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3 **Application of field-portable-XRF for the determination**
4 **of trace elements in deciduous leaves from a mine-**
5 **impacted region**
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7

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22 **Abstract**

23 Deciduous leaves ($n = 87$) from beech (*Fagus sylvatica*), birch (*Betula* spp.) and oak
24 (*Quercus* spp.) trees have been collected from three metal mine-impacted sites in southwest
25 England and tested for concentrations of trace elements (As, Cu, Pb and Zn) using a field-
26 portable-x-ray fluorescence (FP-XRF) spectrometer configured in a low density mode and
27 housed in a stand. When intact leaves were analysed directly, mean detection limits ranged
28 from about 10 (As) to 70 $\mu\text{g g}^{-1}$ (Cu) on a fresh weight basis; after freeze-drying, respective
29 limits increased to about 20 and 120 $\mu\text{g g}^{-1}$ on a dry weight basis. Within these constraints,
30 As and Zn were detected in samples from all genera, with concentration differences between
31 fresh and dry states attributed to the mass of water present and its propensity to attenuate x-
32 rays. A comparison with As and Zn concentrations in local soils and determined by XRF in a
33 higher density mode revealed different accumulation and exclusion characteristics among the
34 three genera of tree. In contrast, and despite soil concentrations that were similar to those of
35 Zn, Cu was detected in only two dried leaves and Pb evaded detection throughout. Pooled
36 results from the study showed good agreement with independent results derived from ICP
37 following acid digestion, with a slope that was close to unit value. Accordingly, the XRF
38 approach is able to provide a rapid assessment of the levels of certain trace elements in
39 leaves from contaminated sites, with the configuration deployed on site having potential to
40 deliver immediate results.

41

42 **Keywords:** deciduous leaves; field-portable-XRF; arsenic; zinc; soils; biomonitoring

43 **1. Introduction**

44 Although mosses and epiphytic lichens have gained widespread use in biomonitoring of
45 airborne trace element pollution, their absence in some urban and industrialised areas has
46 resulted in the study of higher plants (Bargagli et al., 2003; Aničić et al., 2011; Serbula et al.,
47 2014). Many tree species are tolerant of high environmental concentrations of trace elements
48 and leaves are often the main sink for pollutants (Bargagli, 1998). Thus, as well as providing
49 a means of increasing the trace element content of top soils through leaf deposition-
50 decomposition and increasing trace element exposure to consuming organisms, leaves act as
51 potential indicators of both contaminated soil, through uptake via the root system, and
52 polluted air, through wet and dry deposition (Franiel and Babczyńska, 2011; Dimitrijević et
53 al., 2016; Pająk et al., 2017).

54

55 At sites impacted by contemporary or historical metalliferous mining, and where the
56 presence of elevated environmental concentrations of many trace elements is a concern,
57 deciduous and evergreen tree leaves have been commonly employed as indicators of soil
58 contamination (Unterbrunner et al., 2007; Dmuchowski et al., 2014; Nirola et al., 2015;
59 Stefanowicz et al., 2016). Here, capture and uptake of a trace element is from a large soil
60 volume, with the concentration of an element in the leaves reflecting the availability of the
61 element in the soil rather than its total concentration. Strictly, and to act as a true
62 bioindicator, uptake through the roots should be relatively constant over a wide gradient of
63 trace element concentrations in the soil such that there should be a linear relationship
64 between concentrations in the leaves and in the soil (Baker et al., 2000; Kabata-Pendias and
65 Pendias, 2001). However, some trees may act as excluders for certain elements by inhibiting
66 their uptake into roots, even at high external concentrations in the soil, while others may act
67 as hyper-accumulators which are able to tolerate high concentrations in their leaves, even at
68 low external concentrations (Madejón et al., 2004; Schmidt et al., 2016); the latter are,

69 therefore, particularly attractive as phytoremediators of highly contaminated soils
70 (Antosiewicz et al., 2008; Dimitrijević et al., 2016).

71

72 With different possible sources of trace elements (from the atmosphere and soil) and
73 potentially confounding issues of hyper-accumulation and exclusion, the analysis of a large
74 number of leaf samples is often required at contaminated sites, with repeat visits sometimes
75 necessary. Conventional analysis of leaves involves digestion of the matrix in concentrated
76 mineral acid followed by trace element determination by, for example, inductively coupled
77 plasma spectrometry or atomic absorption spectrometry, but this approach can be time- and
78 resource-consuming and generate large quantities of hazardous waste. As an alternative,
79 plant material may be determined non-destructively by x-ray fluorescence (XRF)
80 spectrometry. Here, samples are typically dried, milled and packed before being excited by
81 an x-ray beam, with the expulsion of inner electrons of an atom accompanied by electrons
82 cascading from higher orbitals and the emission of characteristic fluorescent x-rays
83 (Sacristán et al., 2016; Towett et al., 2016).

84

85 In order to further minimise sample preparation, it may be assumed that the plant matrix has
86 similar characteristics to thin plastic films in terms of the absorption, scattering and
87 fluorescence of x-rays, and analyse material intact (i.e. without milling or packing) using an
88 XRF algorithm that is calibrated for low density matter. This approach was recently tested
89 and validated both in the laboratory and in the field on coastal and marine macroalgae (Bull
90 et al., 2017; Turner et al., 2017) and is trialled in the present study on deciduous leaves.
91 Specifically, the current investigation focuses on the trace metalloid, As, and the trace metals
92 Cu, Pb and Zn, in both common leaves and in soils (with the latter employing more
93 established XRF protocols) at three sites impacted by historical, non-ferrous mine waste.

