Mg, P, K, Ca and S, which were measured in ICP-OES, all concentrations were measured by ICP-MS.

		Mg	Al	P	S	K	Ca	V	Mn	Fe	Ni	Cu	Zn	As	Y	Mo	Sb	Ba	Ce	Tl
<i>Biscutella laevigata</i>																				
n=4	Mean	3781 cde	522 abc	1954 abcd	6022 abcd	14251 d	23583 abc	0.828 abc	71.3 a	589 abc	1.68 a	2.59 a	173 ab	15.9 abcd	0.396 abc	0.951 ab	5.80 ab	568 a	0.637 abc	256 a
	SD	881	385	698	1937	1091	9538	0.669	51.5	406	0.317	0.458	129	9.61	0.275	0.176	3.24	299	0.579	224
<i>Dactylis glomerata</i>																				
n=3	Mean	1838 abcd	189 a	471 ab	1375 cb	7315 abcd	4911 ab	0.320 a	230 ab	324 ab	1.84 a	3.47 ab	37.9 ab	9.34 ab	0.267 abc	1.77 a	3.29 ab	193 a	0.263 ab	5.85 a
	SD	247	72.2	123	112	2630	1566	0.119	84.6	168	0.395	0.421	27.3	5.79	0.073	0.300	1.92	124	0.118	4.07
<i>Dianthus sylvestris</i>																				
n=3	Mean	2672 abcde	659 abc	526 b	1243 abc	7520 abcd	25214 abc	1.10 abc	219 ab	797 abc	2.19 a	4.69 ab	64.3 ab	20.3 abcd	0.445 abc	0.982 ab	6.14 ab	948 a	0.887 abcd	6.68 a
	SD	931	686	85.7	231	1938	6796	1.18	91.7	870	0.949	2.10	10.7	24.3	0.420	0.385	6.86	439	0.902	3.17
<i>Euphorbia cyparissias</i>																				
n=7	Mean	2995 ce	405 ab	1818 cd	2401 bd	16302 cde	12840 bc	0.667 ab	77.5 a	517 ab	1.73 a	5.07 b	94.9 b	14.2 ab	0.230 ab	0.594 ab	4.28 ab	642 a	0.440 ab	26.9 a
	SD	858	433	504	716	5342	4526	0.727	31.8	500	0.689	1.38	44.3	12.2	0.224	0.213	3.51	469	0.461	31.5
<i>Festuca violacea</i>																				
n=7	Mean	917 b	377 ab	613 ab	1558 abc	8885 bc	3715 a	0.616 ab	80.5 a	506 ab	2.04 a	2.60 a	29.0 ab	13.4 ab	0.320 ab	1.87 a	3.53 ab	310 a	0.500 ab	6.87 a
	SD	379	327	285	549	1943	1817	0.557	49.2	504	2.34	0.556	14.8	11.2	0.274	0.786	3.18	279	0.477	10.5
<i>Galium corrudifolium</i>																				
n=6	Mean	1903 adc	550 abc	810 abcd	1939 abcd	10407 abcd	15129 c	0.941 abc	149 ab	753 abc	3.98 a	5.29 ab	79.0 ab	21.4 abcd	0.532 abc	1.27 ab	6.63 ab	627 a	0.732 abc	254 a
	SD	220	464	422	660	4019	4398	0.740	100	570	6.73	2.58	39.1	14.4	0.643	0.567	4.80	363	0.541	410
<i>Globularia cordifolia</i>																				
n=3	Mean	1734 abcd	1111 abc	734 abcd	2598 d	5378 ab	13248 bc	1.80 abc	235 ab	1249 abc	3.33 a	5.50 ab	36.9 ab	37.7 abcd	0.749 abc	0.859 ab	10.8 ab	838 a	1.57 abcd	5.56 a
	SD	337	794	298	123	1184	2191	1.38	207	1054	1.25	0.990	5.90	35.6	0.590	0.117	10.1	298	1.25	2.42
<i>Hippocrepis comosa</i>																				
n=3	Mean	1313 abd	1336 c	903 a	1622 c	7089 abc	14237 c	2.05 c	241 b	1686 c	8.89 a	7.22 ab	39.4 b	55.9 cd	0.954 c	30.8 ab	16.1 ab	1029 a	1.72 cd	18.1 a
	SD	196	118	52.0	143	1124	1883	0.190	25.8	274	5.24	2.28	4.57	10.4	0.151	19.9	4.05	354	0.168	14.6
<i>Juniperus communis ssp. nana</i>																				
n=3	Mean	789 ab	178 a	1010 ac	885 a	5272 a	10831 bc	0.292 a	102 a	203 a	1.49 a	2.31 a	14.1 a	2.61 ac	0.127 a	0.514 ab	0.888 a	249 a	0.227 a	2.18 a
	SD	321	79.3	34.5	69.2	390	582	0.127	25.4	71.3	0.039	0.169	2.81	1.59	0.042	0.128	0.497	96.8	0.094	0.345
<i>Pilosella peleteriana</i>																				
n=7	Mean	1952 acd	563 b	1391 d	1852 cd	22525 e	9919 bc	0.893 b	144 ab	575 bc	4.39 a	11.7 ab	49.5 b	16.2 bd	0.442 bc	0.873 ab	4.56 b	492 a	0.709 b	6.50 a
	SD	184	130	184	447	2935	1933	0.210	69.7	146	1.81	5.99	14.6	7.41	0.169	0.559	1.73	166	0.182	3.16
<i>Saxifraga paniculata</i>																				
n=7	Mean	4056 e	604 ab	773 ab	995 ab	5914 ab	52243 d	0.423 ab	1282 c	318 ab	36.6 b	3.12 ab	636 ab	7.63 abcd	3.31 d	0.445 b	2.41 ab	2323 a	2.67 d	28.6 a
	SD	695	275	256	128	1931	10053	0.407	356	219	11.6	0.535	405	3.81	1.18	0.362	1.53	1359	0.974	18.5
<i>Veronica fruticans</i>																				
n=2	Mean	1309 NA	711 NA	534 NA	1195 NA	6816 NA	6569 NA	1.16 NA	194 NA	876 NA	3.31 NA	10.1 NA	83.9 NA	22.5 NA	0.535 NA	1.50 NA	6.95 NA	625 NA	1.07 NA	10.2 NA
	SD	171	109	85.1	265	525	674	0.236	82.4	130	0.023	4.18	24.5	11.0	0.087	0.090	2.65	68.5	0.300	6.37

