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Selenium distribution and speciation in waters of pristine alpine lakes from central-western Pyrenees (France-Spain)

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Abstract

The speciation of both redox reactive and volatile selenium (Se) compounds, barely reported in pristine aquatic environments, has never been investigated in remote alpine lakes, considered as sensitive ecosystems to detect the effect of global change. This work presents an integrated investigation on Se distribution and speciation conducted in 20 high altitude pristine lakes from the central-western Pyrenees. Five seasonal sampling campaigns were carried out after snowmelt (June/July) and in early fall (October) for the period 2017–2019. Concentrations of total dissolved Se (TDSe) ranged from 7 to 78 ng L\(^{-1}\), being selenate ubiquitously observed in most cases (median of 61\% of TDSe). Selenite was only occasionally detected up to 4 ng L\(^{-1}\), therefore a fraction of TDSe was presumably in the forms of elemental Se(0) and/or selenides. Depth profiles carried out in different lakes showed the occurrence of such Se(-II, 0) pool in bottom hypoxic to anoxic waters. The production of volatile Se compounds presented a low median total concentration (TVSe) of 33 pg L\(^{-1}\) (range 3–120 pg L\(^{-1}\)), mainly in the form of dimethylselenide in sub-surface samples (median of 82\% of TVSe). Se concentration in lake waters was significantly correlated with sulphate concentration (\(\rho=0.93, p<0.0001\)), demonstrating that it is influenced by erosion and dissolution of Se and S-enriched parent bedrocks. In addition, for Se depleted alpine lake-bedrock systems, long-range transport and wet atmospheric depositions represent a major source of Se for lake waters.
1. Introduction

High-mountain lakes are pristine ecosystems characterized by extreme environmental conditions. No data on selenium behaviour have been reported in these aquatic environments. The existing data about Se biogeochemistry in lakes are mainly provided by research in low altitude temperate and tropical lakes and have been reviewed elsewhere (Conde & Sanz Alaejos, 1997; Simmons & Wallschläger, 2005; Ponton et al., 2020). In unpolluted waters, total Se concentrations are typically below 200 ng L$^{-1}$ (Ponton et al., 2020). In more extreme environments, reported total Se concentrations in western Siberian lakes range from 12 to 311 ng L$^{-1}$ (Pokrovsky et al., 2018). In Canadian subarctic thermokarst ponds, total dissolved Se ranged from 18 to 67 ng L$^{-1}$ (Lanceleur et al., 2019), similarly to values from 20 to 60 ng L$^{-1}$ reported in Norwegian (Økelsrud et al., 2016) or Finish (Wang et al., 1994; 1995) lakes. In North Carolina, Se concentrations in lakes impacted by coal combustion effluents were between 0.3 and 2 µg L$^{-1}$ while Se was not detected (< 0.22 µg L$^{-1}$) in reference lakes (Brandt et al., 2017). Among its four oxidation states (-II, 0, IV and VI), Se oxyanions, particularly selenate, are expected to be the major species in well-aerated surface waters and oligotrophic environments (Sharma et al., 2014). Aqueous selenium speciation measurements in lake waters indicated the predominance of selenite (Robberecht et al., 1983; Ponton & Hare, 2013; Tanzer & Heumann, 1991) or selenate (Lindström, 1980; Nishri et al., 1999; Ponton & Hare, 2013). Despite the lack of analytical procedures to identify and quantify elemental selenium and selenide forms, several authors have reported the presence of such Se pool in lake waters. For example in Finish lakes, the sum of selenite and selenate represented less than 20% of total Se (Wang et al., 1994; 1995). In Lake Kinneret up to 40% of total Se was associated with colloidal elemental Se and selenide forms (Nishri et al., 1999). In Canadian lakes, organo-Se proportion could attain 95% of total Se (Ponton & Hare, 2013).

Reduced Se include also volatile selenides seldom tested and reported in lake waters. Reported total volatile Se concentrations varied in a wide range (1 pg L$^{-1}$ to 109-ng L$^{-1}$), being dimethylselenide...
DMSe) the major species (≥49%), followed by dimethyl selenide sulphide (DMSeS) (4–41%) and dimethyl diselenide (DMDSe) (≤7%) (Lanceleur et al., 2019 and references therein). In a mountain minerotrophic peatland (elevation 972 m a.s.l.) a DMSe concentration of 10 ng L\(^{-1}\) was reported in surface water samples (Vriens et al., 2014). In Canadian thermokarst ponds, Lanceleur et al. (2019) found DMSe to be the main volatile species with concentrations between <0.17 to 31 pg Se L\(^{-1}\), while in European estuaries, volatile Se ranged from 22–8067 pg L\(^{-1}\), being DMSe the main species found (Amouroux & Donard, 1997; Tessier et al., 2002).

