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Toxicity assessment of polycyclic aromatic hydrocarbons in sediments from European high mountain lakes

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ABSTRACT

Sediment quality guidelines and toxic equivalent factors have been used for assessment of the toxicity of sedimentary long-range atmospherically transported polycyclic aromatic hydrocarbons (PAHs) to the organisms living in high mountain European lakes. This method has provided indices that are consistent with experimental studies evaluating in situ sedimentary estrogenic activity or physiological response to AhR binding in fish from the same lakes. All examined lakes in north, central, west, northeast and southeast European mountains have shown sedimentary PAH concentrations that are above thresholds of no effect but only those situated in the southeast lakes district exhibited concentrations above the indices of probable effects. These mountains, Tatras, are also those having PAH concentrations of highest activity for AhR binding. Chrysene+triphenylene, dibenz[a]anthracene, benzo[k]fluoranthene and indeno[1,2,3-cd]pyrene are the main compounds responsible for the observed toxic effects.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) occur in remote environments as consequence of incomplete combustion of fossil fuels or organic materials during industrial, urban or rural activities. Due to their continuous emission and chemical properties, i.e., semi-volatility and hydrophobicity, they occur ubiquitously in the environment and can be transported to long distances (Ding et al., 2007; Ravindra et al., 2008).

Recent reports have shown that remote ecosystems such as high mountain lakes may trap PAHs and other atmospherically transported pollutants (Fernández et al., 1999; Fernández et al., 2000; Barra et al., 2006). In these environments the PAH concentrations found in lake sediments are sometimes comparable to those found in soils or lake sediments located close to urban or industrial areas. These mountain ecosystems contain plant and animal communities of great ecological value that are sensible to external alterations by stressors such as pollutants (Sommaruga, 2001).

PAHs have well documented toxicity in a variety of organisms. However, the degree of understanding of the toxic effects of these pollutants in high mountain ecosystems is very limited. Garcia-Reyero et al. (2005) evaluated the estrogenic activity of surficial and bottom sediments from European mountain lakes using a recombinant yeast assay. They found that sediments showing highly estrogenic activity were significantly enriched in PAHs. This approach involved great technical and logistic efforts.

Other procedures encompass the use of sedimentary species that are sensitive to specific pollutant exposures and examination of their survival thresholds. Thus, *Chydorus sphaericus* had been proposed as test species for exposure to diverse metals (Koivisto et al., 1992; Dekker et al., 2002; Bossuyt and Janssen, 2005). This type of toxicity information is generally limited to effects of metals.

Ecological risk assessment provides another framework for estimating the toxic effect of sedimentary pollutants in lake organisms. Sediment quality guidelines (SQGs; CCME, 1999) have recently been used for identification of zones with major toxicological risk (Qiao et al., 2006; Colombo et al., 2005; Gómez-Gutiérrez et al., 2007). A procedure for toxicity estimation of high mountain lakes based on SQGs is proposed in the present study. Additionally the dioxin-like toxicity ratios based on binding to Ah receptor (Barron et al., 2004) and expressed in toxic equivalent factors (TEFs) have also been used. This approach has allowed the comparison of the potential toxicological effect of PAHs in sediment from European mountain lakes and the identification of lakes and zones of highest toxicological risk.

2. Methods

2.1. PAH concentrations

PAH concentrations (10 compounds) in surficial sediments of twelve high mountain lakes (Fig. 1) determined in Fernández et al. (1999) have been used to

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estimate sediment toxicity based on SQGs and TEFs methods. PAH concentrations in surficial sediment of the lakes Negru and Bubreka have been analyzed additionally in the present study because PAH data on high mountain lakes from southeast Europe were not available.

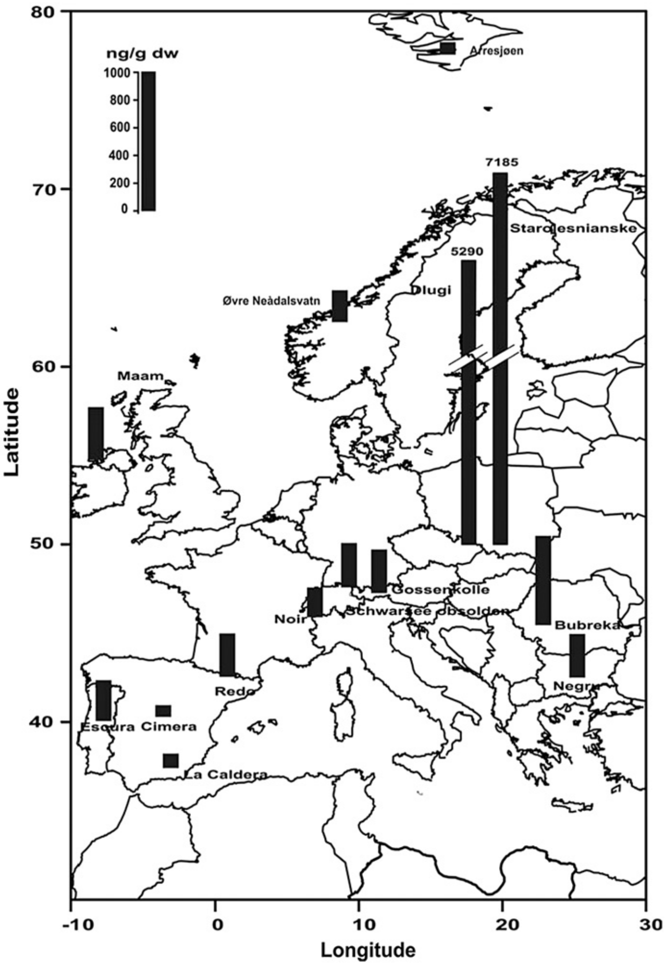

Fig. 1. Studied lakes and PAH concentrations Σ(Flu+Ant+Phe+Fla+Acy+Pyr+(Cry+T)+BaP+DahA). Abbreviations in Table 1.