Table 4: Average concentrations (mg/kg DW) and standard deviation (SD) in the roots of each plant species analysed. The *highest* and *lowest* concentrations of each element are highlighted. The statistical difference between the species ($\alpha=0.05$), is shown for each element by a sequence of letters within which the absence of matches indicates significant difference (NA=not available). Except for Mg, P, K, Ca and S, which were measured in ICP-OES, all concentrations were measured by ICP-MS.

		Mg	Al	P	S	K	Ca	V	Mn	Fe	Ni	Cu	Zn	As	Y	Mo	Sb	Ba	Ce	Tl
<i>Biscutella laevigata</i>																				
n=3	Mean	1654 abc	704 a	1068 ab	5100 ab	12449 abc	7720 bc	<u>0.913</u> a	<u>133</u> a	1124 ab	7.37 a	<u>4.60</u> a	241 a	31.3 a	<u>0.804</u> ab	<u>0.609</u> a	11.9 a	1073 a	1.19 a	23.1 ab
	SD	295	555	305	1058	3469	2199	0.660	87.7	1173	4.45	3.30	210	32.7	0.717	0.229	13.3	1339	1.09	18.9
<i>Dactylis glomerata</i>																				
n=3	Mean	1000 ab	2241 ab	591 ab	2991 ab	3485 a	13851 abcd	4.34 abc	339 ab	5449 ab	8.60 a	19.1 a	95.3 a	<u>210</u> ab	2.23 abc	3.58 ab	39.1 a	<u>4155</u> a	2.98 ab	125 ab
	SD	369	1867	146	2047	867	1476	4.35	191	6101	4.09	11.0	47.5	274	1.08	1.50	26.5	5324	2.35	43.4
<i>Dianthus sylvestris</i>																				
n=3	Mean	1588 ab	<u>640</u> a	454 a	1242 a	3760 a	10156 abcde	1.02 ab	171 a	<u>785</u> a	<u>4.80</u> a	21.6 a	<u>52.4</u> a	<u>21.3</u> a	0.881 a	3.52 ab	<u>7.77</u> a	1321 a	<u>1.08</u> a	<u>6.89</u> a
	SD	189	528	132	305	904	5642	0.876	91.0	658	2.25	23.2	20.1	19.9	0.341	0.747	5.84	710	0.794	2.80
<i>Euphorbia cyparissias</i>																				
n=7	Mean	2697 c	2397 ab	1293 b	3041 b	8129 b	8735 ab	3.94 bc	273 a	3641 ab	7.05 a	13.7 a	169 a	156 ab	1.78 abc	2.14 b	47.6 a	3913 a	3.07 ab	174 ab
	SD	585	746	392	710	1740	3179	1.44	121	1801	1.96	6.87	109	150	0.614	0.683	37.2	4295	1.13	156
<i>Festuca violacea</i>																				
n=6	Mean	1040 a	3446 ab	608 ab	1880 ab	4430 a	9012 ab	5.50 abc	335 a	4280 ab	9.04 a	17.5 a	84.5 a	141 ab	3.08 bc	2.65 b	35.6 a	2413 a	4.88 ab	71.3 ab
	SD	167	1746	256	813	1132	1398	3.07	173	2297	1.95	10.8	31.3	75.9	1.07	0.627	15.2	1609	2.34	63.2
