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► **To cite this version:**

Sophia V. Hansson, Youen Grusson, Marianna Chimienti, Adrien Claustres, Séverine Jean, et al.. Legacy Pb pollution in the contemporary environment and its potential bioavailability in three mountain catchments. *Science of the Total Environment*, 2019, 671, pp.1227-1236. <10.1016/J.SCITOTENV.2019.03.403>. <hal-02353213>

HAL Id: hal-02353213

<https://hal.science/hal-02353213v1>

Submitted on 22 Oct 2021

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1 Legacy Pb pollution in the contemporary environment and its potential
2 bioavailability in three mountain catchments

3
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22 **Abstract**

23
24 Historical mining has a millennial scale history on the globe often leaving a long-lasting imprint
25 on the environment. Previous results on trace metal concentrations in the Pyrenees, where extensive
26 mining (Ag, Fe) occurred from the Antiquity to the 19th century, suggest that ≥ 600 tons of
27 anthropogenic lead (Pb) is stored in soils in the Haut-Videssos area (France). Yet the potential
28 bioavailability of this legacy contamination to contemporary biota remains unclear. We therefore
29 asked if previously reported high-levels of legacy Pb can be seen in other environmental
30 compartments including aquatic biota, and how these are distributed within the biota. Based on Pb-
31 isotopic data, we also assessed if any Pb contamination found in contemporary biota can be linked
32 to local/regional mining. Samples of sphagnum, soil, sediment, biofilm, and fish (*Salmo trutta* and
33 *Phoxinus phoxinus*) were collected from three adjacent valleys in the Haut-Videssos area. Pb
34 concentrations varied both between sites (i.e. decreasing concentrations with increasing distance
35 from the former mine) and between within-site environmental compartments (i.e. soil > biofilm \geq
36 sediment > sphagnum > fish) as well as within organisms (i.e. entire organism>liver>muscle).
37 Further, Pb-isotopic ratios ($^{206}\text{Pb}/^{207}\text{Pb}$, $^{208}\text{Pb}/^{207}\text{Pb}$ and $^{208}\text{Pb}/^{206}\text{Pb}$) measured in soil, biofilm, and
38 fish indicated both natural (weathering bedrock) and anthropogenic (industrial, transportation
39 and/or former mining activities) sources of Pb-deposition to the area. Generally, body Pb-
40 concentrations were within regulatory guidelines, yet contemporary biota in the upper Haut-
41 Videssos area, and their prey, still showed a large range of Pb isotopic signatures, of which former
42 mining activities appeared to have a strong influence. Our study showed that mining derived legacy
43 Pb continues to affect onsite biota even if mining activities ceased >100 years ago, thus reflecting
44 the long-lasting impact of human-environment interaction, suggesting that ecosystem conditions
45 may remain impaired centuries after activities have ceased.

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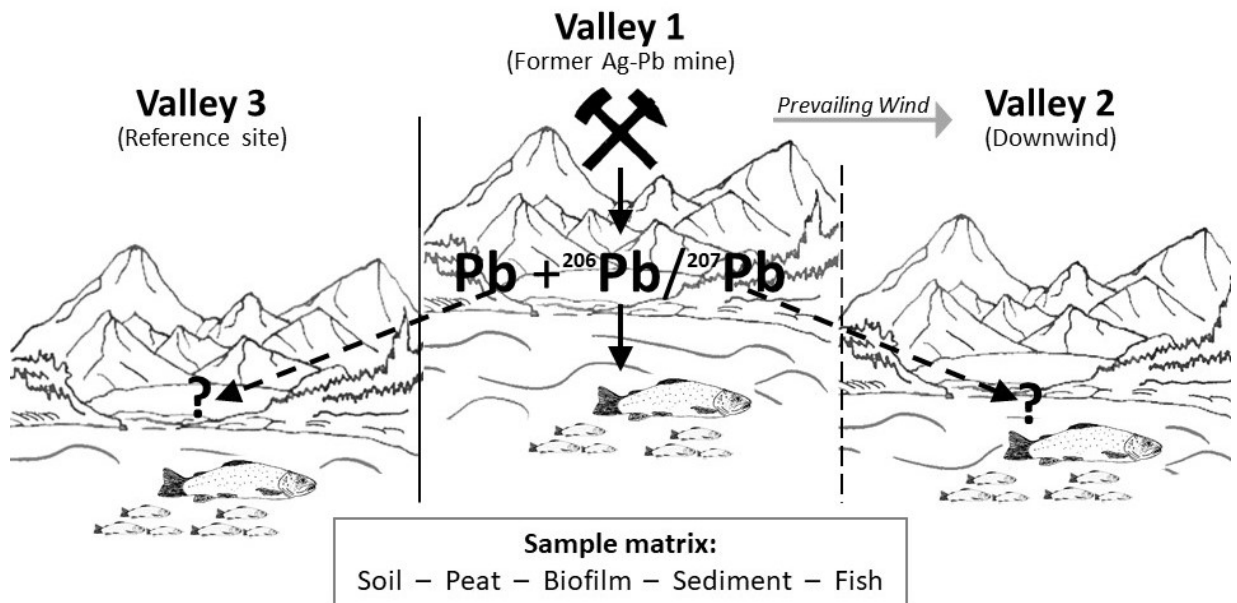
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52 **Keywords:** Mining, Pb, Pb-isotopes, Soil, Biofilm, Fish

53 Graphical Abstract



54

55 **Highlights**

- 56 • Historical mining is still a major source of Pb to contemporary aquatic ecosystems
- 57 • Concentrations of Pb decreases with increasing distance from a former Ag-Pb mine
- 58 • Pb concentrations were in the order soil>biofilm>sediment>sphagnum>fish
- 59 • Soils and biofilm prevents further dispersal and increased loadings of Pb to biota
- 60 • Fish Pb concentrations were in the order minnow>trout & whole fish>liver>muscle
- 61

62 **1. Introduction**

63
64 Although we largely associate the impacts from mining on the surrounding environment with the
65 industrial era and its advancements in mining technology, historical mining has a millennial scale
66 history in many parts of the globe often leaving a long-lasting imprint on the environment (Settle
67 and Patterson, 1980; Nriagu, 1983; Renberg et al. 1994; Nriagu, 1996; Cooke et al. 2009; Camizuli
68 et al., 2018). Studies of metal pollution using natural archives, such as peat or lake sediment
69 records, emphasized the significance of pre-industrial pollution and its impact on the environment
70 in France (Monna et al. 2004; Forel et al. 2010; Hansson et al. 2017) and elsewhere (Renberg et al.
71 1994; Weiss et al. 1997; Mighall et al. 2009; Martinez Cortizas et al. 2012). Pollution from pre-
72 industrial times caused by mining activities, such as ore exploitation and processing, when there
73 were few if any environmental controls, could be substantial (Ek et al. 2001a, 2001b). As mining
74 also entailed widespread land use that included forest disturbance and agriculture, small-scale but
75 generally widespread mining activities thus amounted to a geographically large-scale impact on
76 the landscape (Bindler et al. 2009; 2012).

