1 **Altitudinal distributions of BDE-209 and other polybromodiphenyl ethers**

2 **in high mountain lakes**

3

4

5

6

7

8

9

10 Mireia Bartrons[a,b], Joan O. Grimalt[a]* and Jordi Catalan[b]

11

12

13 [a] Department of Environmental Chemistry. Institute of Environmental Assessment and Water

14 Research (IDÆA-CSIC). Jordi Girona, 18. 08034 - Barcelona, Catalonia, Spain

15 [b] Limnology Unit (CSIC-UB). Centre for Advanced Studies of Blanes (CEAB-CSIC). Accés

16 Cala St. Francesc, 14. 17300 - Blanes, Catalonia, Spain.

17

18 *Corresponding author. Phone: +34934006118. Fax: +34932045904. E-mail address:

19 joan.grimalt@idaea.csic.es (J.O. Grimalt)

20
21
22
23

26   ABSTRACT

27

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.

41

42   Capsule: Decabromodiphenyl ether and the other polybromodiphenyl congeners show vertical

43   distributions in high mountains due to temperature effects and microbial degradation.

1. Introduction

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

In the environment, BDE-209 is susceptible to degradation via debromination, due to exposure to UV light (Bezares-Cruz et al., 2004), to reaction to radicals (Ter Schure et al., 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   Llebreta (42.55ºN 0.89ºE, 1620 m a.s.l.), Llong (42.57ºN 0.95ºE, 2000 m), Xic de Colomina

86   (42.52ºN 0.99ºE, 2425 m) and Vidal d'Amunt (42.53ºN 0.99ºE, 2688 m) from the Pyrenees

87   (Catalonia, Spain) and Vel'ké Hinçovo (49.18ºN 20.06ºE, 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.

4

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.

112

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.

132

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 ºC) 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 550ºC, 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 140ºC (held for 1 min) to 325ºC at $10ºC·min^{-1}$

167 (held for 10 min). Helium was used as a carrier gas ($1 mL·min^{-1}$) and ammonia as ionization

168 gas ($2.4·10^{-4}$ Pa). Their purity was 99.999% in both cases. Transfer line temperature was

169 300ºC. Quantification was performed from the intensities of the m/z 79 ion [Br]$^-$.

170 Confirmation ions were m/z 81 [Br]$^-$, 161 [HBr$_2$]$^-$ and 327, 405, 483, 563 and 643,

171 corresponding to either [M]$^-$ or [M-HBr$_2$]$^-$. BDE-209 was measured from the intensities of the

172 m/z 487 ion [Br]$^-$ and the confirmation ion was m/z 489 [Br]$^-$.

173

174 *2.5. Quality assurance*

175

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^{-1}, 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

3.1. Biofilm concentrations

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

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

Silt biofilms showed lower BDE-209 concentrations than rock biofilms (Table 2). This difference could reflect dilution effects due to sediment slicing, since 1 cm of top sediment sections in these lakes could represent an interval of between 5 and 40 years, probably including long sedimentation periods in which deca-BDE was not used. The silt sediment concentrations of BDE-209 in the lakes of these two mountain ranges (Table 2) are similar to those found in Arctic lake sediments such as Char and AX-AJ lakes (Canada; de Wit et al., 2006), 0.042 and 0.075 ng g^{-1} dw, respectively, and lower than those found in Lake Winnipeg (0.63 ng g^{-1} 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^{-1} 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 Hinçovo (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 Hinçovo 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^{-1} 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 $10^{-6.0}$

269 $^{6.0}$ 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^{-1} for BDE-209 or 500-

315   690 kJ mol^{-1} for BDE-47 (Table 3). The differences between theoretical and apparent

316   enthapies are extremely high, e.g. 570-780 kJ mol^{-1} 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   1ºC, and less than 20% BDE-209 at temperatures higher than 4ºC (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

385

386

387   **References**

388   Bacardit, M., Camarero, L. 2010. Atmospherically deposited major and trace elements in the

389   winter snowpack along a gradient of altitude in the Central Pyrenees: The seasonal

390   record of long-range fluxes over SW Europe. Atmospheric Environment 44, 582-595.

391   Bartrons, M., Camarero, L., Catalan, J. 2010. Isotopic composition of dissolved inorganic

392   nitrogen in high mountain lakes: variation with altitude in the Pyrenees.

