

1 **Altitudinal distributions of BDE-209 and other polybromodiphenyl ethers**  
2 **in high mountain lakes**

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Keywords: polybromodiphenyl ethers, organohalogen compounds, high mountains, lakes, temperature dependence, microbial biofilms, long range transport

26 ABSTRACT

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28         The present study shows the occurrence of 2,2',3,3',4,4',5,5',6,6'-decabromodiphenyl  
29 ether (BDE-209) in microbial biofilms of Pyrenean and Tatra high mountain lakes despite its  
30 low vapor pressure and high hydrophobicity. Aerosol air transport is therefore a feasible  
31 mechanism for BDE-209 accumulation in sites up to 2688 m above sea level. This compound  
32 and other PBDEs exhibit altitudinally-dependent distribution involving higher concentrations  
33 with increasing mountain lake elevation. However, the apparently very high enthalpies of the  
34 concentration gradients observed, including BDE-209, suggest that bacterial anaerobic  
35 debromination also plays a significant role in the resulting altitudinal distributions. This  
36 microbial mechanism explains the relative abundances of PBDEs and their within lake  
37 differences between rocky and sediment microbial biofilms, thereby showing that the  
38 altitudinal pattern observed is not purely due to water temperature control on bacterial activity  
39 but also to changes in the availability of anaerobic microenvironments which increase with  
40 increasing lake productivity at lower altitudes.

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42         Capsule: Decabromodiphenyl ether and the other polybromodiphenyl congeners show vertical  
43 distributions in high mountains due to temperature effects and microbial degradation.

## 44 **1. Introduction**

45

46 Polybromodiphenyl ethers (PBDEs) are common flame-retardant products currently in  
47 use in household and workplace materials. They have been produced commercially in the  
48 form of diphenyl ether mixtures with three degrees of bromination: nominally penta-, octa-  
49 and deca-BDE. The last formulation currently involves 83% of the PBDE global market  
50 demand, followed by penta- and octa-BDE at 11% and 6%, respectively. Due to health  
51 concerns and their widespread occurrence in environmental and human samples, the  
52 pentaBDE and octaBDE technical mixtures were banned by the European Union (EU) in  
53 2004 and their production ceased in North America. Recently, the Stockholm Convention on  
54 Persistent Organic Pollutants (SCPOPs) decided to include the following mixtures in their list:  
55 tetraBDE (defined as BDE 47), pentaBDE (defined as BDE 99), hexaBDE (defined as BDE  
56 153 and BDE 154) and heptaBDE (defined as BDE 175 and BDE 183) (SCPOPS,2010).  
57 Deca-BDE is essentially composed of 2,2',3,3',4,4',5,5',6,6'-decabromodiphenyl ether (BDE-  
58 209) where it constitutes more than 97% of the congener mixture (Sjodin, 2000). Almost half  
59 of the annual global deca-BDE demand (more than 56,000 metric tonnes in 2003; Bromine  
60 Science and Environmental Forum, 2007) is used in flame retarding high impact polystyrene,  
61 the plastic resin commonly used in TV and radio cabinets. Other applications of deca-BDE  
62 include use in polyester fiber additives, coatings for automobiles and acrylonitrile-butadiene-  
63 styrene rubber, in which deca-BDE can be found in concentrations between 6% and 22%  
64 (Environmental Health Criteria, 1994). Production and use of decaBDE has continued  
65 although in 2009 its use was restricted in Europe (EBFRIP, 2009).

66 In the environment, BDE-209 is susceptible to degradation via debromination, due to  
67 exposure to UV light (Bezares-Cruz et al., 2004), to reaction to radicals (Ter Schure et al.,  
68 2004) and/or by microorganism activity (Rayne et al., 2003; Gerecke et al., 2005; He et al.,

69 2006; Vonderheide et al., 2006; Tokarz et al., 2008; La Guardia et al., 2007). These three  
70 debromination pathways can all potentially lead to the formation of PBDE congeners, which  
71 are similar to the congeners found in the now banned penta- and octa-BDE commercial  
72 mixtures. Nevertheless, the occurrence of BDE-209 has been reported in air, sediments, biota,  
73 and house dust (Stapleton et al., 2005; Webster et al., 2009).