94 Although tests were performed solely in the laboratory, the potential for applying the
95 approach in the field is also addressed.

96

97 **2. Materials and methods**

98 *2.1. Sampling*

99 Sampling of leaves and soils was undertaken at three sites impacted by historical (19th
100 century), non-ferrous metal mining activities in west Devon, south west England. The
101 geology of the region is dominated by fine-grained sedimentary sequences and chert but with
102 outcrops of granite and slates, and soils are mainly brown earths that are well-drained but
103 subject to slight seasonal waterlogging (Rawlins et al., 2003). The region is sparsely
104 populated, with occasional small settlements and farms, and current land use is dominated by
105 agriculture and managed woodland. The first site (S1) was on Dartmoor National Park in the
106 vicinity of a series of relatively small, disused copper mines (digital co-ordinates: 50.5130, -
107 4.1116; 85 m asl). The second and third sites were within the UNESCO district of the Tamar
108 Valley at locations influenced by more extensive mining and processing facilities for both
109 copper and arsenic; specifically, S2 was adjacent to an old but functional adit (50.5361, -
110 4.2081; 50 m asl) and S3 was along the northern edge of a large spoil tip (50.5385, -4.2214;
111 78 m asl).

112

113 Each site was visited during mid-autumn (early November) in 2016, following a period of
114 dry weather and as deciduous foliage was being shed. A total of 87 leaves were collected by
115 hand and using plastic tweezers from trees that were common to all sites and that usually
116 occurred in clusters but were occasionally solitary: namely, beech (*Fagus sylvatica*), birch
117 (*Betula* spp., including *B. pendula*) and oak (*Quercus* spp.). Specifically, three leaves were
118 taken from lateral branches at a height of about 2 m from between one and five trees of each
119 genus (depending on their abundance and accessibility) and stored in individual zip-lock

120 specimen bags in a dark polyethylene box. Soil samples, of about 300 g and to depths of
121 around 10 cm, were collected from under the canopies of three trees from each location
122 using a plastic trowel and were stored likewise.

123

124 *2.2. Leaf sample processing and XRF analysis*

125 On return to the laboratory, leaf surfaces were wiped gently with three-ply blue roll to
126 remove any visibly adherent material and weighed on a five-figure Sartorius balance. Fresh
127 samples were then analysed for a suite of elements, of which As, Cu, Pb and Zn as important
128 contaminants of non-ferrous mining are the focus of the present study, by energy dispersive,
129 field-portable (FP-)XRF using a battery-powered Niton XRF analyser (model XL3t 950 He
130 GOLDD+) which was configured, nose-upwards, in a bench-top accessory stand and
131 activated remotely by a laptop connected via USB. In order to minimise any artefacts arising
132 from variations in geometry or thickness, an area of the upper blade midway between the
133 mid-rib and margin, and free from any sign of infection, was selected for analysis. This area
134 was measured for thickness using Allendale digital callipers before being positioned directly
135 above the XRF detection window, a process aided by imagery from an integrated CCD
136 camera and, where necessary, polyethylene blocks outside of the x-ray beam acting as
137 weights. Once the shield of the stand was closed, measurements, with appropriate thickness
138 correction and in a low-density plastics mode, were undertaken for equal counting periods in
139 a main energy range (50 kV/40 μ A) and low energy range (20 kV/100 μ A). Counting was
140 trialled up to ten minutes but a total period of 120 seconds was selected as a suitable
141 timescale that appeared to provide sufficiently low counting errors yet maximise the number
142 of elements detected. Spectra arising from both energy ranges were quantified by
143 fundamental parameters to yield elemental concentrations in $\mu\text{g g}^{-1}$ and a counting error of
144 2σ (95% confidence) that were downloaded to the laptop using Niton Data Transfer
145 software.

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In order to correct for the mass contribution of water and to evaluate the effects of the aqueous phase on x-ray absorption, leaves were frozen, dried for 24 h in an Edwards Super Modulyo freeze-drier and reweighed before being re-analysed at the approximate positions measured while fresh. With no suitable biological reference films available, performance and precision were evaluated by analysing a Niton plastic reference disc (PN 180-619, LOT#T-18) certified for a suite of elements (including As and Pb) at regular intervals during each measurement session.

2.3. Soil sample processing, characterisation and XRF analysis

Soil samples were freeze-dried for 48 h before being ground with a ceramic pestle and mortar and sieved through a 63 μm stainless steel sieve. A sufficient quantity of each fractionated sample was then used to completely fill a series of Chemplex series 1400 XRF sample cups (21-mm internal diameter) that were collar-sealed with 3.6 μm SpectraCertified Mylar polyester film. For XRF analysis, cups were placed centrally over the detector window with the collar-sealed Mylar surface face-down, and measurements were conducted for 60 seconds in a higher density mining mode, comprising successive counting periods of 20 s each in a main (50 kV/40 μA), low (20 kV/100 μA) and high (50 kV/40 μA) energy range. Spectra were quantified and concentrations downloaded to the laptop as above. For quality assurance purposes, sufficient quantities of a Sigma-Aldrich RTC loam certified for As and Zn (MSL-MSL101) and a National Institute of Standards and Technology soil certified for a broader suite of elements (SRM 2709a) were packed into XRF cups and analysed likewise.