Ninety two per cent of Pyrenean lakes are classified as ultraoligotrophic or oligotrophic (Catalan et al., 2006). Bedrock composition associated with slow weathering and limited soil development determine waters with low mineralization (Catalan et al., 2006). In high altitude mountain lakes, Se can originate either from local (geogenic) or remote sources (atmospheric transport). In the Pyrenees, the main groups of rocks are granites, sedimentary limestones, metamorphic slates and schists, detrital sandstones (Catalan et al., 2006). These rocks generally contain low selenium concentrations (0.01 – 0.08 mg kg\(^{-1}\)) although shales can show variable concentrations worldwide (0.1 – 675 mg kg\(^{-1}\)) (Plant et al., 2014; Fernández-Martínez & Charlet, 2009). Previous studies have estimated that Se wet deposition (7.4–20.0 Gg Se yr\(^{-1}\)) was responsible for 80% of its global atmospheric deposition and was thus dominant over its dry deposition (1.1–5.0 Gg Se yr\(^{-1}\)) (Blazina et al., 2017; Pan & Wang, 2015). Suess et al. (2019) reported Se concentration values in rain samples from Pic du Midi (central Pyrenees) between 13–184 ng L\(^{-1}\). The authors determined that the dominant source of Se in rain at the Pic du Midi was the North Atlantic Ocean (54%) with a contribution of terrestrial emissions of 40% with seasonal fluctuation.

Pyrenean lakes are also characterized by surface frost periods comprised generally between November and May (Gascoin et al., 2015; López-Moreno & Vicente-Serrano, 2007). During these ice-covered periods, high mountain lakes have shown oxygen depletion leading to anoxic conditions in bottom waters (Catalan et al., 2002). These winter conditions, when oxygen is consumed, may
promote conditions favourable to microbial (and possibly sequential) reduction of Se oxyanions to elemental Se, process which has been shown to be environmentally significant (Nancharaiah & Lens, 2015) and may lead to selenium settle out of water column. Although situated in different climatic conditions, depletion of total dissolved selenium and selenate concentrations were previously observed in Lake Kinneret bottom waters when O$_2$ and SO$_4^{2-}$ were consumed (Nishri et al., 1999; Be’eri-Shlevin et al., 2021).

In this study, we surveyed during three years the seasonal concentrations of total Se and its species (including non-volatile and volatile dissolved species) in twenty alpine lakes of the central-western Pyrenees. To this end, sub-surface water samples were collected in June/July 2017–2019 (late spring–early summer) and October 2017–2018 (early fall). Additionally, depth-profiles and temporal monitoring were conducted at some lakes in 2018 and 2019 to investigate the effects of water stratification and day-time sampling on Se distribution. To our knowledge, nos studies or data are available about Se in such aquatic systems. Our aim was thus both to contribute to comprehensive data set of selenium occurrence and speciation in such pristine ecosystems and to provide a unique “baseline” (Se background level) that allows to evaluate respective contribution of headwaters and atmospheric inputs versus the impact of land uses in a specific watershed (Adour river watershed on the French slope).

2. Materials and Methods

2.1. Study site

All the lakes considered in this study are located along a north-south transect of the Pyrenees (Fig. 1). Lakes can be divided into three sectors according to their location: Cauterets, Panticosa, and Ossau/Ayous Valleys. Most of the studied lakes are located on granitic rocks (GR). The study area lays on three different geological periods: Devonian, Permo-Triassic and Cretaceous (Zaharescu et al., 2016; Gleizes et al., 1998; Zwart & De Sitter, 1979) which may impact Se bedrock concentration. The geology of the Central Pyrenees has been described in detail elsewhere (Van
In brief, granite (pDe-GR) originated during mountain formation in the pre-Devonian geological age are found ubiquitously. Devonian sedimentary rocks (De-SR) consisted of limestone, marble, shale and sandstone; Permo-Triassic bedrock (PT-SR) contains sedimentary rocks in the form of conglomerates, sandstone and red or green shales; and, the most recent formations, Cretaceous sedimentary rocks (Cr-SR) consist of limestone and sandstone.

Studied lakes are ice-covered during 5-6 months per year (Santoloria et al., 2015). Late spring sampling (June/July) was thus scheduled to coincide with the thawing phase, to probe the effect of recent ice melting and early lake productivity. Late summer-early fall sampling preceded holomixis phase, to probe the effect of summer time stratification and whole biological production (both auto and heterotrophic).

2.2. Sampling lake waters and in-situ sample treatments

A total of 71 subsurface water samples (0.2 – 0.5 m) were collected in 20 lakes during June/July and October 2017–2019. Details of the sampling locations are summarized in Table 1. In brief, five sampling campaigns were carried out in June/July 2017, October 2017, June 2018, October 2018 and June 2019. In the two first sampling campaigns of June and October 2017, three replicate subsurface samples were collected in the upstream, central and downstream sectors of each lake to examine intra-lake variabilities. Diurnal and depth profiles from lakes Gentau and Sabocos were sampled in June 2018, October 2018 and June 2019. Azules diurnal and depth profiles were sampled in June and October 2018, while Arratille profiles were sampled only in June 2018. The diurnal cycle consisted in the acquisition of subsurface samples carried out every few hours from dawn to sunset mainly to evaluate the effect of light in Se speciation.

For the Bachimaña reservoir and other lakes of difficult access, water samples were collected from the shore, below the surface, using nitrile gloves to avoid contamination. For all other lakes, samples were collected onboard an inflatable rubber boat using a non-metallic and PTFE coated sampler (5 L Go-Flo; General Oceanic). The sampler was operated with gloves for subsurface waters and fixed
on a Kevlar cable (scaled every meter from 0 to 40 m) for samples collected at depth. One litre of
the sample was immediately transferred into two 500 mL Teflon bottles with a pre-cleaned silicone
tubing, avoiding bubbles and head-space as for dissolved oxygen sampling, and stored in the dark
as fresh as possible for its subsequent purge and trap of the gaseous species.