74 Generally, it is believed that the large molecular size, low vapor pressure (subcooled  
75 vapor pressure =  $10^{-8.3}$  Pa; Wania & Dugani, 2003) and high hydrophobicity (log Kow = 8.7;  
76 Wania & Dugani, 2003) of BDE-209 will result in limited capacity for long-range  
77 atmospheric transport (Wania & Dugani, 2003; Strandberg et al., 2001) and low  
78 bioavailability (Hardy, 2002). However, recent studies suggest that BDE-209 is also likely to  
79 be transported to remote areas in association to particles under dry conditions and high wind  
80 speed (Breivik et al., 2006) or, perhaps less likely, via the temperature-dependent grasshopper  
81 effect (Gouin & Harner, 2003). The presence of this compound in Arctic mosses and marine  
82 organisms has recently been reported (de Wit et al., 2006).

83 In this paper, we report the presence of BDE-209 in high mountain lakes situated far  
84 from pollution emission sources, with hydrology dominated by atmospheric processes:  
85 Llebretra (42.55°N 0.89°E, 1620 m a.s.l.), Llong (42.57°N 0.95°E, 2000 m), Xic de Colomina  
86 (42.52°N 0.99°E, 2425 m) and Vidal d'Amunt (42.53°N 0.99°E, 2688 m) from the Pyrenees  
87 (Catalonia, Spain) and Vel'ké Hinčovo (49.18°N 20.06°E, 1946 m) from the Tatras (Slovakia)  
88 (Figure 1). They are softwater oligotrophic lakes, with long ice cover periods (from ca. 4 to 7  
89 months) and cold water temperature during ice-free periods. These conditions involve  
90 relatively scarce biomass and simple food webs. The lakes from the Pyrenean transect are  
91 situated in a maximal planar projection distance of 9.2 km. Thus, their differences in PBDE  
92 accumulation capacity will essentially depend on altitudinal differences, e.g. 1068 m between  
93 those two situated at highest and lowest elevation.

94 BDE-209 could only reach these sites by long-range atmospheric transport (Vilanova  
95 et al., 2001; Grimalt et al., 2001; Carrera et al., 2002; Ribes et al., 2002), thereby providing  
96 solid proof of the feasibility of the transfer of this compound from sources to remote areas  
97 through the atmosphere. In addition, we found that PBDE increases in accordance with lake  
98 altitude. We studied the presence of PBDE in microbial biofilms in an altitudinal gradient of  
99 lakes (Figure 1) and within each lake we studied biofilms on different substrates, namely rock  
100 and silt biofilms. Rock biofilms are present in littoral lake areas, whereas silt biofilms are  
101 mostly found in the top sediment of deep lake areas. In these relatively shallow mountain  
102 lakes, even the deep layers receive enough light for light-based autotrophic growth; thus all  
103 microbial biofilms are an amalgam of microalgae (mainly diatoms) and heterotrophic  
104 bacteria. However, they differ in the relative proportions of these components or, in terms of  
105 processes, in the intensity and duration of autotrophic vs heterotrophic activities and  
106 particularly in the time periods in which bacterial activity takes place under anoxic conditions.  
107 Anaerobic bacterial activity has been identified as the main pathway for PBDE  
108 biodebromination (He et al., 2006; Tokarz et al., 2008). Therefore, comparison of PBDE  
109 distribution in biofilms of lakes at different altitudes enables us to elucidate between air-water  
110 exchange and biodegradation as the main mechanism responsible for the altitudinal patterns  
111 observed.

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## 113 **2. Materials and methods**

114

### 115 *2.1. Chemicals and materials*

116

117 Trace analysis (SupraSolv) isooctane, n-hexane, dichloromethane, and acetone were  
118 from Merck (Darmstadt, Germany). Concentrated sulfuric acid ProAnalysis grade 95-97%

119 was also purchased from Merck (Darmstadt, Germany). PCB-200 and PCB-209 were  
120 purchased from Dr. Ehrenstoffer (Augsburg, Germany). The standards solution EO-5099 and  
121 BDE-209 in iso-octane were purchased from Cambridge Isotope Laboratories (Andover, MA,  
122 USA). Helium and ammonia were from Air Liquide (Barcelona, Catalonia, Spain). Their  
123 purity was 99.999%.

124 Copper for desulphurization was activated by sonication with 35.5% hydrochloric acid  
125 (3 x 3 mL), and then it was rinsed several times with Milli-Q water to neutral pH and,  
126 subsequently, with acetone for water removal. This powder was stored under n-hexane prior  
127 to use (not later than 2 days after activation).