77
78 High altitude soils are often shallow and thus fragile to erosional processes. Mountainous areas are
79 therefore sensitive to environmental changes (Whitehead et al. 2009), both human induced (e.g.
80 agriculture, pastoralism, mining, clear-cutting), as well as to the impact of long-term climate
81 change and rapid environmental changes (e.g. flooding). Further, peatlands, a reoccurring feature
82 in mountainous environments, acts as reservoirs of organic matter. Due to peats ability to retain
83 trace metals and radionuclides (Pourcelot et al. 2003; Hansson et al. 2015) these natural
84 environments can be considered as a pollutant “sponge” which have accumulated contaminants
85 since the beginning of the metallurgy (Bindler, 2006), and have acted as natural filters for toxic

86 elements (e.g. As, Hg, Pb) since the last deglaciation (Gonzalez et al., 2006). Similar conclusions
87 have been drawn in aquatic environments for organic rich substrates such as biofilm (Farag et al.,
88 1998; Singh et al., 2006). That is, biofilm, a mixture of bacteria, algae and fine detrital material
89 (Ruttner, 1968), have been shown to be a key link between particle-bound metals, algae, and biota
90 in aquatic ecosystems (Kimball et al., 1995; Farag et al., 1998), and can act to promote
91 bioremediation of polluted waters near mine sites (Singh et al., 2006).

92
93 Previous work on trace metal concentrations in the upper valley of Vicdessos (Central Pyrenees,
94 France) estimated that ≥ 600 tons of anthropogenic lead (Pb) is stored in organic soils on the
95 northern slope of the Pyrenees and that 85% of the Pb stored in the studied peatlands was deposited
96 prior to industrialization (Claustres, 2016; Hansson et al. 2017). Further, Hansson et al. (2017) also
97 showed that deposited Pb derived from historic mining activities, was later on remobilized due to
98 intense pastoralism and grazing activities, causing a secondary pollution input. In a climate change
99 scenario, with increased risks of precipitation and heightened erosion rates, the fate of pre-industrial
100 legacy pollutants, such as Pb, is unknown. If released, legacy pollutants stored in these high altitude
101 organic soils could pose a high risk to the surrounding environment. For example, Monna et al.
102 (2011) showed a clear link between mining heritage and high levels of Pb and cadmium (Cd) in
103 liver and muscles of brown trout (*Salmo Trutta*) at a French historical mine site, i.e. ~60% of their
104 biotic samples exceeded the European Union (EU) maximum levels even though the studied area
105 is currently one of the least industrialized areas in France (Monna et al., 2011). Further, a recent
106 study by Yang et al. (2018) showed that legacy Pb stored in soils in surrounding catchments is the
107 dominant source of Pb to lakes in the United Kingdom today, far exceeding any atmospheric
108 deposition as catchment Pb input accounts for >95% of the total Pb entering the lakes. Similar
109 conclusions have also been shown in the French Pyrenees (Bacardit and Camarero, 2010).

110
111 By comparing concentrations of Pb and Pb-isotopes ($^{206}\text{Pb}/^{207}\text{Pb}$, $^{208}\text{Pb}/^{207}\text{Pb}$ and $^{208}\text{Pb}/^{206}\text{Pb}$) in
112 samples of sphagnum growth tips (*Sphagnum*), soil, sediment, biofilm, and fish (*Salmo Trutta* and
113 *Phoxinus Phoxinus*), including different tissues within the fish (entire organism versus liver versus
114 muscle) we aimed to determine the Pb contamination status in three adjacent valleys either directly
115 connected to, or in the vicinity of, the historical silver (Ag) and Pb mine l'Argentière (i.e. silver in
116 French; Figure 1). Further, we aimed to assess if the high levels of Pb in organic soils, as indicated
117 in earlier studies (Claustres, 2016; Hansson et al. 2017), also could be seen in the on-site aquatic
118 biota, i.e. to investigate the distribution of Pb both between and within species. More specifically,
119 we asked the questions; 1) Are the previously established high concentrations of Pb in organic soil
120 also seen in other compartments, including the aquatic biota?; and if so, 2) How is this Pb
121 distributed within the biota? Finally, we asked 3) Based on Pb-isotopic data, can any Pb potentially
122 found in contemporary aquatic biota be linked to former local and/or regional mining activities?

123

124

125 **2. Material & Methods**

126

127 2.1 Site description

128 All of our study sites are located in upper Vicdessos (Ariège region; Figure 1) approximately 150
129 km south of the city Toulouse, France.

130 L'Argentière Valley (ARG; 42°46'40.09''N, 001°23'30.99''E, 1551 m a.s.l.) hosts an historical
131 Ag-Pb-mine, where mining activities have been dated back to the Antiquity (Dubois, 1999). The
132 vein deposit at l'Argentière-mine consist of galena (PbS) and sphalerite ((Zn, Fe)S) with low Fe
133 content, as well as minor occurrence of chalcopyrite (CuFeS₂), pyrite (FeS₂) and silver bearing

134 sulfosalt from the tetrahedrite series ($\text{Cu}_6[\text{Cu}_4(\text{Fe},\text{Zn})_2]\text{Sb}_4\text{S}_{13}$; Munoz et al. (2016) and references
135 therein). Onsite mining activities were mainly focused on retrieving Pb and Ag, but later on the
136 ores were also mined for Zn (Dubois, 1999). More detailed description of local and regional mining
137 activities can be found in e.g. Verna (1996), Dubois (1999), Munoz et al. (2016), Py-Saragaglia et
138 al. (2017) and references therein. The adjacent valley, Bassiès, is located south-east of l'Argentière
139 following the prevailing wind-direction (Hansson et al. 2017), and have previously been studied in
140 an aspect of long-term metal contamination reconstructions (Claustres, 2016; Hansson et al., 2017).
141 In Bassiès, three separate water bodies were included; Pla de la Font (PDF; $42^\circ45'52.79''\text{N}$,
142 $001^\circ25'09.77''\text{E}$, 1653 m a.s.l.), Etang Mort (ETM; $42^\circ45'52.77''\text{N}$, $001^\circ25'28.86''\text{E}$, 1676 m
143 a.s.l.), and Legunabens (LEG; $42^\circ45'52.85''\text{N}$, $001^\circ25'52.25''\text{E}$, 1675 m a.s.l.). Finally,
144 Bernadouze (BER; $42^\circ48'10.59''\text{N}$, $001^\circ25'26.09''\text{E}$, 1349 m a.s.l.) consists of a stream that runs
145 through a peatland, and is within the same region as l'Argentière and Bassiès, but upwind the
146 prevailing wind direction (i.e. north-east), and thus believed to be less effected by the mining
147 activities near l'Argentière-mine, and therefore included as a reference site.

148

149 2.2 Sampling procedures

150 All fieldwork and sample collection were carried out in September 2012 (sphagnum growth tips),
151 October 2014 (trout) and in October 2015 (soil, sediment, biofilm, common minnow and trout).