<i>Galium corrudifolium</i>																				
n=6	Mean	1341 ab	2355 ab	692 ab	2133 ab	5846 ab	16531 cde	4.04 abc	312 a	3171 ab	6.95 a	9.62 a	149 a	99.7 ab	2.34 abc	2.16 ab	32.5 a	2730 a	3.41 ab	137 ab
	SD	506	2200	126	1047	1981	3479	4.04	130	2494	3.50	2.88	75.2	81.1	1.48	0.777	29.7	2683	2.95	223
<i>Globularia cordifolia</i>																				
n=3	Mean	1095 abc	2252 ab	430 a	1404 a	3831 ab	<u>7257</u> ab	3.70 abc	417 ab	2728 ab	8.17 a	21.0 a	84.1 a	86.8 ab	2.10 abc	2.76 ab	27.9 a	1192 a	3.28 ab	31.5 ab
	SD	591	1263	198	108	2731	796	2.14	225	1586	0.688	9.27	29.9	57.7	0.808	0.778	17.2	290	1.57	11.9
<i>Hippocrepis comosa</i>																				
n=3	Mean	<u>763</u> a	3362 ab	<u>410</u> a	1546 a	<u>3232</u> ab	9595 abcd	5.48 abc	624 ab	4558 ab	13.8 ab	13.0 a	77.6 a	175 ab	2.70 abc	14.3 ab	46.2 a	2143 a	4.70 ab	47.6 ab
	SD	187	1433	85.0	150	1476	1772	2.68	280	2101	6.50	4.18	20.6	90.2	1.12	11.1	25.6	1554	2.02	44.0
<i>Juniperus communis ssp. nana</i>																				
n=3	Mean	1017 ab	1852 ab	628 ab	1635 a	3972 ab	25231 ade	3.11 abc	201 a	2009 ab	7.79 a	25.8 a	105 a	51.4 a	1.95 abc	2.60 ab	30.1 a	1179 a	2.24 a	38.4 ab
	SD	170	158	110	192	1374	3922	0.293	50.5	268	1.62	5.02	43.0	8.25	0.481	0.550	11.1	166	0.240	14.7
<i>Pilosella peleteriana</i>																				
n=7	Mean	1690 bc	1360 a	1380 ab	1236 ab	24555 c	9481 abcd	2.38 abc	231 a	1478 a	6.01 a	20.0 a	70.0 a	42.2 a	1.55 ab	1.44 ab	11.3 a	1746 a	2.10 a	54.7 b
	SD	354	964	973	462	6961	2683	1.83	101	1145	2.53	16.1	33.9	35.8	0.808	0.666	8.68	830	1.37	23.5
<i>Saxifraga paniculata</i>																				
n=7	Mean	2048 abc	6453 b	623 ab	1888 ab	4081 a	26773 e	<u>9.34</u> c	<u>1252</u> b	4851 b	30.9 b	32.4 a	<u>795</u> a	177 b	<u>7.37</u> c	2.12 b	41.3 a	3802 a	<u>8.07</u> b	50.4 ab
	SD	756	2688	171	534	1345	6062	4.42	510	1806	8.07	17.6	616	68.5	3.24	0.721	18.9	1843	3.27	30.0
<i>Veronica fruticans</i>																				
n=2	Mean	1002 NA	1149 NA	434 NA	<u>900</u> NA	5819 NA	7097 NA	1.81 NA	273 NA	1261 NA	8.48 NA	33.9 NA	122 NA	36.8 NA	1.27 NA	2.20 NA	12.5 NA	<u>889</u> NA	1.74 NA	44.5 NA
	SD	18.4	217	124	183	1012	1470	0.596	21.7	449	6.28	27.3	83.0	22.4	0.071	0.077	5.70	205	0.504	47.4