Sampling and analysis were as described in Fernández et al. (1999). Briefly, sediment cores were divided in 0.25 cm sections (0.1–0.5 g), wrapped in aluminium foil and stored frozen at −20 °C. They were extracted by sonication with methanol (1 × 20 ml; 20 min) and subsequently with dichloromethane-methanol (2:1; 3 × 20 ml; 20 min). The combined extracts were spiked with perdeuterated PAHs: anthracene-d_{10}, pyrene-d_{10}, and benzo[*ghi*]perylene-d_{12}. Then, they were vacuum evaporated to 10 ml and hydrolyzed overnight (20 ml of 6% KOH in methanol). The neutral fractions were recovered with n-hexane (3 × 10 ml), vacuum evaporated and transferred to a glass column (35 cm × 0.9 i.d.) packed with 2 g of activated aluminium oxide (120 °C overnight). Two fractions were collected: 5 ml of n-hexane:dichloromethane (19:1) and 10 ml of n-hexane:dichloromethane (1:2; PAH). The PAH fractions were vacuum and nitrogen concentrated and redissolved in iso-octane for instrumental analysis. Perylene-d_{12} was added as internal standard for the injection.

The aromatic fractions were analysed by GC (Carlo Erba GC8000 Series) coupled to a mass spectrometer (Fisons MD800). A 30 m HP-5MS column (0.25 mm i.d. × 0.25 μm film thickness) was used. The oven temperature program was started at 90 °C (held for 1 min) and increased to 120 °C at 15 °C/min, and then to 300 °C at 4 °C/min, held for 10 min. Injector, transfer line, and ion source temperatures were 280, 300, and 200 °C, respectively. Helium was the carrier gas (1.1 ml/min). The injector operated in the splitless mode (48 s). Data were acquired in the electron impact mode (EI, 70 eV ionization energy). PAHs were determined in selected ion recording mode. The following diagnostic ions were chosen: m/z 166, 178, 202, 228, 252, 276, and 278 for PAH and 188, 212, 264, and 288 for perdeuterated standards (dwell time 40 ms per single ion, mass windows defined according to the retention times of individual PAH in the standard mixture). PAH identification was performed by m/z peak matching to retention time to reference standards, and comparison to literature Lee retention indices (Benner et al., 1995; Lee et al., 1979). Quantitative data were obtained by the external standard method (EPA mix 16, Dr Ehrenstorfer). Compounds lacking reference standard (acephenanthrylene, benzo[*e*]pyrene, and perylene) were quantified using the response factor of the standard exhibiting the closest retention time. Reported values were corrected by surrogate recoveries as follows: anthracene-d_{10} for compounds having signals at ions m/z 166 and 178; pyrene-d_{10} for compounds at m/z 202 and 228; and benzo[*ghi*]perylene-d_{12} for compounds at m/z 252, 276 and 278. Recoveries for the overall procedure based on surrogate data were typically anthracene-d_{10} 62 ± 18%, pyrene-d_{10} 70 ± 18%, and benzo[*ghi*]perylene-d_{12} 72 ± 12% (*n*=37).

The whole analytical procedure was successfully calibrated with a standard reference material with certified PAH values (marine sediment HS-4, Institute for Marine Biosciences, Canadian National Research Council).

2.2. *Sediment quality guidelines*

Ecological risk was assessed by comparison with SQGs of the Canadian Council Minister of Environment (CCME, 1999). The concentration values of the interim sediment quality guidelines (ISQGs) and probable effect levels (ng/g dw) for freshwater sediment are shown in Table 1. According to this table acephenanthrylene and dibenz[*ah*]anthracene are the compounds with highest toxicity potential. Two toxicity unit indices per lake sediment have been generated from these reference values. TU1 was calculated from the summed ratios between each individual PAH concentrations and the corresponding ISQGs and TU2 from the summed ratios between individual PAH concentrations and the probable effects

Table 1
Canadian freshwater sediment quality guidelines for polycyclic aromatic hydrocarbons (PAH) in ng/g dw and Toxic Equivalent Factor (TEF) of PAHs.

PAHs	Acronym	number rings	ISQGs[a] ng/g dw	PEL[b] ng/g dw	TEF[c]
Fluorene	Flu	3	21	144	–
Phenanthrene	Phe	3	42	515	–
Anthracene	Ant	3	47	245	–
Fluoranthene	Fla	4	111	2355	2.0E-09
Acephenanthrylene	Acy	4	6	128	–
Pyrene	Pyr	4	53	875	3.9E-07
benz[*a*]anthracene	BaA	4	32	385	2.0E-04
chrysene+triphenylene	Cry+T	4	57	862	5.6E-05
benzo[b+j]fluoranthene	Bb+jF	5	–	–	1.7E-04
benzo[k]fluoranthene	BkF	5	–	–	1.3E-03
benzo[e]pyrene	BeP	5	–	–	2.7E-05
benzo[a]pyrene	BaP	5	32	782	2.4E-04
Perylene	Per	5	–	–	2.3E-05
indeno[1,2,3-cd]pyrene	I123P	6	–	–	1.9E-03
benzo[ghi]perylene	BghiP	6	–	–	1.0E-05
dibenz[*ah*]anthracene	DahA	6	6	135	2.7E-04

[a] Interim sediment quality guideline (ISQG).
[b] Probable effect levels (PEL).
[c] Barron et al., 2004.