152

153 2.2.1 *Sphagnum*

154 All sphagnum samples were collected following the ICP vegetation protocol (Harmens et al.,
155 2010). Each sample was collected in areas with a minimum distance to tree canopy vegetation set
156 to 10 m. Within a 40 x 40 cm square, three 10 x 10 cm sub-squares were collected of surface
157 sphagnum samples using a ceramic knife pre-cleaned with 2% HNO_3 and milliQ H_2O . Within each

158 10 x 10 cm, samples of *Sphagnum*-stems, branch leaves and terminal (apical) buds were picked
159 using nitrile gloves, ceramic scissors, and the fresh bud (outermost 1 cm of each stem) were
160 collected and placed in clean plastic zip-lock bags. The bags were then placed in a cooler and kept
161 in cold and dark condition during transport back to the laboratory where they were immediately
162 placed in a freezer at -20°C.

163

164 2.2.2 Soil, Sediment and Biofilm

165 Using a pre-cleaned stainless steel garden shovel, three samples of soil were collected from the A-
166 horizon (2-10 cm depth), placed into one individual plastic bag, mixed and homogenized into one
167 composite sample of ~500 g. This procedure was repeated on five different locations at each site
168 to ensure spatial representability of final results. Sediment samples were collected using either pre-
169 cleaned stainless steel scoop and grab sample technique (US EPA Method 5035; l'Argentière,
170 Etang Mort and Bernadouze) or with a Universal Percussion Corer (Aquatic Research Instrument;
171 www.aquaticresearch.com/universal_core_head.htm) sediment corer with a 10 cm diameter PVC
172 sample tube (Pla de la Fond and Etang Legunabens) of which the top 5 cm were retrieved and
173 mixed into one composite sample. Using waders and plastic gloves, approximately 5-10 rocks were
174 collected close the shore, removed from the water, and gently brushed clean using toothbrushes,
175 after which the biofilms were transferred to acid-washed 50 mL Falcon tubes filled with 10 mL
176 mQ H₂O. This procedure was repeated on five different locations (seven for PDF) at each site to
177 ensure representative results of each site. All samples were kept in cool and dark conditions during
178 transport back to the laboratory where they were immediately frozen at -20°C.

179

180 2.2.3 Biota

181 Fish samples, i.e. brown trout (*Salmo Trutta Fario*), were collected in October 2014 (Bassiès
182 Valley) and October 2015 (l'Argentière, Bassiès and Bernadouze Valleys) using either electric
183 (n=31 for ARG; n=40 for PDF; n=19 for BER) or net (n=8 for ETM; n=4 for LEG) fishing. In
184 addition, Common minnow (*Phoxinus Phoxinus*) were collected from PDF (n=12) and ETM
185 (n=12) in October 2015 using electric fishing. Upon capture all fish were subjected to an overdose
186 of anesthetic solution (120 mg/L Benzocaïne) and sacrificed in accordance with local guidelines
187 and regulations. Length and weight of each fish was recorded (Table S1), before the fish were
188 placed in pre-labeled plastic zip lock bags and placed on a mixture of ice and dry ice to ensure cold
189 conditions during transport back to the laboratory. Once back at the laboratory dissections,
190 following the protocol by ICP Waters report 105/2010, commenced immediately during which
191 liver and muscle tissue were collected, i.e. liver tissues were collected to represent estimated health
192 of the fish whereas muscle tissues were collected to represent estimated risk towards humans via
193 dietary consumption. Due to their limited size, only five common minnow were dissected for
194 muscle tissues, all others were frozen intact and treated as a bulk sample of the entire organism.
195 The same procedure was applied to brown trout which were deemed too small to dissect (n = 10).
196 Liver and muscle samples, as well as bulk samples of entire individuals, were weighed, recorded
197 and placed in 50 mL Falcon tubes, then transferred to a -20°C freezer, where samples were kept
198 until further analysis.

199
200 2.3 Sample preparation and digestion

201 All samples were lyophilized using a Christ Alpha 1-2 freeze drier. For soils and sediments, all
202 coarse particles (gravels) and organic debris (roots) were removed after freeze-drying (not
203 applicable for biofilm and fish samples). Samples were then gently crushed and sieved at <2 mm
204 (soil) or homogenized into a powder by manually grinding each sample using an acid cleaned agate

205 mortar (sediment, biofilm, bulk of entire individual, liver and muscle tissues), then placed in falcon
206 tubes and stored under dark and cool conditions until further processing.

207
208 All sphagnum samples (n = 10; ~100 mg) were digested in PFA vials using a HF – HNO₃ – H₂O₂
209 digestion, following the procedure described in Hansson et al. (2017). For digestion of soil (n =
210 25) and sediment samples (n = 10), approximately 100 mg of dried and homogenized material was
211 placed in acid cleaned digitubes (SCP Sciences 010-500-263) together with 3 mL of ultrapure nitric
212 acid (HNO₃; ~67-69%), closed with airtight caps, and digested overnight at 90°C. Digestions were
213 cooled down to room temperature and then transferred to 50 mL Falcon tubes and initially diluted
214 with mQ H₂O (3:22 digest:mQ H₂O), after which 10 mL were transferred to 15 mL Falcon tubes
215 (tot 10 mL solution equal to a 1.2:8.8 digest:mQ H₂O ratio) for further analysis.

216
217 Digestions of biofilm (n = 27), bulk of entire individual (n = 10 for *S. trutta* and n = 24 for
218 *P. phoxinus*), liver (n = 25 for *S. trutta*) and muscle (n = 91 for *S. trutta* and n = 5 for *P. phoxinus*),
219 followed the same initial procedure as described above but were diluted in two steps; the digest
220 was first transferred to 15 mL Falcon tubes and diluted with mQ H₂O (3:7 digest:mQ H₂O for Q-
221 ICP-MS) to create a mother solution (MS). The MS was then diluted to 1:9 MS:mQ H₂O and spiked
222 with 50 µL of a mixed natural In/Re solution of known concentration (0.4037 ppm) and masses
223 (¹¹⁵Indium and ¹⁸⁷Rhenium) were monitored for internal standardization. No ¹¹⁵In/¹⁸⁷Re was added
224 to dilutions for HR-ICP-MS analysis. All sample preparations were performed under clean
225 laboratory conditions (Clean room class 10000, ISO 7) using acid cleaned lab ware.

226

227 2.4. Chemical analysis

228 Leachate samples of sediment and soil samples were analyzed for Pb using an Inductively Coupled
229 Plasma Optical Emission Spectroscopy, ICP-OES, (Thermo Electron IRIS Intrepid II XLD,
230 EcoLab, Toulouse, France). The accuracy of digestion and analysis protocol was checked using
231 certified standards; i.e. LKSD-4, WQB-1, SUD1 and IAES SL1 (all = lake sediment). Detailed
232 information can be found in Table S2.