Two aliquots either unfiltered, for bulk, or filtered, for dissolved, total Se analyses were collected
in 15 mL polypropylene tubes and acidified to 1% v/v HNO$_3$ (69%, trace metal grade). For total
dissolved Se (TDSe), samples were filtered (PVDF Sterivex filter units, 0.22μm, Millipore) prior to
their acidification. To study Se speciation, filtered water samples were stored without headspace in
50 mL Falcon tubes, as fresh as possible (4–10 °C) in the field in the dark. Once in the lab, all
samples were stored at ca. 4 °C in the dark until the analysis (less than two weeks). Additional
samples were collected and analyzed at the University of the Basque Country (Bilbao) for the main
anions, non-purgeable organic carbon (NPOC), and dissolved inorganic carbon (DIC) as previously
described elsewhere (Kortazar et al., 2020). Samples of depth profiles (3 ≤ n ≤ 6) and temporal
monitoring were treated following the same protocols detailed here and all procedures were
conducted trying to minimize their exposition to daylight.

The physicochemical parameters in water lakes were measured simultaneously to the sampling
using a multiparametric probe YSI EXO2 (temperature, conductivity, oxidation-reduction potential,
dissolved oxygen, turbidity). The pH was calculated according to Kortazar et al. (2020) with the
help of the CO2SYS software, this methodology providing a better precision and accuracy in
comparison with the pH measured by the probe.

2.2. Total Se analysis

Bulk total Se (TSe Bulk) was determined in unfiltered bulk samples (15 mL) after digestion with
300 μL HNO$_3$ (69%, tracepure grade) and 150 μL HCl (37%, tracepur grade) in sealed tubes and
incubated for 3 h at 90 °C in a hot block (DIGIPREP, SCP Science). The dilution factor was
corrected by weighting samples before and after the digestion.
Total Se concentrations were measured in bulk (TSe Bulk) and filtered (TDSe) samples with an Agilent 7900x Series inductively coupled plasma mass spectrometer (ICP-MS) system (Agilent Technologies, Tokyo, Japan) equipped with an octopole reaction cell, concentric nebulizer and a Scott double pass spray chamber cooled to 2 °C. Argon-based polyatomic interferences were reduced by using H₂ as cell gas at a flow rate of 5 mL min⁻¹ as previously optimised and validated (Darrouzes et al., 2005). The parameters settings were as follow: Ar plasma gas flow, 15 L min⁻¹; Ar auxiliary gas flow, 0.86 L min⁻¹; Ar nebulizer gas flow, 1–1.1 L min⁻¹; radio frequency (RF) forward power, 1550 W. Acquisition parameters consisted of 10 replicates with 50 sweeps/replicate and integration time of 2s per isotope; m/z monitored ratios were 77 and 78. External calibration (range 5 – 100 ng L⁻¹ from single element Se standard 1000 mg L⁻¹ SCP Science) was performed. Quality control used for quantification methodology was based on several repetitions (3 to 4 times) of standard measurements during the day of analysis that led to calibration line slope relative standard deviation <10%. Limits of quantification (based on ⁷⁸Se and according to IUPAC recommendations) were between 0.7–2.1 ng L⁻¹. Typical analytical precision was <12% in the range of measured concentrations of dozen ng Se L⁻¹ (relative standard deviation of the 10 replicates).

2.3. Dissolved non-volatile Se speciation analysis

Chromatographic separation was carried out with an Agilent 1200 HPLC pump hyphenated to ICP-MS. Most samples were analyzed using a porous graphitic carbon stationary phase (Thermo Hypercarb column 10 cm × 4.6 mm i.d) with a formic acid mobile phase (240 mmol L⁻¹, 1% methanol and pH 2.4 adjusted with ammonia) delivered at 1 mL min⁻¹ flow rate (Dauthieu et al., 2006). Chromatographic conditions allowed the separation of inorganic selenite and selenate and, organic species (trimethylselenonium ion, methane seleninic acid, selenomethionine and selenocystine) (Lanceleur et al., 2019; Be-eri-Shlevin et al., 2021). Standard addition was used for quantification using daily diluted certified aqueous standards of 1 g L⁻¹ Se as selenite and 0.1 g L⁻¹
Se as selenate (Spectracer) (see example chromatogram Fig. SI 1). Quantification limits (LoQ) were 3.6 and 2.2 ng Se L$^{-1}$ for selenite (Se(IV)) and selenate (Se(VI)) respectively for 200 µl injected volumes. Exceptionally, samples of October 2017 and part of those of October 2018 were analyzed using the mixed-mode column OmniPac PAX-500 (Thermo, 25 cm x 4 mm i.d.) with a 20 mmol L$^{-1}$ ammonium nitrate mobile phase containing 2% methanol at pH 8.0 (adjusted with ammonia), delivered at 1 ml min$^{-1}$ flow rate. In this case, LoQ were 12 and 10 ng Se L$^{-1}$ for Se(IV) and Se(VI), respectively, for 100 µl injected volumes. Duplicates of all samples were injected obtaining a relative standard deviation below 10%, except for some samples close to the LoQ for which relative standard deviation was up to 15%. Only selenate and eventually selenite were detected. The operationally defined Se ($\text{Se}^{2-}$, 0) fraction was then calculated by subtraction of species concentrations from the total dissolved Se concentration.