170 Ten g portions of the remaining fractionated soil samples were equilibrated with 25 ml
171 aliquots of deionised water in a series of 50 ml polypropylene centrifuge tubes and the pH
172 measured using a Meterlab PHM210 pH meter and Hach pH2051-8 electrode.

173

174 *2.4. Leaf digestion and analysis by ICP*

175 As an independent and more sensitive measure of the metal and metalloid content of leaves,
176 dried and infection-free whole leaf samples ($n = 28$) from different trees and sites and, in
177 triplicate, a powdered leaf reference material (GBW 08501, peach leaves) were acid-digested
178 and analysed by inductively coupled plasma-mass spectrometry (ICP-MS). Thus, samples
179 were accurately weighed into individual Teflon tubes to which 6 ml aliquots of HNO₃
180 (Fisher Chemical TraceMetal™ Grade) were added. The contents were digested in a CCEM
181 MARS 5 XPRESS microwave at 1600 W for 45 min before being allowed to cool. Digests
182 were then washed into individual 25 ml volumetric flasks and diluted to mark with Millipore
183 Milli-Q water.

184

185 Digests were analysed for As, Cu, Pb and Zn using a collision cell-ICP-MS (Thermo X-
186 series II, Thermoelemental, Winsford, UK) with a concentric glass nebuliser and conical
187 spray chamber. RF power was set at 1400 W and coolant, auxiliary, nebuliser and collision
188 cell gas flows rates were 13 L Ar min⁻¹, 0.70 L Ar min⁻¹, 0.786 L Ar min⁻¹ and 3.5 mL 7%
189 H₂ in He min⁻¹, respectively. The instrument was calibrated externally using four standards
190 prepared by dilutions of a QC 26 multi-element solution (CPI International, Amsterdam) in
191 0.1 M HNO₃, and internally by the addition of 100 µg L⁻¹ of In and Ir to all samples and
192 standards. Data were acquired over a dwell period of 10 ms, with 50 sweeps per reading and
193 three replicates and were converted to dry weight concentrations (in µg g⁻¹ dw) from the
194 volume of diluted digest and mass of leaf digested. Limits of detection on this basis were <
195 0.5 µg g⁻¹ for all elements analysed.

196

197 *2.5. Quality assurance*

198 Dry weight concentrations arising from XRF or ICP analyses of the various reference
199 materials (plastic disc, soils and leaves) are compared with certified or indicative values in
200 Table 1. Thus, within the levels of uncertainty and error, agreement was accomplished by
201 XRF for As and Pb in polyethylene and As and Zn in NIST soil and by ICP for As, Pb and
202 Zn in peach leaves, with mean measured concentrations in the remaining cases within 25%
203 of the respective mean certified values. Precision, as one standard deviation relative to the
204 mean, was < 5% in all cases with the exception of Zn in peach leaves (15%) and As, Cu and
205 Pb in NIST soil (between 13 and 24%).

206

207

208 **3. Results and Discussion**

209 *3.1. Leaf and soil characteristics*

210 Table 2 shows the number of leaves taken from each genus of tree at each site, where
211 multiples of three reflect the number of trees sampled. Overall, 87 samples were collected
212 for analysis with a roughly equal distribution among genera but differences between sites
213 that reflected tree abundance and accessibility. At S1, average leaf size, based on fresh
214 weight, decreased in the following order: *Quercus* > *Fagus* > *Betula*; at S2 and S3, tree
215 growth appeared to be more stunted and the size of oak and beech leaves were reduced
216 considerably such that average leaf size across the three genera was similar. The percentage
217 contribution of water to leaf mass averaged 42% for *Fagus* and *Betula* and 21% for *Quercus*,
218 with leaves in the latter genus being distinctly drier at S2 and S3. Leaf thickness through the
219 measurement area was not significantly different ($p > 0.05$ according to a series of paired or
220 two-sample *t*-tests performed in Minitab v17) between genus, sampling site, or fresh and dry

221 state, with overall averages of 0.26 mm, 0.27 mm and 0.34 mm for *Betula*, *Fagus* and
222 *Quercus*, respectively.

223

224 The pH of the soil samples, together with trace element content determined by XRF in a
225 conventional and established soils mode (Kalnicky and Singhvi, 2001; Radu and Diamond,
226 2009), are summarised in Table 3. Thus, average pH ranged from about 4.9 to 5.5, with
227 individual measurements ranging from about 3.9 at S2 to 6.3 at S1. Average concentrations
228 of Pb and Zn were similar among the three sites, with individual concentrations ranging from
229 about 70 to 300 $\mu\text{g g}^{-1}$ dw and 90 to 290 $\mu\text{g g}^{-1}$ dw, respectively. In contrast, and consistent
230 with the relative significance of historical mining for Cu and As and legacy contamination,
231 mean concentrations of Cu were three- to four-fold higher and mean concentrations of As
232 about 50 and 130 times higher at S2 and S3 than corresponding mean concentrations at S1.