233
234 Sphagnum (total digestions) and leachate samples of biofilm and fish were analyzed for Pb with a
235 Quadrupole Inductively Coupled Plasma Mass Spectrometer, Q-ICP-MS, (7500 ce, Agilent
236 Technologies, OMP-GET, Toulouse, France) using Indium (^{115}In)/Rhenium (^{187}Re) as internal
237 standard. The accuracy of digestion and analysis protocol was checked using blanks and certified
238 standards; NIMT, NJV 942 and IAEA 336 for sphagnum samples; NJV 941, NJV 942 and IPE 176
239 for biofilm samples; DORM-4, DOLT-2 and TORT-3 for fish tissues. Detailed information can be
240 found in Table S3a-c in the supplementary information file.

241
242 Pb isotopic signatures (^{206}Pb , ^{207}Pb and ^{208}Pb) in soils, biofilm and trout muscle tissues were
243 analyzed using a High Resolution ICP-MS (Thermo Element II XR; OMP service ICP-MS,
244 Toulouse, France). Individual sample bracketing with certified and adequately diluted (125 ppb to
245 250 ppb) NIST NBS-981 was performed to correct for mass shift according to Krachler et al.
246 (2004). To assure quality standards, certified reference material (NJV 952) was included in the
247 analysis (i.e. $^{208}\text{Pb}/^{206}\text{Pb} = 2.101 \pm 0.002$ (2 s.d.) and $^{206}\text{Pb}/^{207}\text{Pb} = 1.165 \pm 0.001$ (2 s.d.) which
248 aligned with previous NJV-942 measurements reported in Hansson et al. (2017)).

249

250 2.5 Data analysis

251 To estimate the overall health status of the collected fish, Fulton's condition factors (FCF) were
252 calculated for all individuals using the formula (Ricker, 1975);

$$253 \quad FCF = \frac{W}{L^3} \times 100$$

254 in which W is measured body weight (g) and L is measured body length (jaw tip to fork of the tail;
255 cm).

256 Further, multiple comparisons of means in Pb levels across compartments, sites and valleys were
257 estimated through anova and post hoc tests with "bonferroni" correction. Results are showed in
258 Table S4 and summarized in Results and Discussion section. The analysis was performed in R
259 version 3.4.3 (R Core team, 2017).

260

261

262 **3. Results and Discussion**

263

264 Measured Pb concentrations ($\mu\text{g g}^{-1}$) in all samples are reported in Figure 2 (environmental
265 samples), Figure 3 (fish) and in Table S5 and Table S6.

266

267 **3.1 Distribution of Pb between sites and environmental compartments**

268 The lack of significant differences between the three sites (i.e. Pla du la Fond, Etang Mort and
269 Legunabens, $p\text{-value} > 0.05$) in Bassiès Valley (Table S4) allowed for grouping them together in a
270 comparison between l'Argentière, Bassiès, and Bernadouze Valleys.

271 As expected, l'Argentière Valley consistently showed the highest Pb-concentrations in all
272 compartments (i.e. sphagnum, soil, biofilm, sediment, fish), followed by Bassiès Valley, whereas
273 Bernadouze Valley consistently displayed the lowest concentrations (Figure 2; Table S5). The

274 exception to this is the sediment-compartment where LEGs showed the highest average Pb-
275 concentration ($110.8 \mu\text{g g}^{-1}$; similar to values reported by Bacardit et al. (2012)), exceeding average
276 Pb-concentrations seen in ARG ($80 \pm 72 \mu\text{g g}^{-1}$), although ARG still showed the overall highest
277 concentration ($161 \mu\text{g g}^{-1}$ in ARG vs $110.8 \mu\text{g g}^{-1}$ in LEG; Figure 2; Table S5). This trend of
278 decreasing concentrations with increasing distance to the former mine, was also reflected in the
279 statistical analysis. That is, there was a significant difference in Pb concentrations (based on
280 comparison of soil, biofilm and liver samples) between l'Argentière versus Bassiès and versus
281 Bernadouze, but there was no significant difference between Bassiès and Bernadouze (Table S5).
282 However, the lack of significant difference between Bassiès and Bernadouze, is more likely
283 explained by the low sample count than a true reflection of contamination gradient.

284
285 The current Pb-deposition to the three studied valleys is represented here by *Sphagnum* growth
286 tips, yet a more detailed description of Pb-deposition to this region over time (i.e. the last 3000
287 years) can be found in Hansson et al. (2017). Average Pb concentration in sphagnum were $3.36 \mu\text{g}$
288 g^{-1} (one sample) for ARG, $1.69 \pm 0.88 \mu\text{g g}^{-1}$ for PDF, $1.58 \pm 0.43 \mu\text{g g}^{-1}$ for ETM, $1.31 \pm 0.08 \mu\text{g}$
289 g^{-1} for LEG respectively (Figure 2). No data were available from BER. Compared to Pb deposition-
290 trends seen elsewhere (e.g. EU-moss survey as reported in Harmens et al., 2015), the Pb found in
291 sphagnum from all sites here are below the EU-average of $3.69 \mu\text{g g}^{-1}$ (Harmens et al., 2015),
292 indicating a low contemporary Pb-deposition to these sites. With the overall reduction of Pb
293 emission to air masses and subsequent reduction in atmospheric Pb deposition (Thomas et al.,
294 1999), it is clear that catchment Pb input far exceeds that of atmospheric Pb deposition (Bacardit
295 and Camarero, 2010; Yang et al., 2018). Based on the long history of mining activities in this region
296 (Verna, 1996; Dubois, 1999; Munoz et al., 2016; Py-Saragaglia et al., 2017), and the previously

297 documented re-mobilization of legacy Pb (Bacardit and Camarero, 2010; Hansson et al., 2017) it
298 is therefore plausible that Pb found in contemporary samples (e.g. fish) in these valleys are likely
299 derived from legacy Pb pollution rather than from modern Pb pollution input.

300
301 Average Pb concentration in top soil samples were $164 \pm 85.7 \mu\text{g g}^{-1}$ for ARG, $44.4 \pm 24.6 \mu\text{g g}^{-1}$
302 for PDF, $41.2 \pm 14.2 \mu\text{g g}^{-1}$ for ETM, $67.5 \pm 31.5 \mu\text{g g}^{-1}$ for LEG and $19.1 \pm 10.8 \mu\text{g g}^{-1}$ for BER
303 respectively (Figure 2), and in the case of LEG falls within the range of previously reported
304 concentrations (Bacardit et al., 2012). With the exception of BER, the average Pb soil
305 concentrations at all studied sites exceeded the French national average of 28 mg kg^{-1} (Villanneau
306 et al., 2008) and were either close to (PDF and ETM) or exceeded (LEG and ARG) reported
307 threshold values of soil quality, i.e. $\geq 50\text{-}60 \text{ mg kg}^{-1}$ (MEF, 2007; Villanneau et al. 2008; van der
308 Voet et al., 2013; Toht et al., 2016a; 2016b), and at least one sample at each site exceeded this
309 threshold value (Table S5). This indicated both a large within and between site distribution in top
310 soil Pb concentrations, where l'Argentière showed significantly higher concentrations than Bassiès
311 and Bernadouze, whereas no significant difference was found between Bassiès and Bernadouze
312 (Table S5). More importantly, however, these results also showed a clear presence of Pb, and an
313 inherit environmental risk, in the area.