2.4. Dissolved gaseous Se speciation analysis

Dissolved gaseous Se species were purged and trapped within the day of sampling in the field lab set in a mountain hut. This allowed the pre-concentration of dissolved gaseous Se compounds and the preservation of their speciation as described by Lanceleur et al. (2019). Samples (2x500 mL) were purged with pure N$_2$ (500 mL min$^{-1}$) for 45 min. The resulting water vapour during the purge was removed from the gas stream in a moisture trap maintained at −20 °C. The gas stream was then carried through a volatile Se compounds trap (glass tube packed with Carbotrap sorbent). After the purge, the glass columns were tightly closed with Teflon lined plugs and stored in the dark at 4 °C in a sealed double PE bags until analysis. In the laboratory, samples were analyzed within a week after sampling using a cryogenic GC-ICP/MS set-up. Samples were thermo-desorbed from carbotraps at 250 °C for 2 minutes under He flow (100 mL min$^{-1}$). Samples were flushed and trapped on the head of a Cryo GC column submerged in liquid N$_2$, prior to GC elution on Chromosorb SP2100 (Amouroux et al., 1998). Quantification was obtained by external calibration. The limits of
quantification were between 1.3–3.8 pg Se L⁻¹ for DMSe and DMSeS and between 1.3–5.4 pg Se L⁻¹ for DMDS. Thermodesorption efficiency was controlled by carrying out two consecutive analyses of the same carbotrap column. TVSe content was determined as the sum of DMSe, DMSeS and DMDS concentrations.

2.5. Data processing and statistics

In samples that did not contain quantifiable selenite and/or selenate (<LoQ), a value of one half their quantification limit (with associated error of 100%) was used for Se (-II, 0) fraction calculation. The limit of quantification of Se (-II, 0) fraction was calculated as the square root of the sum of the squares of TDSe, Se(IV) and Se(VI) limits of quantification and, was in the range 4.5–15 ng Se L⁻¹. For the statistical treatment, a value of one half the quantification limit (with associated error of 100%) was also assigned to the species (selenate, volatile species) and the Se (-II, 0) fraction concentrations in samples that did not contain quantifiable levels.

All data sets were tested for normality using the Shapiro-Wilk test. The non-parametric pairwise Wilcoxon test or Kruskal-Wallis followed by Conover-Iman tests for multiple, two-tailed, comparisons were performed. Spearman coefficients were used for correlation analysis. These analyses were carried out using using R Commander package (R Core Team, 2021, version 4.0.5) and XLSTAT sofware (version 2021.2.1).

3. Results and Discussion

3.1. Subsurface lake waters

Detailed data for physicochemical caracteristics and selenium concentrations are available in Supporting information Tables SI 1 and SI 2. In June and October 2017 sampling campaigns, the triplicate samples, collected in each lake to check intra-lake variabilities, exhibited no remarkable differences between replicates (relative standard error of the mean was ≤13, 11 and, 9 %, respectively, for bulk and dissolved total Se and Se(VI), data not shown). The diurnal cycle
measurements (June 2018, October 2018, and June 2019) also did not show significant variability along the day for total Se, nor for speciation (data not shown). These results indicated primarily a slow reactivity of Se compounds and Se turnover in the surface waters. We can thus assume that no apparent bias was due to the sampling time of the day and that our measurements were representative of sampled lakes in a given seasonal period.