128 The gas chromatograph used for the PBDE analyses was a Trace GC Ultra (Thermo  
129 Electron, Milan, Italy). This instrument was coupled to a negative ion chemical ionization  
130 mass spectrometer MS DSQ (Thermo Electron, Austin, Texas, USA; Eljarrat et al., 2004).  
131 The DB5-MS capillary column was from J&W Scientific, Folsom, CA, USA.

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## 133 2.2. *Sampling*

134

135 A full description of the analytical method used for analysis of PBDEs is described  
136 elsewhere (Vizcaino et al., 2009). Sampling in the Pyrenean and Tatra lakes was performed in  
137 July 2004 and September 2005, respectively. Two main types of samples were considered:  
138 rock and silt biofilms. Each rock biofilm sample was obtained by scrapping ten stones  
139 representing the littoral shore of each lake, at 1 m deep in the shoreline of the lake. A metallic  
140 ultra – cleaned brush was used. Stone tops and bottoms were scraped separately in order to  
141 identify potential effects due to exposition to UV radiation. Silt biofilms were sampled with  
142 an Uwitec gravity core sampler (Mondsee, Austria). Top layers of 1 cm thickness were sliced  
143 from the sediment cores. Samples were kept frozen (-20 °C) until analysis. Sample dry weight

144 was estimated by drying in a vacuum sealed-drier at 20°C until constant weight. Sediment  
145 loss on ignition was carried out at 550°C, as an estimation of the organic content of the  
146 sediment samples (Heiri et al., 2001).

147

### 148 *2.3. Extraction and clean-up*

149

150 Samples were fortified with PCB-209 standards and extracted by sonication with 20  
151 mL hexane-dichloroethane (4:1) for 15 minutes. This step was repeated four times and all the  
152 extracts (80 mL) were combined and concentrated to a small volumes (2-4 mL) by vacuum  
153 rotary evaporation. Samples were redissolved in n-hexane and cleaning-up was performed by  
154 four successive oxidation steps with sulfuric acid (Eljarrat et al., 2004). About 0.5 g of  
155 activated copper was added to the extracts containing sulfur. After manual stirring the  
156 suspensions were kept overnight at room temperature. Finally, the extract was concentrated to  
157 near dryness under a gentle flow of nitrogen and redissolved in 25 µl of isooctane. An internal  
158 standard of PCB-200 was added before chromatographic analysis.

159

### 160 *2.4. Instrumental analysis*

161

162 Extracts were analyzed by gas chromatography coupled to negative ion chemical  
163 ionization mass spectrometry. The system was equipped with a DB5-MS capillary column (15  
164 m x 0.25 mm x 0.1 µm film thickness) coated with phenyl arylene polymer that is virtually  
165 equivalent to 5%-phenyl/95% methylpolysiloxane stationary phase. The oven temperature  
166 program for GC-NICI-MS analysis was from 140°C (held for 1 min) to 325°C at 10°C·min<sup>-1</sup>  
167 (held for 10 min). Helium was used as a carrier gas (1 mL·min<sup>-1</sup>) and ammonia as ionization  
168 gas (2.4·10<sup>-4</sup> Pa). Their purity was 99.999% in both cases. Transfer line temperature was

169 300°C. Quantification was performed from the intensities of the m/z 79 ion [Br]<sup>-</sup>.  
170 Confirmation ions were m/z 81 [Br]<sup>-</sup>, 161 [HBr<sub>2</sub>]<sup>-</sup> and 327, 405, 483, 563 and 643,  
171 corresponding to either [M]<sup>-</sup> or [M-HBr<sub>2</sub>]<sup>-</sup>. BDE-209 was measured from the intensities of the  
172 m/z 487 ion [Br]<sup>-</sup> and the confirmation ion was m/z 489 [Br]<sup>-</sup>.