314
315 Average Pb concentration in surface sediment samples were $80.1 \mu\text{g g}^{-1}$ for ARG, $56.1 \mu\text{g g}^{-1}$ for
316 PDF, $36.3 \mu\text{g g}^{-1}$ for ETM, $110.8 \mu\text{g g}^{-1}$ for LEG and $21.1 \mu\text{g g}^{-1}$ for BER respectively (Figure 2),
317 of which only LEG exceeds the 100 mg kg^{-1} Pb threshold level for sediment quality in freshwater
318 ecosystems (JORF, 2014). Compared to data reported in similar studies elsewhere (Monna et al.,
319 2011; Camizuli et al., 2014), the sediment Pb-levels seen in the three valleys here are generally

320 much lower, i.e. total range of 24.6 – 161.3 mg kg⁻¹ Pb vs 26 -1588 mg kg⁻¹ Pb (Cévennes National
321 Park, Monna et al., 2011) and 67 – 25 000 mg kg⁻¹ Pb (Morvan, Camizuli et al., 2014). Even if data
322 reported here are well in agreement of previous studies in the area (Bacardit and Camareo, 2010:
323 Bacardit et al., 2012), both PDF and LEG were only represented here by one single sediment
324 sample (bulk of top 5 cm of a single core) that limits any large-scale conclusions, yet combined the
325 results still indicated that the environmental status in the studied sediments are generally good and
326 pose no immediate risk to the health of aquatic biota.

327
328 This, however, was not fully reflected in the Pb-concentrations in biofilm, i.e. average Pb
329 concentration in biofilm samples were 153 ± 81.7 µg g⁻¹ for ARG, 92.9 ± 52.3 µg g⁻¹ for PDF, 67.1
330 ± 27.8 µg g⁻¹ for ETM, 92.6 ± 29.7 µg g⁻¹ for LEG and 3.94 ± 2.12 µg g⁻¹ for BER, thus showing
331 higher Pb average concentrations than sediment (Figure 2; Table S5). Based on the previously
332 reported high affinity of Pb to organic material (Bunzl et al., 1976; Kerndorff and Schnitzer, 1980;
333 Hansson et al., 2015), the reported association of metals to both biotic and abiotic components of
334 biofilm (Farag et al., 1998), and that biofilm can act as a bioremediation of polluted waters near
335 mine sites (Singh et al., 2006), we therefore suggest that the organic rich soils and the biofilm
336 compartments in the three studied valleys act as filters, allowing less Pb to be deposited to, and
337 sequentially buried in, the sediment.

338
339 This is also the likely reason why the average Pb concentration in the fish compartment, represented
340 here by liver tissues from brown trout (*Salmo trutta*), were generally low, i.e. significantly lower
341 than Pb concentrations in soil and biofilm (all three valleys; Table S6). That is, average Pb
342 concentrations in trout livers were 1.25 ± 1.257 µg g⁻¹ for ARG, 0.17 ± 0.146 µg g⁻¹ for PDF, 0.11

343 $\pm 0.056 \mu\text{g g}^{-1}$ for ETM, $0.19 \pm 0.030 \mu\text{g g}^{-1}$ for LEG and $0.01 \pm 0.003 \mu\text{g g}^{-1}$ for BER (Figure 2).
344 Compared to brown trout caught near other historical mine sites in France (i.e. Cévennes National
345 Park and Morvan), the trout caught in the three valleys here all displayed either similar to or lower
346 concentrations of Pb (Table S6; Table S7; Monna et al. 2011; Camizuli et al. 2014), indicating that
347 the previously reported high Pb-concentrations in the area (Claustres, 2016; Hansson et al., 2017)
348 is likely retained in the organic fractions of surrounding soils and biofilms, and thus not easily
349 bioavailable. This is also reflected in the calculated FCFs (Table S1) as >70% of all studied fish
350 displayed FCF greater than 1, and > 90% displayed FCF greater than 0.8, indicating that with
351 respect to weight and length, fish from the three studied valleys are in generally good condition
352 (Fulton, 1904), and thus not likely exposed to high contamination loads. It should be noted
353 however, that calculated FCF for fish in this study are generally lower than FCF reported in Monna
354 et al. (2010), i.e. mean FCF 1.08 vs 1.28, indicating that some environmental and/or physiological
355 stressors may still influence the health of the fish in the three studied valleys.

356

357 3.2 Distribution of Pb within fish

358 Measured Pb concentrations in fish (i.e. bulk of entire organism, liver and muscle) are presented in
359 Table S6. In spite of repeated sampling attempts, the two targeted species, i.e. brown trout (*Salmo*
360 *trutta fario*) and common minnow (*Phoxinus phoxinus*), were both only collected in PDF and ETM.
361 In ARG, LEG and BER, only brown trout were caught. Further, in the initial stages of this project,
362 analysis of Pb within the different organs were focused on samples collected in Bassiès only (no
363 significant difference was seen in biota between the three sites; Table S4). The comparison between
364 species, and between organs, were therefore focused on fish caught in Bassiès only (Figure 3).

365 Average Pb concentrations in bulk of entire organisms were $0.08 \pm 0.04 \mu\text{g g}^{-1}$ for trout ($n = 2$) and
366 $0.14 \pm 0.11 \mu\text{g g}^{-1}$ for minnow ($n = 24$), whereas Pb concentrations in muscle tissues were $0.01 \pm$
367 $0.01 \mu\text{g g}^{-1}$ for trout ($n = 25$) and $0.05 \pm 0.04 \mu\text{g g}^{-1}$ for minnow ($n = 5$; Figure 3). This indicated
368 that there is a clear difference in Pb concentrations between the two species, which could be
369 explained either by a difference in direct environmental and/or dietary exposure, or by differences
370 in species-specific accumulation- and regulatory-capacities. That is, it is possible that minnow,
371 being a bottom feeder, are more exposed to Pb from the surrounding environment (i.e. sediment
372 and biofilm) than trout, which resides higher in the water column (Klemetsen et al., 2003). Further,
373 Pb concentrations in fish tissues have been linked with Pb concentrations in their diet (Luszczek-
374 Tronjar et al., 2013), thus indicating that the two species may feed off different dietary sources.
375 That is, although minnow often act as prey for trout (i.e. trout being considered higher on the
376 aquatic food chain) and should therefore be considered part of the trout's diet, this is not exclusively
377 the case (Borgstrøm et al., 1985). Finally, differences in accumulation and/or excretion efficiencies
378 of accumulated Pb between the two species could also explain why trout exhibited lower Pb
379 concentrations than minnow. For example, it has been shown that although Pb may be accumulated,
380 it does not biomagnify within aquatic food chains (Cardell et al., 2013; Won et al., 2018; Quintela
381 et al 2019). The data on Pb in minnow and trout presented here support these findings.