3.1.1 Physicochemical characteristics and selenium total concentrations

Lake waters presented temperatures ranging from 2 to 19 °C (Table SI 1) and subsurface samples were always oxygenated (>62% of oxygen saturation, data not shown). Conductivity ranged between 4.5 and 130 μS cm⁻¹. The pH varied from slightly alkaline (7.91) to acidic values (4.87). Oxidation-reduction potential (E_H) ranged from slightly reducing (36 mV) to oxidising (285 mV) conditions. No significant differences were observed for conductivity, pH and E_H values between June and October (Wilcoxon test, p>0.05). Some of the anions measured, such as fluoride (F⁻), bromide (Br⁻), nitrites (NO₂⁻) or phosphates (PO₄³⁻), were frequently not detected (<LoQ, Table SI 1). Sulphate (≤7.56 mg L⁻¹) was the main anion found in waters with a median value of 1.6 mg L⁻¹ (n=71). The highest sulphate concentrations were observed in Azul Superior (from 4.84 to 7.56 mg L⁻¹) and Badète (from 2.96 to 5.43 mg L⁻¹) (Table SI 1). The median value found in October (1.89 mg L⁻¹, n=27) was slightly higher than in June (1.36 mg L⁻¹, n=44) but statistically not significantly different (Wilcoxon test, p=0.48). Nitrate concentration was ≤1.16 mg L⁻¹ at all sites with highest values found at Azul Superior (June 2019, 1.16 mg L⁻¹), Badète (June 2018, 1.13 mg L⁻¹) and Bachimaña (June 2019, 1.08 mg L⁻¹) (Table SI 1). Nitrate median concentration of sub-surface samples was 0.46 mg L⁻¹ (n=71). The median value in June samples (0.56 mg L⁻¹, n=44) was almost double that in October (0.29 mg L⁻¹, n=27) and significantly different (Wilcoxon test, p=0.004). Dissolved organic matter ranged from 0.63 to 5.3 mg L⁻¹ and median values were significantly different between June (1.03 mg L⁻¹, n=43) and October (1.72 mg L⁻¹, n=26) (Wilcoxon test, p=0.0006).
Total Se bulk concentrations ranged from $9 \pm 1$ to $82 \pm 5$ ng L$^{-1}$ while dissolved Se ranged between $7 \pm 1$ and $78 \pm 7$ ng L$^{-1}$ (Table SI 2). No significant differences were found for both concentrations between June and October sampling campaigns (Wilcoxon test, $p>0.05$) but bulk and filtered Se concentrations were significantly different (Wilcoxon test, $p<0.0001$). However most of the Se was in the dissolved or colloidal phase in these lakes (Fig. SI 2). Measured dissolved Se concentrations in Pyrenean lakes were similar to those reported in Norwegian lakes located on granitic gneisses (16–75 ng L$^{-1}$) (Økelsrud et al., 2016). The highest total Se concentrations were found at Badète (59.3 ng L$^{-1}$ on average) and Azul Superior lakes (57.8 ng L$^{-1}$ on average). Grouping lakes according to underlying bedrock, the average dissolved Se concentration was 19 ± 6 ng L$^{-1}$ for lakes located at the granitic core (pDe-GR) (n=31), 48 ± 18 ng L$^{-1}$ for Devonian sedimentary bedrocks (De-SR) lakes (n=21), 12 ± 1 ng L$^{-1}$ at lakes located over Permo-Triassic sedimentary bedrock (PT-SR) (n=9) and 14 ± 2 ng L$^{-1}$ for lake Sabocos (n=3) (Cretaceous prevailing bedrock, Cr-SR).

Selenium concentrations (bulk and dissolved) were thus significantly higher in De-SR lakes compared to other bedrock lakes (Kruskall-Wallis and Conover-Iman tests, $p<0.0001$) (Fig. 2A, Table SI 2).

No relationship was found between total Se concentration and lake or catchment surface area. Lake surface area demonstrating a linear relationship with water residence time (personal communication, Lluis Camarero, CSIC, Spain), the lack of correlation between Se concentration and surface area indicated that Se contents in lake waters were not drastically affected by water residence times. Correlation analysis showed that total dissolved selenium concentrations were positively correlated with those of sulphate, dissolved inorganic carbon and nitrates (Table SI 3, Fig. SI 3). Among them, sulphate and dissolved inorganic carbon concentrations were also correlated (Table SI 3). The strong link between total dissolved selenium concentrations and those of sulphate has been previously reported in freshwaters with higher Se concentrations (> 100 ng L$^{-1}$, Ponton et al., 2020) and will be further discussed in Paragraph 3.3. TDSe did not correlate with NPOC, meaning that Se was
weakly incorporated or bound to organic matter in such oligotrophic lakes. Very weak or no correlation of TDS\textsubscript{e} with dissolved organic matter was previously reported in Siberian thaw lake waters in spring time corresponding to frozen underlying peat preventing leaching of dissolved organic matter and Se from peat layers (Pokrovsky et al., 2018).

### 3.1.2 Selenium speciation

Among non-volatile dissolved species, selenate was most often the only species detected with no significant difference between June and October (Wilcoxon test, p>0.05). The average values within each lake group followed the same trend as for total dissolved Se (Fig. 2A), and were: 12 ± 7 (n=21), 42 ± 25 (n=15), 8 ± 2 (n=6) and 10.3 ± 0.4 (mean deviation, n=2) ng Se L\textsuperscript{-1} for pDe-GR, De-SR, PT-SR and Cr-SR groups, respectively for June sampling campaigns. After summer, selenate concentration values were slightly lower: 10 ± 5 (n=14), 37 ± 17 (n=9), <10 (n=3) and 6 (n=1) ng Se L\textsuperscript{-1}, respectively. Spring to late summer selenate concentrations decrease in meso-eutrophic freshwater lake has been related to phytoplankton uptake, and was associated also to selenite concentration decrease (Be’eri-Shleven et al., 2021). Selenate, which is the stable form of Se in oxic waters (Belzile et al., 2000), accounted for 64 ± 28 % of total dissolved Se, and both concentrations were thus significantly different (Wilcoxon test, p<0.0001). This was particularly the case for pDe-GR, PT-SR and Cr-SR lake groups (Fig. SI 4) where other species may thus coexist at extremely low concentration. This may be the case of selenite, which was typically undetected (<limit of quantification, in the range 3.6–12 ng Se L\textsuperscript{-1}) and the Se(-II, 0) fraction (LoQ in the range 4.5–15 ng Se L\textsuperscript{-1}). Pyrenean lakes are usually oligotrophic due to low contents of nutrient and organic matter (Catalan et al., 2006), the presence of organic selenide was thus not expected as main component of Se(-II, 0) fraction and no unknow selenium-containing compound could be chromatographically detected (see example chromatogram Fig. SI 1). Considering E\textsubscript{H} and pH values observed in lake waters, elemental Se should be thermodynamically stable in most lakes (Seby et al., 2001). Estimation of Se(-II, 0) fraction (Table SI 2) indicated the presence of such fraction in both seasons in the range 4.7–17 ng L\textsuperscript{-1} in subsurface waters at some lakes (Bachimaña Bajo,
Coanga and Baños de Panticosa pDe-GR lakes and, Ordicuso De-SR lake). Bibliographic data for
Se speciation in lakes is scarce, especially in remote lakes. Existing publications report selenate
concentrations in the range of 4–17 ng Se L\(^{-1}\) in Finish lakes representing 6–12% of total Se, while
most of Se present was linked to organic matter (Wang et al., 1994; 1995). In Canadian lakes,
selenate was predominant (≥60%) in lakes where Se inputs occur principally in this form (Ponton
et al., 2013).

