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 a 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; Gomez-Gutierrez et al., 2007). A procedure for toxicity estimation of high mountain lakes based on SQCs 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 (Figure 1) determined in Fernandez at al. (1999) have been used to estimate sediment toxicity based on SQCs 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.

Sampling and analysis were as described in Fernandez 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 –20ºC. They were extracted by sonication with methanol (1x20 ml;
20 min) and subsequently with dichloromethane-methanol (2:1; 3x20 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 (3x10 ml), vacuum evaporated and transferred to a glass column (35 cm x 0.9 i.d.) packed with 2 g of activated aluminium oxide (120ºC 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. x 0.25 μm film thickness) was used. The oven temperature program was started at 90ºC (held for 1 min) and increased to 120ºC at 15ºC/min, and then to 300 ºC at 4ºC/min, held for 10 min. Injector, transfer line, and ion source temperatures were 280, 300, and 200ºC, 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 SQCs 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 levels (PELs). Summation gives a rough estimate of the toxicity of the whole PAH distribution. This approach assumes that toxic PAH effects are additive (DiToro and McGrath, 2000). While TU1 is measuring the deviation of the distribution from a reference of no effect, the second is assessing whether toxic effects will be observed according to the sedimentary PAH content.
2.3. Dioxin-like toxicity approach

TEFs (Barron et al., 2004) have also been used to estimate dioxin-like toxicity expressed in toxic PAH equivalents (TEQs-PAHs). These factors have been calculated from the ratio between the activities of each individual PAH and that of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) as AhR agonists. Activity values were obtained from data on CYP1A induction or AhR binding for 74 PAHs in teleost avian or mammalian systems. The reference activity of TCDD was set to one (Barron et al., 2004). In each lake sediment TEQs-PAHs were calculated from the sum of the products of pollutant concentrations of each individual PAH and the corresponding TEFs.

3. Result

Lakes were grouped in European districts according to geographical location: west (Maam, Escura, Cimera, La Caldera and Redo), central (Noir, Schwarsee ob Solden and Gossenkollesee), northeast (Dlugi and Starolesnianske), southeast (Bubreka and Negru) and north (Øvre Neadalsvatn and Arresjøen) (Figure 1).

The PAH distributions are dominated by parent compounds, from phenanthrene to coronene, with a predominance of high molecular weight compounds of catacondensed structures (Fernandez et al., 1999; 2000).

3.1. Toxicity levels

The TU1 values for each high mountain lake included in Figure 1 have been calculated from the ISCQs (Figure 2). This index indicates the deviation of sedimentary PAH content from the level of no effect. All lakes had total TU1 values higher that one
indicating that PAH concentrations in these sediments exceeded the levels of no effect.
The TU1 values were lower in lakes from central, west and north lakes districts, between 2 and 10. The highest values were found in the northeast lakes district, 132 and 173 for Dlugi and Starolesnianske Pleso, respectively. The lakes in the southeast lakes district, Bubreka and Negru, exhibited intermediate values, 8 and 16, respectively.

The TU2 values for each high mountain lake included in Figure 1 have been calculated from the PELs (Figure 3). This index reveals PAH contents for which effects on organisms are likely. Total TU2 values in central, north and west lakes districts were lower than 1 indicating unlikely PAH effects on organisms. The only lakes in which total TU2 levels were higher than 1 were those in the northeast district, 7.8 and 10.0 in Dlugi and Starolesnianske Pleso, respectively. In the southeast lakes district TU2 values showed variable results between lakes, lower than 1 in Negru but nearly 1 in Brubeka. These results indicate probable toxic PAH effects in the lakes from the northeast district and possible toxicity related with these hydrocarbons in Brubeka.

Calculation of the TEQs-PAHs for the lakes included in Figure 1 shows that the sediments from the lakes located in the northeast district are those with highest activity for AhR binding (Figure 4). The lakes from the north, west, central and southeast districts exhibit much lower values. Benzo[k]fluoranthene and indeno[1,2,3-cd]pyrene are the compounds contributing the most to the activity for AhR binding in these lakes, representing about 84% of total activity (Figure 4).

4. Discussion

4.1. PAHs concentrations in high mountain lakes.
The uniform sedimentary PAH profile found in all lakes exhibits a high parallelism with the PAH composition in the atmospheric aerosols collected at these high altitude sites (Fernandez et al., 2003). This profile is quite ubiquitous and has been reported in sediments from remote/rural areas (Sanders et al., 1993) or in sites where organic pollutant inputs are mainly related to atmospheric transport and deposition (Simcik et al., 1996; Simo et al., 1997). The lakes with lower levels were those located at the periphery, e.g. Arresjøen, Cimera and La Caldera (150-210 ng/g). The lakes situated in more central sites, Pyrenees, Alps, Scandinavia, Retezat and also some of the periphery –Escura and Maam-, exhibited concentrations in the 430-910 ng/g range. These levels are significantly lower than those in the Tatra mountains, 13,000-18,000 ng/g. Brubeka, in the Rila mountains, exhibited intermediate values, 1,300 ng/g, among these two last groups (23 PAH, Fernandez et al., 1999).