382
383 What this data also show, is the clear difference in Pb concentrations between different organs.
384 That is, average Pb concentrations in Bassiès trout were $0.08 \pm 0.04 \mu\text{g g}^{-1}$ for bulk of entire
385 organism ($n = 2$), $0.05 \pm 0.03 \mu\text{g g}^{-1}$ for liver ($n = 15$) and $0.01 \pm 0.01 \mu\text{g g}^{-1}$ for muscle tissues (n
386 $= 25$) respectively (Figure 3). Large differences in contaminant concentrations between tissues of
387 the same individuals have been reported elsewhere, with highest Pb concentrations often seen in

388 bone, spleen or kidney (Camusso et al., 1995, Vinodhini and Narayanan, 2008; Monna et al., 2011).
389 Yet, literature often reports values in either liver (representing the overall health status of the fish)
390 or in muscle tissues (for comparison to official guidelines and directives such as EU foodstuffs
391 levels), e.g. Pyle et al. (2005), Monna et al. (2011), and Camizuli et al. (2014). For example, Monna
392 et al (2011) showed that Pb-concentrations varied on a 1000-fold scale ranging from 0.01 to 5.98
393 in liver tissues versus 0.03 to 0.27 in muscle tissues of brown trout caught in the Cévennes National
394 Park, France (Table S7). Although this study here was merely designed as an initial test (hence the
395 relatively large variation in numbers of samples included), the data clearly points to the strong
396 variation of Pb concentrations between organs from the same species collected from the same site.
397 The implication of this is twofold; first, that great care must be taken not just to which species
398 should be collected (selection based on habitat and species specific physiological properties) but
399 also to which organ should be targeted for analysis; and second, it also shows the need for better
400 guideline and regulatory values of contamination levels in fish, adapted to organs that reflect the
401 overall health status of the fish (i.e. liver) rather than organs that reflect risk of exposure to human
402 health via dietary ingestion (i.e. muscle tissues).

403

404 3.3 Origin of Pb and potential pollution sources

405 The detected Pb measured in environmental samples (sphagnum, soil, sediment and biofilm) as
406 well as fish, only states a presence of contamination in this area, but provides no information of
407 potential sources. In an attempt to decipher potential sources of Pb to the three studied valleys, soil,
408 biofilm and fish (entire organism and/or muscle tissues) were therefore analyzed for Pb-isotopic
409 ratios (i.e. $^{206}\text{Pb}/^{207}\text{Pb}$, $^{208}\text{Pb}/^{207}\text{Pb}$ and $^{208}\text{Pb}/^{206}\text{Pb}$), as Pb-isotopic signatures are controlled by the
410 ore from which the Pb originates (Patterson et al., 1955; Chow and Patterson, 1962). All results of
411 Pb-isotopic analysis are presented in Figure 4, Figure S1 and Table S8. It is generally accepted that

412 Pb derived from historical mining activities across mainland Europe shows $^{206}\text{Pb}/^{207}\text{Pb}$ in the range
413 of 1.16 – 1.18 (Cooke and Bindler, 2015). For example, Munoz et al. (2016) reported Pb-isotopic
414 signatures of the local l'Argentière ore as $^{206}\text{Pb}/^{207}\text{Pb}$: 1.171 ± 0.001 (n=3). These signatures can
415 also be seen as atmospherically derived Pb in nearby peatlands, i.e. Pb-isotopic signatures found
416 in peat samples in Bassiès (dated to 1200-1950) is similar to Pb-isotopic signatures seen in local
417 and regional ores (Hansson et al., 2017). If former mining activities is still a source of Pb to the
418 three studied valleys, this mining derived signature should be seen in collected contemporary
419 samples. However, results here (Figure 4, Figure S1 and Table S8) showed a wide range in Pb
420 isotopic signatures, which indicated that several sources of Pb-accumulation to the area must exist.
421

422 In l'Argentière Valley, average Pb-isotopic signatures of all samples (i.e. soil, biofilm, and trout)
423 fell in a narrow range of $^{206}\text{Pb}/^{207}\text{Pb}$: 1.167 – 1.172 (Figure 4), which overlapped with the Pb-
424 isotopic signatures of the local l'Argentière ore deposit (Munoz et al., 2016), indicating that the
425 Argentière ore-Pb dominates the soil-Pb and acts as an upper endmember in the mixture of Pb seen
426 in the l'Argentière fish. However, samples from this area may also be influenced by former mining
427 activities elsewhere in the region (Munoz et al., 2016; Py-Saragaglia et al., 2017), such as the Castel
428 Minier ore deposit ($^{206}\text{Pb}/^{207}\text{Pb}$: 1.176 ± 0.007 , Figure S1; Hansson et al., 2017 and references
429 therein). As the Pb-isotopic signature of the l'Argentière ore fell within the reported range of the
430 Castel minier ores (Hansson et al., 2017 and references therein), we cannot distinguish a clear
431 difference from local (l'Argentière) to regional (e.g. Castel Minier or other mines in the Aulus-les-
432 Bains Mining District) sources of legacy Pb, only conclude that in samples of soil, biofilm and fish
433 from the l'Argentière Valley, local to regional ores appears to have had a major influence on the
434 Pb-isotopic signatures. This in turn shows that historical mining, although no longer active, still

435 acts as a dominant source of Pb to contemporary aquatic biota to this day, which also explains the
436 narrow ranges of Pb-isotopic signatures found in the different environmental compartments in
437 l'Argentière Valley (Figure 4).

438
439 This mining derived Pb-isotopic signature was also seen in the Bassiès Valley, but to a lesser extent
440 than in l'Argentière (Figure 4). Here, wider range of $^{206}\text{Pb}/^{207}\text{Pb}$ (as well as $^{208}\text{Pb}/^{207}\text{Pb}$ and
441 $^{208}\text{Pb}/^{206}\text{Pb}$) could be seen, indicating a mixture of natural (weathering of local bedrock; Bassiès
442 granite, Figure S1) and anthropogenic sources (legacy contamination from mining activities, as
443 well as modern contamination from industry and transportation). These different Pb signatures
444 were also found in various layers of peat samples (dated 1300 B.C. to A.D. 1990) from peatlands
445 surrounding the lakes in the Bassiès Valley (Figure S1; Hansson et al., 2017). Modern European
446 Pb contamination are known to show lower $^{206}\text{Pb}/^{207}\text{Pb}$ (and higher $^{208}\text{Pb}/^{206}\text{Pb}$) signatures than Pb
447 derived from mined ores (Bindler et al., 2008 and references therein), e.g. leaded gasoline have
448 reported Pb-isotopic signatures of $^{206}\text{Pb}/^{207}\text{Pb}$: $1,097 \pm 0,025$ (n=12), whereas industrial sources
449 such as urban incinerators and smelters have reported signatures of $^{206}\text{Pb}/^{207}\text{Pb}$: $1,158 \pm 0,035$
450 (n=15) (Monna et al., 1997; Véron et al, 1999). These trends are in agreement with the atmospheric
451 signature found locally and recorded in ombrotrophic peatlands (Figure S1; Hansson et al. 2017).
452 Average Pb-isotopic signatures in soil, biofilm and biota in the Bassiès Valley showed a range of
453 $^{206}\text{Pb}/^{207}\text{Pb}$: 1.168 – 1.178, which indicated that both mining derived Pb, as well as more modern
454 contamination must be supplying Pb to this valley, which was also seen in onsite peat records (see
455 Hansson et al., 2017 for detailed discussion). Yet, the high $^{206}\text{Pb}/^{207}\text{Pb}$ seen in e.g. PDF and ETM
456 soils, indicated that also naturally occurring, and more radiogenic, Pb derived from weathering of
457 local bedrock was influencing the Pb-isotopic signatures in this system, as the host bedrock often

458 present higher Pb-isotopic signatures than ore intrusions that are mined (Bindler et al., 2008; Cooke
459 and Bindler, 2015).