393   Biogeosciences 7, 1469-1479.

394   Bezares-Cruz, J., Jafvert, C.T., Hua, I., 2004. Solar photodecomposition of

395   decabromodiphenyl ether: products and quantum yield. Environmental Science and

396   Technology 38, 4149-4156.

397   Blais, J.M., Schindler, D.W., Derek, C.G., Muir, D.C.G., Kimpe, L.E., Donald, D.B.,

398   Rosenberg, B. 1998. Accumulation of persistent organochlorine compounds in

399   mountains of Western Canada. Nature 395, 585-588.

400   Breivik, K., Wania, F., Muir, D.C.G., Alaee, M., Backus, S., Pacepavicius, G. 2006.

401   Empirical and modeling evidence of the long-range atmospheric transport of

402   decabromodiphenyl ether. Environmental Science and Technology 40, 4612-4618.

403   Bromine Science and Environmental Forum Home Page; 2007. http://www.bsef.com

404   [accessed 17 July 2010]

405   Camarero, L., Catalan, J. 1996. Variability in the chemistry of precipitation in the Pyrenees

406   (northeastern Spain): Dominance of storm origin and lack of altitude influence.

407   Journal of Geophysical Research 101, 29491-29498.

408   Carrera, G., Fernández, P., Vilanova, R.M., Grimalt, J.O. 2001. Persistent organic pollutants

409   in snow from European high mountain areas. Atmospheric Environment 35, 245-254.

410   Carrera, G., Fernandez, P., Grimalt, J.O., Ventura, M., Camarero, L., Catalan, J., Nickus, U.,

411   Thies, H., Psenner, R. 2002. Atmospheric deposition of organochlorine compounds to

412   remote high mountain lakes of Europe. Environmental Science and Technology 36,
413   2581-2588.

414   Chen, J.W., Yang, P., Chen, S., Quan, X., Yuan, X., Schramm, K.W., Kettrup, A. 2003.
415   Quantitative structure-property relationships for vapor pressures of polybrominated
416   diphenyl ethers. SAR QSAR Environmental Research 14, 97-111.

417   Cox, P., Ethymiou, P. 2003. Directive 2003/11/EC of the European parliament and of the
418   council of February 6, 2003 amending for the 24th time Council Directive
419   76/669/EEC relating to restrictions on the marketing and use of certain dangerous
420   substances and preparations (pentabromodiphenyl ether, octabromodiphenyl ether).
421   Off J Eur Union OJ L 42, 36-45.

422   Daly, G., Lei, Y., Teixeira, C., Muir, D.G., Castillo, L., Wania, F. 2007. Accumulation of
423   current-use pesticides in neotropical montane forests. Environmental Science and
424   Technology 41, 1118-1123.

425   de Wit, C.A., Alaee, M., Muir, D.C.G. 2006. Levels and trends of brominated flame
426   retardants in the Arctic. Chemosphere 64, 209-233.

427   Demers, M.J., Kelly, E.N., Blais, J.M., Pick, F.R., St. Louis, V.L., Schindler, D.W. 2007.
428   Organochlorine compounds in trout from lakes over a 1600 meter elevation gradient in
429   the Canadian Rocky Mountains. Environmental Science and Technology 41, 2723-
430   2729.

431   Dickhut, R.M., Andren, A.W., Armstrong, D.E. 1986. Aqueous solubilities of six
432   polychlorinated biphenyl congeners at four temperatures. Environmental Science and
433   Technology 20, 807-810.

434   Eljarrat, E., de la Cal, A., Barcelo, D. 2004. Determination of decabromodiphenyl ether in
435   sediments using selective pressurized liquid extraction followed by GC-NCI-MS.
436   Analytical and Bioanalytical Chemistry 378, 610-614.

437 Environmental Health Criteria. Brominated Diphenyl Ethers. 1994. WHO/IPCS 162-347.

438 European Brominated Flame Retardant Industry Panel (EBFRIP). The RoHS Directive and
439   Deca-BDE. 2009. http://www.ebfrip.org/main-nav/european-regulatory-centre/rohs-
440   directive-restriction-of-the-use-of-certain-hazardous-substances-in-electrical-and-
441   electronic-equipment/the-rohs-directive-and-deca-bde/.