Figures (pdf)

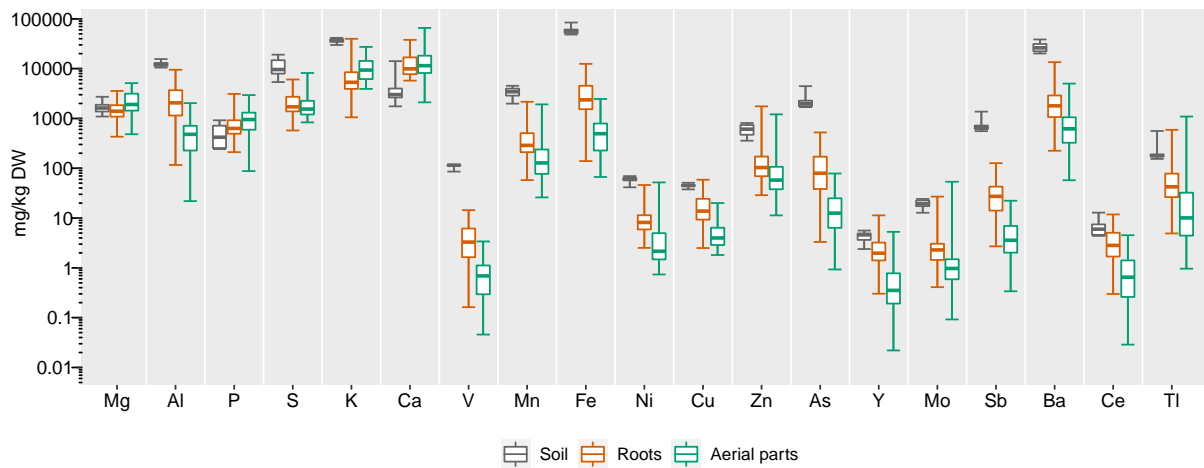


Figure 1: Boxplot with whiskers (minimum to maximum) of elemental concentrations in aerial plant parts (n=55) and roots (n=55) of all individuals of the 12 species compared to the total concentrations in the soil (n=10) in mg/kg DW. The bottom and top of the boxes represent the lower and upper quartile and the median is represented by the horizontal line inside each box.

