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

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Abstract

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

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

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

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1. Introduction

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

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

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

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

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

2. Material & Methods

2.1 Site description

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

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

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 therein). Onsite mining activities were mainly focused on retrieving Pb and Ag, but later on the ores were also mined for Zn (Dubois, 1999). More detailed description of local and regional mining activities can be found in e.g. Verna (1996), Dubois (1999), Munoz et al. (2016), Py-Saragaglia et al. (2017) and references therein. The adjacent valley, Bassiès, is located south-east of l'Argentière following the prevailing wind-direction (Hansson et al. 2017), and have previously been studied in an aspect of long-term metal contamination reconstructions (Claustres, 2016; Hansson et al., 2017). In Bassiès, three separate water bodies were included; Pla de la Font (PDF; $42^\circ 45' 52.79''\text{N}$, $001^\circ 25' 09.77''\text{E}$, 1653 m a.s.l.), Etang Mort (ETM; $42^\circ 45' 52.77''\text{N}$, $001^\circ 25' 28.86''\text{E}$, 1676 m a.s.l.), and Legunabens (LEG; $42^\circ 45' 52.85''\text{N}$, $001^\circ 25' 52.25''\text{E}$, 1675 m a.s.l.). Finally, Bernadouze (BER; $42^\circ 48' 10.59''\text{N}$, $001^\circ 25' 26.09''\text{E}$, 1349 m a.s.l.) consists of a stream that runs through a peatland, and is within the same region as l'Argentière and Bassiès, but upwind the prevailing wind direction (i.e. north-east), and thus believed to be less effected by the mining activities near l'Argentière-mine, and therefore included as a reference site.

2.2 Sampling procedures

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

2.2.1 Sphagnum

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

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

2.2.2 Soil, Sediment and Biofilm

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

2.2.3 Biota

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

2.3 Sample preparation and digestion

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

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

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

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

2.4. Chemical analysis

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

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

Pb isotopic signatures (^{206}Pb , ^{207}Pb and ^{208}Pb) in soils, biofilm and trout muscle tissues were analyzed using a High Resolution ICP-MS (Thermo Element II XR; OMP service ICP-MS, Toulouse, France). Individual sample bracketing with certified and adequately diluted (125 ppb to 250 ppb) NIST NBS-981 was performed to correct for mass shift according to Krachler et al. (2004). To assure quality standards, certified reference material (NJV 952) was included in the 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 aligned with previous NJV-942 measurements reported in Hansson et al. (2017)).

2.5 Data analysis

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

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

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

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

3. Results and Discussion

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

3.1 Distribution of Pb between sites and environmental compartments

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

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

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

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

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

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

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 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), of which only LEG exceeds the 100 mg kg^{-1} Pb threshold level for sediment quality in freshwater ecosystems (JORF, 2014). Compared to data reported in similar studies elsewhere (Monna et al., 2011; Camizuli et al., 2014), the sediment Pb-levels seen in the three valleys here are generally

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

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

This is also the likely reason why the average Pb concentration in the fish compartment, represented here by liver tissues from brown trout (*Salmo trutta*), were generally low, i.e. significantly lower than Pb concentrations in soil and biofilm (all three valleys; Table S6). That is, average Pb 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

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

3.2 Distribution of Pb within fish

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

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

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

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

3.3 Origin of Pb and potential pollution sources

The detected Pb measured in environmental samples (sphagnum, soil, sediment and biofilm) as well as fish, only states a presence of contamination in this area, but provides no information of potential sources. In an attempt to decipher potential sources of Pb to the three studied valleys, soil, biofilm and fish (entire organism and/or muscle tissues) were therefore analyzed for Pb-isotopic 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 ore from which the Pb originates (Patterson et al., 1955; Chow and Patterson, 1962). All results of Pb-isotopic analysis are presented in Figure 4, Figure S1 and Table S8. It is generally accepted that

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

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

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

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

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

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

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

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

4. Conclusion

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

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

5. End Notes

Additional information

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

Associated Content

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

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

Author Contribution

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

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Figures and Tables

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

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

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

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

1°25'E

1°27'E

source: image USGS earth explorer

42°48'N

42°47'N

42°46'N

- (1) L'Arganbière
- (2) Pla du Fond
- (3) Etang Mort
- (4) Legunabens
- (5) Bernadouze

1 Km

Argentière

ARG

Bassières

ETM

LEG

Bernadouze

BER

Argentière

Bassiès

Bernadouze

All sites combined