233

234 3.2. XRF detection limits in leaves

235 For a specific counting time and mode of application, the Niton FP-XRF provides
236 measurement limits of detection (LODs) for each element that are dependent on the
237 characteristics of the sample, including its density, thickness and chemical composition, and
238 that are calculated from three counting errors arising from the analysis (or $2\sigma \times 1.5$;
239 99.7% confidence interval). LODs for As, Cu, Pb and Zn for all samples analysed in the
240 present study are summarised in Table 4, with concentrations given on a fresh weight (fw) or
241 dry weight (dw) basis depending on the nature of sample processing. Thus, for a given
242 element, LODs span about an order of magnitude, with values generally related to the
243 reciprocal of sample thickness, but that were not significantly different ($p > 0.05$ according
244 to one-way ANOVA performed in Minitab v17) between the different genera of tree. For all
245 elements, average LODs were at least 50% greater for leaves analysed freeze-dried than
246 when fresh. With thicknesses that were statistically indistinguishable between the dry and

247 fresh states and the propensity of water to absorb low energy x-rays, this observation is,
248 perhaps, counterintuitive. However, based on similar observations made during the XRF
249 analysis of fresh and dried macroalgae (Turner et al., 2017), we attribute the discrepancy to a
250 greater flexibility of biological material when fresh, allowing blades to be positioned closer
251 to the detector window of the instrument. This effect also resulted in precisions, derived
252 from repeat measurements of the same leaf area, that were better when samples were
253 analysed in the fresh state (between 10 and 20%) than when analysed after freeze-drying
254 (between 15 and 25%).

255

256 *3.3. Elemental concentrations in leaves*

257 Table 5 gives the number of cases that As, Cu, Pb and Zn were detected in the leaf samples
258 along with summary statistics for the resulting concentrations. Using FP-XRF, As was
259 detected in 13 samples when analysed dry with concentrations ranging from about 13 to 360
260 $\mu\text{g g}^{-1}$ dw, and detection was limited to samples taken adjacent to the spoil heap (S3) and
261 was dominated by oak leaves ($n = 8$). The metalloid was detected in 19 samples when
262 analysed fresh, with concentrations ranging from about 5 to 450 $\mu\text{g g}^{-1}$ fw and, after
263 correction for the amount of water present, from about 12 to 500 $\mu\text{g g}^{-1}$ dw. Detection
264 exhibited a similar site and genus distribution to the dried samples but a number of positives
265 were returned for samples of birch taken at S1.

266

267 Zinc was detected in 47 samples analysed dry with concentrations ranging from about 30 to
268 660 $\mu\text{g g}^{-1}$ dw, and both detection and the highest concentrations were most frequent among
269 birch leaves from S1 and S3. When analysed fresh, Zn was detected in 40 samples and at
270 concentrations ranging from about 20 to 280 $\mu\text{g g}^{-1}$ fw and, after correction for water
271 content, from about 30 to 440 $\mu\text{g g}^{-1}$ dw, with a similar site and genus distribution to that
272 derived from analysis of the dried samples. Copper was detected by XRF in just two dried

273 oak leaves from S3, despite lower measurement detection limits for the metal in fresh leaves
274 (Table 4), and Pb was never detected by the instrument.

275

276 Also shown in Table 5 are concentrations of As, Cu, Pb and Zn returned by ICP following
277 acid digestion of a selection of whole, freeze-dried leaves ($n = 28$) that encompassed each
278 genera from all three sites. Here, each element was detected in all samples tested, with
279 results spanning greater concentration ranges than the corresponding results returned by
280 XRF.

281

282 *3.4. Comparison of concentrations derived from fresh and dry analyses of leaves*

283 In order to evaluate the impacts of internal water on the results, hence the feasibility of
284 measuring elements in fresh leaves by XRF on site, concentrations returned directly for
285 freeze-dried leaves are compared with dry weight concentrations derived indirectly from the
286 analysis of fresh samples and after correction for water content in Figure 1 (note that the
287 comparison is restricted to cases where detection was accomplished by both approaches on
288 the same sample; $n = 11$ for As and $n = 32$ for Zn). Thus, despite different geometries and
289 distances from the detector window incurred by the two states, potential differences in the
290 precise location of sample analysed on successive occasions and the heterogeneous
291 distribution of water within leaves, both data sets exhibited a high degree of association
292 when subject to Pearson's moment correlation analysis performed using the data analysis
293 ToolPak in Excel 2016 ($r > 0.85$, $p < 0.001$). Linear regression analysis performed in Excel
294 2016 also revealed equations of best fit with either a y-intercept or that were forced through
295 the origin that were highly significant ($p < 0.001$), but regression slopes indicated
296 concentrations that were not equivalent. Specifically, the gradient defining the fresh to dry
297 converted concentrations versus dry concentrations determined directly were either above
298 (As) or below (Zn) unit value.