(coloured print)

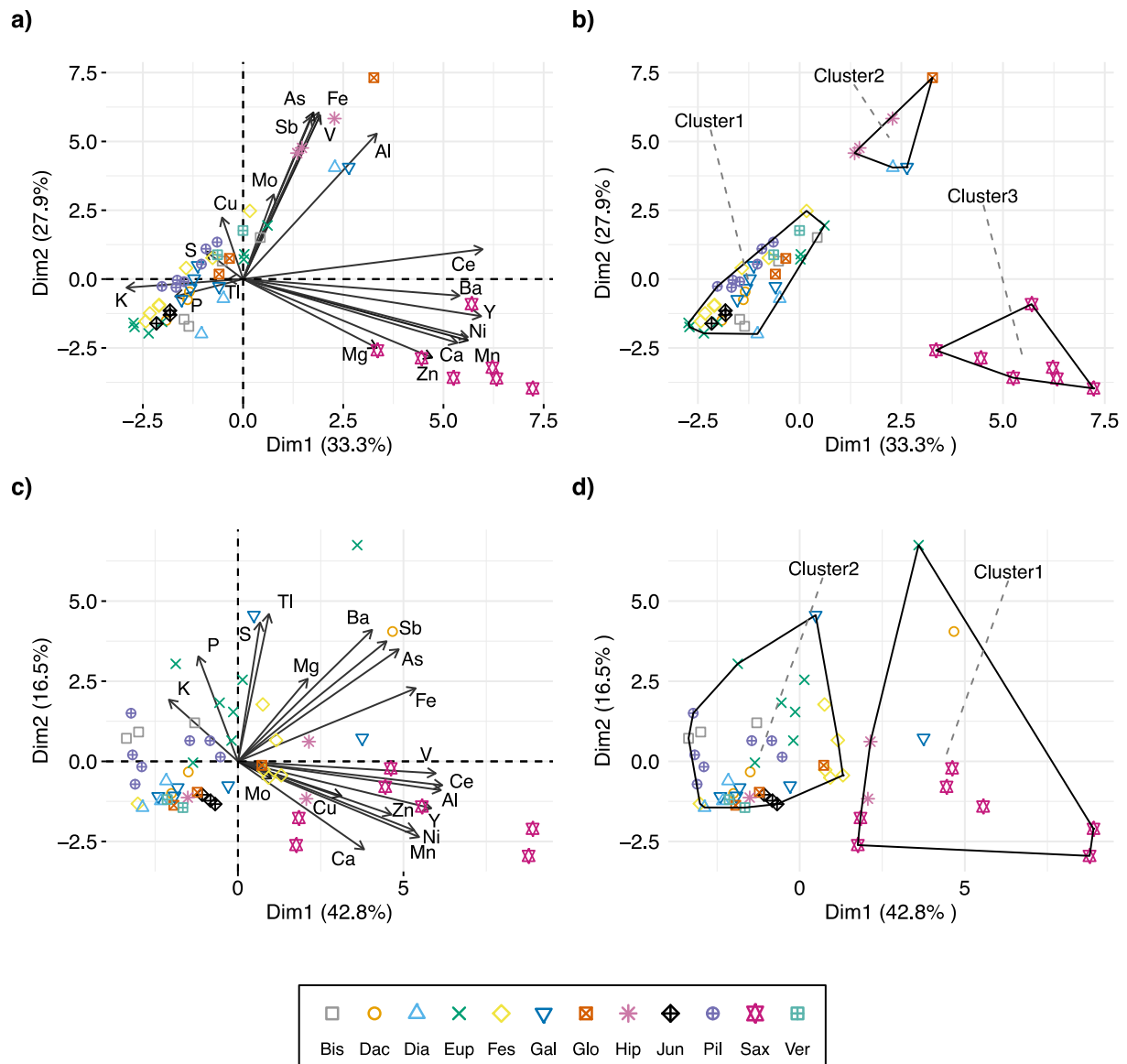


Figure 2: Biplot of the results of a primary component analysis of the elemental concentrations in aerial parts (a) and roots (c) including the individuals according to their species and results of partitioning clustering (K-means) on elemental concentrations in aerial plant parts (b) and roots (d) with a Hopkins clustering tendency of respectively 0.19 and 0.20. Coloured symbols representing the 12 plant species Bis (*Biscutella laevigata*), Dac (*Dactylis glomerata*), Dia (*Dianthus sylvestris*), Eup (*Euphorbia cyparissias*), Fes (*Festuca laevigata*), Gal (*Galium corrudifolium*), Glo (*Globularia cordifolia*), Hip (*Hippocrepis comosa*), Jun (*Juniperus communis subsp. nana*), Pil (*Pilosella peleteriana*), Sax (*Saxifraga paniculata*) and Ver (*Veronica fruticans*).

(coloured print)

		Mg	Al	P	S	K	Ca	V	Mn	Fe	Ni	Cu	Zn	As	Y	Mo	Sb	Ba	Ce	Tl
Bis	Mean	2.2	2.0	1.6	1.3	1.2	3.8	2.0	1.0	1.5	0.4	0.7	1.0	1.7	1.0	1.6	1.4	1.6	1.3	19.4
	SD	0.8	2.2	0.4	0.7	0.3	2.3	2.0	0.8	1.3	0.3	0.5	0.3	1.9	0.8	0.3	1.2	1.3	1.1	17.2
Dac	Mean	1.9	0.1	0.8	0.6	2.1	0.4	0.1	0.8	0.1	0.2	0.2	0.4	0.1	0.1	0.6	0.1	0.1	0.1	0.0
	SD	0.4	0.1	0.1	0.4	0.4	0.0	0.1	0.6	0.1	0.1	0.2	0.3	0.1	0.1	0.5	0.0	0.0	0.1	0.0
Dia	Mean	1.7	1.6	1.2	1.0	2.1	2.8	1.8	1.6	1.6	0.6	0.4	1.5	1.5	0.5	0.3	0.8	0.9	1.1	1.1
	SD	0.7	1.7	0.4	0.2	0.8	1.1	2.0	0.9	1.6	0.4	0.2	0.9	1.5	0.3	0.1	0.6	0.6	0.9	0.7
Eup	Mean	1.1	0.2	1.5	0.8	2.1	1.5	0.1	0.3	0.2	0.3	0.4	0.6	0.1	0.1	0.3	0.1	0.3	0.1	0.2