173

### 174 2.5. *Quality assurance*

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176 Procedural blanks were analyzed for every set of six samples which correspond to  
177 periods between twelve hours and two days of sample handling, depending on the matrix. The  
178 recoveries of the surrogate standard, PCB-209, were calculated for each sample, being 69 ±  
179 22% (average ± standard deviation). The recovery of this surrogate was used for correction of  
180 all PBDE concentrations in each sample. Identification and quantification of PBDEs was  
181 performed by injection of external standards at different concentrations. Relative responses to  
182 PCB-200 were used in order to correct for instrumental variability, and this value was also  
183 corrected by the recovery of the surrogate standards. Limits of detection and quantification  
184 were calculated from real samples as the mean of noise signal plus 3 and 5 times respectively  
185 the standard deviation. They ranged between 0.1-0.8 and 0.2-1.3 pg g<sup>-1</sup>, respectively. Final  
186 validation was made by analysis of reference material obtained from the Arctic Monitoring  
187 and Assessment Program (AMAP). We participate regularly in the AMAP Ring Test  
188 Proficiency Program for POPs (Centre de Toxicologie Institut National de Santé Publique du  
189 Québec, Québec, Canada) and the laboratory results usually were within 20% of the  
190 consensus values, including BDE 209 concentrations.

191

## 192 **3. Results and discussion**

193

194 *3.1. Biofilm concentrations*

195

196 Silt biofilms are relatively similar among lakes. They are composed of large masses of  
197 mostly empty diatom valves, amorphous organic matter and bacteria. These biofilms  
198 experience large periods of anaerobiosis during long ice-cover periods, and the temperature of  
199 their overlying waters (ca. 4°C) varies only slightly between one lake and another throughout  
200 the year. In contrast, rock biofilms are diverse and rich in microalgae. At higher lake altitudes,  
201 they undergo shorter growth periods, higher UV radiation and colder temperatures which  
202 result in less productive and thus thinner biofilms. Higher accumulation of organic matter in  
203 rock biofilms at low altitude lakes enhances the formation of anoxic microenvironments, as  
204 reflected by the presence of sulfur generated by sulfate reduction.

205 BDE-209 was the most abundant congener in the samples, representing about 50% of  
206 total PBDE (Table 1). BDE-47, 99, 183 and 190 were the other main congeners but their  
207 concentrations were much lower. These results evidence the long-range atmospheric transport  
208 and deposition of this low volatile and highly hydrophobic brominated compound to remote  
209 European areas, including high mountains. The BDE-209 dominance agrees with the present  
210 use of deca-BDE as the only PBDE mixture freely traded in Europe (Cox & Ethymiou, 2003).

211 Silt biofilms showed lower BDE-209 concentrations than rock biofilms (Table 2). This  
212 difference could reflect dilution effects due to sediment slicing, since 1 cm of top sediment  
213 sections in these lakes could represent an interval of between 5 and 40 years, probably  
214 including long sedimentation periods in which deca-BDE was not used. The silt sediment  
215 concentrations of BDE-209 in the lakes of these two mountain ranges (Table 2) are similar to  
216 those found in Arctic lake sediments such as Char and AX-AJ lakes (Canada; de Wit et al.,  
217 2006), 0.042 and 0.075 ng g<sup>-1</sup> dw, respectively, and lower than those found in Lake Winnipeg  
218 (0.63 ng g<sup>-1</sup> dw; Law et al., 2006). Comparison of the BDE-209 concentrations in the lake

219 biofilms of these European mountains with those found in Arctic mosses from Skoganvarre  
220 and Valvik (Norway), 0.025 and 0.12 ng g<sup>-1</sup> dw (de Wit et al., 2006) respectively, show  
221 comparable levels in the case of the Pyrenean sites (Table 2). However, the biofilm BDE-209  
222 concentrations in Vel'ké Hinčovo (Table 2) are between one and two orders of magnitude  
223 higher than in these mosses. All PBDE congener concentrations in all biofilms were higher in  
224 Vel'ké Hinčovo than in the Pyrenean lakes ( $p < 0.01$ , Wilcoxon rank-sum test).

225         Comparison between top and bottom rock surfaces, i.e. between exposed and protected  
226 biofilms respectively, does not show consistent significant differences in BDE-209  
227 concentrations (Table 2). Thus, UV radiation of lake surfaces does not seem to be a major  
228 cause of the concentration differences of this flame retardant after deposition.

229

### 230 *3.2. Altitudinal dependence*

231

232         The biofilm concentrations of the most abundant PBDE congeners in the Pyrenean  
233 lakes exhibit altitudinal gradients in most cases. These gradients involve concentration  
234 increases of about two orders of magnitude between the highest and the lowest altitude lakes  
235 but in some compounds and types of samples it may be four orders of magnitude. The  
236 compounds represented in Figure 2 are those with mean PBDE concentrations higher than  
237 0.015 ng g<sup>-1</sup> OM.