460
461 Such naturally occurring Pb was also found in Bernadouze, as high $^{206}\text{Pb}/^{207}\text{Pb}$ ratios could be seen
462 in both soil, biofilm and fish (Figure 4) that is likely caused by weathering of bedrock with high
463 U/Pb and low Th/Pb ratios, such as carbonate rocks. Yet, the isotopic signature seen in the
464 Bernadouze biofilm is indeed quite exotic with a high $^{206}\text{Pb}/^{207}\text{Pb}$ ratio, even superior to the
465 background value given by local bedrock (Bassiès granite) or the deeper and oldest layers of peat
466 (Figure 4 and Figure S1). Part of the Bernadouze watershed is underlain by karstic bedrock (Jalut
467 et al., 1982) with high occurrence of carbonates and uranium, that will produce more ^{206}Pb than
468 ^{207}Pb , and thus yielding a more radiogenic $^{206}\text{Pb}/^{207}\text{Pb}$ ratio (Bindler et al., 2008 and references
469 therein; Cuvier et al. 2016 and references therein). Pb-isotopic signatures in the Bernadouze fish
470 however, did not appear to be influenced solely by this highly radiogenic signature, which indicated
471 a difference of incorporation of Pb between biofilms and fish, where the biofilm may act as
472 bioindicator of dissolved Pb and fish as bioindicators of particle Pb. We therefore conclude that Pb
473 found within the Bernadouze Watershed, is likely derived from weathering of the surrounding
474 bedrock (thus considered as natural origin), and that soil-Pb act as the upper endmember of the Pb
475 mixture seen in Bernadouze fish. Yet, the $^{206}\text{Pb}/^{207}\text{Pb}$ signature of 1.16-1.17 seen in some fish
476 indicated that also anthropogenic Pb is influencing this system, even if not considered a major
477 contributor. The combination of natural weathered bedrock and high occurrences of organic soil in
478 the immediate area (i.e. a peatland), explains the overall low Pb concentrations seen in all
479 environmental compartments in Bernadouze, including the onsite biota (Figure 2). It should also
480 be noted that Bernadouze fish were mainly represented here by samples of entire organisms,

481 whereas Argentière and Bassiès were represented by both muscle tissues and entire organisms that
482 could influence the Pb-isotopic signature detected.

483
484 In summary, the Pb-isotopic signatures indicated that; the l'Argentière Watershed is being directly
485 influenced by mining derived legacy Pb contamination, where ore derived Pb dominates the Pb in
486 soil which, in turn, acts as upper endmember of the Pb mixture seen in l'Argentière fish; Bassiès
487 Valley (PDF, LEG and ETM) is mainly influenced by atmospheric contamination of both local,
488 regional and long-distance sources, i.e. a mixture of more radiogenic, naturally weathered Pb as
489 well as ore derived Pb which acts as upper endmember whereas modern Pb pollution from e.g.
490 gasoline or industry represent the lower endmember of the Pb mixture found in fish; and finally,
491 Bernadouze Valley is relatively unaffected by any contamination as Pb appears to be derived from
492 geological sources, i.e. weathering of carbonate rocks with high U/Pb and low Th/Pb ratios (Figure
493 4, Figure S1 and Table S8).

494

495

496 **4. Conclusion**

497

498 Based on the concentrations and distribution of Pb between sites, environmental compartments and
499 organism tissues, historical mining activities have left a long-lasting impact on the surrounding
500 environment, and that much of this pollution remained either stored in organic-rich soils or in the
501 biofilm compartment. The generally low Pb concentrations (below guideline values) seen here,
502 therefore indicated that organic rich soils and aquatic biofilm acts as filters which reduces
503 contaminant loading to, and sequential burial in, sediments. Based on the strong binding affinity
504 of e.g. Pb to organic substrate, this also reduced the overall bioavailability of mining derived legacy

505 pollution to onsite aquatic biota. However, although bodily Pb-concentrations were low (within
506 regulatory guidelines), contemporary biota in the upper Haut-Videssos area, and their prey, still
507 showed a large range of Pb isotopic signatures, of which former mining activities appeared to have
508 a strong influence. We therefore concluded that even though mining in the area ceased over 100
509 years ago, mining derived legacy Pb pollution continues to affect onsite biota, which clearly reflects
510 the long-lasting impact of human-environment interaction, and that ecosystem conditions may
511 remain impaired centuries after activities have ceased.

512

513

514 **5. End Notes**

515

516 *Additional information*

517 The authors declare that collection and sacrificing of all fish included in this study was performed in
518 accordance to local guidelines and regulations, i.e. authorized by Direction départementale des
519 territoires de l'Ariège, with input from Fédération de l'Ariège de pêche et de protection du milieu
520 aquatique and Service départemental de l'Office national de l'eau et des milieux aquatiques. Further, the
521 authors declare no competing financial interest. All correspondence and requests for materials should
522 be addressed to SVH (sophia.hansson@bios.au.dk).

523

524 *Associated Content*

525 Information on ecological parameters in studied fish is presented in Table S1. Results of quality
526 control and reproducibility of results is presented in Table S2 (ICP-OES), and Table S3a-c (Q-ICP-
527 MS). Results from statistical analysis is presented in Table S4. All measured Pb concentrations in
528 sphagnum, soil, sediment and biofilm is presented in Table S5. All measured Pb concentrations in

529 fish, i.e. entire organism, liver and muscle from brown trout (*Salmo trutta*) and common minnow
530 (*Phoxinus phoxinus*) is presented in Table S6. Comparison of fish Pb-data included in this study to
531 reported reference values are presented in Table S7. All measured Pb-isotopic ratios ($^{206-208}\text{Pb}$) in
532 samples of soil, biofilm and fish (entire organism and muscle tissue), is presented in Figure S1 and
533 Table S8.

534

535 *Author Contribution*

536 S.V.H. designed the study, and all authors participated in various aspects of the fieldwork and
537 sample collection. S.V.H. and S.J. performed all biotic sample collection and preparations. S.V.H.
538 performed all lab work and analyses, G.L.R. participated in all analyses. The final graph work was
539 created by Y.G. who performed statistical analysis together with M.C. based on input from S.V.H.
540 All authors participated in the interpretation of results. S.V.H. wrote the manuscript with additional
541 input from all authors.