299

300 In addition to its contribution to leaf mass and its alteration of sample geometry, water may
301 influence dry weight concentrations derived from fresh analyses through the absorption of
302 primary and secondary x-rays, an effect whose potential significance can be evaluated
303 theoretically. Thus, the mass attenuation coefficient for water at 10 keV, or the approximate
304 energies of the main K-level emission lines for both As and Zn, is $5.3 \text{ cm}^2 \text{ g}^{-1}$ (Hubbell and
305 Seltzer, 1996). This yields a half-value layer (where 50% of incident radiation is attenuated)
306 of about 1.3 mm, and attenuation of 10% for a layer of about 0.4 mm, or the upper thickness
307 of the leaves tested. Most leaves that were Zn-positive when analysed both fresh and dry
308 (and as plotted in Figure 1) were from *Betula* spp., whose internal water content was about
309 60%, while those that were As-positive when analysed in both states were from *Quercus*
310 spp., whose water content was about 20%. On this basis, therefore, we would predict the
311 analysis of fresh samples of consistent thickness to affect the results for Zn to a greater
312 extent than those of As, largely through the absorption of both irradiating and fluorescent x-
313 rays by samples of a higher water content. This assertion is qualitatively consistent with the
314 data in Figure 1 in that the regression slopes for Zn are smaller than those for As, but does
315 not explain why the slope for As lies above unit value. A confounding issue in this respect is
316 the presentation of a flatter and more uniform surface that is closer to the detector when
317 samples are fresh and more pliable, and as described above.

318

319 *3.5. Comparison of concentrations derived by XRF and ICP analyses of leaves*

320 A comparison of the results obtained by ICP, following digestion in concentrated HNO_3 ,
321 with those returned by the FP-XRF revealed no false negatives or false positives; that is, lack
322 of detection by the XRF was not accompanied by a measurement by ICP that exceeded the
323 corresponding LOD of the Niton XL3t and concentrations returned by the XRF were never
324 accompanied by ICP measurements that were below detection limit of the mass

325 spectrometer. Where concentrations were returned by both ICP and XRF (either on fresh
326 leaves or freeze-dried samples), results are plotted in Figure 2, with As and Zn data shown
327 individually and, with Cu, in combination. Despite the potential limitations and sources of
328 error of XRF outlined above and fundamental discrepancies in the leaf part examined (XRF
329 probes a small area of blade while ICP necessitates digestion of the whole sample), both
330 correlation and linear regression analyses performed in Excel 2016 revealed a significant
331 relationship between the results from both analytical approaches in all cases ($p < 0.001$).
332 Agreement between XRF and ICP, defined as the deviation of the gradient of the regression
333 equation (forced through the origin) from unit slope, was within 30% for both As and Zn and
334 within 10% when data for all trace elements were pooled. In all cases, however, closer
335 agreement resulted when leaves were analysed fresh than when dry, an effect that may be
336 related to the ability to manoeuvre samples closer to the detector window when in the fresh
337 state than after freeze-drying (see above).

338

339 *3.6. Potential application of FP-XRF for biomonitoring*

340 The present study has explored the feasibility of measuring trace elements in common
341 deciduous leaves directly and non-destructively by FP-XRF. The approach is capable of
342 determining many key elements to dry weight concentrations of a few tens of $\mu\text{g g}^{-1}$ and is
343 particularly suited to environments where there are clear and significant sources of
344 contaminants. Of the metals and metalloids considered herein, As, a toxic metalloid, and Zn,
345 a micronutrient, were most readily detected in a variety of leaf samples from three sites,
346 and agreement with concentrations derived independently from ICP following acid digestion
347 was sufficiently strong and significant to at least partly satisfy the EPA definitive level data
348 criterion (Environmental Protection Agency, 2007). Being capable of measuring elements in
349 fresh samples, with conversion of concentrations to a dry weight basis accomplished to a
350 good approximation by correction for internal water content, the method has the potential for

351 monitoring in the field. Here, the instrument would be configured in a portable test stand as
352 described elsewhere (Turner et al., 2017) and activated remotely using a laptop under the
353 operating conditions outlined above. With the simultaneous capability of measuring trace
354 metals and metalloids in soils, the approach can deliver a rapid assessment of site
355 contamination, element compartmentalisation and uptake-exclusion-accumulation.

356

357 Although the principal objectives of the study were to explore the potential application of
358 FP-XRF to the biomonitoring of leaves, the results have demonstrated differences in element
359 accumulation among the three genera of tree that were most significant at S3 and as
360 illustrated by the direct measurements of dried samples in Figure 3. Thus, here, As was
361 highly enriched in local soils (Table 3) because of the historical mining for the metalloid but
362 concentrations were two or three orders of magnitude lower in the leaves analysed;
363 specifically, As was detected in two samples of *Fagus* and *Betula* spp. but was detected in all
364 samples of *Quercus* spp. It would appear, therefore, that at least the first two genera are
365 excluders of As in that, despite extremely high concentrations in local soils, there is active
366 inhibition by the root system. In contrast, Zn at S3 exhibited no elevated contamination in
367 soil relative to concentrations at S2 and S3 (Table 3) and, unlike As, showed no measurable
368 accumulation by *Quercus* spp. leaves. However, the metal was detected in every sample of
369 *Betula* and at concentrations that always exceeded the mean concentration of Zn in local soil
370 and that are considered to exert toxic effects on many species (Barker and Pilbeam, 2010).
371 This observation is consistent with the recent assertion that *Betula* spp., and in particular, *B.*
372 *pendula*, should be classified as hyperaccumulators of Zn, thereby having potential for Zn
373 phytoremediation (Dmuchowski et al., 2014). Presumably, Pb evaded detection throughout
374 the study because translocation from the roots is limited for many tree species, while
375 restricted detection of Cu may be attributable to absorption that is often competitively
376 inhibited by Zn (Kabata-Pendias and Pendias, 2001).

