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Comparison of the Isotopic Composition of Hg and Pb in Two Atmospheric Bioaccumulators in a Pyrenean Beech Forest (Iraty Forest, Western Pyrenees, France/Spain)

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Mercury (Hg) and lead (Pb) isotopic compositions were investigated in mosses and lichens collected in a large mountainous beech forest (Iraty Forest) located on the French-Spanish Pyrenean border. Hg isotopic signature in topsoil samples were also analyzed in selected sampling sites. This is the first work that uses the complementary information of both isotopic systems in two distinct atmospheric bioaccumulators. Mosses and lichens present characteristic accumulation due to their integration times, displaying different information on metal pollution over the area. Hg and Pb concentrations in annual moss shoots represent recent atmospheric accumulation, while whole lichen thalli integrates a process of accumulation over a longer period. Lead isotope ratios in mosses are consistent with reported data corresponding to the actual European atmospheric background ($^{206}Pb/^{207}Pb$ ~ 1.158), while Hg isotopic composition reflects potential uptake of both dry and wet Hg depositions. For lichens, Pb isotopic composition exhibits the contribution of a longer integration period of both industrial Pb emissions and legacy of leaded gasoline pollution. Hg isotopes in lichens discriminate two main groups: a larger one representing the background atmospheric contribution and a second one corresponding to unexpected higher Hg content. The similarities in odd and even Mass-independent fractionation of Hg isotopes between topsoils and lichens from the larger group, support the idea that foliage uptake is the main input of Hg in soils. The second group of lichens exhibits more negative $\delta^{202}Hg$ (down to –4.69‰) suggesting a new source of fractionation in this area, probably related to lichens aging and/or stubble and grass fires due to pastoral activities. This study demonstrates that using both Hg and Pb isotopic signature in lichens and mosses allows to trace atmospheric sources and environmental pathways of these metals in forested ecosystems. This original data set in a remote environment provides also new information on the fate of atmospheric Pb and Hg depositions.

Keywords: bioaccumulator, lichens, mosses, soils, mercury, lead, isotopes, forest ecosystem

1 INTRODUCTION

Lichens and mosses present excellent characteristics when monitoring air pollution by metals (Bargagli, 2016). The two biomonitors have neither cuticles nor roots, which means that the influence of substratum is negligible, and that the metal content of their tissues is supposed to originate exclusively from atmospheric deposition. Several recent studies have evaluated mosses and lichens as biomonitors of metal pollution in forest ecosystems and remote areas (Barre et al., 2013; Barre et al., 2015; Agnan et al., 2017; Fabri et al., 2018; Kłos et al., 2018; Ratier et al., 2018; Panichev et al., 2019; Shotyk and Cuss, 2019; Klapstein et al., 2020). These studies suggest that both bioaccumulators provide complementary information depending on their differences in trace elements uptake and integration times (Bargagli et al., 2002; Szczepaniak, 2003; Giordano et al., 2013). Since the development of analytical tools giving precise and accurate isotopic measurements, the analysis of mercury and lead stable isotopes provide information about pollution sources and environmental pathways of the elements (Lauretta et al., 2001; Hintelmann and Lu, 2003; Gratz et al., 2010; Perrot et al., 2010; Sherman et al., 2012; Barre et al., 2018; Wang et al., 2019).

Mercury (Hg) has seven stable isotopes (196, 198, 199, 200, 201, 202 and 204), which undergo mass dependent and mass independent fractionation (MDF and MIF) processes during their transformations or transfer between different reservoirs. Hg isotopic signature is commonly modified in the atmosphere and by the uptake by vegetation and deposition in soil. Several studies have employed Hg isotopes in lichens and mosses to discriminate between atmospheric pollution sources and environmental pathways (Carignan et al., 2009; Estrade et al., 2010; Estrade et al., 2011; Blum et al., 2012; Barre et al., 2013; Enrico et al., 2016; Barre et al., 2018). MDF is commonly reported with $\delta^{202/198}$Hg notation and can occur during biogeochemical transformations (Bergquist and Blum, 2009). Hg uptake in remote areas undergoes MDF generating lighter isotopic composition in plant leaves (Demers et al., 2013; Enrico et al., 2016; Yu et al., 2016). Odd MIF, reported as Δ^{199}Hg and Δ^{201}Hg, is a more complex mechanism and it is mainly associated with photochemical reduction of Hg(II) (Bergquist and Blum, 2009; Carignan et al., 2009; Wiederhold et al., 2010). In the atmosphere, both particulate bound mercury (PBM) and gaseous oxidized mercury (GOM) have more positive MIF compared to gaseous elemental mercury (GEM), which usually displays negative Δ^{199}Hg (Gratz et al., 2010; Demers et al., 2013; Rolison et al., 2013; Fu et al., 2016a; Zheng et al., 2016).

Isotopic anomalies corresponding to MIF were also observed in several studies for even isotopes (Δ^{200}Hg and Δ^{204}Hg) in atmospheric samples (Gratz et al., 2010; Rolison et al., 2013; Sherman et al., 2013; Enrico et al., 2016; Sun et al., 2016). Although there is not a definitive explanation, the different studies suggest that processes involved in even MIF take place in the tropopause due to redox gas phase reactions (Gratz et al., 2010; Chen et al., 2012; Demers et al., 2013). These processes are supposed to be exclusive of the atmosphere and not related to transformations in the Earth's surface or carried out by biota (Blum et al., 2014). Thus, even-MIF signature can trace the pathways of Hg atmospheric deposition to terrestrial ecosystems (Demers et al., 2013; Enrico et al., 2016; Wang et al., 2019; Fu et al., 2016b). In forested ecosystems, differences in MIF of Hg isotopes depend on the nature and pathway of Hg deposition, and are clear between atmospheric oxidized mercury forms (PBM/GOM) and GEM that may accumulate mainly in vegetation (plants and epiphytes) (Biswas et al., 2008; Demers et al., 2013; Enrico et al., 2016). Deposited oxidized Hg(II) shows positive values of even and odd isotopes, while Hg^0 from dry deposition presents negligible and negative even and odd MIF, respectively. The common conclusion between several studies is that forest ecosystems are the largest sink of atmospheric Hg^0 due to its accumulation in foliage and subsequent deposition in soils as litter (Demers et al., 2013; Jiskra et al., 2015; Enrico et al., 2016; Zheng et al., 2016; Obrist et al., 2017; Wang et al., 2017; Olson et al., 2019). On the other hand, mountainous ecosystems in the Pyrenees could be representative of the free troposphere, so atmospheric samples collected at high altitudes can be good indicators of Hg deposition pathways through even MIF evaluation (Fu et al., 2016a).

Lead is present in nature in four main isotopes: three radiogenic isotopes ^{208}Pb (52%), ^{206}Pb (24%), ^{207}Pb (23%) (which are products of radioactive decay of 232Th, 235U and 238U, respectively), and ^{204}Pb which is the only non-radiogenic isotope. Lead (Pb) isotopes are widely used to trace atmospheric pollution in the environment (Monna et al., 1997; Monna et al., 2011; Carignan et al., 2002; Carignan et al., 2005; Cloquet et al., 2006; Veschambre et al., 2008). The differences in the isotopic abundances allow to discriminate Pb between different sources (Stacey and Kramers, 1975; Spiro et al., 2004). Pb can be emitted into the atmosphere by high temperature anthropogenic processes and transported to remote areas (Gross et al., 2012). Lead isotopes are not significantly fractionated by physicochemical processes, and they reflect the composition of the ore of lead, however different reservoirs on Earth represent a mixture of lead from different sources. Thus, Pb isotopic composition is quite well known and allows to trace the source of Pb. In forest ecosystems, Pb results from the contribution of natural and anthropogenic sources, although atmospheric deposition is recognized as the main input of forest Pb (Zhou et al., 2019). Since the ban of leaded gasoline (major source of Pb in the 60–70 s), European atmospheric signature of Pb is representative of the metallurgical industrial emissions (Cloquet et al., 2006). In the particular case of using lichens as biomonitors, Pb isotopic analysis allows to identify several anthropogenic sources (Carignan and Gariépy, 1995; Cloquet et al., 2006; Dolgopolova et al., 2006; Monna et al., 2011; Barre et al., 2018).