Total volatile Se ranged from 2.6 to 118 pg L\(^{-1}\) with an exceptionally high value observed in June
2017 at Ordicuso (484 pg L\(^{-1}\)) without significant seasonal variation in each lake group (Fig. 2B
and Table SI 2). Concentrations showed similar wider range of values for pDe-GR and De-SR lakes
(4 to 118 pg L\(^{-1}\) excluding Ordicuso in June 2017) compared to PT-SR and Cr-SR lakes (3 to 36 pg
L\(^{-1}\)). In pDe-GR lake groups, although not statistically different, average TVSe concentration in
June samples (66 ± 34 pg L\(^{-1}\)), just after the ice-cover melting, was higher compared to October
samples (41 ± 24 pg L\(^{-1}\)), which may indicate volatile species accumulation during ice capping
period. The main species found in all lakes was DMSe, representing in average 76% of the TVSe
detected. Previous studies in remote arctic thermokarst ponds reported total volatile Se in the range
1–32 pg L\(^{-1}\) including 96% of DMSe (Lanceleur et al., 2019). The low concentrations of TDSe and
sulphate in Pyrenean lakes seem to limit the production of dimethyldiselenide (DMDS) or dimethyl
selenyl sulfide (DMSeS) observed in other environments, such as meso-eutrophic lakes,
minerotrophic peatlands or estuarine and marine systems (Amouroux & Donard, 1997; Tessier et
al., 2002; Vriens et al., 2014; Be'eri-Shlevin et al., 2021). For TVSe, no clear trend was observed
with any of the measured parameters in water samples (Spearman coefficient |ρ|<0.3), most
probably because of the extremely low concentration of gaseous Se species in most lakes. The
limited nutrient supplement and further biological activity of Pyrenean lakes seem to be the main
limit for volatile Se production. In addition, the short lifetime and rapid degradation of volatile Se
compounds in the photic zone could make it difficult to establish correlation trends (Mason et al., 2018).

### 3.2. Profiles of selenium and dissolved species in lake waters

During the sampling campaigns of 2018 and 2019, depth profiles were carried out in four lakes: Arratille and Azul Superior (De-SR lakes), Gentau (PT-SR lake) and Sabocos (Cr-SR lake). Detailed data are available in Table SI 4.

#### 3.2.1 Shallow lakes (maximum depth < 15 m)

Arratille and Azul Superior lakes belong respectively to Cauteret and Panticosa areas of similar geological structure (mostly granitic and, limestone, marble, shale and sandstone sedimentary rocks) but respectively located in the northern and southern slope. Oxygen and temperature depth profiles demonstrate that Arratille exhibited a small oxygen depletion only at the bottom water sample in June, while Azul Superior, with a maximum depth of around 8 m, had a well-mixed water column (Fig. SI 5). In both lakes, total Se concentration did not vary significantly and the main species was selenate with less than 6 and 7% variation, respectively, along the water column. Selenite and Se(-II, 0) fraction were both below the LoQ. The profile of volatile species was different from those of non-volatile selenium species. In June, the concentration of DMSe was increased at half-depth in both lakes. This concentration peak was more visible in Aratille lake where DMSe concentration stayed constant to the bottom. In Azul Superior lake, DMSe profile appeared to be similar to the one of nitrate in June while it was opposite in October where DMSe concentrations were clearly lower. The formation of DMSe has been shown to occur with similar production rates from selenate or selenite in bottom pond waters (i.e oxygen depleted) (Lanceleur et al., 2019). Although, DMSe production in the photic zone could not be excluded, it should be of lesser extent in such oligotrophic lakes if compared to deeper microbial processes in bottom waters and sediments (Amouroux & Donard, 1997; Lanceleur et al., 2019). Minor volatile algal production was also previously observed.
comparing marine algae, bacteria and mixed bacteria-algae culture experiments (Luxem et al., 2017). Observed difference between sub-surface and bottom volatile Se concentrations could thus be indicative of its formation and accumulation at depth during winter-early spring (i.e ice-covered period promoting reducing conditions), upward diffusion and volatilization and/or photodegradation in the surface layer during summer period.