238         Representation of the logarithmic transformation of these concentrations vs lake  
239 altitude (Figure 2) shows a linear trend with statistically significant correlation coefficients in  
240 several cases, e.g. BDE-47 ( $p < 0.05$ ) and BDE-173/190 ( $p < 0.05$ ) in silt biofilms and BDE-  
241 183 ( $p < 0.05$ ) and BDE-209 ( $p < 0.001$ ) in top rock biofilm and BDE-183 in bottom rock  
242 biofilm ( $p < 0.05$ ). These results must be considered tentative in view of the small numbers of  
243 cases defining the correlations. Altitudinal gradients encompassing more cases should be

244 undertaken for confirmation. In these cases, the altitudinal concentration gradients involve  
245 two orders of magnitude for a difference of 1060 m (Figure 2). These gradients evidence the  
246 feasibility of PBDE including BDE-209 for long range atmospheric transport. Thus, despite  
247 its low volatility (Wania & Dugani, 2003), this decabrominated compound can be found in  
248 remote mountain lakes situated at 2688 m above sea level.

249         Increased precipitation and snow accumulation at higher mountain altitudes may be  
250 potential sources for the enhanced deposition of this compound and the less brominated  
251 PBDEs. However, studies in this geographic area have found no consistent patterns  
252 corresponding to altitude or depth of the snow layer in the distribution and chemistry of major  
253 components in winter snowpack in the altitude range of 1820–3200 m above sea level  
254 (Bacardit and Camarero 2010). This result is consistent with a previous study in the area  
255 showing lack of altitudinal influence in the amount and chemistry of precipitation in an  
256 altitude range of 1600-2200 m above sea level (Camarero and Catalan 1996). These previous  
257 results make unlikely that altitudinal changes in precipitation and snow deposition with  
258 altitude may determine the PBDE concentration increase along this Pyrenean lake profile.

259         Previous studies reported altitudinal distributions of organohalogen compounds in  
260 remote mountain areas, either in aquatic organisms (Grimalt et al., 2001; Gallego et al., 2007;  
261 Demers et al., 2007) or adsorbed in soils (Ribes et al., 2002), snow (Blais et al., 1998; Carrera  
262 et al., 2001) or plants (Grimalt et al., 2004), explained by the partial condensation of their  
263 atmospheric gas phase standing reserve. The process is consistent with the global distillation  
264 effect (Wania & Mackay, 1993) and requires gas phase transport of semi-volatile compounds  
265 (Grimalt et al., 2001; 2004; Daly et al., 2007) such as the organochlorine pollutants of these  
266 previous mountain studies. These compounds have volatilities of  $10^{-2.5}$ - $10^{-3.9}$  Pa (Grimalt et  
267 al., 2001) which are similar to those of BDE-47 and BDE-99,  $10^{-3.5}$  and  $10^{-4.2}$  Pa (Chen et

268 al., 2003) respectively and are higher than those of BDE-183 and BDE-173/190,  $10^{-6.1}$  and  $10^{-6.0}$  Pa respectively.

270 In this context, BDE-209 stands out for its low volatility,  $10^{-8.3}$  Pa (Wania & Dugani,  
271 2003), i.e. more than four orders of magnitude lower than that of the organochlorine range.  
272 Accordingly, this compound predominantly occurs in the particulate phase (Gouin & Harner,  
273 2003; Hoh & Hites, 2005; Strandberg et al., 2001; Ten Schure et al., 2004; Breivik et al.,  
274 2006). Despite this association mode, evaluations based on experimentally determined  
275 deposition velocities have pointed to a much higher capacity for long-range atmospheric  
276 transport of this compound than initially estimated (Breivik et al., 2006). In addition, the  
277 association to particles may protect it from significant UV-decomposition on its way to high  
278 mountain lakes, as occurs with polycyclic aromatic hydrocarbons (Fernandez et al., 2000;  
279 2002). In any event, the BDE-209 vertical gradient observed (Figure 2) is challenged by the  
280 predominant particle-association of this compound.