377

378 The observations for As and Zn described above are likely the result of differences in the
379 accumulation, regulation, tolerance, translocation, exclusion and contribution of atmospheric
380 deposition between the two elements and among the tree genera. Although determination of
381 the precise causes for the contrasting behaviours of As and Zn would require further
382 investigation, this study has clearly demonstrated the usefulness of FP-XRF in rapidly
383 identifying such issues and its potential for guiding research iteratively and strategically.

384

385 **Conclusions**

386 This study has shown that FP-XRF configured in a low density mode is capable of
387 determining certain trace elements, and in particular, As and Zn, on intact leaves from
388 contaminated sites. With detection limits of a few tens of $\mu\text{g g}^{-1}$ in both the fresh and dry
389 states and good agreement with independent results from ICP analysis, the approach is
390 suitable for the rapid throughput of samples with minimal preparation. As such, and when
391 combined with a portable test stand, it also has the potential to be applied on site as a
392 biomonitoring tool or for directly guiding a research plan strategically.

393

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397

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496 Table 1: A comparison of measured and certified elemental concentrations in plastic, leaves
 497 and soils. Errors represent the 95% confidence interval, with weighting for inter-method
 498 variance where appropriate (certified), or two standard deviations about the mean (measured
 499 n times); asterisks denote indicative values.

		certified, $\mu\text{g g}^{-1}$ dw	measured, $\mu\text{g g}^{-1}$ dw
polyethylene disc, PN 180-619 XRF; $n = 10$	As	51 \pm 7.0	46 \pm 4.4
	Pb	150 \pm 12	145 \pm 9.2
peach leaves, GBW 08501 ICP; $n = 3$	As	0.34 \pm 0.06	0.44 \pm 0.04
	Cu	10.4 \pm 1.6	8.0 \pm 0.42
	Pb	0.99 \pm 0.08	0.96 \pm 0.04
	Zn	22.8 \pm 2.5	23.3 \pm 7.4
soil, NIST 2709 XRF; $n = 5$	As	10.5 \pm 0.3*	9.3 \pm 2.3
	Cu	33.9 \pm 0.5*	26.3 \pm 6.1
	Pb	17.3 \pm 0.1	12.9 \pm 3.4
	Zn	103 \pm 4.0*	97.5 \pm 8.1
soil, MSH-100 XRF; $n = 5$	As	1090 \pm 16.7	999 \pm 24.0
	Zn	1100 \pm 16.8	1047 \pm 36.0

501

502 Table 2: Number and distribution of leaf samples used in the present study.

sampling site	<i>Fagus</i>	<i>Betula</i> spp.	<i>Quercus</i> spp.
S1	15	9	9
S2	3	3	12
S3	9	18	9
total	27	30	30

503

504

505 Table 3: A summary of the characteristics of the soil samples from the three sites, shown as
 506 the mean and one standard deviation for each parameter.

sampling site	pH	As, $\mu\text{g g}^{-1}$ dw	Cu, $\mu\text{g g}^{-1}$ dw	Pb, $\mu\text{g g}^{-1}$ dw	Zn, $\mu\text{g g}^{-1}$ dw
S1 ($n = 3$)	5.53 \pm 0.68	183 \pm 60	356 \pm 192	103 \pm 34.1	192 \pm 35.3
S2 ($n = 3$)	4.89 \pm 0.83	9310 \pm 8470	1070 \pm 858	159 \pm 73	125 \pm 33.9
S3 ($n = 3$)	5.40 \pm 0.74	24,200 \pm 6620	1380 \pm 453	254 \pm 44.9	187 \pm 89

507

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509

510 Table 4: Mean and, in parentheses, minimum and maximum XRF measurement detection
 511 limits for trace elements in fresh and freeze-dried leaf samples.

	As	Cu	Pb	Zn
fresh ($n = 87$), $\mu\text{g g}^{-1}$ fw	12.9 (4.5-47.9)	71.3 (29.2-215)	26.7 (10.9-83.4)	33.5 (15.6-113)
512 dry ($n = 87$), $\mu\text{g g}^{-1}$ dw	21.2 (9.9-52.9)	116 (59.1-353)	42.9 (19.2-118)	56.9 (22.6-189)