542

543 *Acknowledgement*

544 The authors wishes to thank; F. Dauba, and A. Perrault for their assistance in both the field and in
545 the lab; J. Carrillo, F. De Vleeschouwer, S. Ferrant, P-A. Herrault, L. Jaquin, C. Li, T. Rosset, and
546 V. Thierion for their assistance in the field; and D. Baque, F. Candaudap, J. Chmeleff, P. Durantez,
547 A. Lanzanova, E. Omparet, and M.J. Tavella for their assistance in the lab. The authors also want
548 to thank D. and P. Dupui (Refuge de Bassiès) for their warm hospitality and assistance during all
549 fieldwork, and S.V.H. also want to thank F. M. van Beest for fruitful discussions during the writing
550 process of this manuscript. Further, the Préfecture de l'Ariège and DDT Ariège is gratefully
551 acknowledged for granting fishing permission.

552

553 *Funding*

554 This project was made possible thanks to the generous support by AXA Research Fund –
555 Postdoctoral fellowship (14-AXA-PDOC-030), and by the support from Prestige/Campus France,
556 co-funded by Marie Curie, Postdoctoral fellowship (PRESTIGE-2014-1-0037), both awarded to
557 S.V. Hansson. G. Le Roux was funded by ANR JCJC TRAM (ANR-15-CE01-0008). Additional
558 support from Labex DRIIHM ANR-11-LABX0010 OHM Vicdessos are also acknowledged and
559 much appreciated.

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737 **Figures and Tables**

738
739 **Figure 1.** Location of the three studied valleys; l'Argentière, Bassiès (Pla de la Fond, Etang Mort
740 and Legunabens), and Bernadouze. All sites are located in the upper Ariège region, French
741 Pyrenees and ~100 km south of Toulouse, France.

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743
744 **Figure 2.** Pb concentrations (mg kg^{-1}) in sphagnum, soil, sediment, biofilm and fish (here
745 represented by liver tissues of brown trout; *Salmo trutta fario*). Pb is presented as dry weight (d.w.)
746 for sphagnum, soil, sediment and biofilm and in wet weight (w.w.) for fish. ARG for l'Argentière,
747 PDF for Pla de la Fond, ETM for Etang Mort, LEG for Legunabens and BER for Bernadouze. Note
748 that Pb concentrations are presented on a logarithmic scale.

749
750
751 **Figure 3.** Pb concentrations (mg kg^{-1} w.w.) in common minnow (*Phoxinus phoxinus*), i.e. entire
752 organism and muscle tissue, as well as in brown trout (*Salmo trutta fario*), i.e. entire organism,
753 liver and muscle tissues. All samples are from Bassiès Valley. Note that Pb is presented on a
754 logarithmic scale.

755
756
757 **Figure 4.** Lead isotopic signatures of $^{208}\text{Pb}/^{207}\text{Pb}$ vs $^{206}\text{Pb}/^{207}\text{Pb}$ in soil (grey filled circle), biofilm
758 (grey filled triangle) and fish (grey thick cross) collected from the valleys l'Argentière (top-left
759 panel), Bassiès (top-center panel) and Bernadouze (top-right panel). Bottom panel: Lead isotopic
760 signatures of $^{208}\text{Pb}/^{207}\text{Pb}$ vs $^{206}\text{Pb}/^{207}\text{Pb}$ in all samples and all sites, i.e. muscle tissues (filled circles)
761 and entire organism (filled square) in brown trout (T.; *Salmo trutta fario*); muscle tissues (thick
762 cross) and entire organism (filled star) in common minnow (M.; *Phoxinus phoxinus*); soil samples
763 (filled diamond); and in biofilm samples (filled upward triangle). The average Pb-isotopic signature
764 of the local ore (Argentière-mine; purple cross) and the regional ore (Aulus-les-Bains-mining
765 district; light-pink cross) are marked to aid comparison. ARG for Argentière (grey), PDF for Pla
766 de la Fond (blue), EM for Etang Mort (red), LEG for Legunabens (dark green) and BER for
767 Bernadouze (yellow). Note that $^{206}\text{Pb}/^{207}\text{Pb}$ in biofilm samples from Bernadouze is presented on its
768 own (extended) axis.

1°25'E

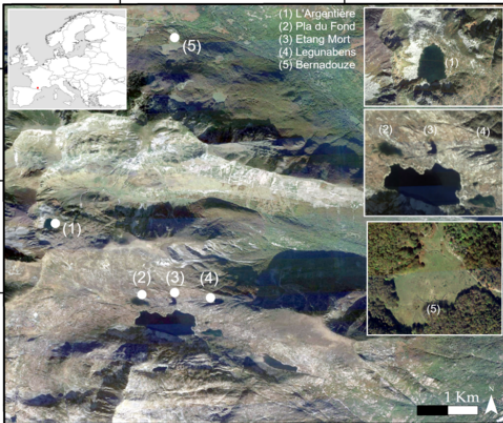
1°27'E

source: image USGS earth explorer

42°48'N

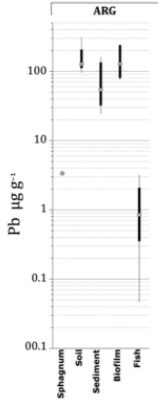
42°47'N

42°46'N



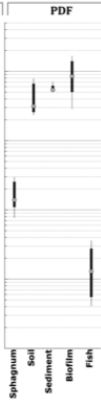
Argentière

ARG



Bassières

PDF



ETM



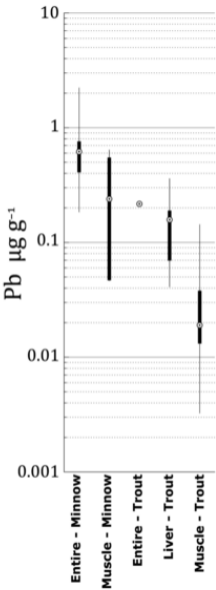
LEG



Bernadouze

BER

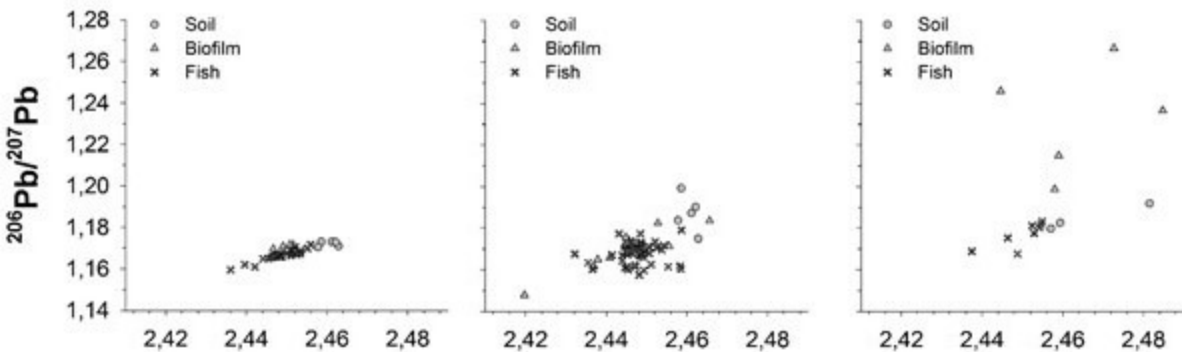




Argentière

Bassiès

Bernadouze



All sites combined