3.2.2 Deep lakes

Gentau and Sabocos lakes are located at lower elevation and belong respectively to Ayous and Panticosa areas where agropastoralism activities can represent a source of organic matter and nutrients (Kortazar et al., 2020). Both lakes are affected by thermal and oxygen stratification and during 2018 samplings, the deepening of thermocline and oxycline was observed in both lakes from late spring to autumn (Fig. 3). Hypoxic conditions (DO < 4 mg L\(^{-1}\)) were maintained in the deepest 5 m. Nitrate and sulphate concentrations in Sabocos were a little lower than the mean values (≈ 1 and 4 mg L\(^{-1}\), respectively) measured in 2012 (Santoloria et al., 2015) and, higher compared to Gentau. This was particularly the case for sulphate concentration that was extremely low (≤0.7 mg L\(^{-1}\)) in Gentau. After summer, a nitrate depletion was observed in the photic zone (4–8 m depth in Sabocos, up to 12 m depth in Gentau). Conductivity values were also higher in Sabocos due to its calcareous basin (Santoloria et al., 2015; Kortazar et al., 2020). An increase of conductivity values from the surface to the bottom was observed in Gentau. With the exception of two deviant values observed in Gentau (abnormal high values for TDSe at 0.5 m depth in June 18 and, TSe bulk at 18 m depth in June 19), total Se concentrations did not vary significantly along the water column (<14% variation) as previously observed in shallower lakes.

Selenite concentration was in both lake profiles below the LoQ and, selenate demonstrated opposite profiles. Selenate was not detected in October profile of Gentau lake which may be related to the high LoQ of 10 ng L\(^{-1}\) of the chromatographic conditions used. However, reproducible profiles were observed in June where selenate concentration decreased with increasing depth, resulting in
quantifiable Se(-II, 0) fraction in hypoxic deepest 5 m. These data suggested either abiotic and/or microbial reduction of selenate or its slower re-oxidation in bottom waters of Gentau lake as observed in other lentic systems (Lanceleur et al., 2019; Be’eri-Shlevin et al., 2021). In Sabocos, however, selenate proportion in June profiles did not vary significantly along the water column (67 ± 7 and 90 ± 9 % of total dissolved Se in 2018 and 2019, respectively). The lower proportion of selenate in June 2018 resulted in occasional quantifiable Se(-II, 0) fraction at values marginally above the LoQ. In October, despite an oxygen saturation above 70%, Se(VI) depletion was observed in the photic zone as was the case for nitrate. These data could indicate biological uptake of selenate as previously suggested elsewhere (Harrison et al., 1988; Duan et al., 2010; Be’eri-Shlevin et al., 2021). Observed differences between selenate proportion in oxygen-depleted bottom waters of both lakes may be related to the fact that Gentau lake receives mineral inputs, mainly iron (Kortazar et al., 2020), which may promote the existence of Fe-selenides or colloidal elemental selenium at the sediment-water interface (Fernández-Martínez & Charlet, 2009).

The total volatile Se concentration sharply peaked in both bottom waters in June 2018 at values that were not measured afterward. This increase mainly resulted from DMDS contribution (43 to 76% of TVSe). In comparison with shallower lakes, DMSeS contribution to total volatile selenium was non negligible in the photic zone of Gentau and Sabocos lakes. In addition to enhanced production of DMSe, Lanceleur et al. (2019) have reported that DMDS was formed in bottom pond waters enriched with local biofilm with a production rate approximately 100 times lower compared to the one of DMSe. The significant proportion of DMDS with respect to DMSe observed in-depth of these oligotrophic aquatic systems may thus be related to their respective volatility (Karlson et al., 1994) and dark conditions favourable to formation and stability of diselenide bond (Amouroux et al., 2000). The presence of DMSeS in shallower waters suggested its possible formation from DMDS and sulphur-containing volatile compounds (Chasteen, 1993; Swearingen et al., 2006). Likewise previous observations in shallow lakes, enhanced concentration levels of TVSe were
observed in springtime after the ice coverage period preserving such compounds from volatilization and photodegradation that seem to control TVSe in sub-surface waters.

3.3. Sources of Selenium in alpine lake waters: bedrocks leaching/erosion versus wet depositions inputs

Because most of the investigated lakes have a limited watershed size (Table 1), main Se inputs are suggested to be related to the elemental composition of the prevailing bedrocks through dissolution and erosion pathways, and direct atmospheric inputs. Among major anions and considering all lakes and sampling dates, correlation analysis showed a very strong positive relationship between total dissolved Se and sulphate (Fig. SI 3). Sulphate’s concentration mainly depends on the geological substrate while the intensive study of Camarero et al. (2009) in European mountain lakes revealed that lakes having sulphate concentration < 2.3 mg L⁻¹ could be considered as mainly controlled by S atmospheric inputs. During our recent field sampling program, a similar threshold sulphate concentration value of 0.7 mg L⁻¹ has been observed and proposed to distinguish between atmospheric or geological sources in lakes of studied zone (Duval, 2020). This value agrees with the annual sulphate concentration of 0.67 mg L⁻¹ reported by Suess et al. (2019) at the Pic du Midi de Bigorre high altitude station. In the western Pyrenees region, shales formed during the Devonian geological period are the main source of SO₄²⁻ (Van Lith, 1968). Lakes presenting the highest sulphate and selenium concentrations are those located over sedimentary rocks containing shales (De-SR lakes group). Their SO₄²⁻ concentration is higher than concentrations found in rainwaters (Roulier et al., 2021; Suess et al., 2019) that may thus, similarly to S, indicate selenium geological supply as selenium concentrations are typically higher in shales than in granite, limestone or sandstone (Plant et al., 2014; Fernandez-Martinez & Charlet, 2009).