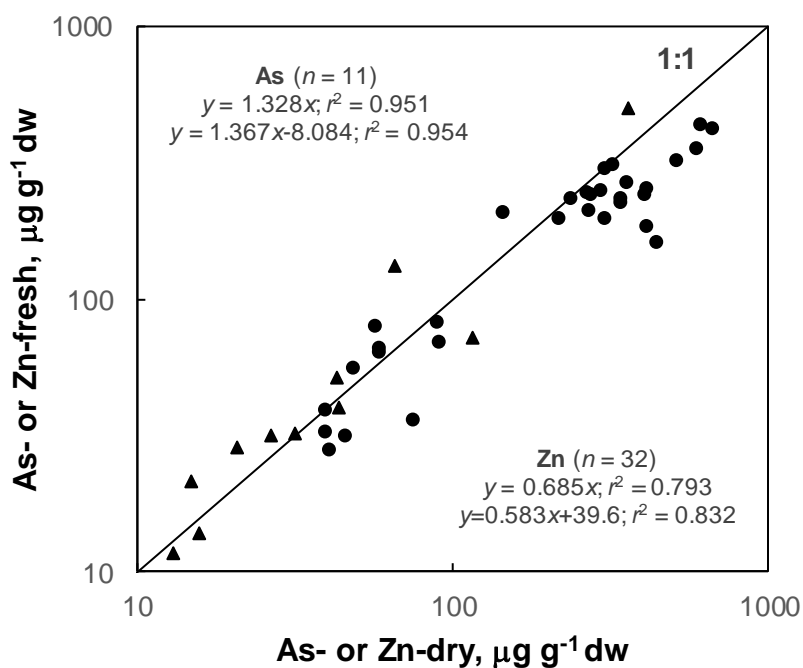
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514 Table 5: The number of cases that each element was detected among the leaf samples
 515 analysed (n) and summary statistics for the resulting concentrations.

		As	Cu	Pb	Zn
XRF-fresh, $\mu\text{g g}^{-1}$ fw	n	19	0	0	40
	mean	47.5			92.4
	median	20.3			89.3
	min	5.2			21.0
	max	450			280
XRF-fresh, $\mu\text{g g}^{-1}$ dw	n	19	0	0	40
	mean	60.0			169
	median	28.4			192
	min	11.7			28.0
	max	501			436
XRF-dry, $\mu\text{g g}^{-1}$ dw	n	13	2	0	47
	mean	63.5	71.3		208
	median	37.6	71.3		175
	min	12.9	62.0		27.6
	max	361	80.6		661
ICP, $\mu\text{g g}^{-1}$ dw	n	28	28	28	28
	mean	28.0	12.9	4.4	76.6
	median	1.4	8.7	2.4	23.1
	min	0.04	3.7	0.52	2.7
	516 max	503	85.6	25.6	344

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518



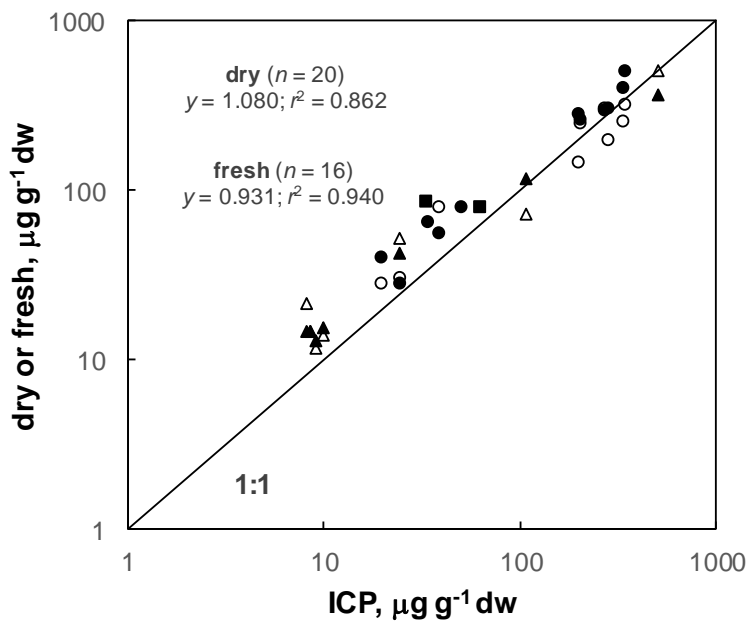
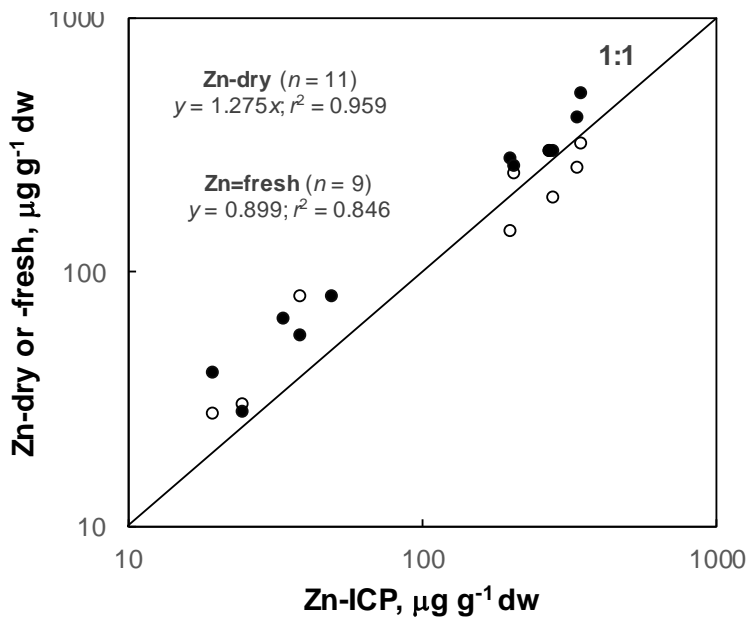
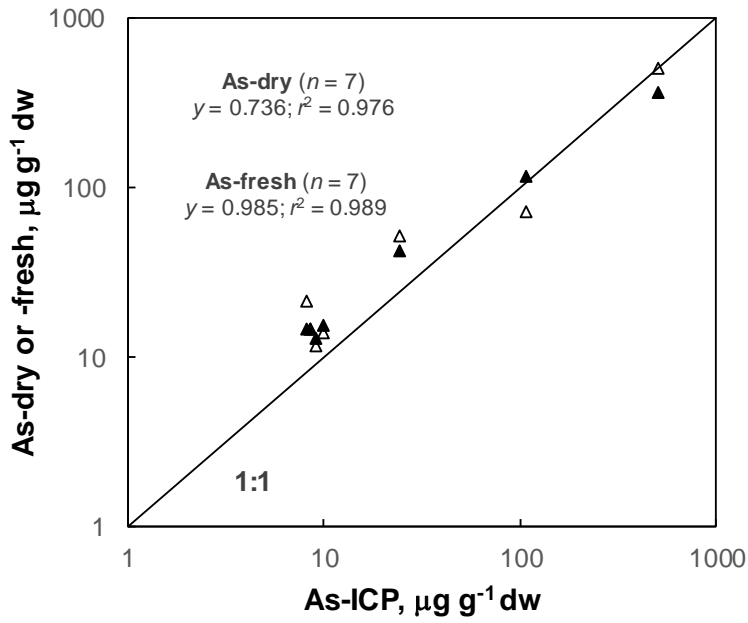
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522 Figure 1: A comparison of dry weight leaf concentrations of As (\blacktriangle) and Zn (\bullet) derived from
 523 fresh analysis by XRF and correction for water content and returned by direct analysis by
 524 XRF. The solid line denotes unit slope and regression equations and coefficients of
 525 determination defining the data sets are annotated.