442 Fernandez, P., Vilanova, R.M., Martínez, C., Appleby, P., Grimalt, J.O. 2000. The historical
443   record of atmospheric pyrolytic pollution over Europe registered in the sedimentary
444   PAH from remote mountain lakes. Environmental Science and Technology 34, 1906-
445   1913.

446 Fernandez, P., Grimalt, J.O., Vilanova, R.M. 2002. Atmospheric Gas-Particle Partitioning of
447   Polycyclic Aromatic Hydrocarbons in High Mountain Regions of Europe.
448   Environmental Science and Technology 36, 1162-1168.

449 Gallego, E., Grimalt, J.O., Bartrons, M., Lopez, J.F., Camarero, L., Catalan, J. 2007.
450   Altitudinal gradients of PBDEs and PCBs in fish from European high mountain lakes.
451   Environmental Science and Technology 41, 2196-2202.

452 Gerecke, A.C., Hartmann, P.C., Heeb, N.V., Kohler, H.P.E., Giger, W., Schmid, P., Zenneg
453   M., Kohler, M. 2005. Anaerobic degradation of decabromodiphenyl ether.
454   Environmental Science and Technology 39, 1078-1083.

455 Gouin, T., Harner, T. 2003. Modelling the environmental fate of the polybrominated diphenyl
456   ethers. Environment International 29, 717-724.

457 Grimalt, J.O., Fernandez, P., Berdie, L., Vilanova, R.M., Catalan, J., Psenner, R., Hofer, R.,
458   Appleby, P.G., Rosseland, B.O., Lien, L., Massabuau, J.C., Battarbee, R.W. 2001.
459   Selective trapping of organochlorine compounds in mountain lakes of temperate areas.
460   Environmental Science and Technology 35, 2690-2697.

461 Grimalt, J.O., Borghini, F., Sanchez-Hernandez, J.C., Barra, R., Torres Garcia, C., Focardi, S.
462   2004. Temperature dependence of the distribution of organochlorine compounds in the

463   mosses of the Andean mountains. Environmental Science and Technology 38, 5386-

464   5392.

465   Hardy, M.L. 2002. A comparison of the properties of the major commercial PBDPO/PBDE

466   product to those of major PBB and PCB products. Chemosphere 46, 717-728.

467   Harner, T., Shoheib, M., 2002. Measurements of Octanol-Air Partition Coefficients (KOA)

468   for Polybrominated Diphenyl Ethers (PBDEs): Predicting Partitioning in the

469   Environment. Journal of Chemical Engineering Data 47, 228-232.

470   He, J., Robrock, K., Alvarez Cohen, L. 2006. Microbial reductive debromination of

471   polybrominated diphenyl ethers (PBDEs). Environmental Science and Technology 40,

472   4429-4434.

473   Heiri, O., Lotter, A.F., Lemcke, G. 2001. Loss on ignition as a method for estimating organic

474   and carbonate content in sediments: reproducibility and comparability of results. Journal

475   of Paleolimnology 25, 101-110.

476   Hoh, E., Hites, R.A. 2005. Brominated flame retardants in the atmosphere of the east-central

477   United States. Environmental Science and Technology 39, 7794-7802.

478   La Guardia, M.J., Hale, M.J., Harvey, E. 2007. Evidence of debromination of

479   decabromodiphenyl ether (BDE-209) in biota from a wastewater receiving stream.

480   Environmental Science and Technology 41, 6663-6670.

481   Law, K., Halldorson, T., Danell, R., Stern, G., Gewurtz, S., Alaee, M., Marvin, C., Whittle,

482   M., Gregg, T. 2006. Bioaccumulation and trophic transfer of some brominated flame

483   retardants in a lake Winnipeg (Canada) food web. Environmental Toxicology and

484   Chemistry 25, 2177-2186.

485   Rayne, S., Ikonomou, M.G., Whale, M.D. 2003. Anaerobic microbial and photochemical

486   degradation of 4,4'-dibromodiphenyl ether. Water Research 37, 551-560.

487   Ribes, A., Grimalt, J.O., Garcia, C.J.T., Cuevas, E. 2002. Temperature and organic matter

488   dependence of the distribution of organochlorine compounds in mountain soils from

489   the subtropical Atlantic (Teide, Tenerife Island). Environmental Science and

490   Technology 36, 1879-1885.

491   Robrock, K.R., Korytar, P., Alvarez-Cohen, L. 2006. Rapid breakdown of brominated flame

492   retardants by soil microorganisms. Journal of Analytical Atomic Spectrometry 21,

493   1232-1239.