	SD	0.2	0.2	0.6	0.3	0.7	0.3	0.1	0.1	0.1	0.1	0.3	0.2	0.1	0.1	0.1	0.1	0.3	0.1	0.1
Fes	Mean	0.9	0.4	1.0	0.8	2.2	0.4	0.6	0.6	0.6	0.3	0.2	0.4	0.6	0.2	0.7	0.2	0.4	0.4	0.1
	SD	0.5	0.7	0.3	0.4	1.2	0.2	1.2	0.8	1.2	0.5	0.1	0.3	1.2	0.2	0.4	0.3	0.8	0.6	0.1
Gal	Mean	1.6	0.4	1.2	1.1	1.8	1.0	0.4	0.6	0.4	0.7	0.6	0.6	0.3	0.3	0.7	0.3	0.5	0.3	2.0
	SD	0.5	0.3	0.6	0.6	0.5	0.6	0.4	0.4	0.3	1.1	0.4	0.4	0.3	0.5	0.4	0.3	0.4	0.3	0.5
Glo	Mean	2.4	0.5	1.8	1.9	2.1	1.8	0.5	0.5	0.4	0.4	0.3	0.5	0.4	0.3	0.3	0.3	0.7	0.4	0.2
	SD	2.2	0.1	0.3	0.1	1.5	0.2	0.1	0.3	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.3	0.1	0.1
Hip	Mean	1.8	0.5	2.3	1.1	2.5	1.5	0.5	0.4	0.5	0.7	0.6	0.5	0.4	0.4	3.8	0.4	0.6	0.4	0.7
	SD	0.7	0.2	0.4	0.1	1.1	0.5	0.3	0.2	0.3	0.2	0.0	0.2	0.3	0.3	4.4	0.3	0.3	0.3	0.7
Jun	Mean	0.8	0.1	1.6	0.5	1.5	0.4	0.1	0.5	0.1	0.2	0.1	0.2	0.0	0.1	0.2	0.0	0.2	0.1	0.1
	SD	0.5	0.1	0.3	0.1	0.5	0.1	0.1	0.3	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.0
Pil	Mean	1.2	0.7	1.5	1.7	1.0	1.1	0.7	0.7	0.7	0.9	0.7	0.8	0.6	0.4	0.6	0.6	0.3	0.5	0.1
	SD	0.2	0.5	0.9	1.0	0.2	0.3	0.5	0.4	0.4	0.2	0.3	0.2	0.4	0.3	0.4	0.3	0.2	0.3	0.1
Sax	Mean	2.2	0.1	1.3	0.6	1.6	2.1	0.1	1.2	0.1	1.2	0.1	1.0	0.1	0.5	0.3	0.1	0.8	0.4	0.7
	SD	1.0	0.1	0.6	0.2	0.8	0.8	0.1	0.5	0.1	0.5	0.1	0.9	0.1	0.3	0.3	0.1	0.6	0.2	0.3
Ver	Mean	1.3	0.6	1.3	1.4	1.2	1.0	0.7	0.7	0.7	0.5	0.4	0.8	0.6	0.4	0.7	0.6	0.7	0.6	0.4
	SD	0.2	0.0	0.6	0.6	0.3	0.3	0.1	0.2	0.2	0.4	0.2	0.3	0.1	0.1	0.1	0.0	0.1	0.0	0.2