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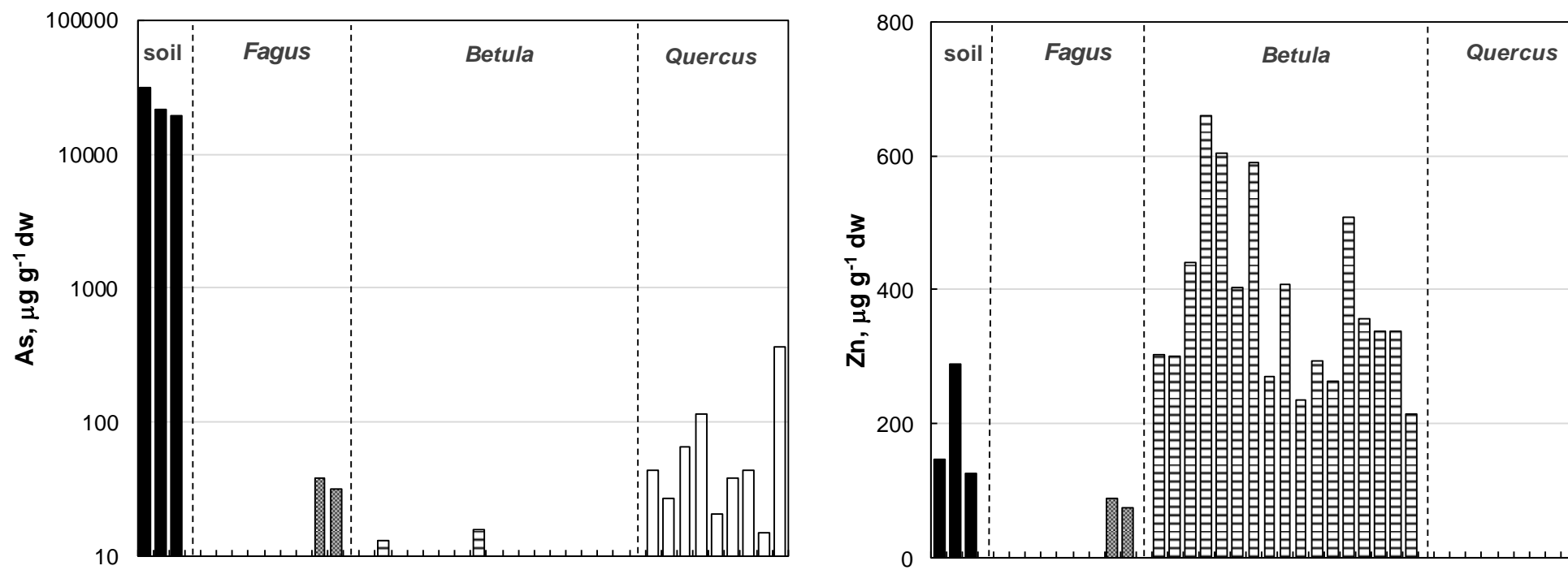
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531 Figure 2: A comparison of the dry weight concentrations of trace elements in leaves
532 determined by ICP following acid digestion and by XRF (As analysed fresh, Δ , and dry, \blacktriangle ;
533 Zn analysed fresh, \circ , and dry, \bullet ; Cu analysed dry, \blacksquare). The solid line denotes unit slope and
534 regression equations and coefficients of determination defining the data sets are annotated.

535 Figure 3: Concentrations of As and Zn, where detected, in the individual soil and tree leaf samples from S3.



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