281

### 282 *3.3. Transport and transformation mechanisms*

283

284 The cold condensation theory referred to above is consistent with the annual average  
285 air temperatures of mountain sites in Europe (Grimalt et al., 2001; Vives et al., 2004; Gallego  
286 et al., 2007) and the Andes (Grimalt et al., 2004). Transformation of the altitudinal  
287 distributions of Figure 2 into equivalent temperatures using the annual average temperatures  
288 of each lake shows concentration gradients in line with temperature dependence (Figure 3).  
289 This approach allows joint representation of the BDE concentrations from both the Pyrenean  
290 and Tatra lakes vs the reciprocal of temperature (Figure 3). Vél'ke Hinçovo is the lake with  
291 lower air temperatures and consequently higher PBDE concentrations. In nearly all cases,  
292 lower temperatures correspond to higher BDE concentrations involving differences between

293 one and five orders of magnitude. The correlations are statistically significant ( $p < 0.05$ ) in  
294 some of these environmental matrices, e.g. BDE-47 in top rock biofilm and silt biofilm, BDE-  
295 183 in top and bottom rock biofilm, BDE-173/190 in top rock biofilm and silt biofilm and  
296 BDE-209 in top and bottom rock biofilm (Table 3). Calculation of these correlations over the  
297 Pyrenean transect alone also shows statistically significant correlation coefficients, e.g. BDE-  
298 47 and BDE-173/190 in silt biofilm, BDE-183 in bottom rock biofilm and BDE-183 and  
299 BDE-209 in top rock biofilm. The lower number of cases with statistically significant  
300 temperature dependence in the Pyrenean series alone can be explained by loss of statistical  
301 power because of the lower number of lakes and elimination of the case with highest  
302 concentrations from the correlations. However, in practically all cases, PBDE concentrations  
303 increase with the altitude of the lake.

304         Possible changes in retention capacity of the lake catchments at increasing altitude  
305 could be considered for wash out differences into the lakes and therefore causes of altitudinal  
306 dependence in PBDE accumulation. However, the altitudinal pattern of relative abundances of  
307 these compounds (Figure 4) and the differences in PBDE distributions between biofilm types  
308 (Figure 4) cannot be explained by this hypothesis. Conversely, they are consistent with a  
309 temperature-dependent effect.

310         Calculation of the apparent enthalpies derived from the slopes of the statistically  
311 significant correlations and comparison with the theoretical vaporization and solubilization  
312 enthalpies provides further insight into the processes involved in these altitudinal trends. In  
313 most cases, the experimental values are much higher than the theoretical estimates from the  
314 physical-chemical constants of the compounds, e.g. 980-760 kJ mol<sup>-1</sup> for BDE-209 or 500-  
315 690 kJ mol<sup>-1</sup> for BDE-47 (Table 3). The differences between theoretical and apparent  
316 enthalpies are extremely high, e.g. 570-780 kJ mol<sup>-1</sup> in the case of BDE-209 (Table 3),

317 indicating that processes other than condensation and solubilization must determine the air  
318 temperature-dependent accumulation of PBDE in the biofilms of high mountain lakes.

319 Microbial reductive debromination is one of the most important routes for  
320 environmental transformation of persistent halogenated compounds. Penta-BDE  
321 (Vonderheide et al., 2006), octa-BDE (He et al., 2006), deca-BDE (He et al., 2006;  
322 Vonderheide et al., 2006) technical mixtures or individual congeners such as BDE-15 (Rayne  
323 et al., 2003) and BDE-206, BDE-207, BDE-208 and BDE-209 (Gerecke et al., 2005) have  
324 been reported to be degraded under anaerobic conditions. These processes may also occur in  
325 lake biofilms after long-range atmospheric transport of these compounds to these sites.

326 Increasing air temperatures generate longer growing seasons and ice-free periods;  
327 productivity rises and littoral environments become richer in organic matter. Higher biofilm  
328 thickness and organic matter content increase the formation of anoxic microenvironments  
329 during less productive periods of the year in the rock biofilms of the lower lakes. Higher  
330 PBDE biodegradation may therefore occur in the lower altitude lakes paralleling the  
331 temperature differences. Alternatively, higher rock biofilm thickness could be attributed to  
332 higher PBDE dilution and therefore lower concentrations at lower altitudes. However, this  
333 hypothesis contradicts the observations of silt biofilms whose thickness is not altitudinal-  
334 dependent and their PBDE concentrations also show altitudinal concentration gradients. In  
335 this context, the differences in PBDE distributions between silt and rock biofilms are key  
336 findings suggesting that reductive dehalogenation of PBDE by anaerobic bacteria, e.g.  
337 *Dehalococcoides* sp, is responsible for the patterns obtained. In fact, silt biofilms with higher  
338 periods of anaerobiosis and similar organic matter content along the altitudinal gradient  
339 (Bartrons et al., 2010) show the same PBDE distribution than rock biofilms from low altitude  
340 lakes.