494   Sjödin, A. 2000. Occupational and dietary exposure to organohalogen substances, with

495   special emphasis on polybrominated diphenyl ethers. Stockholm University,

496   Stockholm, Sweden.

497   Stapleton, H.M., Dodder, N.G., Offenberg, J.H., Schantz, M.M., Wise, S.A. 2004.

498   Polybrominated diphenyl ethers in house dust and clothes dryer lint. Environmental

499   Science & Technology 39, 925-931.

500   Stockholm convention on Persistent Organic Pollutants (POPs). Programmes. New

501   POPs.http://chm.pops.int/Programmes/NewPOPs/The9newPOPs/tabid/672/language/e

502   n-US/Default.aspx

503   Strandberg, B., Dodder, N.G., Basu, I., Hites, R.A. 2001. Concentrations and spatial

504   variations of polybrominated diphenyl ethers and other organohalogen compounds in

505   Great Lakes air. Environmental Science and Technology 35, 1078-1083.

506   Ter Schure, A.F.H., Larsson, P., Agrell, C., Boon, J.P. 2004. Atmospheric transport of

507   polybrominated diphenyl ethers and polychlorinated biphenyls to the Baltic sea.

508   Environmental Science and Technology 38, 1282-1287.

509   Tittlemier, S.A., Halldorson, T., Stern, G.A., Tomy, G.T. 2002. Vapor pressures, aqueous

510   solubilities, and Henry's law constants of some brominated flame retardants.

511   Environmental Toxicology and Chemistry 21, 1804-1810.

512   Tokarz III, J., Younahn, M., Leng, J., Filley, T.R., Nies, L. 2008. Reductive debromination of

513   polybrominated diphenyl ethers in anaerobic sediment and a biomimetic system.

514   Environmental Science and Technology 42, 1157-1164.

515   Vilanova, R.M., Fernandez, P., Martinez, C., Grimalt, J.O. 2001. Organochlorine pollutants in

516   remote mountain lake waters. Journal of Environmental Quality 30, 1286-1295.

517   Vives, I., Grimalt, J.O., Lacorte, S., Guillamón, M., Barceló, D. 2004. Polybromodiphenyl

518   ether flame retardants in fish from lakes in European high mountains and Greenland.

519   Environmental Science and Technology 38, 2338-2344.

520   Vives, I., Grimalt, J.O. 2002. Method for integrated analysis of polycyclic aromatic

521   hydrocarbons and organochlorine compounds in fish liver. Journal of Chromatography

522   B 768, 247-254.

523   Vizcaino, E., Arellano, L., Fernandez P., Grimalt J.O. 2009. Analysis of whole congener

524   mixtures of polybromodiphenyl ethers by gas chromatography–mass spectrometry in

525   both environmental and biological samples at femtogram levels. Journal of

526   Chromatography A 1216, 5045-5051

527   Vonderheide, A.P., Mueller-Spitz, S.R., Meija, J., Welsh, G.L., Mueller, K.E., Kinkle, B.K.

528   Shann, J.R., Caruso, J.A. 2006. Rapid breakdown of brominated flame retardants by

529   soil microorganisms. Journal of Analytical Atomic Spectrometry 21,1232-1239.

530   Wania, F., Mackay, D. 1993. Global fractionation and cold condensation of low volatility

531   organo-chlorine compounds in polar regions. Ambio 22, 10-8.

532   Wania, F., Dugani, C.G. 2003. Assessing the long-range transport potential of polybrominated

533   diphenyl ethers: A comparison of four multimedia models. Environmental Toxicology

534   and Chemistry 22, 1252-1261.

535   Webster, T.F., Harrad, S. Millette, J.R., Holbrook, R.D., Davis, J.M., Stapleton, H.M., Allen,

536   J.G., McClean, M.D., Ibarra, C., Abdallah, M.A.-E., Covaci, A. 2009. Identifying

537   Transfer Mechanisms and Sources of Decabromodiphenyl Ether (BDE 209) in Indoor

538   Environments Using Environmental Forensic Microscopy. Environmental Science and

539   Technology 43, 3067-3072.

540   **Figure Legends**

541

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