This study reports for the first time the combination of Hg and Pb isotopic systems in mosses, lichens and soils collected at different altitudes in the French (North) and Spanish (South) slopes of the Iraty Forest (western Pyrenees), one of the largest beech forests in the south of Europe. This mountainous ecosystem is impacted by local anthropogenic sources (road traffic, local industrial emissions, agricultural and livestock

activities), but also by the long-range atmospheric transport (Moldovan et al., 2007; Veschambre et al., 2008; Ezcurra et al., 2013; Agnan et al., 2015; Corella et al., 2017). The objective of the study is to compare the isotopic signature of Hg and Pb measured in both biomonitors and to differentiate the spatial information provided at different time scale. Hg and Pb isotopic signatures are complementary since Hg is able to trace the atmospheric processes, while Pb isotopic information is related to the elemental source. For the two atmospheric biomonitors, isotopic signatures demonstrate that the integration time is critical to evaluate main sources of Pb and deposition pathways of Hg. As it was previously demonstrated (Barre et al., 2018), the employment of two isotopic systems (stable and radiogenic isotopes) enhance the understanding of atmospheric and deposition pathways of the pollutants. Therefore, this work has an important implication for future research conducted on the use of such biomonitors to set up atmospheric monitoring programs at large scale.

2 MATERIALS AND METHODS

Sampling Site

The study was performed for biomonitors and topsoils collected in one of the largest beech forests in the south of Europe: the Iraty forest. This forest is located on the border between France and Spain, one part belonging to the Spanish province of Navarra and the other to the French Basque Country, with a total surface area of 17,300 ha. The Iraty forest is located in a mountainous area far from urban and industrial regions, whose main activity is forestry and animal breeding in pastures (e.g., cows, sheep and horses). The Iraty forest receives major inputs from free tropospheric air mass circulation (Ezcurra et al., 2013), being considered a suitable monitoring site for long-range transport of air pollutants (i.e., monitoring of European background pollution). In the study, 51 sampling sites were selected in an area of 30×30 km^2 in the center of the Iraty forest (**Supplementary Figure S1**). The sampling region was defined according to the extension of the Iraty forest and considering that each sampling site should have the following requirements: i) located within a beech wood, ii) at least 100 m away from any kind of road or building to avoid direct contamination effects, and iii) including a significant population of moss or lichen species. More details about the procedure of selection and design of the sampling area and sampling sites are described in Bustamante Alonso (2014). From these sampling sites, 30 were selected on the Spanish side of the border and 21 on the French side. On the Spanish side, the sampling area covers all the forest and altitudes range from 800 to more than 1,400 m. The difference in altitude in the areas of the French side is larger, ranging from 400 up to 1,400 m. In order to obtain the best representation of the study area, the location of the sampling points was determined according to the elevation and sorted in different ranges: below 400, 400–800, 800–1,000, 1,000–1,200 and above 1,200 m. In the Iraty area, highest sampling points (>1,200 m a.s.l.) were characterized as sites where free tropospheric air masses circulate, while downhill sampling sites were connected to local air masses circulation areas (Ezcurra et al., 2013).

Sample Collection and Preparation

The sampling campaign was performed during fall 2011 (three consecutive days in November). Seasonal effect on sampling for Pb and Hg was also tested in a previous study (Barre et al., 2018), and the conclusion was that it is negligible for remote locations and especially for Hg. The sampling strategy for lichens and mosses was similar to the one performed in other study for geographically close areas (Barre et al., 2013). Therefore, the collection consisted in samples of lichens (n = 30), mosses (n = 21), and topsoils only for several selected sites (n = 23). Samples were collected with gloves and stainless-steel tools and immediately packed in zip-lock PE bags and placed into a freezer (–20°C) to transport them to the laboratory.

The epiphytic lichens were collected at a height of 1–2 m on the tree bark, according to the French norm NF X903–43 (AFNOR, 2008). Three ubiquitous and very similar foliose lichens were sampled: *Parmelia caperata* (L.) Ach., *Parmelia sulcata* Taylor and *Hypogymnia physodes* (L.) Nyl. These lichens taken on the tree barks represent an integration period of up to a few years (Walther et al., 1990; Szczepaniak and Biziuk, 2003; Bačkor and Loppi, 2009). Lichens were separated by hand from their substratum and ground in a mixer mill (MM200, Retsch) in Teflon bowls, using to that end balls of the same material. The homogenized powder was transferred into glass vials and stored at −18°C before mineralization. About 0.5–1 g of sample was digested in a High Pressure Asher (HPA-S, Anton Paar) with 5 ml of sub-boiled nitric acid for 3 h at 300°C and 130 bar, according to the protocol published in Barre et al., 2018 and Estrade et al., 2009. For mosses, the pleurocarpous species *Hypnum cupressiforme* Hedw. was collected in soils and tree stumps. This species is fairly tolerant to air pollution and has been widely used in biomonitoring surveys (González-Miqueo et al., 2009; Harmens et al., 2010; Harmens et al., 2013; Schröder et al., 2013). Samples were taken following the guidelines of the UNECE ICP Vegetation Moss Manual (Vegetation Coordination Center, 2010) avoiding places close to roads, populated areas or sites under the influence of tree canopies when possible. Due to its structure forming dense mats of interwoven filaments, *Hypnum cupressiforme* can be easily confused with other moss species. For this reason all the samples collected were identified and separated at the laboratory of the University of Navarra (Spain) with a long experience in the use of this species for similar surveys (González-Miqueo et al., 2009; González-Miqueo et al., 2010; Izquieta-Rojano et al., 2018). Moss samples were oven-dried at 40°C for 72 h and the extreme apices (3 cm) of mosses were cut and ground. Sample digestion applied on mosses is the same as previously detailed for lichens. Surface organic forest soils samples (organo-mineral horizon) were collected at the sampling site after removing the top litter layer (sampling depth 1–5 cm) (Rodriguez Iruretagoiena, 2015). Surface soils (10 cm) were collected in the same area than mosses and lichens. Once in the laboratory, soils were lyophilized (150 mTorr, 52°C, 48 h) in a Cryodos 50 (Telstar, Spain)

freeze-dryer. Then soils were sieved to <2 mm and different objects such as bones or stones were removed. After air dried in a fume hood during 24 h soils were ground in a planetary ball mill, and digested in a 4 ml of reverse aqua regia (HNO3:HCl, 3:1) in 50 ml polyethylene tubes at 85°C. To assess the quantitative recovery of the trace elements under this procedure, reference material IAEA 405 (sediments) were digested in the same way.

Hg and Pb Quantification Analyses

Hg concentrations in lichens, mosses and soils were measured by Atomic Absorption in an AMA-254 (LECO). About 30 mg of sample powder were added to a nickel boat for each analysis. The sample is dried for 60 s at 120°C and pyrolyzed at 750°C for 120 s under an oxygen flow. The resulting mercury in the gaseous form is amalgamated on a gold trap and subsequently released by thermal desorption toward the detector, where quantification takes place. The concentration of Pb in lichens was determined by Neb-ICP-MS (Thermo X2, ThermoFisher). Daily optimization of the ICP-MS parameters was performed by using tune solution of 1 ng g^{-1} of Li, Y, Tl and Ce standard. The quantification was performed by direct calibration, with internal standard (^{115}In and ^{191}Ir). The control of the methodological blanks allows to determine the detection limits that range from 1 to 10 ng L^{-1}. More details on the analytical performance are presented in previous works (Barre et al., 2013; Barre et al., 2018). The QA/QC of the analytical procedures were evaluated by the analysis of two reference materials using the same procedures applied to lichens samples, BCR CRM 482 (Pseudevernia furfuracea) and IAEA 336 (E. prunastri).

Hg and Pb Isotopic Analyses

Mercury isotopes analyses were performed according to previous works (Lauretta et al., 2001; Bergquist and Blum, 2007; Gehrke et al., 2009), by using a cold vapor generation (CVG) with $SnCl_2$ reduction, coupled to a Multicollector (MC) - ICP-MS (Nu Plasma, Nu Instrument). For samples with low concentrations of Hg, isotopic composition was determined using dual gold amalgamation technique (DGA) coupled to CVG by MC-ICP-MS (Bérail et al., 2017). This system works with two gold traps to pre-concentrate Hg and decrease the detection limits. In order to correct the instrumental mass-bias, internal standard of Tl (NIST 997, ^{205}TL/^{203}TL = 2.38714) and sample standard bracketing with NIST 3133 standard solution were used. All the samples and standards were measured at a concentration of 1 ng g^{-1} of Hg, to avoid any bias caused by differences in concentration. Mass-dependent fractionation (MDF) is reported as recommended by Bergquist and Blum (2007) relative to the NIST 3133 Hg solution as follows in **Eq. 1**:

$$\delta^{xxx}Hg = \left(\frac{^{xxx/198}Hg_{sample}}{^{xxx/198}Hg_{NIST\ 3133}} - 1 \right) * 1000 \ \text{‰} \quad (1)$$

where xxx is the studied isotope. Mass-independent fractionation (MIF) of Hg is reported as the difference between the theoretical value predicted by MDF of δ^{199}Hg and δ^{201}Hg and the measured values as Δ^{199}Hg and Δ^{201}Hg in ‰, according to the protocol suggested by Bergquist and Blum (2007):

$$\delta^{xxx}Hg = \delta^{xxx}Hg - \left(\delta^{202}Hg \times \beta_{xxx}\right) \quad (2)$$

where β_{xxx} is the kinetic mass-dependence scale factor, characteristic of the isotope which values are 0.2520 for ^{199}Hg, 0.5024 for ^{200}Hg, 0.7520 for ^{201}Hg and 1.493 for ^{204}Hg (Blum and Bergquist, 2007).

For CVG-DGA/MC-ICP-MS, samples were analyzed at 50 pg ml^{-1} and NIST RM 8610 (n = 43) and BCR-482 (n = 45) were used as secondary standards with values of δ^{202}Hg −0.57 ± 0.27‰ and −1.70 ± 0.33‰ and for Δ^{199}Hg 0.00 ± 0.21‰ and −0.64 ± 0.15‰ and for Δ^{200}Hg 0.02 ± 0.14‰ and 0.06 ± 0.14‰ respectively (see **Supplementary Table S1**). Analytical uncertainty for CVG/MC-ICP-MS was evaluated by multiple measurements of the secondary standard NIST RM 8610 (former UM-Almadén), and the certified reference material BCR-482 (*lichen*), with certified and consensus values for δ^{202}Hg of −0.61 ± 0.26‰ and −1.69 ± 0.28‰, for Δ^{199}Hg 0.00 ± 0.13‰ and −0.65 ± 0.1‰ and for Δ^{200}Hg 0.01 ± 0.10‰ and 0.05 ± 0.09‰, respectively (uncertainties expressed as 2 SD). The comparison of long-term reproducibility for the measurement of NIST RM 8610 (former UM-Almadén) and BCR 482 (*Lichen*) for CVG-MC-ICP-MS and DGA/CVG-MC-ICP-MS, did not show any deviation (**Table S1**).

For Pb isotopic analysis, Pb was firstly pre-concentrated on ion exchange resin (Dowex 1 × 8, Acros organics 100–200 mesh) according to a previously published procedure (Manhes et al., 1980; Cloquet et al., 2006). Recoveries obtained after Pb isolation were comprised between 85 and 110% and fractionation of Pb isotopes during pre-concentration step was also controlled with NIST-981 and BCR-482 (*Lichen*). Pb isotopes were measured using MC-ICP-MS (Nu Instrument) coupled with a desolvator nebulizer unit (DSN-100, Nu Instrument). Analytical precision obtained for reference materials after column separation were as follows: NIST-981 (and BCR-482), 687 ppm (633 ppm) for ^{208}Pb/^{204}Pb, 498 ppm (307 ppm) for ^{207}Pb/^{204}Pb, and 380 ppm (865 ppm) for ^{206}Pb/^{204}Pb. In the same way as Hg isotopes, instrumental mass-bias was corrected with Tl internal standard added directly in the Pb solution (Pb/Tl = 10) and NIST-981 was analyzed every two samples for sample-standard bracketing correction.

Statistical Analysis

All the statistical calculations were performed using the software Origin (OriginLab corporation, Northampton, Massachusetts, USA). Since data set is not normally distributed for all the samples according to the results of Shapiro-Wilk test, non-parametric Kruskal-Wallis test was used at the $p < 0.05$ level of significance for all tests. Pearson's correlation test was also performed to decide if the correlation between two variables was significantly different from zero or not.

3 RESULTS

Pb Concentration in Bioaccumulators

The average concentrations of Pb in mosses and lichens are presented in **Table 1** (the complementary raw data are shown

TABLE 1 | Average values for concentrations, isotopic ratios and delta values of Hg and Pb for the samples of lichens, mosses and soils collected in the Iraty forest (Uncertainties are expressed as 1 SD). Results are compared with data from previous publications of samples collected in areas close to Iraty.

			N	Hg (mg kg^{-1})	Pb (mg kg^{-1})	δ^{202}Hg (‰)	Δ^{199}Hg (‰)	Δ^{200}Hg (‰)	Δ^{201}Hg (‰)	Δ^{204}Hg (‰)	$^{206}Pb/^{207}Pb$
Lichens	Spanish sites	This study	30	0.24 ± 0.11	15.4 ± 15.5	−2.39 ± 0.60	−0.17 ± 0.14	0.01 ± 0.04	−0.21 ± 0.12	−0.02 ± 0.09	1.1519 ± 0.0041
	French sites		21	0.46 ± 0.30	11.1 ± 5.53	−2.98 ± 0.83	−0.12 ± 0.15	0.01 ± 0.03	−0.17 ± 0.13	−0.02 ± 0.08	1.1524 ± 0.0032
	All		51	0.33 ± 0.23	13.6 ± 12.5	−2.68 ± 0.78	−0.15 ± 0.14	0.01 ± 0.04	−0.19 ± 0.13	−0.02 ± 0.09	1.1521 ± 0.0037
	Urban area	Barre et al., 2018	20	0.12 ± 0.04	11.1 ± 8.4	−1.72 ± 0.11	−0.31 ± 0.02	—	—	—	1.1460 ± 0.0057
	Industrial area		22	0.15 ± 0.06	12.7 ± 11.2	−1.55 ± 0.64	−0.31 ± 0.04	—	—	—	1.1475 ± 0.0021
	Agricultural area		24	0.14 ± 0.04	5.9 ± 5.1	−2.11 ± 0.23	−0.33 ± 0.05	—	—	—	1.1505 ± 0.0035
	Forested area		24	0.14 ± 0.03	6.1 ± 4.8	−2.06 ± 0.19	−0.32 ± 0.04	—	—	—	1.1525 ± 0.0021
	Contaminated area		4	0.43 ± 0.34	124 ± 116	−0.68 ± 0.32	0.10 ± 0.18	—	—	—	1.1493 ± 0.0015
	Lichens at Pinet	Enrico et al., 2016	1	—	—	−1.62	−0.44	−0.06	−0.47	−0.01	—
Mosses	Spanish sites	This study	22	0.04 ± 0.13	3.47 ± 2.13	−1.61 ± 0.22	0.00 ± 0.07	0.04 ± 0.06	0.06 ± 0.15	−0.14 ± 0.21	1.1627 ± 0.0070
	French sites		21	0.04 ± 0.20	4.45 ± 2.70	−1.43 ± 0.36	−0.02 ± 0.13	0.03 ± 0.06	0.05 ± 0.13	−0.17 ± 0.15	1.1583 ± 0.0070
	All		43	0.04 ± 0.15	3.90 ± 2.42	−1.53 ± 0.30	−0.01 ± 0.10	0.04 ± 0.06	0.06 ± 0.14	−0.15 ± 0.18	1.1611 ± 0.0068
	Sphagnum mosses	Enrico et al., 2016	41	—	—	−1.53 ± 0.49	−0.17 ± 0.11	0.01 ± 0.04	−0.22 ± 0.09	−0.02 ± 0.05	—
Soils	Spanish sites	This study	7	0.10 ± 0.04	—	−1.62 ± 0.25	−0.27 ± 0.04	0.00 ± 0.03	−0.28 ± 0.06	0.00 ± 0.05	—
	French sites		16	0.13 ± 0.05	—	−1.60 ± 0.38	−0.25 ± 0.09	0.01 ± 0.03	−0.21 ± 0.08	−0.04 ± 0.05	—
	All		23	0.12 ± 0.05	—	−1.61 ± 0.34	−0.26 ± 0.07	0.01 ± 0.03	−0.23 ± 0.08	−0.03 ± 0.05	—

in **Supplementary Table S2**). The concentrations range from 1.64 to 87.5 mg kg^{-1} in lichens and from 0.98 to 12.3 mg kg^{-1} in mosses. Generally, lichens have four times more Pb than mosses. The average value of Pb concentration for lichens in the Iraty forest (13.6 ± 12.5 mg kg^{-1}) is consistent with the data previously published for samples of the Atlantic Pyrenees (Barre et al., 2018). Background Pb concentrations in lichens usually range from 4 to 6.4 mg kg^{-1} (Loppi and Pirintsos, 2003; Basile et al., 2008; Veschambre et al., 2008; Agnan et al., 2013; Barre et al., 2013). The comparison with these values shows that the concentration of Pb in lichens from the Iraty forest is two-fold higher than the previously reported background concentrations. The average Pb concentration in mosses is 3.9 ± 2.4 mg kg^{-1}. Five of the mosses (4F, 5F, 19F, 13E and 17E), however, exhibit higher concentrations of Pb up to 12.31 mg kg^{-1}. The average concentrations of Pb in lichens and mosses between north and south sides of the border are not statistically different, suggesting that the concentrations of Pb are representative of the whole sampling area. The Pb concentrations found in our study fall in the range of unpolluted sites reported in previous biomonitoring studies in France or Spain (Galsomiès et al., 1999; Fernández et al., 2002; Carballeira et al., 2008; González-Miqueo et al., 2010). Pb concentrations are represented in **Supplementary Figure S2** (S.2.c and d) classified according to the altitudes of the sampling sites for mosses and lichens.

Hg Concentration in Bioaccumulators and Soils

The average concentrations of Hg obtained for lichens, mosses and soils are presented in **Table 1** (complete raw data is shown in **Supplementary Table S2**). Hg concentrations in epiphytic lichens of the Iraty forest range from 0.12–1.4 mg kg^{-1}. These values are in agreement with the average concentrations of Hg found in other French forest areas located close to the Iraty forest (0.14 ± 0.03 mg kg^{-1}) (Barre et al., 2018). In the same region Barre and coworkers, determined Hg concentrations up to 0.85 mg kg^{-1} in lichens contaminated by industrial metallurgical inputs (Barre et al., 2018). According to Garty et al. (2001), areas free of pollution can reveal concentrations ranging from 0.02 to 3 mg kg^{-1}. Some lichens sampled in Patagonia showed Hg concentrations ranging from 0.07 to 0.57 mg kg^{1} (Ribeiro Guevara et al., 2004). In Europe, lichens sampled in a forested ecosystem from Italy showed concentrations ranging from 0.10 to 0.22 mg kg^{-1} (Loppi and Pirintsos, 2003). In our work, samples from the northern slope (French sites, 0.46 ± 0.30 mg kg^{-1}) presented Hg concentrations significantly higher than samples from the southern slope

(Spanish sites, 0.24 ± 0.11 mg kg^{-1}). The average values of both groups are statistically different (Kruskal-Wallis, H = 10.3, p = 0.013)), suggesting differences in the inputs or uptake of atmospheric Hg between lichens in both slopes of the forest. Hg concentrations in mosses are significantly lower compared to lichens ranging from 0.02 to 0.07 mg kg^{-1}. The concentrations in mosses are not statistically different between north and south sides of the border (Kruskal-Wallis, H = 0.0997, p = 0.752 thus they are representative of the whole sampling area. For forested areas in France, Hg concentrations in mosses are between 0.03 and 0.1 mg kg^{-1} (Galsomiès et al., 1999). The Hg concentrations found in mosses from the Iraty forest are the characteristic ones in rural to remote areas (Barre et al., 2018). Similar concentrations were also measured in mosses from Navarra, with mean concentrations of 0.12 ± 0.12 mg kg^{-1} (González-Miqueo et al., 2009).

Forest soil samples were analyzed in order to compare Hg concentrations and evaluate sources in lichens and mosses. The concentrations of Hg found in the 23 soil samples (see **Supplementary Table S2**) are quite homogeneous within the studied area (0.052–0.244 mg kg^{-1}), with average values of 0.12 ± 0.05 mg kg^{-1} (**Table 1**). A negative correlation (Pearson, r = –0.31 at 0.05 significance level) was found between Hg concentrations in soil and mosses collected at the same sites. The correlation can be consistent with the fact that Hg in soils mainly originate from its atmospheric uptake by vegetation and the long-term decomposition of the litter (Zheng et al., 2016; Obrist et al., 2017; Jiskra et al., 2018; Huang et al., 2020). Hg concentrations as a function of altitude are presented in **Supplementary Figure S3A,B**, both for mosses and lichens. Hg concentrations are statistically correlated with altitude above sea level (Pearson, r = -0.47, at 0.05 level of significance), which is in agreement with the observation that the highest Hg concentrations measured in this study were found in some lichens collected at the lowest elevation (<800 m a.s.l.).

Pb Isotopes in Lichens and Mosses

The average isotope ratios of $^{206}Pb/^{207}Pb$ for mosses and lichens are summarized in **Table 1**. As previously shown, isotopic ratios using the non-radiogenic ^{204}Pb did not show better discrimination of lichen samples collected in the same region (Barre et al., 2018). Moreover, $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{206}Pb$ ratios were also preferred in this report in order to be compared with previously published studies that did not measure ^{204}Pb. The complete raw data and $^{208}Pb/^{206}Pb$ and $^{206}Pb/^{207}Pb$ ratios for lichens and mosses are presented in **Supplementary Table S3**. $^{206}Pb/^{207}Pb$ isotopic signature range from 1.1465 to 1.1640 in lichens and from 1.1420 to 1.1801 in mosses. Box plots diagrams for Pb isotope ratios are presented in **Supplementary Figure S4** in function of the altitude. In similar way as for Pb concentrations, Pb isotopic fingerprint seems to be rather homogeneous along the whole area (see **Supplementary Figure S3**). The results from mosses are consistent with values reported for Pb in the mountainous ecosystem of the Alps (1.150–1.170 for $^{206}Pb/^{207}Pb$ and 2.090–2.115 $^{208}Pb/^{206}Pb$) (Schnyder et al., 2018). Mosses on sites 19E, 22E and 25E (i.e., Spanish slope) present $^{206}Pb/^{207}Pb$ values of about 1.18, suggesting a more crustal signature. These samples, located in Spanish sites for altitudes higher than 1000 m, strongly suggest crustal absorbed inputs from soil particles on their surface. Many studies have reported increased values of $^{206}Pb/^{207}Pb$ since the leaded petrol ban, because atmospheric samples have decreased their contribution and therefore their origin is closer to that of lead in rock ores (Kunert et al., 1999; Komárek et al., 2008). The isotopic signature of Pb in lichens found here is similar to that reported in previous studies conducted in 2004 in the Aspe Valley (Western Pyrenees) for atmospheric particles ($^{206}Pb/^{207}Pb$ 1,152 ± 0.009) (Veschambre et al., 2008), and in peat bogs from the Basque Country (Monna et al., 2004).

Hg Isotopic Composition in Biomonitors and Soils

Lichens and Mosses

The average values for the isotopic composition of Hg in lichens and mosses are presented in **Table 1**. Values reported in this study are in good agreement with those found for lichens, mosses or other atmospheric biomonitors in literature (Ghosh et al., 2008; Carignan et al., 2009; Estrade et al., 2010; Blum et al., 2012; Demers et al., 2013; Yin et al., 2013; Barre et al., 2018). The values of $\delta^{202}Hg$ measured in lichens range from –1.71 to –4.69‰. A few sampling sites of lower altitude (under 800 m), mainly located on the French side, are characterized by significant negative $\delta^{202}Hg$ signatures, with average values of -2.98 ± 0.83‰ (as 1 SD). The odd isotopes of Hg exhibit anomalies ranging from -0.43 to 0.08‰ for the $\Delta^{199}Hg$. On the contrary, $\Delta^{199}Hg$ split in two groups, one of them indistinguishable from zero, and the other one with negative MIF values. Site 4F displayed the most negative values of $\delta^{202}Hg$ (–4.69‰) with no odd-MIF ($\Delta^{199}Hg$ = –0.03‰). This site is located at the lowest elevation considered in this study. The results for even-MIF ($\Delta^{200}Hg$) suggest that in the case of lichens it is indistinguishable from zero (average 0.01 ± 0.04‰ as 1 SD). $\delta^{202}Hg$ values in mosses are different than those found in lichens, ranging from –1.99 and –0.86‰ (–1.53 ± 0.30‰ as 1 SD), and $\Delta^{199}Hg$ from –0.38 to +0.20‰ (–0.01 ± 0.10‰ as 1 SD). For even-MIF ($\Delta^{200}Hg$) in mosses, we observed larger variability, with slightly positive shift in $\Delta^{200}Hg$ values (average 0.04 ± 0.06‰ as 1 SD). In the case of $\Delta^{204}Hg$ signature, the measurement of isotope ^{204}Hg was not precise enough to establish a clear trend (see complete raw data in **Supplementary Table S3**). According to the box-plot diagrams in **Supplementary Figure S5**, a trend between $\delta^{202}Hg$ and the sampling altitude can be observed (Pearson, r = 0.427, at a 0.05 significance level), while Hg isotopic composition obtained at higher altitudes is consistent with values previously reported in forested areas of the same region (Barre et al., 2018). $\delta^{202}Hg$ between mosses and lichens are statistically different (Kruskal-Wallis, H = 69.4, p < 0.0001). A similar difference can be also observed for odd-MIF ($\Delta^{199}Hg$ H = 20.8, p < 0.0001 and $\Delta^{201}Hg$ H = 53.5, p < 0.0001). Therefore, Hg isotopic composition allows to discriminate the sources and uptake processes of Hg between both biomonitors. In addition, the results exhibit clear differences for $\delta^{202}Hg$ in lichens between the Spanish and French slopes, due to an unknown source of

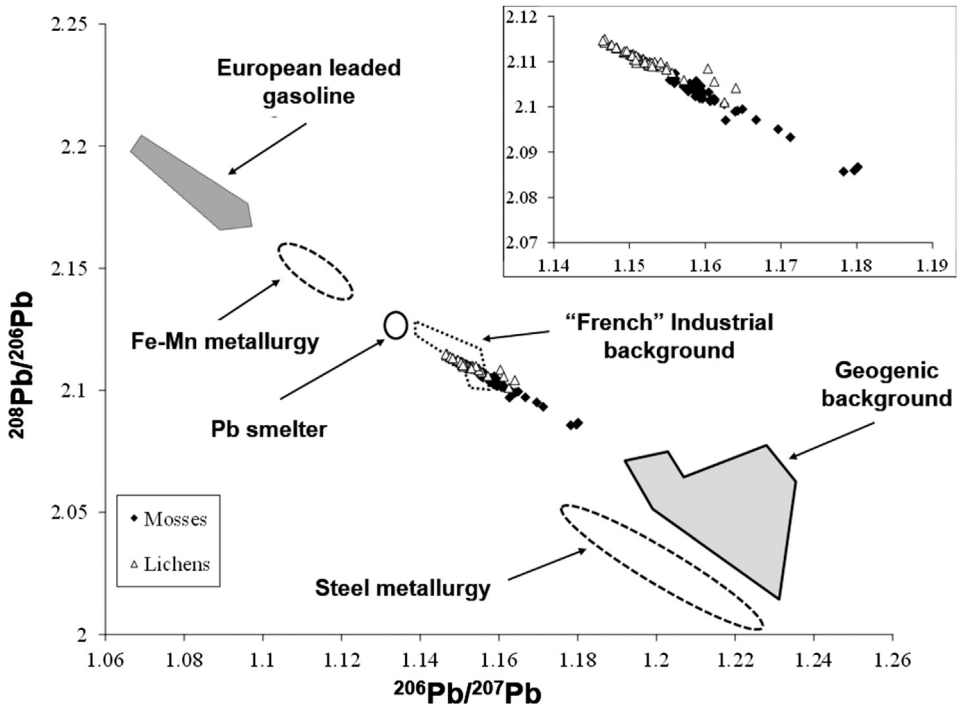

FIGURE 1 | Tri-isotopic diagram ($^{208}Pb/^{206}Pb$ vs. $^{206}Pb/^{207}Pb$) of lichens and mosses compared with different European sources and geogenic background. (Elbaz-Poulichet et al., 1984; Grousset et al., 1994; Monna et al., 1997; Weiss et al., 1999; Millot et al., 2004; Monna et al., 2004; Carignan et al., 2005; Cloquet et al., 2006; Geagea et al., 2008; Elbaz-Poulichet et al., 2011).

fractionation leading to more negative values mainly in French samples (**Supplementary Figure S6**).

Soils

The soils analyzed displayed $\delta^{202}Hg$ ranging from −2.06 to −0.36‰, quite similar to the MDF measured in mosses (**Supplementary Figure S6**). Soils exhibit a significant negative MIF ($\Delta^{199}Hg$ between −0.40 and −0.06‰), close to values measured in lichens. The average values for even-MIF in soils ($\Delta^{200}Hg$ = 0.01 ± 0.03‰ as 1 SD) show that they are consistent with the data obtained for lichens with values close to zero. Besides these general trends, the sampling site exhibiting the most negative MDF in lichens (4F, $\delta^{202}Hg$ = −4.69‰) and in mosses (4F, $\delta^{202}Hg$ = −1.99‰) have a soil with the less negative MDF and MIF (respectively, −0.36 and −0.06‰). The results are in good agreement with the data from vegetal biomass and soils obtained for other ecosystems. (Zheng et al., 2016; Obrist et al., 2017; Jiskra et al., 2018; Huang et al., 2020). Values reported in this study are comparable to those obtained for soils of North American forested ecosystems (Biswas et al., 2008; Demers et al., 2013) but having a slightly more negative shift in odd-MIF. The negative Hg isotopes odd-MIF observed in these soils is consistent with the MIF found in lichens. Unpolluted and less organic topsoils from Metz city display odd-MIF close to zero and MDF, ranging from −0.94 to −0.73‰ (Estrade et al., 2011). Demers et al. (2013), have shown that organic soils have more negative $\delta^{202}Hg$ signatures than mineral soils, but similar MIF values. These results suggest that our topsoil samples are mostly representative of the organo-mineral horizon of temperate forest soils. Also, the soil located on site 4F, which has a $\delta^{202}Hg$ comparable to mineral soils, could be representative of the mineral phase of the soils.

4 DISCUSSION

Recent Versus "Older" Lead Isotopes Signature

Regarding to **Figure 1**, lichens and mosses are distributed along the same single line following a binary mixing model between the contribution of the crustal composition (Elbaz-Poulichet et al., 1984; Elbaz-Poulichet et al., 2011) and the so-called "French" industrial lead (Carignan et al., 2005). In a previous study, lichens from forested areas of the French South Aquitaine region, close to the Iraty Forest (Barre et al., 2018), presented a similar binary mixing line. The plot of $^{206}Pb/^{207}Pb$ vs. 1/[Pb] for lichens and mosses (**Figure 2**) allows to define three possible reservoirs of deposited Pb based on the isotopic signature: a local geogenic source, the "European" atmospheric background and a more regional anthropogenic and industrial source ("French" industrial lead). $^{206}Pb/^{207}Pb$ ratio in lichens for the highest concentrations of Pb, is representative of the "French" industrial Pb defined in previous works (Monna et al., 1997; Carignan et al., 2005; Geagea et al., 2008). Geogenic influence of

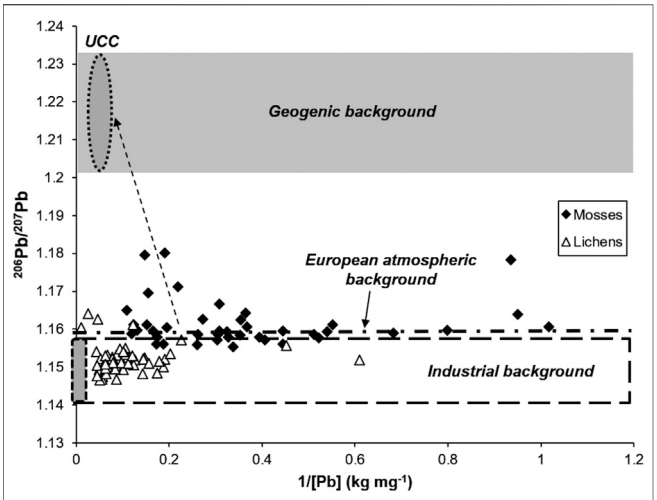

FIGURE 2 | $^{206}Pb/^{207}Pb$ vs. 1/[Pb] in kg/mg diagram for lichens and mosses. Upper continental crust (UCC) data issued from Millot et al. (2004), Wedepohl (1995) et Weiss et al. (1999). Industrial data are from Geagea et al. (2008), Monna et al. (1997) and Cloquet et al. (2006).

soil in mosses is clearly represented with increasing Pb concentration and more radiogenic composition (Wedepohl, 1995; Weiss et al., 1999; Millot et al., 2004). Moreover, Al content in these mosses are particularly high, supporting this crustal origin of Pb (Rodriguez Iruretagoiena, 2015). The decrease in Pb concentration in mosses exhibits an end-member of the isotopic signature with a $^{206}Pb/^{207}Pb$ ratio of about 1.158, which was set as the European atmospheric background (Monna et al., 1997; Geagea et al., 2008). This value is similar to Pb isotopic ratios measured in mosses from the arctic region ($^{206}Pb/^{207}Pb$ = 1.157 ± 0.006) directly influenced by European pollution (Haack et al., 2004). The comparison of the isotopic fingerprint between the two biomonitors (see **Figure 2**) suggests that mosses have a more radiogenic composition than lichens, and closer to the geogenic isotopic signature of Pb. The isotopic measurements support that mosses mostly integrate recent atmospheric Pb during their last year of growth, due to their similarity to the current isotopic signature of the global atmospheric Pb pool in Europe. Lichens exhibit a less radiogenic signature (1.152 ± 0.004 for $^{206}Pb/^{207}Pb$ average ratio) and have an isotopic composition in agreement with atmospheric particles from the Aspe valley collected several years before (Veschambre et al., 2008). These observations can be consistent with data published in a previous work, suggesting that Pb in lichens in nearby territories can be influenced by resuspended particles of dust, still impacted by legacy contamination of leaded gasoline (Barre et al., 2018).

In **Figure 2**, we observed that few lichens reflect more radiogenic composition (sites 5F, 6E, 9E and 23E with $^{206}Pb/^{207}Pb$ close to 1.16) also with increasing concentrations of Pb, which is consistent with a geogenic contribution. According to a previous work made in nearby areas of southern Aquitaine region (Barre et al., 2018), lichens collected in agricultural and forested sites present Pb isotopic compositions closer to pre-industrial values, which could be extended to several sites of the Iraty forest. In order to normalize the data and eliminate most of the variability induced by the different lichens samples, Ti can be used instead of Al, due to its terrigenous origin (Monna et al., 2011). In this way, it is also possible to reduce variability from the use of different lichen species, different ages of growth, different exposure rates or different morphology. **Supplementary Figure S7**, shows that isotopic composition measured in lichens seems to be a mixture between the pool of "French" industrial lead and "old" leaded gasoline, suggesting the reemission of particles containing legacy Pb pollution. Sites 6E and 23E do not follow the same trend than the rest of dataset and seem to be mainly influenced by industrial contamination, presenting lower Ti/(Pb*1,000) ratios.

Lichens collected can have a much longer life span (several years) than the annual shoots mosses collected. The Pb isotopes measured in mosses and lichens suggest that the actual atmospheric Pb (<1 year) accumulated in mosses have a composition close to the European background atmospheric lead. This observation is consistent with one hypothesis of Carignan et al. (2005), suggesting that Pb in mosses is representative of the European atmospheric pool. On the other hand, Pb accumulated in lichens may represent a mixture between actual Pb and "old" Pb due to longer exposure time (several years) within the entire thalli of lichen samples.

Mass Independent Fractionation of Even- and Odd-Hg Isotopes

It has been shown that Hg deposited from precipitation should be affected by even-MIF, which is reflected in the isotopic signature of $\Delta^{200}Hg$ and $\Delta^{204}Hg$ (Demers et al., 2007; Gratz et al., 2010; Chen et al., 2012; Enrico et al., 2016). **Figure 3** shows the plot of $\Delta^{200}Hg$ vs. MDF for mosses, lichens and soils in this study, and the comparison with other data from bibliography with similar values (Fu et al., 2016b; Yuan et al., 2019). Lichens, as well as soils, present a $\Delta^{200}Hg$ close to zero, meaning that the main contribution is the uptake of gaseous elemental Hg (GEM) from dry deposition. The even-MIF signature of Hg in topsoils suggests a dominant influence of Hg inputs from foliage litterfall after leaves uptake of ambient gaseous Hg. Hg inputs to soils in deciduous forests are variable but are generally higher for litterfall than for precipitation (Lindberg, 1996; Grigal et al., 2000; Schwesig and Matzner, 2000; St. Louis et al., 2001; Demers et al., 2007; Demers et al., 2013; Zhou et al., 2013). In some cases they can represent about 93% of total deposition of Hg (Demers et al., 2013). For mosses, the set of samples are divided between $\Delta^{200}Hg$ close to zero, consistent with samples of sphagnum mosses in other Pyrenean areas (Enrico et al., 2016), and some samples with significantly positive values. One hypothesis to explain such difference can be obtained by the respective isotopic composition of Hg in wet deposition and the Total Gaseous Mercury (TGM) in other Pyrenean regions (Fu et al., 2016a; Enrico et al., 2016). The moss samples with slightly positive even-MIF present a stronger contribution of wet deposition, according to the results obtained in previous studies (Gratz et al., 2010; Demers et al., 2013; Enrico et al., 2016).

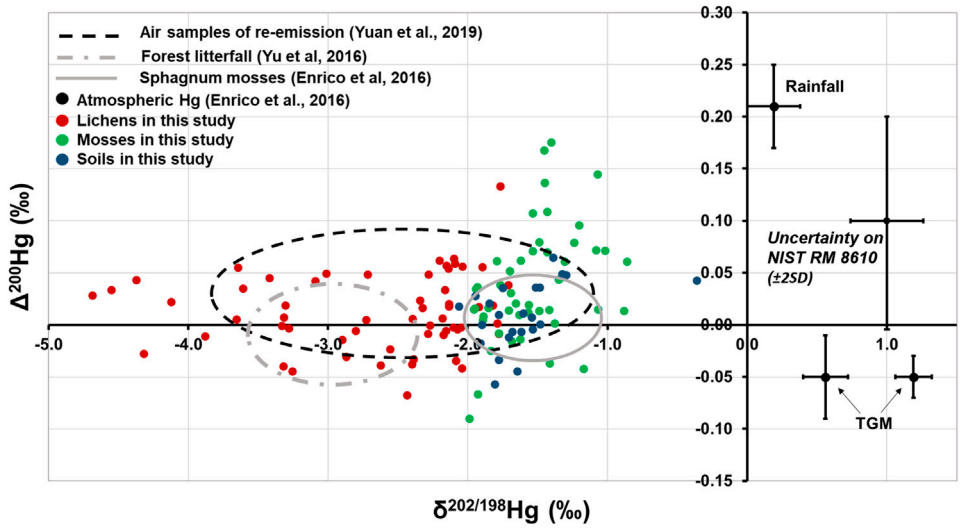

FIGURE 3 | Δ^{200}Hg vs. $\delta^{202/198}$Hg in lichens, mosses and soils. Uncertainty calculated for BCR 482 represent the reproducibility of the NIST RM 8610 (former UM-Almadén) (as 2 SD). The data were compared with other data from bibliography.

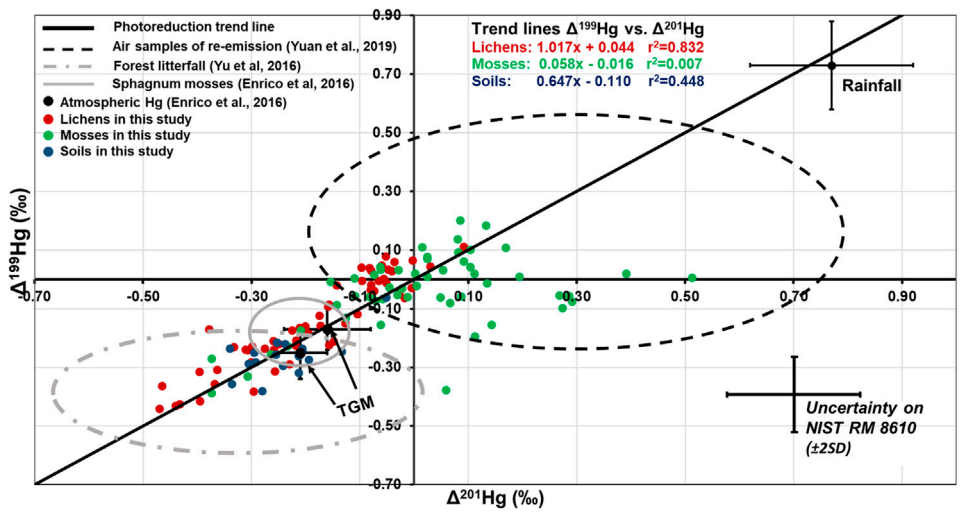

FIGURE 4 | Δ^{199}Hg vs. Δ^{201}Hg in lichens, mosses and soils. Uncertainty calculated for NIST RM 8610 (former UM-Almadén) (as 2 SD) represents the long-term reproducibility of the method for CVG. Hg^{2+} photoreduction line is from Bergquist and Blum, 2009.

As shown in **Figure 4**, two classes of lichens can be discriminated: the ones with odd-MIF close to zero and the other group with negative odd-MIF. Lichens with negative odd-MIF have a Hg isotopic composition close to the TGM (mainly as GEM) samples also from the Pyrenean area (Enrico et al., 2016). The isotopic signature of lichens from background sites is representative of the Hg^0 from the surrounding atmosphere, and beech leaves possibly have a similar isotopic fingerprint. Lichens which have a Δ^{199}Hg close to zero could probably integrate both gaseous Hg^0 (GEM) and Hg(II) (GOM) from dry deposition, or could be influenced by rainfall Hg(II) inputs (Enrico et al., 2016). The plot of Δ^{199}Hg vs. Δ^{201}Hg for lichens shows that all the samples follow a straight line with a slope of 1.017 ± 0.061 (r^2 = 0.832). This slope suggests that both geogenic sources and photoreduction induce a magnetic isotopic effect (MIF), which makes atmospheric Hg^0 the major source of contribution for lichens. The odd-MIF for soils is consistent with the values obtained for most lichens, confirming the values for even-MIF. Atmospheric gaseous Hg exhibits generally negative to close to zero Δ^{199}Hg values (Gratz et al., 2010; Rolison et al., 2013; Fu et al., 2016a; Yu et al., 2016). Wet deposition samples exhibit positive odd-MIF (Gratz et al., 2010; Sherman et al., 2012). Therefore, odd-MIF distribution confirms the binary-mixing model exhibited by even-MIF of Hg inputs from wet

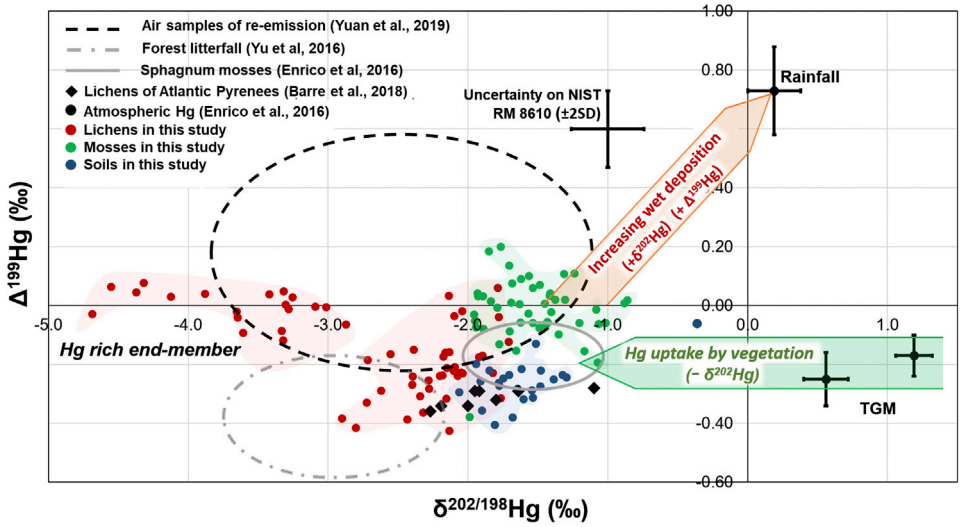

FIGURE 5 | Tri-isotopic plot of Δ^{199}Hg vs. $\delta^{202/198}$Hg (‰) for lichens, mosses and soils from Iraty forest. Uncertainties are expressed for the long-term reproducibility of NIST RM 8610 (former UM-Almadén) (as 2 SD) by CVG.

deposition and from gaseous dry deposition in lichens, mosses and soils. Slightly odd-MIF positive values for mosses imply a more significant uptake of Hg (II) mainly from wet deposition, in opposition to lichens with an odd-MIF composition closer the atmospheric GEM.

Mass Dependent Fractionation of Hg Isotopes

Figure 5 shows the plot of Δ^{199}Hg vs. δ^{202}Hg for lichens, mosses and soil samples collected in this study. The results obtained were compared with other data of vegetation and samples from the same region in previous studies (Enrico et al., 2016; Barre et al., 2018). Regarding the major part of the samples, the results are consistent with other data obtained for lichens in forested areas of the Atlantic Pyrenees on the French side (Barre et al., 2018). The lichens analyzed in this previous work represented a regional background Hg isotopic signature of the gaseous atmospheric Hg uptake by lichens. In the case of mosses, MDF is similar to the values obtained for soil samples, while MIF remains close to zero. Mosses isotopic signatures are also close to the *Sphagnum* sp. samples analyzed by Enrico et al. (2016). The differences between the signatures of the two biomonitors can be explained by their different Hg absorption fraction because of the relative physical configuration (height, substrate, specific surface . . .) of the collected organisms. As was mentioned before, epiphytic lichens represent an integration period of up to few years, while moss shoots samples correspond to their last year of growth. Recent studies have demonstrated that Hg concentrations in mosses are not well correlated with atmospheric Hg^0 uptake by dry deposition, but are strongly influenced by precipitation (Harmens et al., 2010; Schröder et al., 2010). Since the moss samples representative of the last year of growth did not display any significant contamination, this suggests that the isotopic signature obtained in lichens is a past phenomenon or related to lichens aging. Most importantly, we can conclude that Hg isotopic composition in mosses and lichens is significantly different combining both MDF and odd-MIF as it is shown in **Figure 5**. Lichens uptake more atmospheric or oxidized Hg from dry deposition while mosses present evidence of both types of deposition (dry and wet). The values representing MIF and MDF in mosses are between values of rainfall (wet deposition) and TGM (dry deposition) reported by Enrico et al. (2016), leading to more positive MDF values (red arrow). However the Hg uptake by vegetation produces more negative MDF values (green arrow).

The plot of δ^{202}Hg vs. 1/[Hg] for lichens in **Figure 6** shows a correlation between MDF and Hg concentrations typical of a binary mixing model involving a contamination source and the atmospheric background. Through this plot (**Figure 6**), we can clearly differentiate the two groups of lichens, as we mentioned in the previous section. The majority of samples (n = 39) corresponds to the background isotopic signature of Hg in the Iraty forest, which is consistent with results from other studies carried out in Pyrenean regions located close to the Iraty forest (Enrico et al., 2016; Barre et al., 2018). On the other hand, there is a group of samples (n = 19) exhibiting more negative δ^{202}Hg values with increasing Hg concentrations. An extrapolated, so-called, Hg rich end-member would exhibit a δ^{202}Hg of –4.86 ± 0.21‰ (*y*-axis intercept) and follows a linear trend correlated with 1/[Hg] (Pearson, r = 0.88 at 0.05 level of significance). The source of such significant fractionation is difficult to assess, and it is supposed that several fractionation processes could be involved. Very negative values of δ^{202}Hg (–4.37 ± 0.13‰ as a confidence 1 SD) for atmospheric measurement are not common but were already observed near a coal-fired power plant in Florida (Sherman et al., 2012). **Supplementary Figure S8** represents Pb isotope ratios vs. δ^{202}Hg for lichens and mosses in this

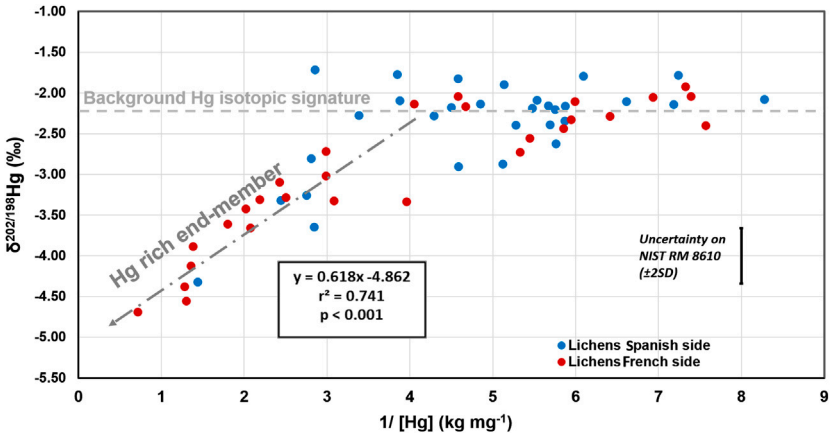

FIGURE 6 | $\delta^{202/198}$Hg vs. 1/[Hg] for lichens from Iraty Forest. Blue dots correspond to lichens of the Spanish sampling sites while red dots correspond to lichens of the French sampling sites. The trend suggests a contamination end member of about –4.86 ± 0.21‰ ($p < 0.001$) for 1/[Hg] = 0.

study and the comparison with lichens of a previous study (Barre et al., 2018). The results suggest that the isotopic composition of the Hg rich end-member does not match the expected values for contaminated samples of lichens geographically equivalents, obtained for anthropogenic or industrial areas. This is consistent with the results of concentrations for Pb and Hg, and other analyzed elements (see more details about other trace elements concentrations in **section S1** in the Supplementary material), that do not exhibit any local metallic pollution source influencing any of the sampling sites.

An unknown anthropogenic emission of Hg or an unidentified source of fractionation must be the origin of the negative δ^{202}Hg values. Natural processes such as re-emission of mercury from soils causing the fractionation of Hg isotopes during its diffusion in the surrounding atmosphere could be involved (Demers et al., 2013). In addition, it has been demonstrated that biologically mediated reduction, which is likely to occur in soils, produces Hg^0 with a lighter signature but without significant MIF (Kritee et al., 2007). Recently, Yuan et al. (2019) have observed that after continuous processes of Hg^0 uptake and re-emission, the mature foliage becomes progressively enriched in lighter Hg isotopes (more negative δ^{202}Hg values) and depleted in odd-mass isotopes (i.e. negative shift of odd-MIF). Lichens from the Hg rich end-member present also a very negative MDF but, on the contrary, a positive shift in odd-MIF. Thus, the balance between accumulation and re-emission of Hg over time in a forest ecosystem leads to vegetal biomass enriched in lighter isotopes. This is consistent with the observation that the samples of lichens with the most negative MDF are also the samples with the highest Hg concentrations (i.e. larger accumulation with time). The isotopic shift to more positive odd-MIF values in those lichens could then be explained if larger re-emitted Hg^0 from lichen surface Hg photoreduction have preferentially preserved odd Hg isotopes (Demers et al., 2013; Yuan et al., 2019). In addition, such lichens may also have accumulated re-emitted Hg^0 enriched with odd Hg isotopes (i.e. from foliage and soil) as a consequence of thiol induced Hg photoreduction (Yuan et al., 2019).

In the area of the Iraty forest, a major anthropogenic activity is related to animal breeding in pastures (e.g. cows, sheep and horses). The burning of natural grasses is largely employed annually to increase pasture renewal and supply. Regarding to our sampling area, uncontrolled grass burning between November and April of 2002 lead to large forest fires just after deciduous trees had lost their foliage (Ministère de l'Agriculture et de la Pêche, 2008). **Supplementary Figure S9** presents a geographical distribution of the Hg isotopic composition of each sampling site compared with the grasslands burned regularly and during the fire event in 2002 along the Iraty forest. We clearly observe on the map that the most negative values of δ^{202}Hg correspond mainly to samples on the French slope, and that grass burning exhibits rather significant spatial overlap with such specific negative isotopic signature of Hg. During the fire, Hg in ashes and Hg^0 are released from soils to the atmosphere (Caldwell et al., 2000; Dicosty et al., 2006; Engle et al., 2006; Biswas et al., 2008). Reactive Hg in ashes may display a lighter isotopic composition (consistent with negative values) as suggested by a binary mixing model set for coal power plant (Sun et al., 2013). Thus, the combination of the effect of re-emission from soil litterfall together with the past effect of grassland and forest fires, could provide an explanation for the isotopic signature of the lichens in the so-called Hg rich end-member.

5 CONCLUSION

This work demonstrates for the first time that the employment of Pb and Hg isotopic systems in mosses and lichens improves the understanding of deposition processes with complementary information between both bioaccumulators. Pb isotopes track well major anthropogenic inputs and background contribution, while Hg isotopes are much more informative for Hg deposition pathways. Since lichens have integrated an isotopic signature of Pb exhibiting a mixture between legacy leaded gasoline and the modern European and Industrial Pb fingerprints, they do not equilibrate with the present surrounding atmosphere and

integrate variations among several years. Hg isotopic composition is more directly influenced by the type of Hg depositions and uptake pathways by biomonitors than by interannual changes. Interestingly, the isotopic fingerprint of Hg in mosses represents a mixture of Hg uptake from the gaseous phase (Hg^0) and from wet deposition (Hg (II)). In this sense, mosses shoots are better indicator for recent depositions, as expected, but can be altered by local soil dust events for Pb and by mixed wet and dry deposition for Hg. In the case of lichens, the isotopic fingerprint is closer to the isotopic composition of top organic soils influenced by tree foliage deposition (e.g., litterfall). In addition, the differences between elemental integration times of both bioaccumulators represent significant differences. Mosses (annual "shoots") represent a shorter integration period than entire lichens thalli for which annual growth cannot be determined. For lichens, integration time is much uncertain and altered but Hg isotopic signature only reflect dry gaseous Hg contribution in this remote location. This confirms that gaseous elemental Hg is the main source of Hg in epiphytic lichens in such temperate forest. This work also exhibits that the spatial distribution of the Hg isotopes composition over a small scale in a forest ecosystem could be altered by natural or anthropogenic events such as climatic conditions, land use and forest fires.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

JB, DV, JS, AD, DA, and GD conceived the scientific questions and objectives, and decided the sampling strategy. JB, SB, ET, DV, DA, JS, AD, and GD performed the sampling strategy and sample conditioning. JB, SQ-A, CS-L, SB, and ET have performed the sample preparation, sample analysis and data treatment. JB, SQ-A, and DA interpreted the data and wrote the first draft, which was also edited and commented on by the other authors.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvc.2020.582001/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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GLOSSARY

A.s.l. Above Sea Level

CVG Cold Vapor Generation

DGA Dual Gold Amalgamation

GEM Gaseous Elemental Mercury

GOM Gaseous Oxidized Mercury

ICP-MS Inductively coupled Plasma Mass Spectrometry

MC Multicollector

MDF Mass Dependent Fractionation

MIF Mass Independent Fractionation

PE Polyethylene