341 Further insight into this degradation effect is obtained when considering the qualitative  
342 PBDE changes by compilation of the ratio between each PBDE and total PBDE  
343 concentrations for the rock biofilms analyzed in the lakes considered for study shows a clear  
344 temperature dependence, i.e. more than 80% BDE-209 at annual average air temperatures of  
345 1°C, and less than 20% BDE-209 at temperatures higher than 4°C (Figure 4). Since anaerobic  
346 microbial degradation of BDE-209 involves the formation of most PBDEs listed in Table 1  
347 (Tokarz et al., 2008) the changes in these ratios may reflect the extent of transformation of  
348 BDE-209 at different temperatures. In contrast, silt biofilms do not show this temperature  
349 dependence, as they are characterized by long anoxic periods, irrespective of the altitude at  
350 which the lake is located, and thus high degradation occurs in all of them (Figure 4). This  
351 higher degradation in silt microbial biofilms is also consistent with their PBDE  
352 concentrations, which are much lower than in rock biofilms (Table 2).

353 BDE-209 may reach high mountain sites in association to particles and be better  
354 preserved at low temperatures (high altitude) than at warm temperatures (low altitude). Since  
355 PBDE microbial degradation involves transformation of the more brominated congeners into  
356 others less brominated, e.g. nona-, octa-, hexabromodiphenyl ethers (Bezares-Cruz et al.,  
357 2004; Tokarz et al., 2008; La Guardia et al., 2007), BDE-47 (He et al., 2006; Tokarz et al.,  
358 2008) and others, this process may also account for the enhanced thermal dependence of  
359 BDE-47, BDE-100, BDE-154 and BDE-173/190 observed in mountains. For compounds such  
360 as BDE-47, the convective diurnal cycles in mountains during summer may add an upwards  
361 transport mechanism to the preservation mechanism.

362

#### 363 **4. Conclusions**

364

365 Climate-related processes which generate altitudinal and latitudinal concentration  
366 gradients of PBDE include biodegradation as well as global distillation long-range transport  
367 effects, e.g. volatilization, cold condensation and lipophilic phase-transfer which depend on  
368 the physical-chemical properties of the compounds. Biodegradation enhancement or depletion  
369 may respond to both direct thermal dependence and availability of anaerobic (micro)  
370 environments. This microbial mechanism is needed to explain the relative abundances of  
371 PBDEs and their within lake differences between rocky and sediment microbial biofilms,  
372 thereby showing that the altitudinal pattern observed is not purely due to water temperature  
373 control on bacterial activity but also to changes in the availability of anaerobic  
374 microenvironments which increase with increasing lake productivity at lower altitudes. To a  
375 significant degree, these microbial processes may also influence the global distribution of  
376 other organohalogen pollutants.

377

378 **Acknowledgements.** This work has been supported by the EU Projects Euro-Limpacs  
379 (GOCE-CT-2003- 505540) and ArcRisk (FP7-ENV-2008-1-226534), Spanish MEC project  
380 Trazas (CGL2004-02989) and Consolider Ingenio “GRACCIE” program (CSD2007-00067).  
381 Technical assistance in instrumental analysis by R. Chaler, D. Fanjul, and R. Mas, analytical  
382 advice by J. López and P. Fernández, field help by L. Camarero, M. Bacardit, G. Mendoza, E.  
383 Gallego, and S. Jarque is acknowledged. One of the authors, M.B., thanks a FPU grant of the  
384 Spanish Ministry of Education and Science.

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540 **Figure Legends**

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542 Fig. 1. Maps showing the location of the lakes chosen for study in the Pyrenees and  
543 the Tatras.

544 Fig. 2. Altitudinal distributions of BDE concentrations in the biofilm samples from the  
545 Pyrenean lakes. Note that the ordinate scale is logarithmic. \* $p < 0.05$ .

546 Fig. 3. BDE concentrations vs reciprocal of annual mean air temperature. Note that the  
547 ordinate scale is logarithmic. \* $p < 0.05$ .

548 Fig. 4. Relative PBDE distribution in the biofilm samples from all lakes

549