Figure 3: Heatmap of average translocation-factors (mean) and standard deviation (SD) for each plant species and chemical element (Concentration in the leaves / Concentration in the roots). The acronyms are standing for the plant species *Biscutella laevigata* (Bis), *Dactylis glomerata* (Dac), *Dianthus sylvestris* (Dia), *Euphorbia cyparissias* (Eup), *Festuca laevigata* (Fes), *Galium corrudifolium* (Gal), *Globularia cordifolia* (Glo), *Hippocrepis comosa* (Hip), *Juniperus communis subsp. nana* (Jun), *Pilosella peleteriana* (Pil), *Saxifraga paniculata* (Sax) and *Veronica fruticans* (Ver).

(coloured print)

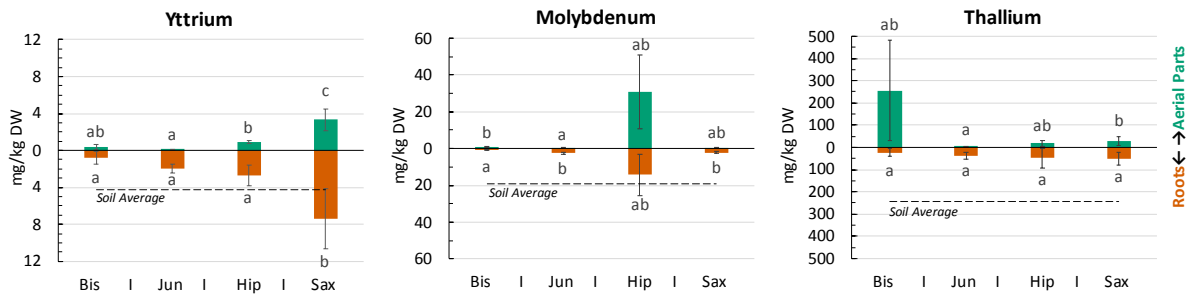


Figure 4: Average concentrations of Y, Mo and Tl in aerial plant parts and root of *Biscutella laevigata* (Bis, n=4), *Juniperus communis* (Jun, n=3), *Hippocrepis comosa* (Hip, n=3) and *Saxifraga paniculata* (Sax, n=4) compared to the average soil concentration (n=10) in mg/kg DW.



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Supplementary Material

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: