



Research project report

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**Biomonitoring of trace metal elements by
lichens of the Western Pyrenees
(Pyrenees-Atlantiques).**

**Study of elemental and isotopic signature
of mercury.**

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Acknowledgments

I would like to express my gratitude to my supervisor Dr. David Amouroux whose expertise and understanding have contributed to my personal developing in the research field. I want to thank him for giving me the opportunity of working and learning in his team during this time.

I also like to thank Julien Barre by his practical lessons and assistance in all the areas of my research project.

Thanks to Dr. Cristina Sola by her invaluable help, without which, the final results would not have been the same.

I would like to acknowledge Dr. Jerome Frayret by his continued attention, although at the end technical problems don't allow to develop the work expected.

I would like to thank the other members of the LCABIE and CNRS for integrating and motivating me concerning to the world of science.

I have to recognize that this stage would not be possible without the financial assistance of CajAstur (Asturias, Spain).

And special thanks for the people who have shared this stage with me: Cris, Jeni, Mónica, Silvia, Vir, Zoyne and Laureant. The experience wouldn't be the same without all of you.

Abstract

The lichens, organisms originated by symbiotic relationship between an algae and a fungus, have shown good properties as biomonitors of pollution from Trace Metal Elements. They can be used as indicators of local and long-range atmospheric pollution.

The assessment of deposition of atmospheric pollutants results difficult to know their long term impact. This happens specially when the study is focalized in remote areas. In this project it has been developed the elemental and isotopic analysis of mercury composition (and lead that will be carried out after presentation of this report) on epiphytic lichens (growing on tree barks). The samples were collected in the mountain ecosystem of Iraty Forest, on both sides of the border between France (Departement des Pyrénées Atlantiques) and Spain (Comunidad de Navarra).

The determination of different trace metal elements (by ICP-MS Analysis) present in the lichens can be used to asses the spatial distribution of this pollutants all over the study area.

The new analytical methodology (Multi-Collection Analysis coupled with ICP-MS) used for isotopic analysis allow to a better discrimination or “fingerprinting” of the pollution sources and environmental pathways followed by mercury in mountain ecosystems.

Regarding to the results of isotopic analysis of mercury, $\delta^{202}\text{Hg}$ values in the lichens of Iraty Forest they are the expected values for background concentration in the case of the Spanish side samples. However French side samples reach lower values ($\delta^{202}\text{Hg} \sim -4.5 \pm 0.08\text{‰}$) that do not correspond to any signatures previously reported. Therefore it can be noted that a local source of mercury occurs for French sampling sites, don't studied yet.

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1. INTRODUCTION

1.1 Atmospheric pollution

In recent decades, the interest on the environment has been growing at the same time that concern over the effects of human activities. Hence also is increasing the need of developing technologies for determining what extent it is, and how pollutants affect. Even also laws have been established to regulate and control the increasing impact of the pollutants.

Air pollution is the development and retention of foreign substances to the natural composition of the environment.

Weber's concept (1982)

“the presence of substances in the atmosphere, resulting from antropogenic activities or natural processes causing adverse effect to man and the environment”.

An important aspect is to reach a better understanding of the distribution of pollution. How pollutants are transported in the atmosphere. This allows to develop dispersion models that report:

- Local sources of pollutants
- Large-scale transport

It is of great interest to study remote areas to know continental and large scale pollution. It allows to develop maps of distribution of pollution in different areas.

1.2 Trace metal elements (TME)

The objective of this study is to asses the pollution caused by TME all over a mountain ecosystem. These measurements have been done using lichens as biomonitors. It is also necessary to control the emissions. Emissions of TME are generally due to coal - fossil combustion, metal industry and transport. These pollutants can't be degradated in the atmosphere and persist during long time, accumulating in living organisms.

There are some of these elements that are considered as anthropic pollutants (Cd, Pb, Cu and Zn) because their presence in the atmosphere is mainly by human contribution. In the case of traffic for example there are many representative TME as Pb, Cu, Cd, Zn, Sb, Ce, Al, Mn, V, Cr, Sn or U. In the Table 1 it is shown a comparison between natural and anthropic emissions of different metals.

The contaminants found in the lichens of the area of study (Iraty Forest) that are indicators of air quality are the following: Mn, Ti, Zn, Sr, Rb, Pb and Cu (in less concentration V, Cr, Ni, Ce, Li, Cd, Hg, Sn). (Pacyna & Pacyna, 2001).

Table 1: Comparison between anthropic and natural sources for different metals and the ratio anthropic/natural source. The emissions are estimated at 10^3 t/year all around the world in the 90s. (Veschambre, 2006). Data for mercury is reported by Pirrone et al., 2010 referred to the year 2008.

	V	Cr	Mn	Cu	Zn	Cd	Sb	Pb	Hg (2008)
Anthropic source	240	14,7	11	25,9	57	5	1,6	119,3	2,3
Natural source	28	44	317	28	45	12	1,4	12	5,2
Ratio	8,6	0,3	0,035	0,9	1,3	0,4	0,7	9,9	0,44

Regarding to the enrichment factor determine by Veschambre et al., 2008 the most part of TME found in the Pyrenees region have a crustal origin. The enrichment factors for the ratio between Rb or Fe and the rest of metals are close to 0 (it is considered that their origin is mineral and they are geochemical tracers). The exception are Cu, Pb, Sb, Zn and Cd for which there are a significant increase (specially for Cd) in both ratios. Therefore these elements are considered as metal pollutants.

1.2.1. Metallic pollutants

Metallic pollutants are those whose anthropogenic contribution in the atmosphere is significant (Table 1). Here it is shown a description of some of this pollutants, their uses and possible anthropic sources and also their toxic effects in human beings.

V⁽²³⁾: its origin is geological, being an essential nutrient for many animals and plants. In the last years vanadium has reach importance in the industry as component in the fabrication of colorants, some types of petrol and its uses as catalyzer, in thermo electrical plants and incinerators. In the living organisms it is incorporated by breath and accumulated in bones, liver and lungs. It has benefits for the organism but the direct exposition of vanadium smokes can cause conjunctivitis, pharyngitis and bronchitis. After long exposition periods digestive, neurological and renal effects could be observed.

Cr⁽²⁴⁾: it is an important constituent of the terrestrial crust but their presence in the atmosphere is generally caused by coal and fossil combustions. In the air is associated to particulated matter and can keep in suspension for long time being transported long distances. Its main applications in industry are: steel alloys, coatings, mineral pigments and electrical resistences. It is not accumulated in organisms, but chromium (VI) and chromium (III) are highly toxic because they can associate with proteins and nucleic acids. Its direct and continuous exposition can caused skin ulcers, dermatitis and inflammation of the airways. In the case of chromium (VI) it has carcinogenic effect.

Ni⁽²⁸⁾: Its origin is mainly anthropic in the atmosphere. It is used in the steel industry, manufacturing vats, catalysts and electrolytic cells from the combustion of coal, fuel oil, traffic and soil where it is adsorbed. It is incorporated into the body by inhalation having a carcinogenic effect. The nickel carbonyl causes chemical pneumonia.

Cu⁽²⁹⁾: the main source of this element in the atmosphere is anthropogenic. It is used in the fabrication of plastics and pesticides and in the metallurgical industry. Also it is

emitted by coal combustions. Copper is often present in soils and sediments bonding with their different components. Its effects are usually consequence of direct exposition. Copper is accumulated in liver and bone marrow and it cause brain diseases and hepatic necrosis.

Zn⁽³⁰⁾: its presence in the terrestrial crust is reduced to the 0,004%. In the environment is associated with particulated matter. Its bioaccumulation isn't common in plants and other terrestrial organisms but it can be found in aquatic organisms. The main industrial uses of zinc are in the fabrication of different products (pigments, galvanized materials, plastics, lubricants, batteries, steel...). It can reach the atmospheric air also by wear of tires and from fossil combustions. The inhalation of smokes from welding process can caused fever, tremors and mental confusion over the workers.

Ag⁽⁴⁷⁾: is a rather scarce element. Sometimes found in nature as a free element (native silver) or mixed with other metals. However, most often found in minerals containing silver compounds. Approximately three-fourths of the silver produced are a byproduct of the extraction of other minerals, especially copper and lead. Silver which has the highest thermal and electrical conductivities of all metals is used in electrical contact points and electronics. It is also widely used in jewelry. Among the alloys are a component of dental amalgam and metals for engine bearings and pistons.

Cd⁽⁴⁸⁾: in nature is associated to zinc through the wurtzite and therefore is a byproduct of the zinc industry. Among its industrial applications are steel alloys, manufacture of electric accumulators, dyes, plastics, nuclear reactors or photovoltaic cells. It is an element with high mobility due to the solubility of compounds. Cadmium is bioaccumulated in the body and is absorbed through respiratory and digestive ways. It is accumulated in various organs (liver, pancreas, kidney, lung, thyroid ...) causing gastric distress, pulmonary irritation, kidney problems, bone lesions and even carcinogenic action.

Sn⁽⁵⁰⁾: The most important tin mineral is cassiterite, SnO₂. There are no known deposits of high quality of this mineral. Most of the world tin ore is obtained from low quality placers. Organic tin compounds may remain in the environment for long periods of time, they are persistent and not readily biodegradable. The organotins can spread through water systems when particles are absorbed by plants.

Sb⁽⁵¹⁾: is not an abundant element in nature and it is rarely found naturally, often as a mixture isomorphous with arsenic: the allemonita. It enters the environment through several human applications. Antimony can be found in water and air in very small quantities, but mainly in soils. It is used in many alloys, accumulators coating cables, anti-friction bearings and various kinds of metals consumption. The human exposure to antimony can occur through breathing, drinking water and food containing it, but also through dermal contact with soil, water and other substances. Exposure to relatively high amounts of antimony (9 mg/m³ of air) over a long period of time can cause eye irritation,

skin and lungs. If the exposure keeps on time the effects are aggravated: lung disease, heart problems, diarrhea, severe vomiting and stomach ulcers.

Hg⁽⁸⁰⁾: can be found in the form of metal salts of mercury or organic mercury. Mercury enters the environment as a result of the breakdown of minerals in rocks and soils through exposure to wind and water. The 95% of mercury is vapor of elemental mercury, that can be transported long distances. The release of mercury from natural sources has remained at the same level throughout the year. Mercury concentrations in the environment are growing, this is due to human activity. The most of mercury from human activities is released into the air through burning fossil fuels, mining, smelting and combustions of solid waste. It's main uses are the fabrication of electrical devices, chlor-alkali industry and pesticides fabrication (already forbidden because of its toxicity). Another sources of mercury are fossil combustions, metallic fusions, extraction of gold or outgassing (from rocks and volcanoes). Every mercury compounds are toxic. It is adsorbed on soils and sediments and can be bioconcentrated in organisms. Inhalation of mercury can cause respiratory inflammation. It can also be a source of kidney and digestive ulcers. Minamata disease is caused by mercury.

Pb⁽⁸²⁾: Lead occurs naturally in the environment, but the highest concentrations are found as result of human activities. It is widespread in the environment and usually associated with particulate matter as oxides and carbonates. Plants and animals can accumulate lead but biomagnification doesn't happen. At an industrial level lead is involved in fabrication of steel, paints, plastics and cristals. Until 2000 year its main use was as component of petrol and this was also the main source of emission.

Due to the application of lead in gasoline an unnatural cycle occurs. It can end up in water and soil through the corrosion of lead pipes in transport systems and by corrosion of lead-containing paints. It cannot be broken, but can be converted to other compounds. Lead is absorbed by breath and accumulate in bones, liver, kidneys and central nervous system and it can cause several unwanted effects, such as: disruption of the biosynthesis of hemoglobin and anemia, increased blood pressure, damage to the kidneys, disturbance of the nervous system and damage to the brain.

1.2.2 Geochemical tracers

This classification is for those metals which main contributions in the environment until now are natural sources. They require a control because their uses are increasing and maybe they are beginning to be present in the atmosphere by anthropic sources. The following are the description of some of that elements analyzed in this study:

Li⁽³⁾: is present in many minerals in different forms and it has a wide variety of applications: aluminum alloys, lithium batteries, glass industries, to increase the viscosity of oils and fats, air conditioning equipments, catalysts, lubricants, nuclear weapons... Lithium has no known biological use although it is present in the organism, and is not

readily absorbed by the human body. After oral intake of lithium may be moderately toxic. Lithium carbonate is applied in psychiatry to treat bipolar disease.

Ti⁽²²⁾: is the second metal more abundant in soils after iron, (reaching contents of 0,5% of the soil) present as titanium oxides. Its industrial uses are pigments fabrication and as catalyzer in some chemical processes. It isn't common its bioaccumulation in living organisms, but it can get into the organism by the breath and caused inflammations in the lungs.

Mn⁽²⁵⁾: is an essential element for living organisms, but its accumulation in the lungs can caused degenerative diseases. It is present in the nature and combined forming oxides. It can be accumulate in different plants and fruits. The main source of manganese oxides is the soil. At industrial level it is involved in different process of fabrication (alloys, colorants, paints, pesticides...) and also is used as substitute of lead in petrol.

Rb⁽³⁷⁾: is considered to be the 16th most abundant element in the earth's crust. The relative abundance of rubidium has been reassessed in recent years and it is now suspected of being more plentiful than previously calculated. The metal is used in the manufacture of photocells and in the removal of residual gases from vacuum tubes, in glasses and also ceramics. Potential uses are in ion engines for space vehicles, as working fluid in vapor turbines. Rubidium readily reacts with skin moisture to form rubidium hydroxide, which causes chemical burns of eyes and skin. The overexposure can cause ataxia, hyper-irritation, skin ulcers, and extreme nervousness and it is aggravated for heart disease patients.

Sr⁽³⁷⁾: Strontium in its elemental form occurs naturally in many compartments of the environment, including rocks, soil, water, and air. Commonly it is found in nature, forming about 0.034% of all igneous rock and in the form of the sulfate mineral celestite (SrSO_4) and the strontium carbonate (SrCO_3). Strontium concentrations in air are increased by human activities, such as coal and oil combustion. This element can end up in water through soils and through weathering of rocks. People are exposed to small levels of (radioactive) strontium by breathing air or dust, eating food, drinking water, or by contact with soil that contains strontium. For children exceeded strontium uptake may be a health risk, because it can cause problems with bone growth.

Ce⁽⁵⁸⁾: is normally found in two different types of minerals. Its applications are increasing due to the fact that is useful for producing catalysts and for polishing glass. This element is released to the environment in many different places, mainly by industries producing oil, and also can enter the environment when household equipment is thrown away. Cerium will gradually accumulate in soils and water soils and this will eventually lead to increased concentration in humans, animals and soil particles.

U⁽⁹²⁾: The total content in the crust to a depth of 25 km is estimated at 1017 kg. In water most of the dissolved uranium is derived from rocks and soil on which the water flows.

Humans add uranium to the soil through industrial activities. The compounds in the soil will be combined with other compounds, which can remain in soil for years. Uranium alloys are useful in the dilution of enriched uranium for reactors and the supply of liquid fuels. It has not been detected harmful effects on the radiation from natural levels of uranium but chemical effects can cause effects such as liver disease or cancer.

1.3 Atmospheric mercury

Mercury is a trace element considered as a priority pollutant because of its high toxicity. But there are also naturally mobilized Hg coming from deep reservoirs emitted by geological and volcanic activity. As it is shown in table 1, and recently published by Pirrone et al., 2010 the main sources of mercury are the oceans. The combustion of fossil fuels represents the major part of mercury released to the atmosphere. In figure 1 the main anthropogenic sources of mercury are shown, and also their contributions.

Atmospheric mercury can be deposited to terrestrial and aquatic ecosystems and it undergoes different transformation pathways depending on the environmental compartment. The global atmospheric Hg cycle is mainly governed by elemental Hg vapor emissions and its deposition pathway is dependent on the oxidation process.

Mercury is emitted in its elemental form Hg^0 by geological sources and also from the oceans, while anthropogenic sources, as coal-fired power plants emit $Hg(II)$ and HgP (associated with particles).

$Hg(II)$ is the most soluble form so usually its presence is predominant in wet and dry deposition. However the lifetime of Hg^0 is higher than $Hg(II)$ (~1 year), so that characteristic makes Hg^0 as a highly globally distributed pollutant due to its toxicity. Because of that the main transformation in the atmosphere is the photo-oxidation of $Hg^0 \rightarrow Hg(II)$ which can be accumulated in the different ecosystems (Figure 2).

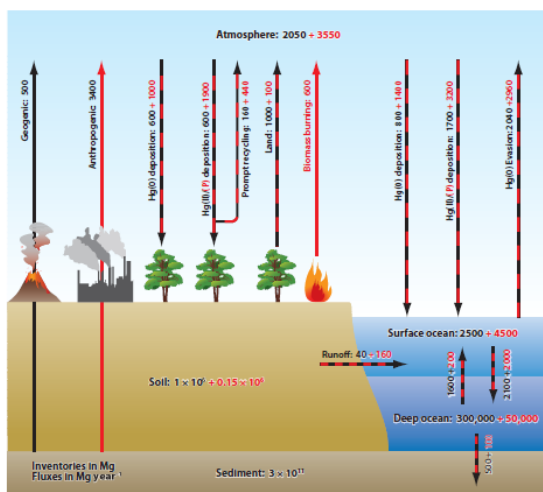


Figure 1: Main atmospheric sources and Contributions of mercury (Sellin, 2009)

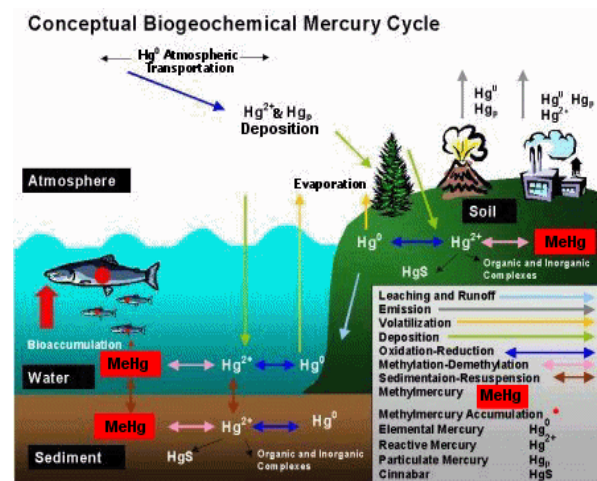


Figure 2: Global atmospheric mercury cycle

1.4 Isotopic composition of mercury

Mercury has 7 stable isotopes (^{196}Hg , ^{198}Hg , ^{199}Hg , ^{200}Hg , ^{201}Hg , ^{202}Hg , ^{204}Hg) with a 4% relative mass difference. The study of the isotopic ratios allows us to understand the sources and transformations of Hg in the environment. Differences in the isotopic ratios from natural ones are consequence of the different transformations that can take place (Table 3). These transformations lead to the fractionation of the mercury.

1.4.1 Isotope fractionation

It is the partitioning of isotopes (1 and 2) from the natural abundances, between 2 substances (a and b) or phases of the same substance.

$$\alpha^{a-b} = \frac{\left(\frac{X_2}{X_1}\right)_a}{\left(\frac{X_2}{X_1}\right)_b} \quad (\text{Isotope fractionation factor})$$

The study of isotope fractionation can provide information about the process that mercury follows in the environment (Table 2). The isotopic fingerprint of mercury offers a new dimension of information to determine environmental processes and sources. Therefore mercury isotopes can be used as tracers of biogeochemical pathways in the environment.

Table 2: Values of mercury isotope fractionation for different biogeochemical processes. (Yin et al.,2010)

Type	A	B	$^{202}\alpha_{A/B}$	Δ_{A-B} (‰)	References
<i>Chemical reactions</i>					
Photo reduction	$\text{CH}_3\text{Hg}^+_{(\text{aq})}$	$\text{Hg}^0_{(\text{g})}$	1.0013–1.0017	0.6	Bergquist and Blum (2007)
Photo reduction	$\text{Hg}^{2+}_{(\text{aq})}$	$\text{Hg}^0_{(\text{g})}$	1.0006	1.5	Bergquist and Blum (2007)
UV photolysis reduction	$\text{Hg}^{2+}_{(\text{aq})}$	$\text{Hg}^0_{(\text{g})}$	1.0006	1.34	Yang and Sturgeon (2009)
Dark organically mediated reduction	$\text{Hg}^{2+}_{(\text{aq})}$	$\text{Hg}^0_{(\text{g})}$	1.0020	1.7	Bergquist and Blum (2007)
Chemical reduction (SnCl_2)	$\text{Hg}^{2+}_{(\text{aq})}$	$\text{Hg}^0_{(\text{g})}$	1.0004	1.17	Yang and Sturgeon (2009)
Chemical reduction (NaBH_4)	$\text{Hg}^{2+}_{(\text{aq})}$	$\text{Hg}^0_{(\text{g})}$	1.0004	1.08	Yang and Sturgeon (2009)
Chemical reduction (ethylation with NaBH_4)	$\text{Hg}^{2+}_{(\text{aq})}$	$\text{Hg}^0_{(\text{g})}$	1.0012	3.59	Yang and Sturgeon (2009)
Volcanic emission	$\text{Hg}^0_{(\text{p})}$	$\text{Hg}^0_{(\text{g})}$	1.00135	1.63	Zambardi et al. (2009)
Absorption	$\text{Hg}^{2+}_{(\text{aq})}$	$\text{Hg}(\text{OH})_2$	- ^a	0.62	Wiederhold et al. (2010)
<i>Biological reactions</i>					
MerA	$\text{Hg}^{2+}_{(\text{aq})}$	$\text{Hg}^0_{(\text{g})}$	1.0013–1.0020	1.6	Kritee et al. (2007, 2008)
MerB	$\text{CH}_3\text{Hg}^{2+}_{(\text{aq})}$	$\text{Hg}^0_{(\text{g})}$	1.0004	0.4	Kritee et al. (2009)
Methylation	$\text{Hg}^{2+}_{(\text{aq})}$	$\text{CH}_3\text{Hg}^+_{(\text{aq})}$	1.0026	1.0	González et al. (2009)
<i>Physical reactions</i>					
Evaporation	$\text{Hg}^0_{(\text{l})}$	$\text{Hg}^0_{(\text{g})}$	1.0067	6.5	Estrade et al. (2009)
Volatilization	$\text{Hg}^0_{(\text{aq})}$	$\text{Hg}^0_{(\text{g})}$	1.0004–1.0005	1.48	Zheng et al. (2007)
Evaporation	$\text{Hg}^0_{(\text{l})}$	$\text{Hg}^0_{(\text{g})}$	1.0009	0.8	Estrade et al. (2009)

^a Lack of data.

1.4.1.1 Mass dependent fractionation (MDF)

Mass dependent processes are diffusion, metabolic transformations or high temperature equilibrium processes (Figure 4). The difference of behavior between molecules with light and heavy isotopes is controlled by vibrational energies. The dissociation energy of light isotopes is less than for heavy ones, so molecules with light isotopes react faster. The products of reaction are enriched in that light isotopes.

Isotope composition is reporting using delta notation δ which is the per mil (‰) and referred to a standard ratio between that isotope and ^{198}Hg . (Blum and Bergquist,2007)

$$\delta^{xxx}\text{Hg} = \left(\frac{\left(\frac{^{xxx}\text{Hg}}{^{198}\text{Hg}} \right)_{\text{sample}}}{\left(\frac{^{xxx}\text{Hg}}{^{198}\text{Hg}} \right)_{\text{NIST 3133}}} - 1 \right) \times 1000\text{‰}$$

(xxx is the mass of each Hg isotope between 199 and 204 amu).

These notations have been accepted to facilitate interlaboratory comparisons. And also it has been accepted the report of $\delta^{202}\text{Hg}$ values due to its abundance and because it doesn't suffer from isobaric interferences (like Hg^{204} with Pb^{204}).

In the case of mercury the reduction process of $\text{Hg(II)} \rightarrow \text{Hg}^0$ follow a MDF, isotopic composition of mercury is enriched in light isotopes:

$$\delta^{202}\text{Hg}^0 < \delta^{202}\text{Hg(II)}$$

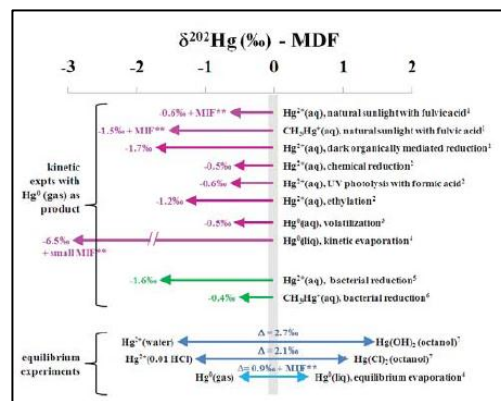


Figure 4 : Theoretical values for Hg isotope fractionation studies (Bergquist & Blum, 2009)

1.4.1.2 Mass independent fractionation (MIF)

These processes are mainly related with photo-reduction processes (Figure 5). When reduction of mercury is carried out by light effect, mercury isotopes also exhibit mass independent fractionation. These anomalies have been found for the odd isotopes and for ^{200}Hg , which delta shows a deviation from the MDF line. Deviation is quantified as Δ :

$$\Delta^{xxx}\text{Hg} \approx \delta^{xxx}\text{Hg} - (\delta^{202}\text{Hg} * \beta_{xxx})$$

where β (Table 3) is a constant extract from the fractionation law for a given system of three isotopes that only depends on the mechanism followed as it is explained in Young et al.,2002.

- kinetic reaction: $\beta_{xxx} = \frac{\text{Ln}(198/xxx)}{\text{Ln}(198/202)}$
- equilibrium mechanism: $\beta_{xxx} = \frac{(1/198)-(1/xxx)}{(1/198)-(1/202)}$

Table 3 : Theoretical MIF values (Bergquist & Blum, 2007) where β is the slope of the three-isotope plots corresponding to MDF line.

$$\Delta^{199}\text{Hg} \approx \delta^{199}\text{Hg} - (\delta^{202}\text{Hg} * 0.252)$$

$$\Delta^{200}\text{Hg} \approx \delta^{200}\text{Hg} - (\delta^{202}\text{Hg} * 0.502)$$

$$\Delta^{201}\text{Hg} \approx \delta^{201}\text{Hg} - (\delta^{202}\text{Hg} * 0.752)$$

$$\Delta^{204}\text{Hg} \approx \delta^{204}\text{Hg} - (\delta^{202}\text{Hg} * 1.492)$$

MIF for the odd isotopes could be explained by two mechanisms:

- Magnetic Isotope Effect (MIE) (Estrade et. al, 2009; Sonke 2011)

It is produced by effect of radical reactions occurring by light exposure. When radical pairs are formed they are in a triplet state of multiplicity they can't convert again in reagents (singlet state of multiplicity) because the transition is "forbidden". However, odd isotopes show a nuclear spin-electron spin that can induce a triplet-singlet conversion. Hence, reagents of reduction will be enriched in odd mercury isotopes while products are enriched in even isotopes.

- Nuclear Volume Effect (NVE) (Schauble, 2007)

For even isotopes r^2 is a factor that increase linearity with the mass. For odd isotopes a distorsion occurs and cause that their r^2 are lower than expected. They have higher charge densities and valence electrons are more attracted from the nuclei. Therefore odd isotopes form less strong bonds and the enrichment of products in light isotopes don't follow the same proportions than MDF.

In summary, because of MIF mechanisms products of mass-independent fractionation processes are enriched in even mercury isotopes and reagents are enriched in odd mercury isotopes.

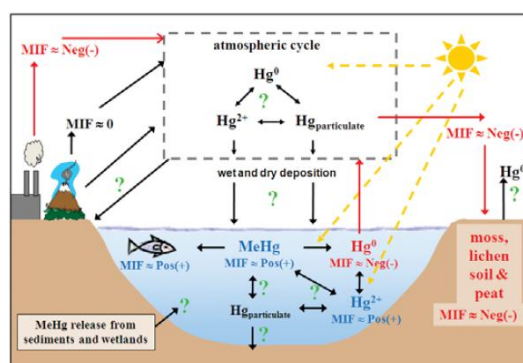


Figure 5: Atmospheric cycle of mercury and MIF trends (Bergquist & Blum, 2009)

1.5 Atmospheric lead

Lead is an anthropic TME that can be used as tracer of pollution sources in the atmosphere. The toxicity of lead in the environment has been known for many years, but

until recently this toxicity has been generally confined to certain occupations. The more recent use (50 to 100 years) of large quantities of lead in gasoline, pigments, batteries, ceramics, and other industrial products has led to a more widespread exposure of populations to lead concentrations well above natural background levels. Table 2 (Veschambre,2006) shows that most of lead present in the atmosphere correspond to anthropogenic sources. The contribution anthropic contribution is almost 10 times higher than natural one.

Lead is released by various natural and anthropogenic sources to the atmosphere and to aquatic and terrestrial environments and there are fluxes between these compartments. Into the atmosphere, it is deposited on land and into aquatic environments and some lead released onto soil over time is also washed out to aquatic environments.

Once emitted to air, lead is subject to atmospheric transport. It is mainly emitted to the atmosphere in particle form. The atmospheric transport of lead is governed by aerosol (particle) transport mechanisms depending on various factors, including particle size, the height of the emission outlet and meteorology. Because it has a relatively short residence time in the atmosphere (days or weeks), this metal is mainly transported over local, national or regional distances.

Regarding at table 1, it can be observed that lead anthropic emissions are almost ten times higher than natural ones. Nowadays the emissions of lead has been reduced since its use in petrol is forbidden. The figure 5 shows the decrease in lead emissions in Europe.

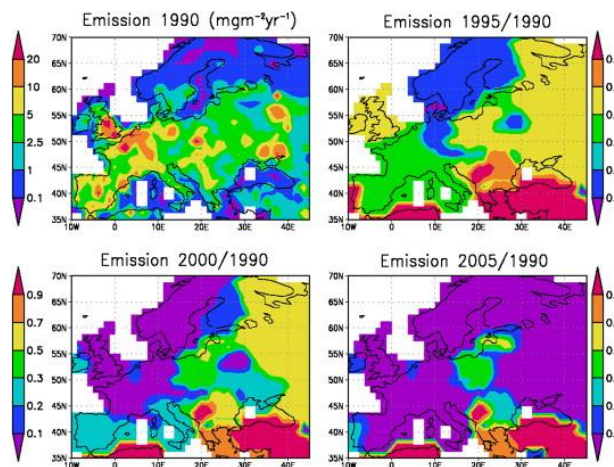


Figure 5: Reported of lead emission flux distribution ($\text{mg m}^{-2} \text{yr}^{-1}$) for Europe in 1990, and the proportions of emissions in 1995, 2000, and 2005 to the emission in 1990. (Niisoe et al.,2010).

1.6 Isotopic composition of lead

Natural lead is normally composed of four stable isotopes of masses 204, 206, 207 and 208. The use of Pb isotope ratios to identify and to distinguish between natural and anthropogenic sources of contamination has become a routine fingerprinting tool in the last few decades.

Where problems of toxicity or pollution occur, tracing the source of the problem can often be difficult because of the multiplicity of sources, both anthropogenic and natural (geogenic). Tracing sources is even more difficult in cases of toxicity where lead from soil, dust, water and air can all contribute to the problem. Lead does have one advantage over other heavy metals as a tracer of pollution and toxicity, since the relative amounts of the lead isotopes in nature vary significantly. Different sources of lead have different isotopic ratios and the variation in these ratios acts as a tracer for the source of lead.

Because every lead ore form are originated in unique conditions of time, process and source, each ore will have a unique set of isotopic ratios. Indeed, so sensitive are the isotopic ratios to small variations in conditions that even within ore bodies' variations in isotopic ratios can be discerned, such variations being the result of differences in the time of formation, differences in source and differences in processes of formation. Such ore bodies are the major sources of lead used by humans. Thus, anthropogenic lead will have isotopic ratios reflecting these sources and in most cases these ratios are different from other geogenic sources such as soil and bedrock. Figure 6 shows signatures of lead isotope ratios for different samples.

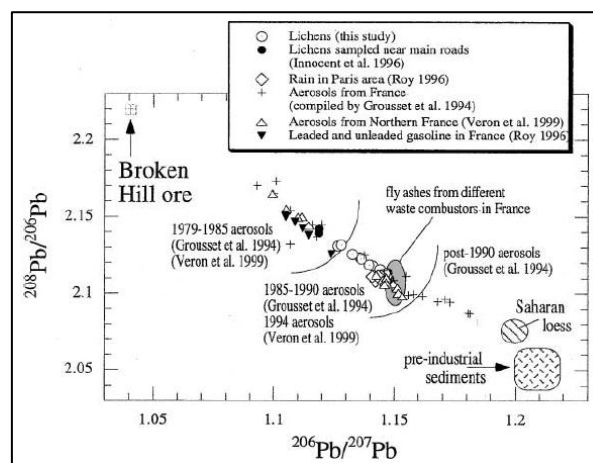


Figure 6: Signatures characteristic of different samples for lead isotopes (Doucet and Carignan,2000)

1.7 Biomonitoring, bioindicators and bioaccumulators

There are many difficulties to measure low concentrations of pollutants in the air. The ability of some organisms to bioaccumulate these pollutants has special interest in the control of atmospheric quality.

Bioindicators are organisms able to show the presence and amount of pollutants by a qualitative way (Sloof et al, 1998). The use of biomonitoring is to assess the quality of the air depending on the degree of disturbance of the system by determining the degree of stress or analyzing the levels of different pollutants. When they exhibit only damage because of the pollutant's presence they can be used as bioindicators. If they can accumulate these pollutants they are bioaccumulators. Following this line, biomonitoring

are bioindicators and bioaccumulators that provide identification and quantitative information in the course of time. (Garty, 2001)

Biomonitoring are term related bioindicators but referred to the quantitative measure of pollutants and its long-term assessment.

Characteristics of a biomonitor: (Martin and Coughtrey, 1982; Sloof et al., 1988)

- Capable of accumulate measurable amounts of pollutants
- Availability of the organism
- Availability independent of seasonal changes
- Repeatability of the measurement
- Low cost of acquisition
- Abundance in the location of the study and independent of local conditions
- Tolerance to pollutants of interest

Advantages of biomonitoring

- Assessment of accumulative processes influenced by several factors
- Study of long and expensive accumulative processes
- Measure of low concentrations when there are difficulties of detection by physico-chemical methods
- Establishment of wide long-scale networks of monitoring with high spatial resolution
- Low cost

1.8 Lichens as biomonitors

Lichens are symbiotic organisms originated by association of fungi and algae. They are able to accumulate metals coming from atmosphere. The different species of lichens are widespread, have longevity and their morphology don't change with seasons, because of that they are considered as well biomonitors of long-term pollution caused by atmospheric deposition. In contrast with higher plants lichens don't have cuticles, which facilitate the penetration of the different substances. As they haven't roots the accumulation doesn't depend on the substratum. There are several difficulties to find lichens with the same characteristics because of the tree barks modified their composition (Szczepaniak 2003).

Accumulation of metals by lichens

Lichens do not contain the cuticles of vascular plant leaves, and elemental exchange occurs across their entire surface. Hence, some lichen species are "sensitive" and die because of air pollution, while others are "tolerant" and develop resistance to pollution. The processes by which lichens develop resistance are various, but this occurs generally

by complexation of heavy metals leading to the synthesis of insoluble oxalate or carboxylic groups. (Sarret et al., 1998)

Metallic ions may exist in different compartments within lichens. Although metal absorption is also influenced by precipitation, geographical effects, temporal changes or local pollution sources, there are mechanisms that can explain why the concentrations in lichens are not uniform: (Garty,2001)

1. Extracellular exchange of metals: Thalli of lichens is the main responsible of the accumulation. It acts like a cation exchange resin for the metals. This is a fast process, passive and reversible. (Figure 7)
2. Intracellular uptake of metals: The intracellular accumulation of lichens is influenced by metabolism. This is a slow process that increases in time and depends on the different species of lichens and the different elements.

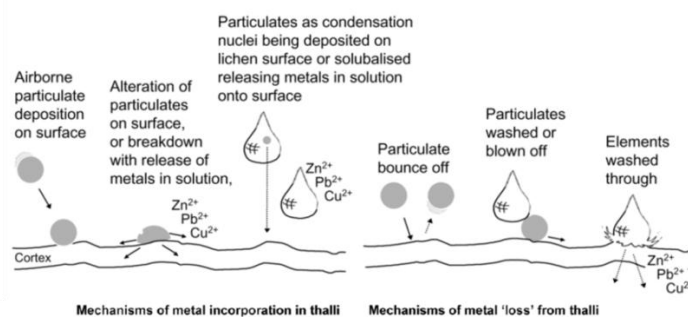


Figure 7: Diagram of the main mechanisms of deposition of traffic TME on lichen thalli (Williamson et al., 2004)

Objective of the use of lichens as biomonitors:

There are some problems associated with the uses of lichens as biomonitors:

- space-time fluctuations
- difficults on understanding of the sporadic emissions
- biological tolerance of the species
- dose-effect response not linear

However they can't carry out precise measurements of pollutants in the air, interesting information can be extract from biomonitoring of lichens:

- Mapping of the species in one area (spatial distribution of pollution)
- Physiological evaluation of parameters due to the concentration of pollutants
- Isotopic analysis of pollutants (Hg, Pb) to a better understanding of the processes and sources.

In this study samples used are epiphytic lichens, which grow in the tree barks. These lichens are expected to be only influenced by air pollutants, not by pollutants present for example in soils. This allow to asses pollution coming directly from the air.

1.9 PYNATEO Project

1.9.1 Communauté de travail des Pyrénées (CTP)



The Pyrenees work community (CTP) was created in 1983 with the support of European Council. The aim was the establishment of a cooperation in the Pyrenees over this border area to preserve and develop it. This allows to the joint treatment of the problems and the carrying out of shared actions.

Due to its specific geographical and political situation (an area built around the massif of the Pyrenees, straddling three states), the CTP must include different laws and work with multiple entities (States, local entities and autonomous French.) In this context, the CTP is intended as a forum for exchange of ideas and also generating proposals in order to solve common problems. The members of the CTP are working for thematic commissions allowing them to compare their experiences and reach innovative solutions.

Within the scope of this project the objectives of the commission are focalized in the environmental studies all over the Pyrenees area.

1.9.2 PYNATEO Project

The CTP Project is based on an assessment and control of air pollution in different areas of the mountains of the Pyrenees. The objective is to carry out, at an environmental level, analytical methods and strategies and the sharing of the results to develop models of air pollution. In this case study focuses on biomonitoring trace metals, HAPs, PCBs and isotopes of C,N,Hg and Pb, through the use of mosses and lichens. Specifically, in the LCABIE, the first part of the project is focused on the preparation and analysis of lichen samples collected earlier in the month of November 2011. These samples were collected in the Iraty Forest, an area of 17000 Ha, inside a mountain ecosystem.

1.9.2.1 Objectives:

- Use of lichens as biomonitors of air pollution
- Analysis of Trace Metal Elements (TME) in remote area (forest ecosystem)
- Isotopic analysis of mercury and lead to trace the environmental sources of pollution

1.9.2.2 Participants

- LABORATOIRE de CHIMIE AGROINDUSTRIELLE - ENSIACET – Toulouse
 - - Valérie Simon



- Universidad del País Vasco - Bilbao
 - Alberto de Diego
 - Agustín Ezcurra
 - Silvia Fernández Ortiz de Vallejuelo



- Universidad de Navarra - Laboratorio Integrado de Calidad Ambiental – Pamplona
 - Jesús Miguel Santamaría
 - David Elustondo



- Université de Pau et des Pays de l'Adour

- IPREM/LCABIE – Pau
 - David Amouroux
 - Hervé Pinaly
 - Emmanuel Tessier
 - Julien Barre
- SET –Pau
 - Gaëlle Deletraz
 - Thao Tran



2. MATERIALS AND METHODS

2.1 Sampling



The samples of lichens have been selected in a mountain ecosystem of the Western Pyrenees (Iraty Forest). A set of 51 samples were collected in both sides of the border between France and Spain (21 in the French side and 30 in the Spanish side) as it is shown in Figure 8.

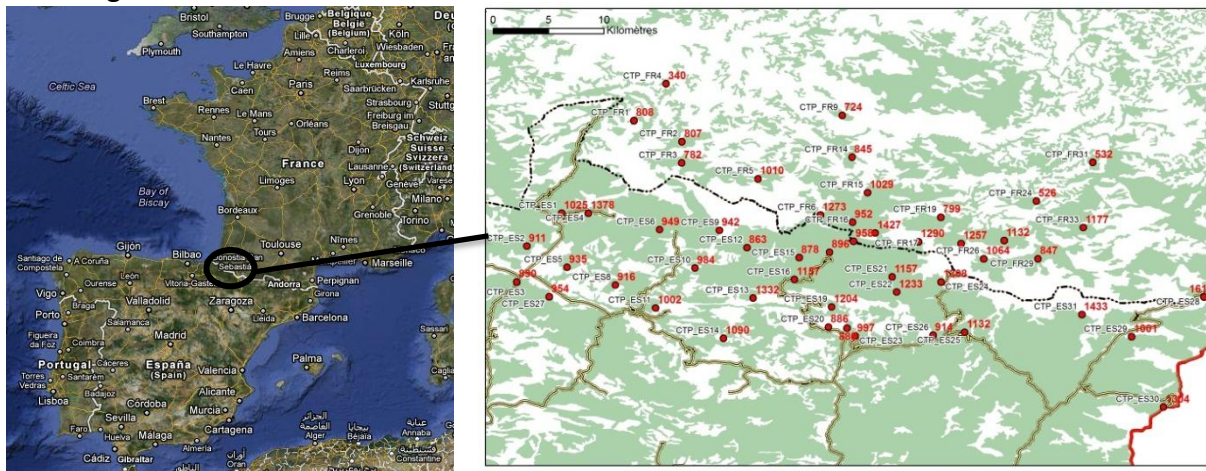


Figure 8: Maps of sampling sites from Iraty Forest

In each location a group of samples were collected in an area of 200 m combining it into an homogeneous sample. The samples were stored in LDPE bags, transported in portable fridges and refrigerated until the first steps of its preparation (to avoid degradation of the lichens).

The species of lichens were *Parmelia caperata*, *Parmelia sulcata* and *Lobaria pulmonaria*. The last one will be also used to asses the different accumulation between species, without finding significant differences.



Parmelia sulcata



Parmelia caperata



Lobaria pulmonaria

Figure 9: Species of lichens collected

The selection of these species of study (Figure 9) was done because of their presence in all the sampling sites, while the rest of species found only grew in a few places. The lichens were collected between 1 and 2 m in the tree barks from the ground, to assure that they are not contaminated by the soil.

2.2 Preparation of samples

2.2.1 Decontamination of material the storage and analysis

Glass and quartz vials

LDPE bottles

LDPE tubes

Eppendorfs

It's necessary to carry out a carefull decontamination procedure for all the material. This process involves several steps following this protocol:

- Inmersion in a RBS bath(liquid detergent, concentrated, alkaline and foaming) for 1 hour of ultrasonic bath
- Wash with ultrapure water
- Inmersion in a HNO₃ bath (Reagent quality 10% v/v(x1)) for 1 hour of ultrasonic bath
- Wash with ultrapure water
- Inmersion in a HNO₃ bath (Instra quality 10% v/v (x1)) for 1 hour of ultrasonic bath
- Inmersion in a Milli-Q water bath($\rho < 18 \text{m}\Omega \text{cm}^{-1}$) for 1 hour (3 times)
- Dry in Laminar flow hood Class 100
- Storage in double LDPE bags

2.2.2 Liophilization, separation and grinding



Figure 10: Separation of mosses and barks from the lichens



Figure 11: Retch mill with Teflon balls and vials

The samples were freeze-dried to eliminate the presence of water. After, a treatment by hand (with gloves and tweezers, as it is shown in Figure 10) allow to separate lichens mainly from mosses and tree barks. Finally, the samples were grinded in a Retch mill (Figure 11) with Teflon balls to obtain an homogeneous fine powder.

2.2.3 Acid digestion of the samples (HPA-S)

The solid samples were mineralized using a HPA-S system before their analysis by Inductively Coupled Plasma coupled with Mass Spectrometry (ICP-MS).

Organic matter of the samples is eliminated by digestion with nitric acid in a High Pressure Asher System (Anton Paar) shown in figure 12. HPA device yield a more efficient digestion than Microwaves system in the case of silicates. These compounds contribute to matrix effects in the analysis mainly when these samples are going to performed precise isotopic analysis. So matrix effects are minimized using high pressure digestion.

This device exert over the samples (introduced in quartz vials closed with Teflon film) a high pressure (130 bars) and also high temperature(300°C). The program of the HPA-S used follows different steps:

1. Room temperature → 80°C
2. 80°C → 120°C (2°C/min)
3. 300°C (2,5 h)
4. Decrease of temperature until room temperature (aprox. 1 h)

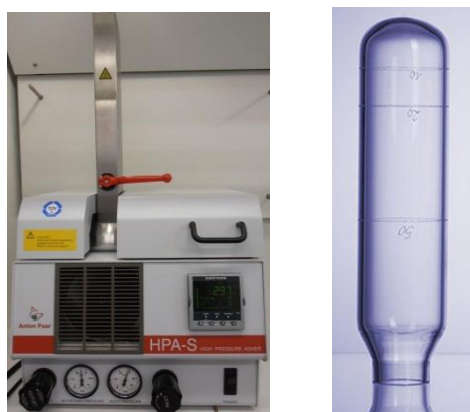


Figure 12: HPA-S from Anton Paar and quartz vials used for the digestion.

Reagents

- HNO₃ sub-boiled*
- Ultrapure Milli-Q water ($\rho > 18 \text{ M}\Omega \text{ cm}^{-1}$)

*Sub-boiled acid is obtained using a DST-1000 Sub-Boiling Distillation System (Savillex, Minnetonka, USA)

Procedure

1. Around 1 g of lichen's powder is introduced into the quartz vials.
2. 5 mL of sub-boiled nitric acid is added to start the pre-digestion 18 hours before the mineralization to facilitate the process in the HPA-S. It's necessary to cover the vials with Teflon film to avoid the loss of acid and to allow the exit of CO₂. The addition takes place in a Clean Room to avoid contamination of the liquid samples.
3. After this time the quartz vial is covered with a quartz disc and sealed with Teflon film.
4. The vials are introduced in the HPA-S for approximately four hours of process.

5. Once the process is finished, the samples carried to the Clean Room are transferred to vials rinsing it with 1 mL of ultrapure Milli-Q water (2 times).

2.3 Determination of Trace Metal Elements (TME)

2.3.1 Inductively coupled plasma coupled with Mass Spectrometry Analysis

2.3.1.1 Principle

The elements are analyzed in an Argon plasma induced by high frequency. The sample transformed in aerosol is introduced by pneumatic nebulization in the plasma where it suffers desolvation, dissociation, atomization and ionization of the elements. The quadrupole mass analyzer is able to separate the different anions by its ratios m/z .

This technique represents multiple advantages in the trace element analysis. It's possible to do multielemental analysis with low limits of detection ($<1\text{ng/g}$) in a wide lineal range. Quantification is carried out by comparison with calibration plots introduced previously to the analysis of samples in the software program.

Metals of main interest analyzed by this technique are: Li, Ti, V, Cr, Mn, Ni, Cu, Rb, Ag, Cd, Sb, Ce, Pb, U, Zn, As, Zr, Sn.

2.3.1.2 Material and equipment

- LDPE tubes (5 mL)
- Micropipettes Eppendorf
- Mass Spectrometer coupled with Inductively Coupled Plasma Thermo Fisher Scientific (Waltham, USA) (Figure 13)



Figure 13: ICP-MS Thermo Fisher Scientific (Waltham, USA)

2.3.1.3 Reagents

- Ir solution (1000 ppm)
- In solution (1000 ppm)
- Tune F multielemental solution
- Tune A F50 multielemental solution
- HNO_3 sub-boiled

2.3.1.4 Experimental procedure

1. Solutions of the samples: (dilutions in HNO₃ 1%)
 - Dilution 1:1000 for Sr, Zn, Mn and Pb
 - Dilution 1:100 for Pb and the rest of metals analysed

A solution of In/Ir (1 ppb) is added to all the samples as an internal standard to correct mass bias.

2. Optimization of the device:

One hour before the analysis it is necessary to start the plasma. The optimization of the parameters of analysis takes place using the multielemental solution Tune A (1 ppb).

3. Quantification:

Calibration plot is prepared using multielemental solutions* of Tune F50 with concentrations of 0;0,1;0,5;1;2 ppb.

*This multielemental solution does not contain Ir, so it is necessary its in addition.

4. Validation

It is carried out with Certified Reference Materials shown in the Annexe 7. In the table 3 it is presented a comparison between certified values and concentrations obtained by measure of CRM.

Table 3: Assesment of the method with CRM of lichens.

	Metals	V	Cr	Mn	Cu	Sr	Ce	Pb
BCR-482	Certified values (mg kg ⁻¹)	-	4,1±0,1	63±7	7,0±0,2	9,3±1,1	1,3±0,2	41±1,4
	n=7 measurements	-	5,4±1,9	44±25	8,0±0,4	9,8±0,9	1,4±0,25	39±1
IAEA-336	Certified values (mg kg ⁻¹)	1,5±0,2	-	-	3,6±0,5	-	-	4,9±0,6
	n=5 measurements	1,3±0,7	-	-	3,8±0,6	-	-	2,6±1,5

2.3.2 Determination of mercury by Atomic Absorption

2.3.2.1 Principle

The total content in Hg of lichens has been analysed by Cold Vapor Atomic Absorption Spectroscopy (CV-AA) using an AMA-254.

The technique is based in the capability of atoms in its fundamental state to absorb light of certain wavelength, specific for each element. By Lambert-Beer law the amount of light absorb can be related with the concentration of the element of interest.

The AMA-254 technique of direct combustion features a combustion/catalyst tube (750°C) that decomposes the sample in an oxygen-rich environment and removes interfering elements. A gold amalgamator trap collects all mercury from the evolved gases. Once the amalgam is formed the temperature increased to 600°C to release the atomic mercury. It arrives to detector where is analyzed at a wavelength of 254 nm.

The concentration is determined by comparison with a calibration plot previously introduced.

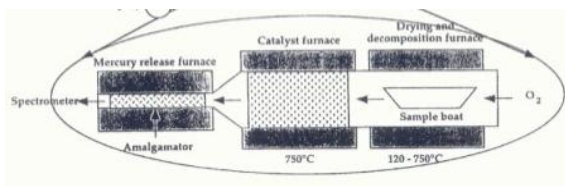


Figure 14: Scheme of AMA-254 analysis steps.

2.3.2.2 Material and equipment

- Nickel boats
- Analytical balance
- LECO AMA 254 Mercury Analyzer

2.3.2.3 Reagents

- Tort II
- BCR-482
- IAEA-336

2.3.2.4 Procedure

The analysis is performed with 30 mg of each powder sample in the nickel boats . After ten samples one standard tort II is introduced (and also at the beginning and the end of the analysis). After each standard 100µL of Milli-Q water are added to a boat to clean the device and reduce memory effects.

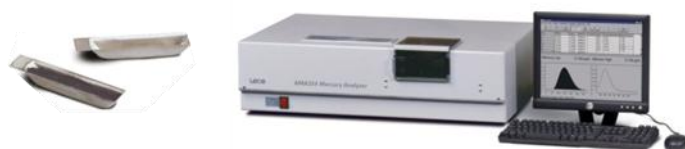


Figure 15: LECO AMA-254 and nickel boats for sample introduction

2.3.2.5 Validation:

It is carried out with Certified Reference Materials shown in the Annexe 7. In the table 4 it is presented a comparison between certified values for mercury and concentrations obtained by measure of CRM.

Table 4: Assesment of the method with certified Hg materials of lichens.

BCR-482	Certified values	0,48±0,02
	n=6 measurements	0,45±0,01
IAEA-336	Certified values	0,20±0,04
	n=4 measurements	0,17±0,01

2.4 Isotopic analysis

The isotopic compositions of mercury and lead are interesting since it can be used as environmental tracer of mercury sources and transformations. The slight difference between isotopic ratios can be analyzed using a Multiple Collector Inductively Coupled Plasma Mass Spectrometer. The high precision of detection for this mass spectrometer opened up a new dimension for environmental investigations.

2.4.1 Principle

Samples in solution (mineralized solution of lichen's powder) were introduced in the nebulizer transforming it into an aerosol. This aerosol passes through a nebulization chamber where only the smallest particles are directed to the plasma.

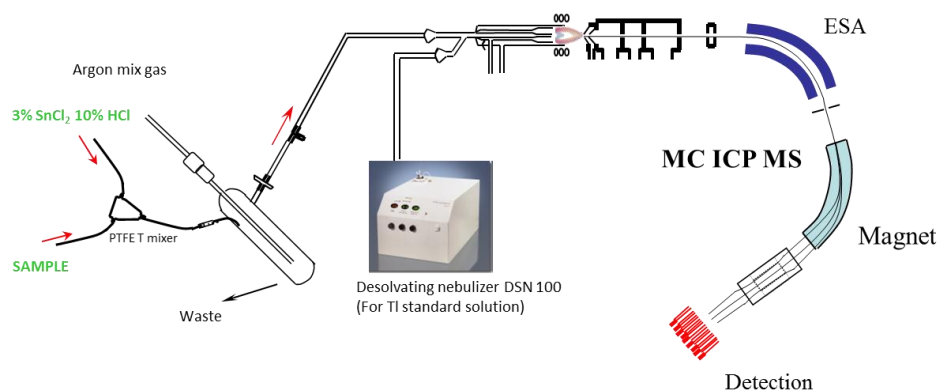


Figure 16: Scheme of MC-ICPMS with desolvating nebulizer for Tl introduction and system introduction of SnCl₂ for mercury analysis.

2.4.2 Multi- Collection

At the exit of the collision cell ions are focalized by a lens system and they pass throughout an electrostatic field and after a magnetic field. This ions arrive to the detector constitute of several cups of Faraday. Ions are separated by their ratio mass-charge and the separation can be quantified by the next equation:

$$\frac{m}{z} = \frac{B^2 r^2}{2V}$$

m = mass of the ion B = magnetic field
z = charge of the ion r = radius
V = voltage difference

A Faraday cup is just an electrode from which electrical current is measured while a charge particle beam (electrons or ions) impinges on it. The shape helps minimize loss of secondary electrons that would alter the current measurement. A deep cup with an electron repeller plate minimizes secondary electron loss. The arrival of positive ions to the detector creates an induced current of electrons in order to neutralize the positive charges.

How the Faraday cup works

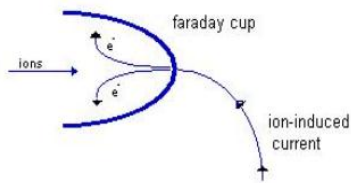


Figure 17: Mechanism of faraday cup to induce ion current.

2.4.3 Equipment

A Nu Plasma HR MC-ICPMS from Nu Instruments (Wrexham, U.K.) was employed to measure the isotopic ratios of Hg and Pb. The detector configuration allows measuring simultaneously the seven stable isotopes of Hg.



Figure 18: Picture of MC-ICP-MS of Nu Instruments

This device incorporates a System for cold vapor generation to produce efficient Hg^0 flow. The DSN-100 Desolvating Nebuliser System (Nu Instruments Wrexham, U.K.) , that is shown in Figure 16, for TI standard solution introduction.

2.4.4 Isotopic analysis of mercury

2.5.4.1 Material and Reagents

- LDPE tubes
- Micropipettes Eppendorf
- SnCl_2 3% /HCl 10% (reductor solution)
- Milli-Q water
- TI standard solution NIST SRM 997
- HNO_3 (2% ultrex)
- HNO_3 10% /HCl 2% (matrix of the samples)
- Hg standard solution NIST SRM 3133
- Hg standard solution UM- Almadén
- Certified reference material IAEA 336 (Annexe 7)
- Certified reference material BCR 482 (Annexe 7)

2.4.4.2 Experimental procedure

(in agreement with the method proposed by [Bergquist and Blum, 2007](#))

Hg(II) of mineralized samples is reduced to Hg⁰ by reaction with SnCl₂ (Cold Vapor Generation CVG). This method is a way to purify Hg for the analysis with generation of a flow of mercury to the plasma and reduced to the minimum matrix effects. The system of sample and SnCl₂ is shown in Figure 16.

All the samples and standards should have the same concentration of Hg (previously analyzed by AMA 254) to be introduced for MC-ICPMS analysis and it is estimated as 1 ppb in a matrix of HNO₃/HCl (10%, 2%% respectively). The sample-standard bracketing requirements are the measurement of NIST 3133 before and after each sample to correct the signal, and the measure of UM-Almadén each ten samples to validate the measurements.

Correction of instrumental mass bias

This correction is done by measurement of the isotopic composition of the certified thallium isotope standard (NIST 997, with ²⁰⁵Tl/²⁰³Tl ratio 2,38714). This solution is introduced in line using the DSN Desolvating System explained above.

Normalization of measurements

As it was recommended by [Bergquist & Blum, 2007](#) all the measurements must be done using the Hg standard NIST 3133. The sample-bracketing consist in the measurement of the standard before and after the sample to refer the value to the NIST 3133.

Reporting of uncertainties

Relative to a Hg standard of the University of Michigan made from metallic Hg mined from Almadén, Spain (UM-Almadén). This standard is introduced each 7 samples during the analysis and it is used to validate the measurements. Uncertainty is quantified as the double of standard deviation from the CRM (BCR-482) unless it is lower than UM-Almadén. In that case we would report the standard deviation from this standard.

Validation of the method

Because of the lack of certified reference materials for isotopic analysis of these samples, the validation of the methodology is carried out by comparison with the values reported by Estrade et al.,2010 (published in Geostandars and Geoanalytical Research). Which ones have been accepted as reference values.

Table 5: Data of δ and Δ obtained in the MC-ICP-MS Analysis compared to those published by Estrade et al., 2010 as reference values.

	n	$\delta^{202/198}$ (‰)	$\delta^{201/198}$ (‰)	$\delta^{200/198}$ (‰)	$\delta^{199/198}$ (‰)	Δ^{201} (‰)	Δ^{199} (‰)	Δ^{200} (‰)
BCR-482 (Estrade et al., 2010)		-1,48±0,24	-1,71±0,24	-0,68±0,16	-0,99±0,17	-0,60±0,08	-0,62±0,12	0,06±0,08
UM-Almadén (Estrade et al., 2010)		-0,51±0,15	-0,41±0,11	-0,26±0,10	-0,14±0,09	-	-	-
BCR-482	10	-1,69±0,15	-1,94±0,11	-0,79±0,07	-1,07±0,11	-0,66±0,06	-0,64±0,07	0,06±0,05
UM-Almadén	7	-0,58±0,16	-0,46±0,11	-0,29±0,07	-0,15±0,08	-0,03±0,04	-0,007±0,06	0,003±0,07

*Uncertainties reported correspond to the double of the standard deviation (2SD ‰).

2.4.5 Isotopic analysis of lead

2.4.5.1 Extraction of lead

As the method to generate a flow of Hg^0 it is necessary to separate lead from the matrix to minimize interferences in multiple collection analysis. The extraction was achieved by ionic exchange with DOWEX 1x8 resine.

a) Materials and equipment

- Eppendorfs
- Tips
- Hot-Block vials
- Hot-Block system (Fig. 19)
- Centrifuge Eppendorf (Fig. 20)



Figure 19: Hot-Block System



Figure 20: Centrifuge Eppendorf

b) Reagents:

- Dowex 1x8 resine
- HBr solution (0,5N)
- HCl solution (6N, Sub-boiled)
- Milli-Q water
-

c) Experimental procedure

1. Preparation of the column

Resine is activated with HCl ultrex. A volume of 200-300 μL of this mixture is added to a tip where a Teflon piece is previously introduced. The column is optimized:

- 500 μL of Milli-Q water
- 1 mL HBr (0,5N)
- 200 μL of Milli-Q water
- 1 mL HCl (6N)
- 200 μL Milli-Q water
- 500 μL HBr (0,5 N)

2. Preparation of the sample

An aliquot of 1.4 mL of mineralized solution is evaporated in the Hot-Block overnight at 85°C. To the residue obtained 0.6 mL of 0.5 N HBr were added and placed in ultrasonic bath for 1h. The solution obtained is transferred to an eppendorf and centrifuged 5 min at 40000 rpm. The supernatant was passed through the column. Introducer is again in 0.6 mL eppendorf HBr (0.5 N), stirred manually and centrifuged under the same conditions. The supernatant was passed through the column along with 0.6 mL initials.

3. Elution of the matrix

To perform this step 0.5 mL of 0.5 N HBr (x2) are added to the column.

4. Recovery of lead

Lead retained on the column and separated from the matrix is recovered with 1 mL of 6N HCl (x3) on Hot-Block vials. The HCl solution was evaporated overnight at 85°C in Hotblock. To the residue was added 0.5 ml of HNO₃ (2%) and sonicated for 1 h. From these solutions are prepared dilutions of 20 ppb of Pb, containing 2 ppb of Tl for Multiple Collection Analysis.

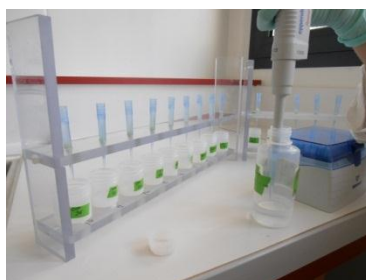


Figure 21: Lead extraction on tips.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Metal concentration

3.1.1.1 Mercury

Concentrations of mercury in the lichens of both sides of the Pyrenees are reported in Annexes 1 and 2. They are found in the order of concentration expected for mountain ecosystems. Minimum and maximum values in the different locations from the border are showed in Table 6. Also a comparison between concentrations found in similar ecosystems and samples has done (Table 7).

Table 6: Minimum and maximum concentrations of mercury find in the lichens of the Iraty Forest at different altitudes.

Location Altitude (m)	Spanish side samples			French side samples		
	n	Median (mgkg ⁻¹)	Min –Max (mgkg ⁻¹)	n	Median (mgkg ⁻¹)	Min-Max (mgkg ⁻¹)
<400	-	-	-	1	1,4	-
400-800	-	-	-	5	0,482	0,135-0,768
800-900	7	0,218	0,121-0,694	4	0,526	0,168-0,738
900-1000	8	0,183	0,138-0,409	1	0,253	-
1000-1200	7	0,181	0,139-0,356	6	0,335	0,167-0,457
>1200	8	0,228	0,176-0,296	4	0,233	0,214-0,781

Table 7: Comparison between concentrations of Hg in the Iraty Forest and others found in similar samples and ecosystems

Reference	Site	Specie of lichen	Hg (mg kg ⁻¹)
Iraty Forest	Near Atlantique Pyrenees	<i>Parmelia caperata</i> , <i>Parmelia sulcata</i> , <i>Lobaria Pulmonaria</i>	0,121-0,781*
Estrade et. al., 2010	Rural area (North-East France)		0,077-0,115
	Urban area (North- East France)	Lichens	0,088-0,151
	Industrial Valley North-East France)		0,082-0,148
Becnel 2004	Near rivers	Lichens	0,013-0,141
Bargagli et al., 2002	Forest	<i>Parmelia caperata</i>	0,05-0,18
Loppi et al.,1994	Forest Italy	<i>Parmelia caperata</i>	0,055-1,38
Ribeiro et al.,2004	Natural Park	<i>Usnea sp. L</i>	0,11-1,17

*Data of the Iraty Forest are reported removing the highest value corresponding to a lower altitude to compare with the rest of similar samples for forest ecosystems.

3.1.1.2 Lead

As shown before, concentrations of lead in the lichens of both sides of the Pyrenees are reported in Annexes 1 and 2, and the minimum and maximum values are shown in the Table 8.

Table 8: Minimum and maximum of concentrations for lead of Iraty Forest in both sides of the border between Spain and France.

Location	Spanish side samples			French side samples		
Altitude	n	Median (mg kg ⁻¹)	Min –Max (mg kg ⁻¹)	n	Median (mg kg ⁻¹)	Min-Max (mg kg ⁻¹)
<400 m	-	-	-	1	9,4	-
400-800 m	-	-	-	5	5,2	1,6-6,9
800-900 m	7	15,5	4,9-87,5	4	14,5	8,3-23,4
900-1000 m	8	8,5	2,2-38,3	1	12,8	-
1000-1200 m	7	9,9	5,7-20,1	6	23,3	6,4-21,8
>1200 m	8	13,1	6,9-22,5	4	9,8	8,1-16,1

The values obtained were compared with bibliography to determine if they are in agreement with the values found for other lichens in similar ecosystems. The comparison is shown in Table 8.

3.1.1.3 Other metals

The concentrations of the other metals analyzed by ICP-MS are reported in Annexes 1 and 2. Concentrations of trace metal elements analyzed can be divided in the samples into high concentrated elements over 5 mg kg⁻¹ (Mn>>Zn>Ti>Sr) and low concentrated elements under 5 mg kg⁻¹ (Rb>Pb>Cu>V>Cr>Ni>Ce). One additional group can be listed with the very low concentrated elements under 1,5 mg kg⁻¹ in which there are placed Li>Cd>Hg>Sn>Ag>Sb>U (the three last elements in a range of concentration of 30-60 ppb).

A comparison has been done between minimum and maximum values found in the Iraty Forest and others published (Table 9).

Table 9: Min-Max values of concentrations for all the elements analyzed (except mercury concentrations, reported before) and comparison with publications of lichen samples.

	PYNATEO Project	Veschambre et al.,2008	Veschambre et al.,2008	Loppi et al.,1994	Rizzio et al.,2001	Koz et al.,2010
mg kg ⁻¹	Iraty Forest	Valle de Aragon (Forest ecosystem)	Valle d'Aspe (Forest ecosystem)	Apeninos (mountain ecosystem)	Natural Park (Italy)	Lichens near highway
Pb	1,6-87,5	0,1-11	0,2-19	5,5-24,8	24-38	22-157
Mn	71,8-497	14-56	6-308		23-47	222-1976
Zn	11,7-55,7	13-41	1-206	43-134,7	47-65	77-432
Sr	8,05-36,5	9-31	7-27			
Rb	4,9-26,03	1,3-5,7	0,2-17		14-26	
Cu	3,15-19,9	1,5-6,5	0,3-11	5,4-16,2	1,2-6,2	112-2140
V	0,81-7,88	1-6,6	0,5-3,5		0,6-1,8	
Ce	0,56-5,93	1-3,6	0,3-1,9		0,14-0,34	
Sn	0,12-6,8	0,11-0,41	0,11-0,37			70-450
Cd	0,16-0,88	0,8-0,38	0,04-0,3	0,24-0,95	0,09-0,37	
U	0,02-0,14	0,02-0,19	0,04-0,12		0,02-0,04	
Sb	0,13-0,67	0,18-0,20	0,006-0,4		3,2-6,6	
Cr	0,49-16,8			0,94-4,07	0,19-0,67	35-191
Ni	0,90-5,			1,19-4,59	0,20-1,10	12,8-62,2
Ti	0614,5-79,7				9-23	3,1-12,3

3.1.2 Isotopic signature of mercury

Results of the analysis of mercury isotope ratios are shown in Annexe 3. The data presented are all δ values for all the isotope ratios determined. The mean values are shown in Table 10.

Table 10: Minimum and maximum values and standard deviations from the mean for mercury isotopic data. Standard deviations are calculated respect to the mean values.

	$\delta^{204/198}$ Hg	$\delta^{202/198}$ Hg	$\delta^{201/198}$ Hg	$\delta^{200/198}$ Hg	$\delta^{199/198}$ Hg	Δ^{201} Hg	Δ^{199} Hg	Δ^{200} Hg
Min	-6,9	-4,69	-3,43	-2,32	-1,22	-0,47	-0,04	-0,04
Max	-2,5	-1,71	-1,39	-0,90	-0,39	0,03	0,08	0,06
SD	1,21	0,81	0,55	0,41	0,17	0,13	0,14	0,04

3.1.2.1 Mass dependent fractionation

Three-isotope representation (Figure 22) of results from multiple collection analysis show expected values and trends for isotopes.

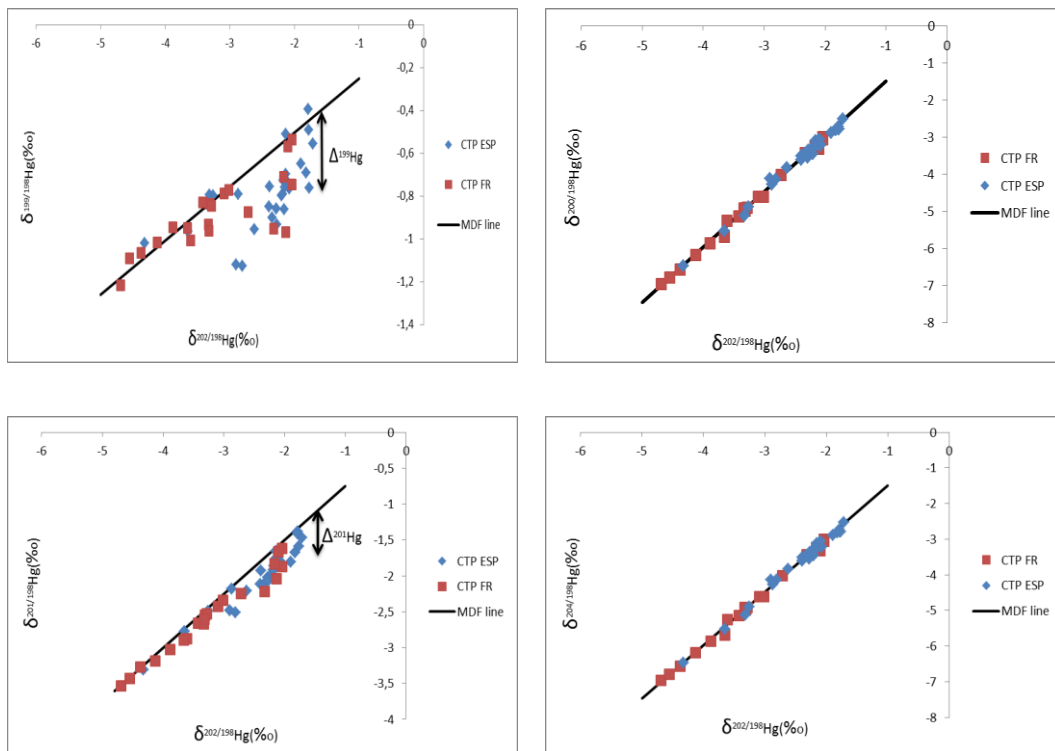


Figure 22: Three-isotope plots for isotopes 199,200,201 and 204 respect 198 vs. 202 respect 198.

Even isotopes show a lineal MDF following the theoretical line, while odd isotopes show deviations from MDF line corresponding to the MIF, which is reported as the deviation in isotope ratios from the theoretical MDF line.

In figure 23 it is shown a comparison between $\delta^{202/198}$ for lichen samples and other different samples.

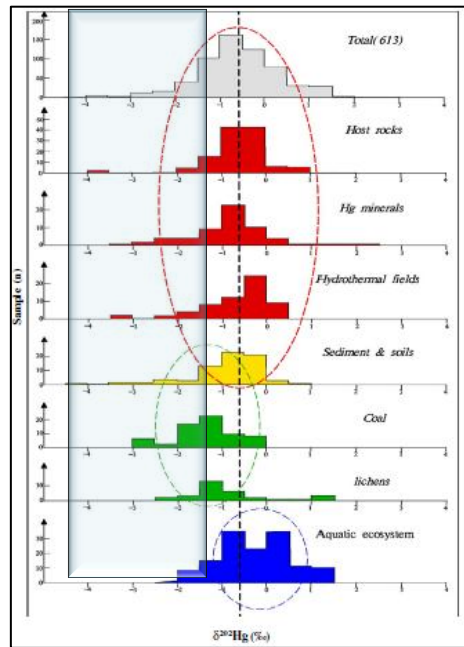


Figure 23 : Comparison between values from literature for $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ for different samples and the values of the samples of lichens in the Iraty Forest (represented in a blue rectangle).

3.1.2.2 Mass Independent Fractionation

The representation of $\Delta^{199}\text{Hg}$ vs. $\Delta^{201}\text{Hg}$ for all the samples shows a lineal trend in the negative region (Figure 24).

The lineal trend of the samples of lichens from Iraty forest were plotted with the theoretical line for photo-reduction processes of Hg^{2+} to Hg^0 according to the experiments carried out by Bergquist & Blum, 2007. In addition, they correspond to the expected values found in literature for other samples of lichens in France (Carignan et al., 2009, Estrade et al., 2010) and also other different natural samples as coal, soil, sediment, peat and mosses (Sherman et al., 2012; Biswas et al., 2008; Ghosh et al., 2008).

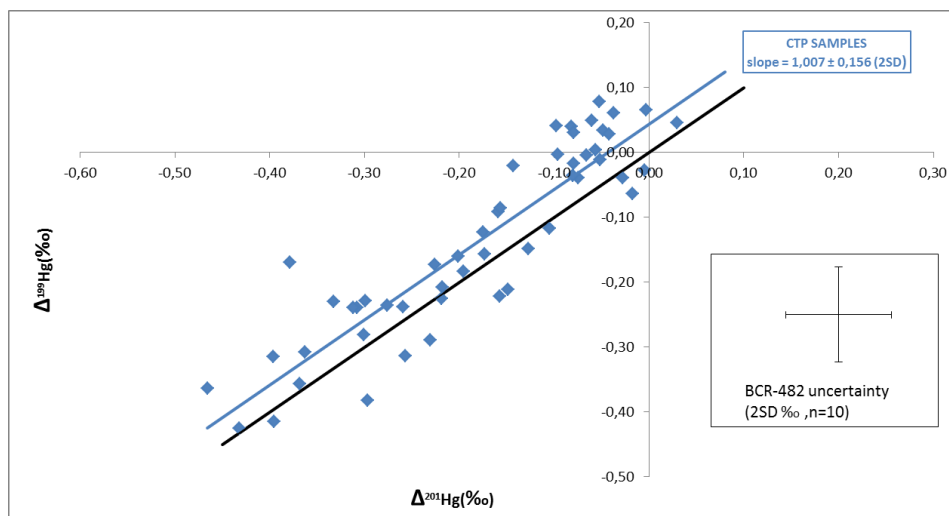


Figure 24: Representation of $\Delta^{199}\text{Hg}$ vs. $\Delta^{201}\text{Hg}$. The uncertainty determined correspond to the double of the standard deviation from the mean for the CRM (BCR-482).

The comparison just with samples of lichens and one set of precipitation samples (Sherman et al., 2012) is shown in Figure 25. The values in red correspond to unpublished data of lichens collected near Iraty Forest (Lichens 64 Project).

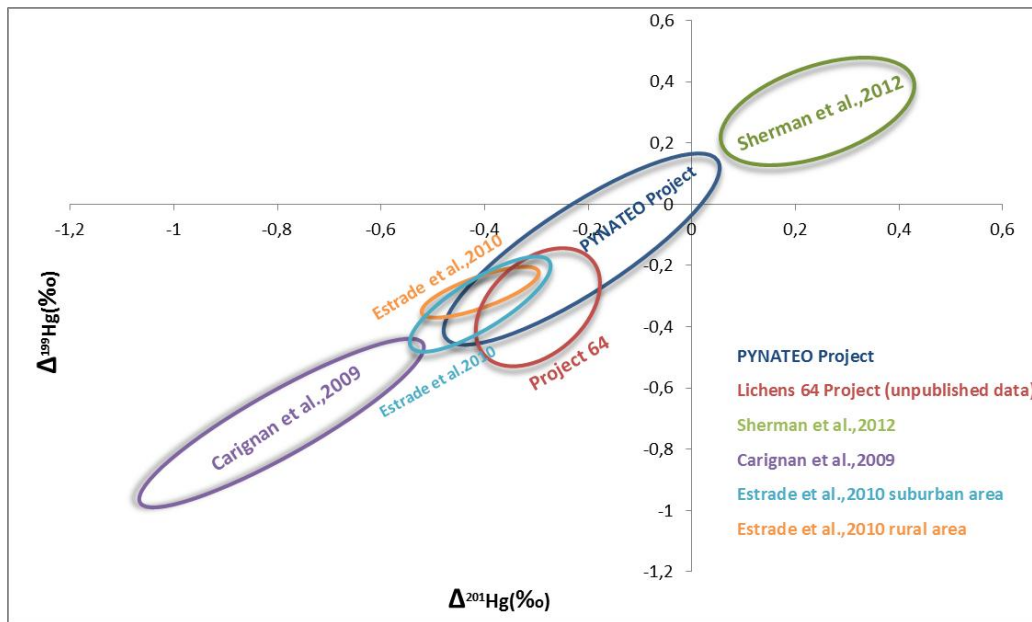


Figure 25: Representation of $\Delta^{199}\text{Hg}$ vs. $\Delta^{201}\text{Hg}$ for comparison between isotopic mercury values of Iraty Forest and others found in lichen samples.

3.2 Discussion

3.2.1 Mercury, lead and other metals

The concentrations are in agreement with others reported for lichens in different forest ecosystems (Table 6).

Mercury concentrations in remote areas determined in the lichen *Parmelia caperata* are reported in the range of 0,11-0,57 mg kg^{-1} , while in contaminated areas the concentrations found were in the order of 0,58-1,17 mg kg^{-1} (Ribeiro et al., 2004). So the values found in the samples of the Iraty Forest are in agreement with that. Highly concentrated samples with values in the order of 0,7 – 1,4 mg kg^{-1} correspond to the lowest sampling sites, maybe in rural areas with activities related to agriculture.

A comparison with literature was carried out to determine if the concentrations found in the lichens of the Iraty Forest are in agreement with others found in similar ecosystems. For the main anthropic elements studied (Cu, Cd, Sb, Sn, Pb, Zn, Ni and also Hg) the results are in the range expected in all the cases for mountain areas (Rizzio et al., 2001).

The main discrepancies have been detected in the case of lead where concentrations can vary from 6 mg kg⁻¹ in the Aspee Valley (Veschambre et al., 2008) to 30 mg kg⁻¹ in a natural park of Italy (Rizzio et al., 2001). Lead concentrations could be explained by long-range transport, because there's no local sources of lead in this ecosystem.

Titanium values were compared with others for contaminated areas showing that concentrations found in lichens from a forest near a motorway (Koz et al, 2010) are more than three times less than Iraty Forest lichens. From these observations, the concentrations of Ti can be attributed to natural sources taking into account that is a highly abundant metal in the terrestrial crust.

In the case of manganese the concentrations found are lower than for the lichens of this study (mean value of ~180 mg kg⁻¹). In the publications cited before (Rizzio 2001, Veschambre et al. 2008) the concentrations of Mn were from 35 to 66 mg kg⁻¹. Higher values are reported for lichens sampling near motorway (Koz et al., 2010) around 600mg kg⁻¹.

3.2.2 Comparison between French and Spanish sites

3.2.1.1 Mercury and other metals

French samples show a general higher level of mercury. (Figure 26). Statistical analysis of data reveals that mean value for French side samples is significantly higher than one for Spanish side (Figure 27).

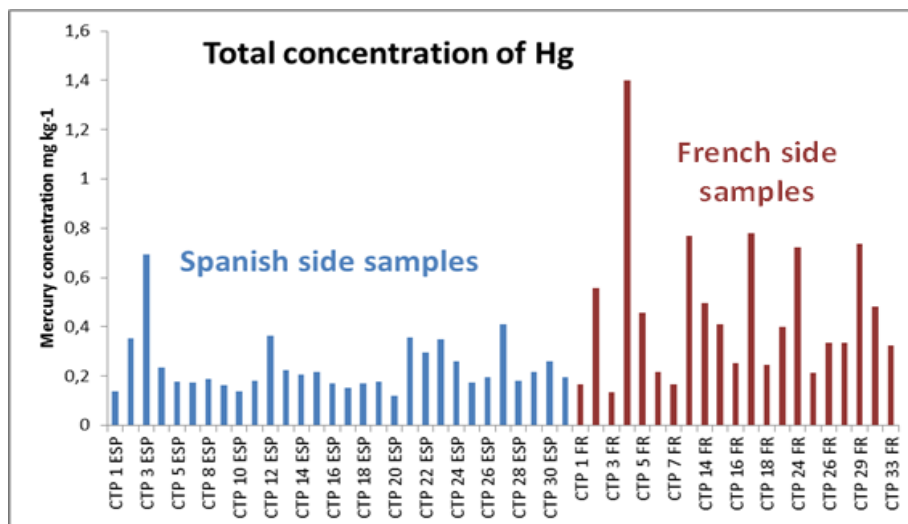


Figure 26 : Concentrations of mercury (mg kg⁻¹) for all the samples.

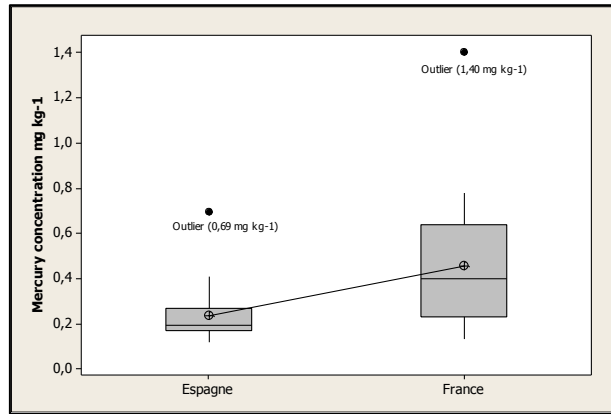


Figure 27 : T-test for the mean concentrations of mercury from Spanish and French samples, $t_{exp}=3,21$ is higher than $t_{theor}=0,22$ in a confidence interval of 95%, so there are significant difference between mean values.

Also standard deviation from the mean of the samples collected in the Spanish and French sides are reported (Figure 28).

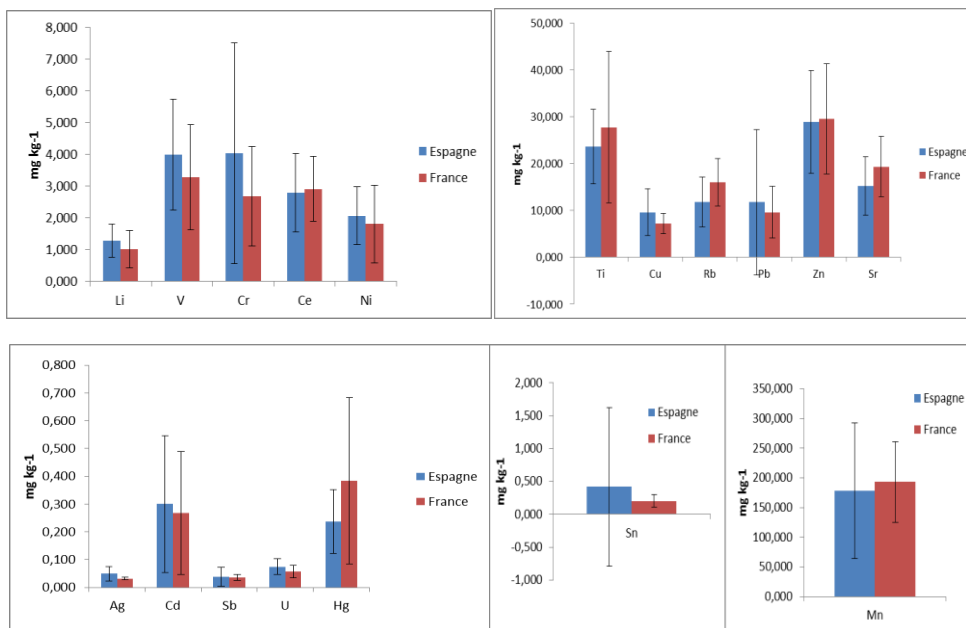


Figure 28: Comparison between Spanish side and French side mean values for the elements

T-test was done for the main anthropic metals: Pb, Cu, Cd and Zn (Figure 29). For lead, copper and zinq no significant differences were found. In a confidence interval of 95% the values were $t_{experimental} < t_{theoretical}$ ($1,40 < 4,29$; $3,17 < 3,26$; $1,16 < 3,40$). Only in the case of cadmium a statistical difference between mean values was found ($t_{exp}=0,16 > t_{theor}=0,01$).

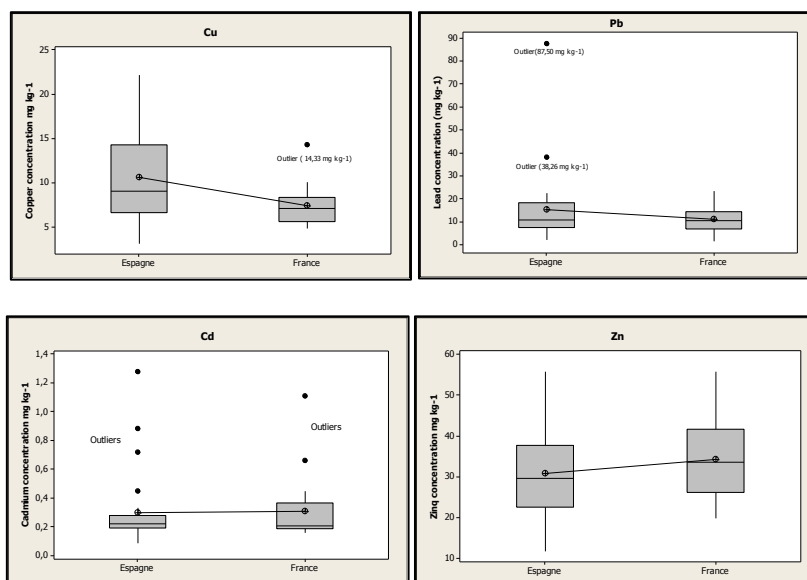


Figure 29: Diagrams of t-test for spanish and french samples of lead and copper.

Neither significant differences were found respecting to the location of the sampling site for statistical analysis of the data by Pearson's correlation or Principal Component Analysis (Annexe 5). However in the case of copper as it happens with mercury (reported before) mean values of Spanish samples were higher than ones for French samples (Figure 29)

3.2.1.2 Mercury isotopes

The coupling of MDF and MIF (Figure 43) anomalies in the plot $\Delta^{199}\text{Hg}$ against $\delta^{202}\text{Hg}$ reflect two general different sources of pollution and also the coincidence of each source with Spanish and French samples respectively. $\Delta^{199}\text{Hg}$ values close to 0‰ reflect the presence of a local anthropic source of mercury for the French sampling sites with lowest $\delta^{202}\text{Hg}$ values found in recent literature. On the other hand, anomalies found for Spanish side samples show that the main fractionation process occurring in those sampling sites is photo-reduction.

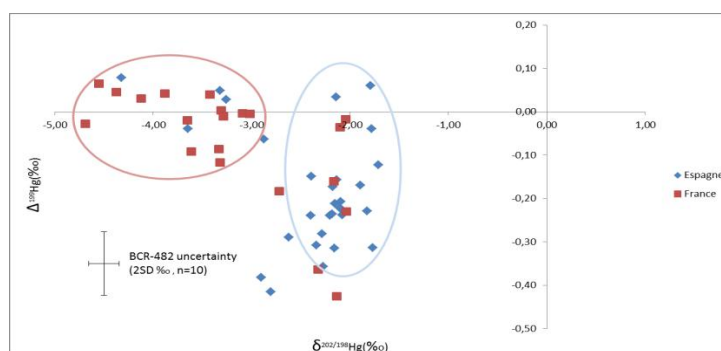


Figure 30: Representation of $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ values. The uncertainty determined corresponds to the double of the standard deviation from the mean for the CRM (BCR-482).

3.2.2 Influence of altitude

3.2.2.1 Mercury and trace metals

Regarding to the different altitudes of the sampling sites significant differences were found. In figure 31 diagrams for anthropic metals are reported.

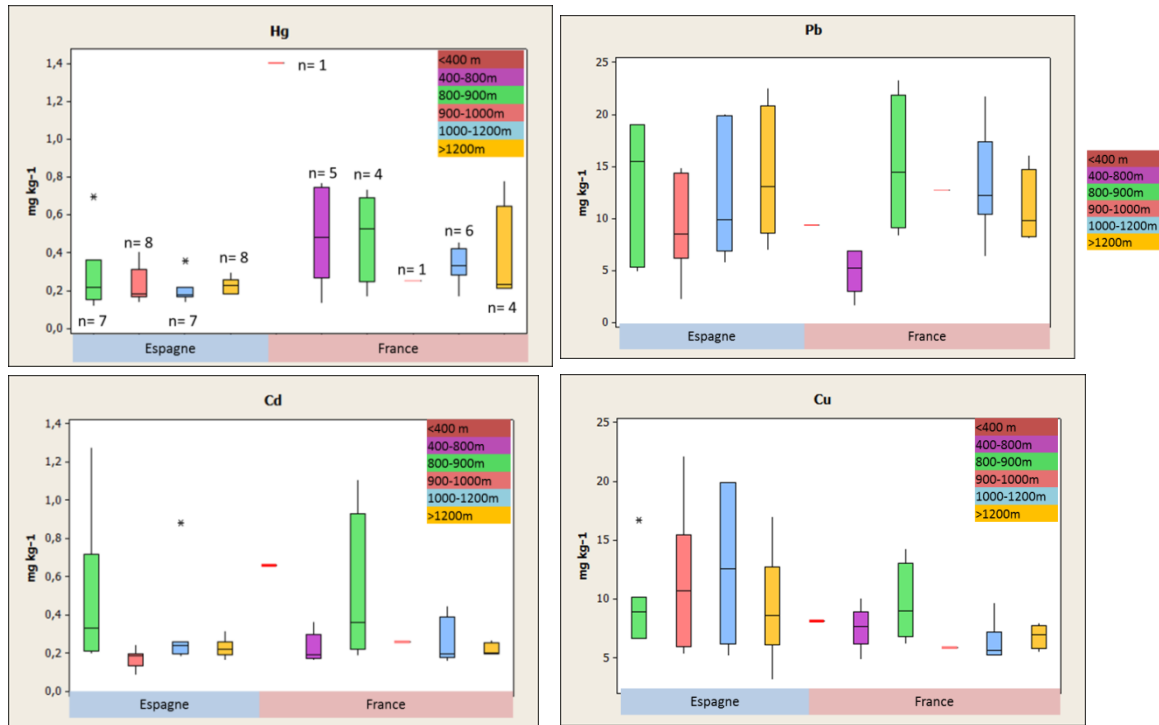


Figure 31: Box diagrams for spanish and french side samples between different altitudes for mercury lead, copper and cadmium.

Regarding to the concentrations in terms of height it can be observed that some of the higher concentration values correspond to samples collected at a lower altitude (Figure 32), while samples collected over 1200 m of altitude show concentrations under 0,4 mg kg⁻¹. Specifically, the highest concentration of mercury corresponds to the only sample collected within 400 m. This sample is probably influenced by some human activities. We can make the hypothesis on the French side that we have a specific source of contamination which has its origin in the piedmont of Iraty forest.

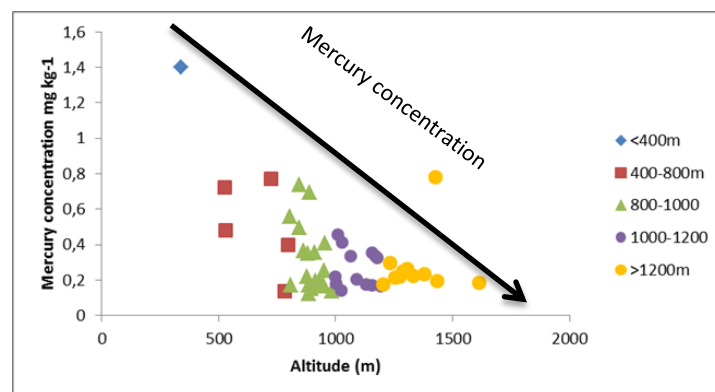


Figure 32: Concentrations of mercury (mg kg⁻¹) in terms of altitude showing a general decreasing trend.

3.2.2.2 Mercury isotopes

Regarding to the $\Delta^{199}\text{Hg}$ vs. $\Delta^{201}\text{Hg}$ with the altitude (Figure 33), the highest values correspond also with the lowest altitude. This fact reveals that MIF values are related with mercury total concentration.

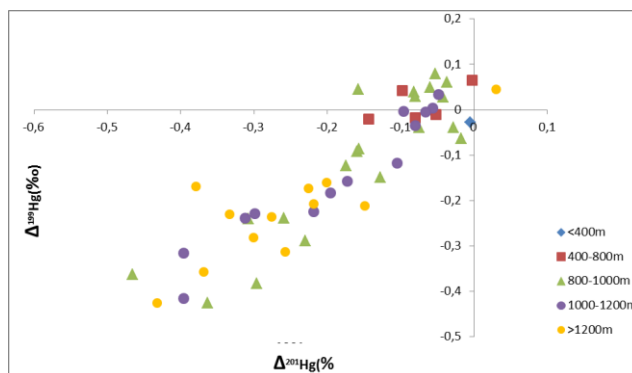


Figure 33: Plot of $\Delta^{199}\text{Hg}$ vs. $\Delta^{201}\text{Hg}$ reported before in Figure 24 in terms of altitude of the sampling sites.

The comparison between MIF and MDF (Figure 34) in terms of altitude show slightly opposite direction trends for all the samples. The largest anomalies found for $\Delta^{199}\text{Hg}$ correspond to the remote areas at the highest sampling sites.

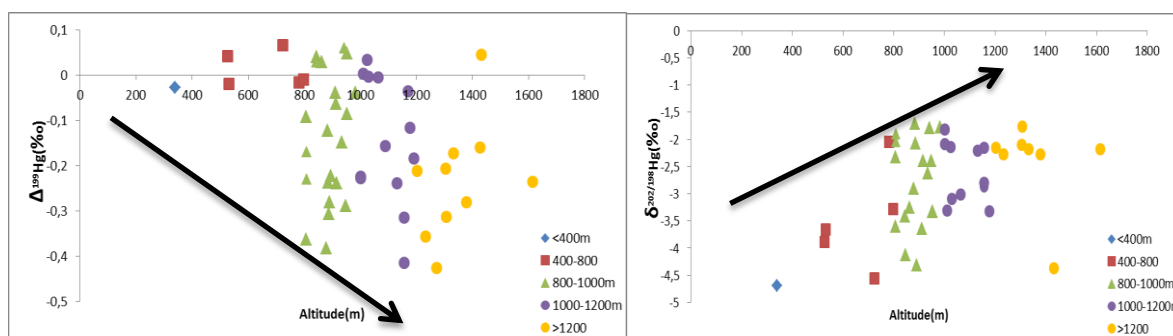


Figure 34 : Representation of $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ vs. altitude.

3.2.3 Relationship between Hg isotope signature and concentration

Diagram of figure 35 suggest the existence of two mercury sources with different isotopic signatures and concentrations. The values of $\delta^{202}\text{Hg}$ higher than -2 ‰ can be attribute to background pollution, and correspond mainly to Spanish side samples. However the most of samples from French side show a lineal trend. These values reflect a local mercury source, unknown until the moment, because there aren't similar values for other samples studied by isotopic analysis. Regarding to the same plot for the MIF(Figure 35) it can observed different trends followed by Spanish side samples and French side.

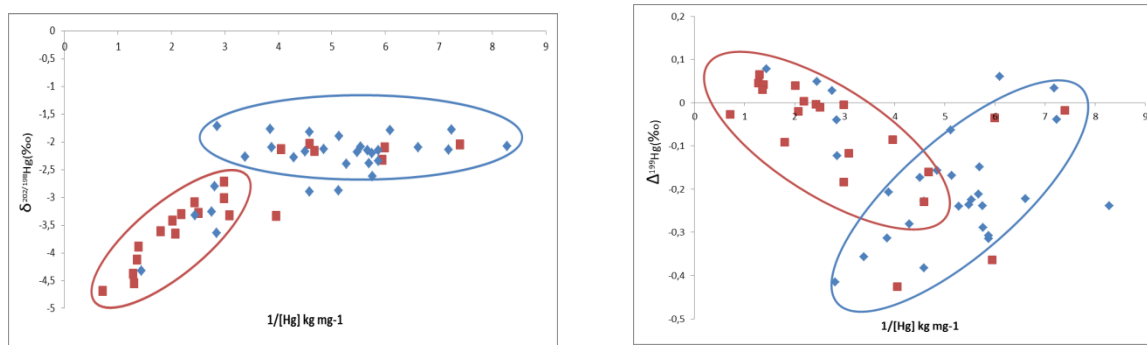


Figure 35: Representations of $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ vs. $1/[\text{Hg}]$ (kg mg^{-1}).

3.2.4 MDF vs. MIF isotope signature

This representation allows determining mercury inputs (Figure 36). Results obtained for other samples of lichens collected in France, near Iraty Forest (yellow circle) correspond to the region of the large density of points from Spanish sides, corresponding to the background signature of mercury in this region. However, the most of the points from the samples collected in the French side follow a different trend. This trend can be classified as a local source of mercury for all that samples. As it is different from background signature can be attribute to an anthropic input of local origin.

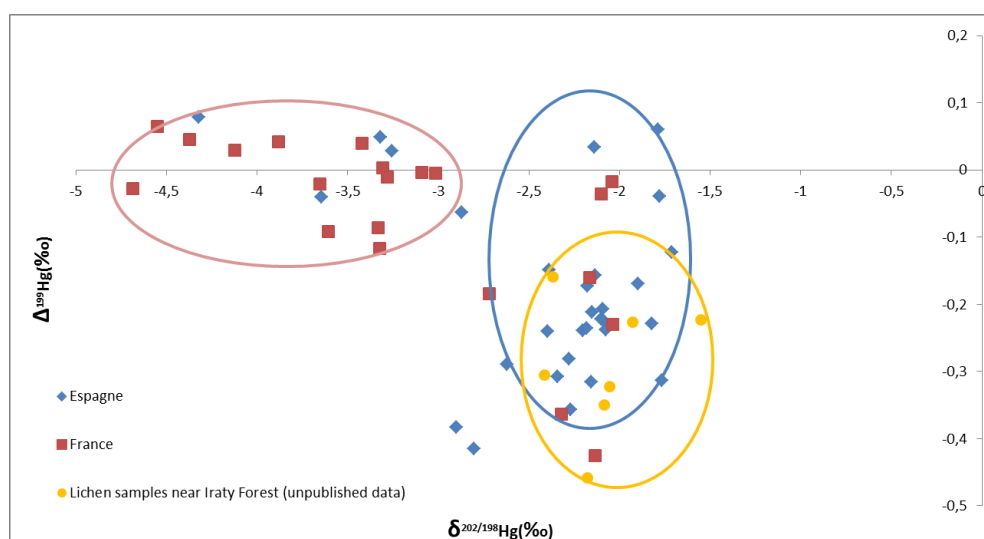


Figure 36: Representation of $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ values from literature. Red circles correspond to French side samples and Blue circles to Spanish side samples from Iraty Forest. The yellow points correspond to unpublished data for points near Iraty Forest (Lichens 64 Project).

After these observations, statistical correlation between all the elements and altitude of the sampling sites, was carried out, but just with the samples exhibiting the $\delta^{202}\text{Hg} < -3$. Some significant correlations between concentrations of geochemical tracers were found. Li, Ce, U and V are correlated also with Ni. Also different metallic pollutant concentrations are correlated as Cr, Sb with Pb or Cd with Cu. Maybe these relationships between concentrations of the metallic pollutants have a common anthropic source.

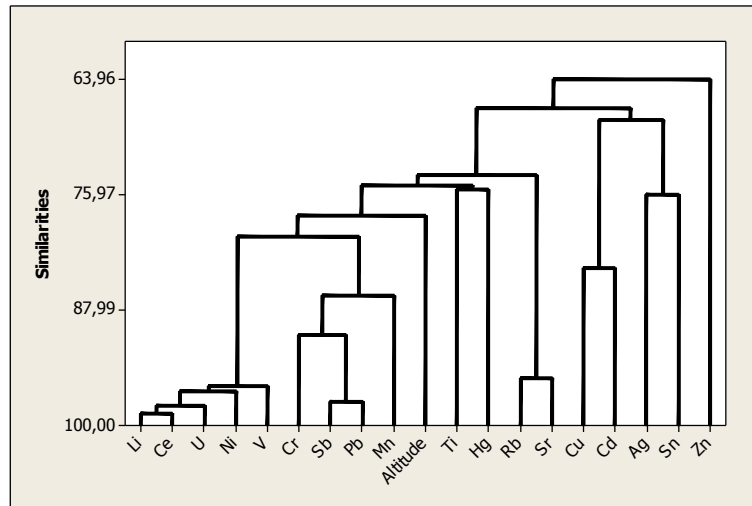


Figure 37 : Cluster dendrogram for the elements concentration with the lower $\delta^{202}\text{Hg}$ values, (those following a trend close to MIF= 0).

Also a comparison with other samples of lichens was carried out in Figure 38. Also it has been incorporated the data some precipitations collected near a Coal-Fired Power Plant in Florida, USA (Sherman et al.,2012). These values are close to the negative values found for the unknown anthropic input in the Iraty Forest.

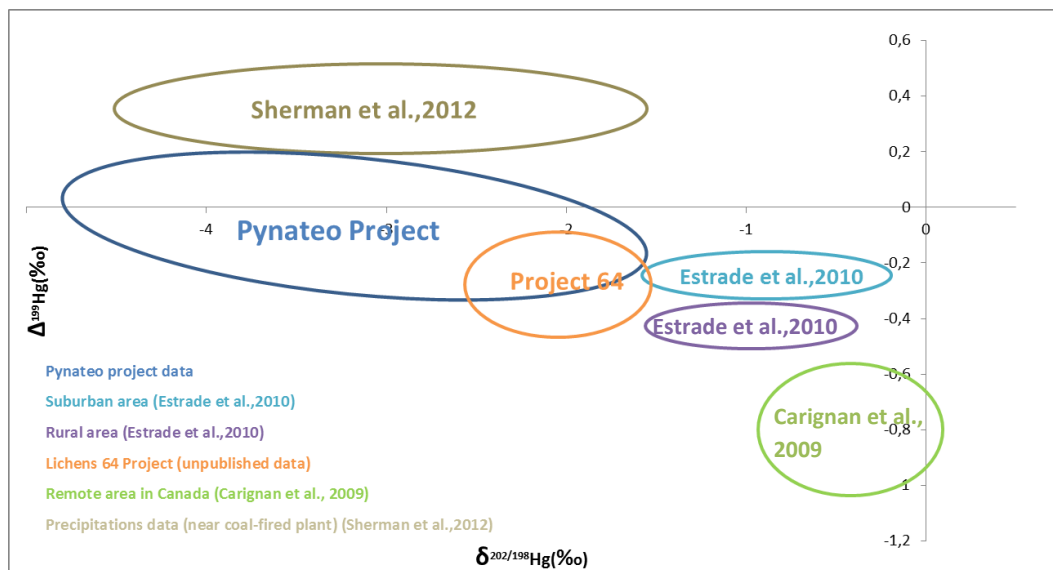


Figure 38 : Comparison between the samples of lichens from Iraty forest (PYNATEO Project) and other samples of lichens (Carignan et al.2009; Estrade et al,2011) and precipitation data (Sherman et al.,2012). The orange circle corresponds to the values obtained in lichens near Iraty Forest (unpublished data of Lichens 64 Project).

4. CONCLUSION

Lichens are revealed as good biomonitors for the measurement of trace metal elements in the Iraty Forest, in both sides of the Pyrenees. Results obtained by the two methods (AMA-254 for mercury and ICP-MS for the rest of elements) are in agreement with other published for similar ecosystems and the same species of lichen (*Parmelia Caperata*). Both methodologies have been validated with CRM from lichens, presented in the Annex 7. The measurements done with these biomonitors allow to develop distribution maps of atmospheric pollution (not presented yet in this report).

Those metals which main contribution in the atmosphere are anthropogenic sources show a lower level of concentration respect to highly contaminated areas. For example for mercury the mean difference between polluted and unpolluted area is about 0,4 mg kg⁻¹ (Published by [Ribeiro et al.,2004](#)), corresponding to the values obtained in Iraty Forest to the ones for unpolluted areas. In addition, the concentrations for mercury showed a general decrease with altitude.

In the case of lead, the values obtained are higher respect to others reported for similar environment (especially the ones reported by [Vescambre et al.,2008](#) also in another area of the Pyrenees). Regarding to the fact that Iraty Forest is a natural ecosystem which hasn't any known source of lead, these concentrations could be caused by long-scale transport.

For the isotopic analysis of mercury the results are also in agreement with others found in closer areas to the Iraty Forest. The methodology was validated also using the reference values reported by [Estrade et al.,2009](#) for the CRM BCR-482 and the Reference Standard UM-Almadén. The value are also correlated with mercury concentrations and, as it was expected, with the altitude.

The most relevant information obtained by this data is the general trend of a set of 20 samples towards $\delta^{202}\text{Hg} \sim -4\%$, which hasn't been published until now. This evidence reveals the presence of an unknown mercury source in the Iraty Forest and also the coincidence that most of those samples correspond to the points with the lowest elevation. For the rest of samples the values are in agreement with other data unpublished yet (Lichens 64 Project) corresponding to background pollution in the forest ecosystem.

In perspective, the study that will be carried out after the presentation of this report will be the isotopic analysis of lead. This analysis could provide more information and maybe reveal the source of mercury found in the Iraty Forest.

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6. Annexes

Annexe 1: Concentrations of trace metal elements analyzed by ICP-MS and mercury analyzed by Atomic Absorption (AMA-254) in Spanish side samples and altitudes.

Samples	Hg		V		Cr		Sn		Ni		Li		Ag		Cd		Sb		Ce		U	
	mg kg ⁻¹	SD	mg kg ⁻¹	SD	mg kg ⁻¹	SD	mg kg ⁻¹	SD	mg kg ⁻¹	SD	mg kg ⁻¹	SD	mg kg ⁻¹	SD	mg kg ⁻¹	SD	mg kg ⁻¹	SD	mg kg ⁻¹	SD	mg kg ⁻¹	SD
CTP 1 ESP	0,139	0,002	2,325	0,010	2,498	0,000	0,255	0,00582	6,148	0,038	0,938	2E-06	0,038	0,019	0,199	0,006	0,044	0,023	1,603	0,632	0,061	9E-07
CTP 2 ESP	0,352	0,038	3,908	0,018	2,891	0,010	0,188	3,9E-06	2,775	0,011	1,184	0,007	0,066	0,033	0,197	4E-06	0,029	0,015	1,960	0,770	0,067	4E-07
CTP 3 ESP	0,694	0,025	4,458	0,014	2,177	0,026	0,192	4,9E-06	2,849	0,027	0,938	4E-06	0,049	0,024	0,199	5E-06	0,039	0,020	1,668	0,663	0,070	7E-07
CTP 4 ESP	0,233	0,015	5,991	0,070	16,836	0,094	0,252	2,9E-06	3,378	0,019	1,538	0,018	0,067	0,033	0,319	3E-06	0,064	0,032	2,710	1,071	0,099	9E-07
CTP 5 ESP	0,176	0,023	0,949	0,008	1,411	0,000	0,115	2,5E-06	1,642	0,000	0,432	2E-06	0,025	0,012	0,122	3E-06	0,035	0,018	0,703	0,276	0,032	1E-07
CTP 6 ESP	0,174	0,007	3,912	0,024	6,581	0,026	0,202	2,9E-06	2,677	0,000	1,563	6E-06	0,042	0,020	0,196	3E-06	0,034	0,018	2,337	0,905	0,079	7E-07
CTP 8 ESP	0,190	0,000	4,035	0,027	2,228	0,000	0,215	3E-06	2,940	0,013	0,986	6E-06	0,037	0,018	0,184	3E-06	0,034	0,017	1,633	0,646	0,065	7E-07
CTP 9 ESP	0,164	0,007	0,818	0,000	0,903	0,000	0,195	1,2E-06	1,342	0,017	0,628	6E-06	0,051	0,025	0,185	1E-06	0,042	0,022	0,794	0,305	0,039	3E-07
CTP 10 ESP	0,138	0,009	2,630	0,018	3,987	0,022	0,227	3,5E-06	2,075	0,019	1,013	0,01	0,060	0,030	0,246	3E-06	0,035	0,018	1,403	0,545	0,064	4E-07
CTP 11 ESP	0,181	0,008	3,432	0,018	2,720	0,000	0,160	3,3E-06	2,249	0,019	1,275	6E-07	0,070	0,035	0,243	3E-06	0,032	0,017	1,946	0,770	0,074	4E-07
CTP 12 ESP	0,364	0,011	2,120	0,010	1,715	0,010	0,211	1,5E-06	1,748	0,010	0,751	5E-06	0,071	0,035	0,451	2E-06	0,029	0,015	1,226	0,483	0,045	4E-07
CTP 13 ESP	0,222	0,020	6,264	0,026	3,398	0,000	0,214	2,7E-06	3,102	0,015	1,488	0,025	0,071	0,035	0,276	3E-06	0,049	0,025	2,554	1,004	0,093	1E-06
CTP 14 ESP	0,206	0,015	6,126	0,052	3,567	0,021	0,233	2,9E-06	3,886	0,014	1,863	0,011	0,052	0,026	0,262	3E-06	0,039	0,020	3,183	1,272	0,113	9E-07
CTP 15 ESP	0,218	0,001	2,025	0,008	1,966	0,018	0,334	0,00248	1,913	0,022	0,640	5E-06	0,040	0,020	1,278	0,002	0,032	0,016	1,134	0,447	0,043	5E-07
CTP 16 ESP	0,170	0,011	5,475	0,000	3,832	0,026	0,248	5,2E-06	3,997	0,028	1,883	0,013	0,040	0,020	0,261	5E-06	0,029	0,015	2,757	1,103	0,094	1E-06
CTP 17 ESP	0,151	0,008	4,678	0,345	2,951	0,229	0,187	0,01508	2,605	0,171	1,189	0,083	0,165	0,089	0,211	0,015	0,033	0,016	2,373	1,052	0,075	5E-06
CTP 18 ESP	0,170	0,011	5,509	0,059	9,578	0,053	0,177	3,8E-06	3,873	0,031	1,825	6E-06	0,037	0,018	0,334	4E-06	0,025	0,012	2,876	1,131	0,092	1E-06
CTP 19 ESP	0,176	0,006	5,194	0,040	2,971	0,035	0,129	2,9E-06	2,601	0,019	1,717	0,016	0,042	0,021	0,220	3E-06	0,020	0,010	2,672	1,052	0,096	9E-07
CTP 20 ESP	0,121	0,007	2,196	0,000	6,204	0,026	6,813	0,02889	1,831	0,000	0,739	3E-06	0,060	0,030	0,720	0,029	0,024	0,012	1,134	0,441	0,039	4E-07
CTP 21 ESP	0,356	0,004	4,571	0,026	3,955	0,030	0,246	3,5E-06	2,509	0,030	1,623	0,009	0,039	0,019	0,885	4E-06	0,031	0,016	2,388	0,935	0,098	1E-06
CTP 22 ESP	0,296	0,019	5,434	0,064	3,561	0,038	0,148	5,7E-06	4,875	0,020	1,496	6E-06	0,053	0,026	0,223	6E-06	0,030	0,015	2,356	0,933	0,089	1E-06
CTP 23 ESP	0,351	0,003	6,276	0,040	6,276	0,008	0,184	3,2E-06	3,822	0,028	2,014	0,013	0,038	0,018	0,295	3E-06	0,214	0,112	3,721	1,470	0,119	5E-07
CTP 24 ESP	0,260	0,017	3,605	0,000	3,443	0,008	0,149	4,1E-06	2,917	0,027	1,448	5E-06	0,034	0,017	0,164	4E-06	0,023	0,012	2,028	0,807	0,076	4E-07
CTP 25 ESP	0,174	0,004	2,456	0,028	2,708	0,029	0,198	3,6E-06	1,222	0,016	0,965	4E-06	0,027	0,013	0,183	4E-06	0,022	0,011	1,543	0,612	0,059	8E-07
CTP 26 ESP	0,195	0,012	1,742	0,000	1,828	0,000	0,122	7,9E-07	1,116	0,011	0,836	2E-06	0,027	0,014	0,086	8E-07	0,013	0,007	1,048	0,410	0,031	7E-07
CTP 27 ESP	0,409	0,035	2,542	0,035	0,489	0,011	0,292	0,00568	1,460	0,014	0,549	7E-06	0,031	0,015	0,197	0,006	0,039	0,020	0,911	0,364	0,033	3E-07
CTP 28 ESP	0,183	0,000	3,406	0,045	3,053	0,035	0,181	0,00307	1,378	0,016	1,125	0,013	0,030	0,015	0,215	0,003	0,043	0,021	1,793	0,715	0,071	2E-06
CTP 29 ESP	0,218	0,007	6,345	0,105	3,287	0,020	0,156	3,8E-06	3,380	0,042	1,503	0,017	0,032	0,016	0,238	4E-06	0,030	0,016	2,876	1,158	0,096	1E-06
CTP 30 ESP	0,258	0,011	7,197	0,027	4,559	0,043	0,151	2,7E-06	5,263	0,189	2,812	0,015	0,041	0,020	0,187	3E-06	0,021	0,011	4,663	1,838	0,144	9E-07
CTP 31 ESP	0,195	0,005	3,946	0,042	2,813	0,017	0,123	2,5E-06	2,054	0,010	1,218	0,017	0,034	0,017	0,221	3E-06	0,018	0,009	2,026	0,802	0,070	1E-06

Annexe 1: Concentrations of trace metal elements analyzed by ICP-MS and mercury analyzed by Atomic Absorption (AMA-254) in Spanish side samples and altitudes

Samples	Mn		Ti		Cu		Rb		Pb		Zn		Sr		Altitude m
	mg kg-1	SD	mg kg-1	SD	mg kg-1	SD	mg kg-1	SD	mg kg-1	SD	mg kg-1	SD	mg kg-1	SD	
CTP 1 ESP	302,166	3,029	21,224	0,680	19,927	0,260	17,631	0,008	9,912	0,029	55,753	0,430	19,906	0,231	1025
CTP 2 ESP	180,961	0,626	31,233	0,392	22,195	0,291	21,341	0,117	12,932	0,059	22,788	0,146	25,250	0,038	911
CTP 3 ESP	453,710	2,296	20,496	0,742	16,743	0,169	13,847	0,072	18,214	0,090	36,659	0,240	15,492	0,162	890
CTP 4 ESP	277,760	1,457	28,354	0,325	17,065	0,119	7,523	0,074	22,558	0,093	41,629	0,262	10,102	0,050	1378
CTP 5 ESP	218,825	1,509	15,206	0,098	5,388	0,035	20,970	0,254	7,856	0,066	26,031	0,134	30,784	0,276	935
CTP 6 ESP	232,443	0,422	19,129	0,472	10,930	0,091	17,804	0,035	38,270	0,143	27,658	0,154	26,168	0,070	949
CTP 8 ESP	251,853	0,998	23,168	0,241	10,539	0,077	11,104	0,035	14,921	0,072	26,391	0,152	14,220	0,016	916
CTP 9 ESP	128,766	0,460	16,085	0,204	12,613	0,042	27,579	0,161	8,173	0,097	30,777	0,285	31,693	0,043	942
CTP 10 ESP	71,879	0,258	16,753	0,266	16,455	0,056	12,286	0,030	5,619	0,020	26,350	0,249	13,177	0,073	984
CTP 11 ESP	176,302	0,370	21,688	0,373	12,623	0,083	11,993	0,050	6,927	0,048	33,490	0,291	13,457	0,093	1002
CTP 12 ESP	116,339	0,771	17,640	0,180	8,978	0,141	22,601	0,132	4,954	0,021	19,780	0,098	26,987	0,138	863
CTP 13 ESP	312,862	0,940	28,881	0,406	13,923	0,076	14,665	0,069	22,309	0,151	38,390	0,237	19,413	0,037	1332
CTP 14 ESP	191,778	0,580	30,253	0,322	19,939	0,144	11,801	0,015	20,110	0,066	36,388	0,157	16,339	0,105	1090
CTP 15 ESP	497,665	4,109	38,139	0,528	8,993	0,074	11,223	0,049	11,495	0,072	42,584	0,290	16,084	0,128	878
CTP 16 ESP	332,687	1,567	30,728	0,607	9,709	0,056	12,394	0,061	14,808	0,056	37,432	0,253	17,855	0,070	1157
CTP 17 ESP	124,010	0,114	20,650	1,344	8,762	0,741	6,072	0,294	15,512	0,083	24,232	0,184	10,719	0,047	896
CTP 18 ESP	200,612	34,147	36,236	0,457	6,599	0,031	9,156	0,071	19,057	0,074	41,692	0,238	16,255	0,025	887
CTP 19 ESP	216,982	1,804	17,975	0,501	8,611	0,093	13,499	0,063	10,329	0,039	28,398	0,275	14,921	0,077	1204
CTP 20 ESP	89,249	0,553	19,605	0,165	6,692	0,038	4,895	0,025	5,328	0,044	24,939	0,252	8,749	0,042	886
CTP 21 ESP	248,525	0,617	46,605	0,838	15,442	0,106	13,729	0,045	9,384	0,034	45,102	0,242	15,990	0,048	1157
CTP 22 ESP	129,609	0,236	26,003	0,609	9,254	0,043	7,582	0,037	16,537	0,060	53,005	0,145	11,539	0,055	1233
CTP 23 ESP	433,206	0,560	33,072	0,593	10,161	0,024	9,785	0,029	87,503	0,228	33,690	0,171	15,110	0,015	883
CTP 24 ESP	102,792	0,800	20,360	0,455	7,127	3,591	5,717	0,039	9,836	0,052	13,615	0,238	9,082	0,060	1308
CTP 25 ESP	71,994	0,532	21,035	0,590	6,178	3,650	12,935	0,149	5,745	0,039	15,727	0,102	12,787	0,076	1132
CTP 26 ESP	74,792	0,114	14,463	0,099	5,528	3,552	8,679	0,072	2,208	0,007	11,750	0,046	8,054	0,035	914
CTP 27 ESP	207,285	0,946	19,797	0,283	7,450	3,495	9,909	0,085	8,934	0,048	33,773	0,251	10,502	0,062	954
CTP 28 ESP	84,876	0,743	22,212	5,771	3,147	0,061	15,330	0,124	6,975	0,014	20,744	0,141	14,919	0,065	1615
CTP 29 ESP	132,526	1,270	27,691	0,373	5,212	0,165	9,086	0,077	19,894	0,122	36,584	0,251	13,140	0,023	1001
CTP 30 ESP	156,535	0,652	37,735	0,514	8,645	0,036	13,891	0,080	15,935	0,094	18,388	0,206	17,269	0,026	1304
CTP 31 ESP	146,531	0,150	17,936	0,663	5,835	0,069	9,079	0,056	8,267	0,028	22,050	0,062	11,233	0,023	1433

Annexe 2: Concentrations of trace metal elements analyzed by ICP-MS and mercury analyzed by Atomic Absorption (AMA-254) in French side samples and altitudes

Samples	Hg		V		Cr		Sn		Ni		Li		Ag		Cd		Sb		Ce		U	
	mg kg ⁻¹	SD	mg kg ⁻¹	SD	mg kg ⁻¹	SD	mg kg ⁻¹	SD	mg kg ⁻¹	SD	mg kg ⁻¹	SD	mg kg ⁻¹	SD	mg kg ⁻¹	SD	mg kg ⁻¹	SD	mg kg ⁻¹	SD	mg kg ⁻¹	SD
CTP 1 FR	0,168	0,012	4,269	0,019	4,101	0,019	0,186	2,1E-06	4,446	0,034	1,023	0,015	0,029	0,015	0,325	2E-06	0,053	0,026	1,937	0,778	0,065	9E-07
CTP 2 FR	0,556	0,096	5,636	0,502	4,396	0,028	0,155	0,01406	5,067	0,449	2,546	0,213	0,034	0,017	0,397	0,035	0,032	0,016	4,714	2,095	0,106	4E-07
CTP 3 FR	0,135	0,009	2,028	0,149	0,732	0,013	0,130	0,0096	0,908	0,065	0,445	0,033	0,032	0,016	0,185	0,013	0,031	0,015	0,569	0,250	0,027	7E-07
CTP 4 FR	1,401	0,153	1,953	0,189	3,874	0,028	0,195	0,02047	3,102	0,283	0,811	0,07	0,038	0,019	0,663	0,058	0,044	0,022	1,415	0,660	0,045	9E-07
CTP 5 FR	0,457	0,005	4,062	0,234	4,524	0,411	0,228	0,01498	3,522	0,200	1,746	0,09	0,030	0,014	0,450	0,023	0,040	0,020	2,481	1,067	0,078	1E-07
CTP 6 FR	0,218	0,004	4,224	0,009	0,713	0,047	0,213	0,0041	3,090	0,012	1,337	7E-06	0,031	0,015	0,206	2E-06	0,051	0,024	2,360	0,937	0,087	7E-07
CTP 7 FR	0,167	0,017	2,424	0,008	2,001	0,166	0,555	0,00273	3,578	0,024	0,762	2E-06	0,024	0,012	0,185	5E-07	0,037	0,018	1,332	0,532	0,045	7E-07
CTP 9 FR	0,768	0,029	2,150	0,014	3,359	0,180	0,231	3,2E-06	3,157	0,034	0,871	0,015	0,032	0,016	0,192	1E-06	0,030	0,015	1,502	0,609	0,047	3E-07
CTP 14 FR	0,496	0,021	3,084	0,273	4,328	0,029	0,158	0,01472	2,322	0,217	0,689	0,056	0,033	0,016	0,188	0,017	0,042	0,021	1,437	0,656	0,044	4E-07
CTP 15 FR	0,412	0,036	3,579	0,395	6,496	0,031	0,251	0,02803	2,253	0,233	0,815	0,082	0,029	0,014	0,204	0,021	0,045	0,022	1,528	0,714	0,057	4E-07
CTP 16 FR	0,253	0,007	4,516	0,286	2,019	0,009	0,284	0,03662	2,960	0,202	0,780	0,043	0,028	0,014	0,262	0,016	0,067	0,033	1,492	0,659	0,053	4E-07
CTP 17 FR	0,781	0,239	4,868	0,529	4,444	0,441	0,171	0,02299	3,359	0,342	1,047	0,105	0,039	0,019	0,272	0,028	0,037	0,018	2,204	1,027	0,066	1E-06
CTP 18 FR	0,247	0,011	5,601	0,362	3,722	0,420	0,166	0,01129	3,770	0,233	1,749	0,119	0,048	0,024	0,196	0,014	0,031	0,015	2,896	1,268	0,102	9E-07
CTP 19 FR	0,399	0,032	1,074	0,053	2,009	0,141	0,126	0,00578	1,782	0,090	0,501	0,025	0,027	0,013	0,166	0,008	0,020	0,010	1,042	0,454	0,032	5E-07
CTP 24 FR	0,723	0,140	1,920	0,157	2,432	0,259	0,232	0,01951	2,749	0,211	0,711	0,055	0,032	0,016	0,234	0,019	0,035	0,017	1,338	0,601	0,039	1E-06
CTP 25 FR	0,214	0,014	7,889	0,359	4,744	0,335	0,263	0,01444	5,365	0,235	2,656	0,115	0,033	0,016	0,200	0,009	0,031	0,016	5,930	2,543	0,102	5E-06
CTP 26 FR	0,335	0,002	3,588	0,011	0,541	0,023	0,150	2,2E-06	2,543	0,009	1,027	5E-07	0,036	0,018	0,188	2E-06	0,031	0,015	1,757	0,707	0,059	1E-06
CTP 28 FR	0,335	0,051	4,435	0,064	2,024	0,176	0,184	0,00382	2,479	0,040	1,092	0,022	0,029	0,014	0,157	2E-06	0,039	0,020	1,924	0,789	0,066	9E-07
CTP 29 FR	0,738	0,083	1,495	0,016	4,560	0,208	0,177	2,7E-06	2,571	0,015	0,998	5E-06	0,032	0,016	1,109	5E-06	0,023	0,011	1,388	0,554	0,050	4E-07
CTP 31 FR	0,482	0,003	3,241	0,379	2,603	0,008	0,312	0,03801	2,700	0,288	1,169	0,125	0,032	0,016	0,365	0,043	0,032	0,016	1,942	0,896	0,059	1E-06
CTP 33 FR	0,325	0,000	4,655	0,254	3,187	0,053	0,141	0,00327	3,006	0,168	0,933	0,039	0,031	0,015	0,372	0,019	0,031	0,015	1,879	0,803	0,064	1E-06

Annexe 2: Concentrations of trace metal elements analyzed by ICP-MS and mercury analyzed by Atomic Absorption (AMA-254) in French side samples and altitudes

Samples	Mn		Ti		Cu		Rb		Pb		Zn		Sr		Altitude
	mg kg ⁻¹	SD	mg kg ⁻¹	SD	mg kg ⁻¹	SD	mg kg ⁻¹	SD	mg kg ⁻¹	SD	mg kg ⁻¹	SD	mg kg ⁻¹	SD	m
CTP 1 FR	207,898	1,345	45,272	0,162	9,341	0,047	16,537	0,033	17,424	0,089	62,743	0,536	24,401	0,035	808
CTP 2 FR	269,378	2,660	50,798	4,578	8,626	0,747	28,583	2,051	23,390	0,187	64,214	0,471	36,454	0,041	807
CTP 3 FR	40,209	0,129	14,881	0,968	7,527	0,511	21,349	1,073	1,642	0,012	44,868	0,168	20,900	0,079	782
CTP 4 FR	219,601	0,925	79,696	7,490	8,176	0,712	9,657	0,720	9,429	0,036	25,625	0,041	23,413	0,136	340
CTP 5 FR	164,136	0,303	50,774	2,439	9,694	0,507	24,221	0,936	21,812	0,077	25,661	0,019	28,892	0,121	1010
CTP 6 FR	217,996	1,016	24,428	0,581	7,110	0,023	11,066	0,074	8,080	0,043	25,950	0,035	12,082	0,108	1273
CTP 7 FR	108,039	0,415	16,153	0,295	6,431	0,057	11,177	0,061	6,366	0,038	31,158	0,039	11,473	0,066	1170
CTP 9 FR	234,764	1,692	19,521	0,249	7,723	0,067	16,003	0,116	6,788	0,025	26,828	0,047	20,134	0,045	724
CTP 14 FR	133,981	0,326	17,704	3,254	6,232	0,528	26,035	1,788	11,554	0,064	25,093	0,037	27,860	0,066	845
CTP 15 FR	269,220	1,329	23,740	2,654	5,305	0,560	15,817	1,344	11,819	0,058	23,403	0,019	17,463	0,082	1029
CTP 16 FR	361,227	3,212	32,881	2,152	5,886	0,377	14,953	0,798	12,794	0,053	25,838	0,036	17,282	0,036	952
CTP 17 FR	224,887	1,216	16,379	4,441	6,887	0,701	10,865	0,950	16,101	0,096	25,582	0,021	11,609	0,015	1427
CTP 18 FR	221,768	0,889	28,660	2,577	8,043	0,473	11,965	0,601	8,986	0,036	24,013	0,045	13,724	0,039	1290
CTP 19 FR	121,300	1,163	18,220	0,836	4,873	0,232	15,366	0,715	6,918	0,037	25,044	0,040	15,001	0,115	799
CTP 24 FR	183,347	0,434	26,888	1,966	7,687	0,612	17,458	1,230	5,245	0,017	25,201	0,026	24,191	0,056	526
CTP 25 FR	239,562	1,243	36,802	1,835	5,487	0,222	21,062	0,740	10,695	0,050	30,574	0,116	26,563	0,241	1257
CTP 26 FR	225,711	0,664	21,011	0,325	5,826	0,030	12,232	0,042	11,980	0,077	31,139	0,040	13,572	0,028	1064
CTP 28 FR	236,943	0,878	23,822	0,550	5,545	0,064	16,341	0,205	12,548	0,058	24,599	0,033	20,184	0,049	1192
CTP 29 FR	275,692	2,418	19,128	0,258	14,328	0,063	17,016	0,076	8,376	0,029	30,285	0,050	18,861	0,170	847
CTP 31 FR	205,252	0,512	40,703	4,899	10,089	1,131	16,373	1,608	4,433	0,037	26,040	0,032	20,440	0,098	532
CTP 33 FR	216,214	1,265	41,996	2,003	5,289	0,242	16,253	0,633	15,905	0,059	27,182	0,035	21,713	0,102	1177

Annexe 3: Data values for mercury isotope ratios and standard deviations (2SD) obtained by MC-ICP-MS Analysis.

Sample	$^{204/198}\delta$ (‰)	$^{202/198}\delta$ (‰)	$^{201/198}\delta$ (‰)	$^{200/198}\delta$ (‰)	$^{199/198}\delta$ (‰)	Δ^{201} (‰)	Δ^{199} (‰)	Δ^{200} (‰)
CTP 1 ESP	-3,14	-2,14	-1,66	-1,02	-0,51	-0,05	0,03	0,05
CTP 2 ESP	-5,53	-3,64	-2,78	-1,78	-0,97	-0,03	-0,04	0,06
CTP 3 ESP	-6,47	-4,32	-3,31	-2,19	-1,02	-0,05	0,08	-0,03
CTP 4 ESP	-3,55	-2,28	-2,02	-1,09	-0,86	-0,30	-0,28	0,05
CTP 5 ESP	-3,61	-2,39	-1,93	-1,23	-0,75	-0,13	-0,15	-0,03
CTP 6 ESP	-3,83	-2,62	-2,21	-1,35	-0,95	-0,23	-0,29	-0,04
CTP 8 ESP	-3,52	-2,39	-2,11	-1,19	-0,85	-0,31	-0,24	0,00
CTP 9 ESP	-2,73	-1,79	-1,38	-0,89	-0,39	-0,04	0,06	0,00
CTP 10 ESP	-2,79	-1,78	-1,42	-0,90	-0,49	-0,07	-0,04	-0,01
CTP 11 ESP	-3,23	-2,08	-1,80	-1,08	-0,75	-0,22	-0,22	-0,03
CTP 12 ESP	-4,89	-3,26	-2,49	-1,68	-0,80	-0,04	0,03	-0,04
CTP 13 ESP	-3,23	-2,17	-1,87	-1,10	-0,73	-0,23	-0,17	-0,01
CTP 14 ESP	-3,14	-2,13	-1,78	-1,05	-0,70	-0,17	-0,16	0,02
CTP 15 ESP	-4,13	-2,90	-2,48	-1,47	-1,12	-0,29	-0,38	-0,01
CTP 16 ESP	-3,22	-2,16	-2,02	-1,09	-0,86	-0,39	-0,31	-0,01
CTP 17 ESP	-3,07	-2,10	-1,74	-1,06	-0,75	-0,16	-0,22	0,00
CTP 18 ESP	-3,47	-2,34	-2,13	-1,15	-0,90	-0,36	-0,31	0,02
CTP 19 ESP	-3,11	-2,15	-1,77	-1,02	-0,76	-0,15	-0,21	0,05
CTP 20 ESP	-3,20	-2,08	-1,82	-1,05	-0,76	-0,26	-0,24	0,00
CTP 21 ESP	-4,14	-2,80	-2,51	-1,41	-1,13	-0,39	-0,41	-0,01
CTP 22 ESP	-3,36	-2,27	-2,08	-1,14	-0,93	-0,37	-0,36	0,00
CTP 23 ESP	-2,52	-1,71	-1,46	-0,82	-0,56	-0,17	-0,12	0,04
CTP 24 ESP	-2,79	-1,77	-1,59	-0,75	-0,76	-0,26	-0,31	0,13
CTP 25 ESP	-3,45	-2,20	-1,97	-1,04	-0,79	-0,31	-0,24	0,06
CTP 26 ESP	-4,28	-2,87	-2,18	-1,47	-0,79	-0,02	-0,06	-0,03
CTP 27 ESP	-5,13	-3,32	-2,56	-1,66	-0,79	-0,06	0,05	0,01
CTP 28 ESP	-3,30	-2,18	-1,92	-1,09	-0,79	-0,27	-0,24	0,01
CTP 29 ESP	-2,82	-1,82	-1,67	-0,89	-0,69	-0,30	-0,23	0,02
CTP 30 ESP	-3,06	-2,09	-1,79	-0,99	-0,74	-0,22	-0,21	0,06
CTP 31 ESP	-2,89	-1,89	-1,81	-0,89	-0,65	-0,38	-0,17	0,05
CTP 1 FR	-3,44	-2,32	-2,22	-1,15	-0,95	-0,47	-0,36	0,02
CTP 2 FR	-5,27	-3,61	-2,88	-1,78	-1,01	-0,16	-0,09	0,03
CTP 3 FR	-3,01	-2,04	-1,62	-1,07	-0,53	-0,08	-0,02	-0,04
CTP 4 FR	-6,97	-4,69	-3,54	-2,32	-1,22	-0,01	-0,03	0,03
CTP 5 FR	-4,98	-3,31	-2,55	-1,64	-0,84	-0,06	0,00	0,02
CTP 6 FR	-3,06	-2,04	-1,87	-0,97	-0,75	-0,33	-0,23	0,06
CTP 7 FR	-3,33	-2,10	-1,66	-0,99	-0,57	-0,08	-0,03	0,06
CTP 9 FR	-6,79	-4,55	-3,43	-2,25	-1,09	-0,00	0,07	0,03
CTP 14 FR	-5,13	-3,42	-2,66	-1,67	-0,83	-0,08	0,04	0,05
CTP 15 FR	-4,61	-3,09	-2,43	-1,51	-0,79	-0,09	-0,00	0,04
CTP 16 FR	-4,92	-3,33	-2,67	-1,67	-0,93	-0,16	-0,09	-0,00
CTP 17 FR	-6,57	-4,37	-3,27	-2,15	-1,07	0,03	0,04	0,04
CTP 18 FR	-3,29	-2,13	-2,04	-1,05	-0,97	-0,43	-0,42	0,02
CTP 19 FR	-4,91	-3,28	-2,53	-1,65	-0,84	-0,05	-0,01	-0,00
CTP 24 FR	-5,86	-3,88	-3,02	-1,96	-0,94	-0,09	0,04	-0,01
CTP 25 FR	-3,23	-2,16	-1,83	-1,05	-0,71	-0,20	-0,16	0,03
CTP 26 FR	-4,61	-3,01	-2,34	-1,46	-0,77	-0,07	-0,01	0,05
CTP 28 FR	-4,03	-2,72	-2,24	-1,32	-0,87	-0,19	-0,18	0,05
CTP 29 FR	-6,19	-4,12	-3,19	-2,05	-1,02	-0,08	0,03	0,02
CTP 31 FR	-5,69	-3,65	-2,90	-1,83	-0,95	-0,14	-0,02	0,01
CTP 33 FR	-4,99	-3,32	-2,61	-1,71	-0,96	-0,11	-0,12	-0,04
2SD(‰)*	0,24	0,15	0,11	0,07	0,11	0,06	0,07	0,05

*Uncertainties correspond to the double of the standard deviation for n=14 measurements of the BCR-482.

Annexe 4: Pearson correlation coefficients of trace metal elements (mg kg⁻¹) and altitudes (m) for all the samples of lichens from Iraty Forest (n=51). The values highlighted correspond to those over 0,600.

	Altitude	Hg	Li	V	Cr	Ti	Ni	Cu	Rb	Ag	Cd	Sb	Ce	U	Sn	Mn	Pb	Zn	Sr
Altitude	1,000																		
Hg	-0,473	1,000																	
Li	0,366	-0,136	1,000																
V	0,479	-0,184	0,841	1,000															
Cr	0,116	0,006	0,416	0,430	1,000														
Ti	-0,287	0,443	0,380	0,307	0,206	1,000													
Ni	0,139	0,052	0,678	0,643	0,302	0,423	1,000												
Cu	-0,068	-0,010	0,137	0,093	0,187	0,064	0,240	1,000											
Rb	-0,242	0,116	-0,020	-0,186	-0,224	0,077	0,009	0,084	1,000										
Ag	0,011	-0,164	0,077	0,163	0,112	-0,105	-0,017	0,346	-0,225	1,000									
Cd	-0,233	0,271	-0,055	-0,170	0,117	0,403	-0,065	0,162	-0,054	0,006	1,000								
Sb	-0,059	0,042	0,140	0,238	0,503	0,147	0,171	0,102	-0,055	-0,012	-0,029	1,000							
Ce	0,334	-0,084	0,958	0,871	0,407	0,387	0,715	0,057	0,021	0,081	-0,087	0,191	1,000						
U	0,475	-0,193	0,928	0,899	0,462	0,318	0,647	0,239	-0,170	0,169	-0,083	0,255	0,877	1,000					
Sn	-0,081	-0,128	-0,131	-0,144	0,126	-0,082	-0,123	-0,077	-0,250	0,112	0,269	-0,066	-0,133	-0,171	1,000				
Mn	-0,072	0,211	0,164	0,280	0,242	0,291	0,325	0,266	-0,006	-0,078	0,350	0,444	0,212	0,236	-0,156	1,000			
Pb	0,022	-0,007	0,433	0,480	0,612	0,202	0,337	0,148	-0,078	0,059	-0,021	0,838	0,441	0,514	-0,099	0,468	1,000		
Zn	0,015	-0,343	0,225	0,221	0,182	0,101	0,290	0,429	-0,010	0,187	0,132	0,113	0,172	0,280	0,006	0,199	0,248	1,000	
Sr	-0,389	0,239	0,074	-0,103	-0,098	0,337	0,153	0,083	0,905	-0,161	0,045	0,013	-0,053	-0,084	-0,204	0,107	0,062	0,070	1,000

Annexe 5: Principal Component Analysis for all the elements analyzed and the altitudes of the sampling sites

Principal Component Analysis								
Correlation matrix								
Auto-value	5,6811	2,9651	2,0642	1,5105	1,4180	1,0513	1,0097	0,7350
Variance %	0,299	0,156	0,109	0,080	0,075	0,055	0,053	0,039
Accumulated %	0,299	0,455	0,564	0,643	0,718	0,773	0,826	0,865
	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Li	0,372	0,078	-0,184	0,146	0,078	-0,130	-0,012	-0,107
V	0,376	0,151	-0,090	0,050	0,061	0,010	0,048	0,001
Cr	0,251	0,013	0,312	-0,143	0,046	-0,268	-0,149	-0,048
Ti	0,187	-0,328	0,026	0,305	0,285	-0,076	0,097	0,072
Ni	0,323	-0,083	-0,125	0,163	-0,019	0,137	-0,033	0,413
Cu	0,112	-0,117	0,140	0,116	-0,610	0,052	0,248	-0,116
Rb	-0,037	-0,350	-0,429	-0,168	-0,200	-0,169	-0,195	-0,267
Ag	0,050	0,149	0,176	0,168	-0,465	-0,427	0,373	0,003
Cd	-0,003	-0,246	0,335	0,422	0,049	0,110	-0,166	-0,508
Sb	0,186	-0,110	0,314	-0,529	0,034	-0,064	-0,067	0,051
Ce	0,375	0,051	-0,194	0,110	0,112	-0,138	-0,022	-0,044
Pb	0,300	-0,092	0,216	-0,397	0,003	-0,128	-0,040	0,002
U	0,387	0,139	-0,080	0,078	0,002	-0,026	0,078	-0,115
Sn	-0,071	0,100	0,300	0,287	0,029	-0,369	-0,579	0,075
Mn	0,185	-0,247	0,254	-0,110	-0,025	0,439	0,050	-0,269
Zn	0,167	-0,223	0,031	0,139	-0,338	0,372	-0,322	0,427
Sr	0,022	-0,433	-0,351	-0,092	-0,128	-0,263	-0,162	-0,149
Hg	-0,032	-0,355	0,098	0,093	0,361	-0,083	0,457	0,064
Altitude	0,135	0,403	-0,142	-0,032	0,007	0,279	-0,103	-0,403

Annexe 6: Cluster dendrograms for all the elements analyzed and the different altitudes of sampling (similarities in order to Pearson correlation).

Diagram A: All the elements analyzed

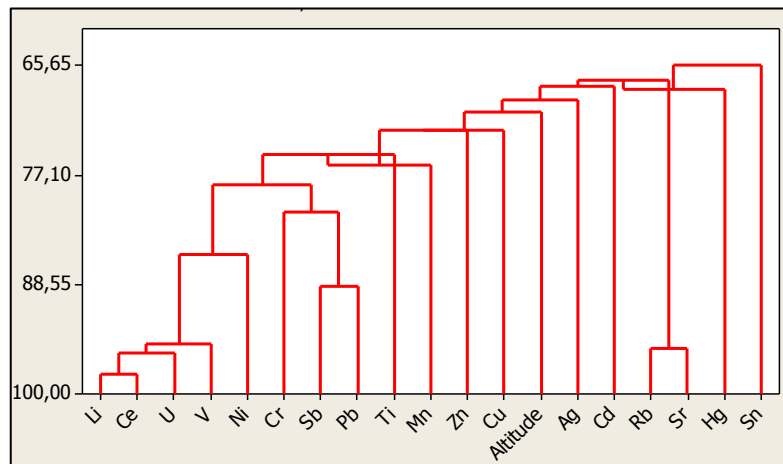


Diagram B: Spanish side data

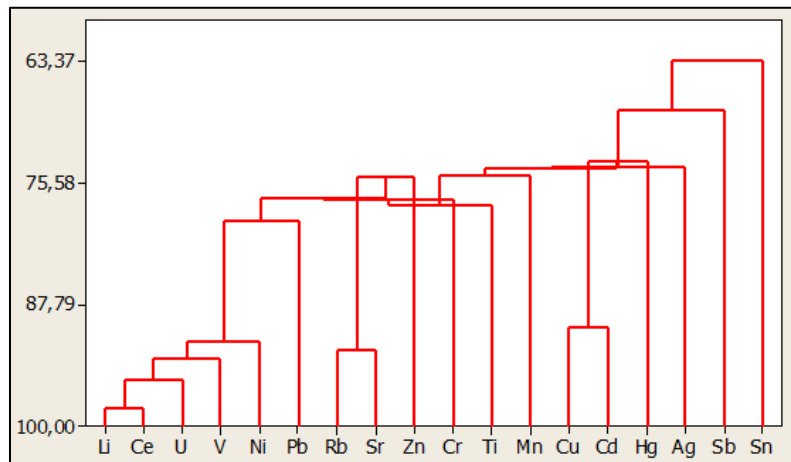
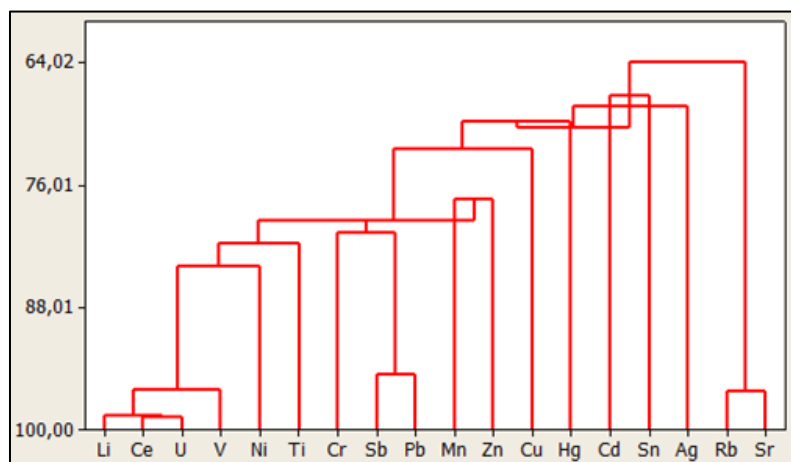
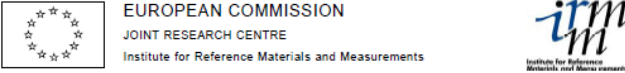


Diagram C: French side data



Annexe 7: Certified Reference Materials used for validation of all the methodologies used in this study. Figure A shows the BCR-482 and Figure B the IAEA-336, both are lichen materials.

Figure A



CERTIFIED REFERENCE MATERIAL
BCR® – 482

CERTIFICATE OF ANALYSIS

LICHEN			
	Mass fraction based on dry mass		Number of accepted sets of data p
	Certified value ¹⁾ [mg/kg]	Uncertainty ²⁾ [mg/kg]	
Al	1103	24	9
As	0.85	0.07	6
Cd	0.56	0.02	8
Cr	4.12	0.15	7
Cu	7.03	0.19	10
Hg	0.48	0.02	8
Ni	2.47	0.07	8
Pb	40.9	1.4	10
Zn	100.6	2.2	13

¹⁾ The certified value was calculated from the unweighted mean of the means of p accepted datasets. The certified value is traceable to the SI.
²⁾ Half-width of the 95 % confidence interval of the mean defined in ¹⁾.

This certificate is valid for one year after purchase.

Sales date:

The minimum amount of sample to be used is 250 mg.

DESCRIPTION OF THE SAMPLE

The material consists of a lichen powder in a glass bottle containing about 15 g of powder. Additional information on the preparation, the certified and indicative values is given in the certification report.

NOTE

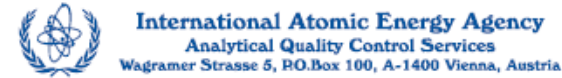
This material has been certified by BCR (Community Bureau of Reference, the former reference materials programme of the European Commission). The certificate has been revised under the responsibility of IRMM.

Brussels, December 1995
Revised: May 2007

Signed: 

Prof. Dr. Hendrik Emons
Unit for Reference Materials
EC-JRC-IRMM
Retieseweg 111
2440 Geel, Belgium

Figure B



REFERENCE SHEET

REFERENCE MATERIAL

IAEA-336

TRACE AND MINOR ELEMENTS IN LICHEN

Date of issue: June 1999[•]

Recommended Values
(Based on dry weight)

Element	Recommended Value mg/kg	95% Confidence Interval* mg/kg	N**
As	0.63	0.55 - 0.71	17
Ba	6.4	5.3 - 7.5	11
Br	12.9	11.2 - 14.6	18
Ce	1.28	1.11 - 1.45	13
Co	0.29	0.24 - 0.34	19
Cs	0.110	0.097 - 0.123	13
Cu	3.6	3.1 - 4.1	21
Fe	430	380 - 480	35
Hg	0.20	0.16 - 0.24	15
K	1840	1640 - 2040	24
La	0.66	0.56 - 0.76	12
Mn	63	56 - 70	29
Na	320	280 - 360	20
Sb	0.073	0.063 - 0.083	12
Se	0.22	0.18 - 0.26	12
Sm	0.106	0.092 - 0.120	15
Sr	9.3	8.2 - 10.4	19
Th	0.14	0.12 - 0.16	16
Zn	30.4	27.0 - 33.8	38
Al	680	570 - 790	15
Cd	0.117	0.100 - 0.134	14
Cl	1900	1600 - 2200	13
Cr	1.06	0.89 - 1.23	22
Eu	0.023	0.019 - 0.027	9
Lu	0.0066	0.0042 - 0.0090	5
Nd	0.60	0.42 - 0.78	5
P	610	490 - 730	12
Pb	4.9	4.3 - 5.5	23
Rb	1.76	1.54 - 1.98	16
Sc	0.17	0.15 - 0.19	13
Tb	0.014	0.012 - 0.016	7
V	1.47	1.25 - 1.69	8
Yb	0.037	0.025 - 0.049	5

* It should be noted that the confidence interval was calculated from the combination of the standard deviation of the mean value and an additional 5% to account for any variation due to sample inhomogeneity.

** Number of accepted laboratory means which were used to calculate the recommended values and confidence intervals.

• Revision of the original reference sheet dated October 1994