To evaluate the contribution of geogenic versus atmospheric Se sources, the molar ratio of Se/S measured in lake waters in this study was compared with Se/S ratios calculated for different rock types using a standard database (Reimann & De Caritat, 1998) as no data was available for local
bedrocks. Molar Se/S ratios measured in lake waters were also compared with Se/S ratios in wet
deposition at the Pic du Midi de Bigorre (calculated from Suess et al., 2019) and Adour watershed
(calculated from Roulier et al., 2021). The molar ratios Se/S in Pyrenean lakes were in the range
(0.7–6.8) · 10^{-5} (average values per lake groups in Table 2) and do not allow to provide clear
discrimination of potential major sources of Se in those lakes. However, considering the threshold
value of sulphate concentration in rain waters (0.7 mg L^{-1}=7.5 µmol L^{-1}) below which wet
depositions is the main source of S (Duval, 2020; Camarero et al., 2009), a strong direct relationship
was obtained between Se/S ratio and sulphate concentration for half of the studied lakes belonging
to pDe-GR and PT-SR groups (Spearman coefficient ρ=-0.75, p<0.0001, Fig. 4). For the other half
of lakes, including all De-SR group lakes, for which sulphate concentration was higher than 7.5
µmol L^{-1} and controlled by geological substrate, only a weak correlation was obtained (Spearman
coefficient ρ=-0.34, p<0.05, Fig. 4).

An estimation of Se input from wet deposition was calculated using the average annual Se flux in
Adour watershed located downstream the northern section of the west Pyrenees (50 ± 20 µg Se m^{-2}
yr^{-1}, Roulier et al., 2021). The estimation considered the surface area and volume of each lake to
compare the input of Se from wet deposition with the stock of Se at each lake. The lake volume and
surface area data were taken from Duval (2020) and the results are presented in Table SI 5. We
found that Se provided from annual wet deposition could account for 12 to 100% (median value of
39%) of Se stored in lake water at a specific time of the year. Thus, wet depositions through rain or
snow events and during the snowmelt period is a non-negligible source of selenium in such alpine
Pyrenee lakes, which can be counterbalanced by a Se-enriched geological substrate.

The emission of volatile Se compounds could then contribute to lower the extent of Se in surface
lake waters, mainly in the form of DMSe. We used our measurements to estimate Se gaseous output
from lake water to the atmosphere, but as they corresponded only to two months of the year the
variability of TVSe concentrations was not well constrained. However, due to the low TVSe
concentrations and low seasonal variability observed before and after summer, together with the low surface area of most lakes (Table 1), low volatilization fluxes are expected. A rough estimation of Se emission was carried out similarly to Lanceleur et al. (2019) using the model of Cole & Caraco (1998) developed for small lakes considering 3 m s\(^{-1}\) as maximum average wind speed, as recorded in those sheltered lake systems (ca. 1–3 m s\(^{-1}\), from Duval et al., in preparation). We assumed that atmospheric exchange occurred for approximately 6 months, while during late fall to late spring the ice cover prevented volatilization. The annual Se emission (Table SI 5) was compared with the estimated total Se stock in the corresponding lake. These values (3.8–31 ng Se day\(^{-1}\) m\(^{-2}\)) were similar to the daily emission reported at thermokarst ponds (1–97 ng Se day\(^{-1}\) m\(^{-2}\)) (Lanceleur et al., 2019). The results indicated that annual Se emission from lakes to the atmosphere approximatively represents 0.2 to 6.4% of the Se stock in lakes at a specific time of the year (median value of 3%), remaining thus a minor pathway for Se removal from lake waters.

4. Conclusions

Pyrenean lakes showed similar bulk and dissolved total Se concentrations, in the low range of a dozen to a few dozen of ng Se L\(^{-1}\). Total dissolved Se varied between 7–78 ng L\(^{-1}\) and, on average, 64% was in the form of selenate in subsurface samples. The extremely low Se concentration in alpine lakes limited the detection of other Se species such as selenite. Volatile Se speciation was mainly limited to the production of DMSe and volatilization appeared to not represent an important removal pathway for Se in Pyrenean lakes.

Since no significant seasonal variations (June versus October) was observed neither for total Se concentrations nor its species in subsurface waters, it is suggested that Se concentrations in lake waters are regulated by downstream export and possible scavenging. The proportion of Se (-II, 0) fraction was for example increased in-depth of deep lakes, such as Gentau lake, together with oxygen depletion probably because of microbial reduction. Further studies are required to ascertain
how this process promotes Se scavenging, precipitation and further sedimentation in those lakes, as shown in thermokarst artic ponds (Lanceleur et al., 2019).

The values of molar ratios Se/S in Pyrenean lakes overlap those for comparable bedrock types and wet depositions. Our interpretation suggests that atmospheric inputs represent an important source of Se to remote lakes when bedrock geochemistry is depleted with Se and sulphate. This study provides a unique set of data that allows to better assess Se biogeochemistry in lakes and watershed mainly affected by global emissions and long-range transport and atmospheric depositions.

Conflicts of interest

There are no conflicts of interests to declare.

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