4.2. Toxicity Unit levels

Phenanthrene, chrysene+triphenylene and dibenz[ah]anthracene were the main PAH determining the TU1 values with average contributions of 14%, 17% and 22%, respectively. Comparison of PAH concentrations and TU1 values according to number of aromatic rings in all lakes (Figure 5) shows higher relative abundance of the PAHs with 3 rings but a more even contribution of all PAHs to the observed TU1 values. Dispersion between PAHs levels was lower than between TU1 values.

Comparison of PAH concentrations and TU2 values according to number of aromatic rings in all lakes (Figure 6) shows higher relative abundance of the PAHs with 3 rings but higher contribution of PAHs with four aromatic rings to TU2 values. The dispersion of values between PAH concentrations was lower than between TU2 levels.
The PAHs contributing the most to the high TU2 values in the northeastern lakes district were chrysene+triphenylene (concentration/PEL 1.7 and 2.1 in Dlugi and Starolesnianske Pleso, respectively), and dibenz[ah]anthracene (concentration/PEL 1.4 and 1.8 in Dlugi and Starolesnianske Pleso, respectively). These two hydrocarbons exhibited concentrations above the PEL values (Table 1) in Dlugi. In Starolesnianske Pleso other PAHs are also above this level such as pyrene and benz[a]anthracene (concentration/PEL 1.5 and 1.1, respectively). The concentration of benzo[a]pyrene is also very close to its PEL value in this lake. In Brubeka no individual PAH concentration is above the corresponding PEL value. The highest concentration/PEL ratios are found for pyrene, phenanthrene and chrysene+triphenylene.

4.3. TEQs-PAHs levels

A study of the physiological response to dioxin-like compounds in fish inhabiting in European high mountain lakes based on the analysis of Cyp1A gene expression showed that among the PAH included in Table 1, chrysene+triphenylene, benzo(ghi)perylene and indeno[1,2,3-cd]pyrene were those with highest correlation to Cyp1A expression levels (Quiroz et al., 2007). Another study in which sediment extracts were assayed by recombinant yeast analysis for estimation of estrogenic activity (Garcia-Reyero et al., 2005) showed that benzo(ghi)perylene, chrysene+triphenylene and indeno[1,2,3-cd]pyrene were the three PAHs in highest concentration in the extracts with highest estrogenicity. The results of these two previous independent experimental studies are in agreement with the estimations of the present one based on TU2 and TEQs-PAH values.

Compilation of these TU1, TU2 and TEQs-PAHs indices has allowed to identifying toxic marine hot spots due to PAH concentrations in the vicinity of
industrial and urban locations (Gomez-Gutierrez et al. 2007). Now, the present study identifies the northeast lakes district in the Tatra mountains as a continental hot spot. These results are consistent with previous reports showing high concentrations of these hydrocarbons in the lakes from this area (Fernández et al., 1999; Fernández et al., 2000) but now their toxicity to organisms is assessed. PAHs are long-range transported through the atmosphere, mostly in association with particles (Masclet et al., 1988; Aceves and Grimalt, 1993; Allen et al., 1996). Emissions of these compounds in the vicinity of these mountains may be responsible for the probable toxic PAH effects resulting from compilation of the TU2 and TEQ-PAH indices.

UV radiation in mountain areas can enhance PAH toxicity due to the formation of polar derivatives that are more toxic than the parent compounds (Sommaruga, 2001). Photoinduced PAH toxicity has not yet been studied in organisms from high mountain lakes but it could enhance even further the environmental risk for the occurrence of these compounds in these ecosystems (Oris et al., 1990).

5. Conclusions

The approach developed in the present study for risk assessment of the PAH exposure in high mountain lake sediments provide indices that are consistent with experimental studies evaluating in situ estrogenic activity in sediment extracts or physiological response of fish inhabiting in these lakes to AhR binding. All examined lakes in north, central, west, northeast and southeast European mountains have shown sedimentary PAH concentrations that are above thresholds of no effect. However, only those situated in the northeast lakes district exhibit concentrations above the indices of
probable effects levels. These mountains, Tatras, are also those having PAH concentrations of highest activity for AhR binding.

PAH present in these environments have been incorporated from long-range atmospheric transport. The high concentrations of these hydrocarbons in the northeast lakes district may be originated from regional sources. Chrysene+triphenylene, dibenz[a]anthracene, benzo[k]fluoranthene and indeno[1,2,3-cd]pyrene are the main compounds responsible for these toxic effects.

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References


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1. Interim sediment quality guideline (ISQG); 2. Probable effect levels (PEL); 3. Barron et al., 2004
Figure captions

Figure 1. Studied lakes and PAH concentrations ($\Sigma$ Flu+ Ant+ Phe+ Fla+ Acy+ Pyr+ (Cry+T)+BaP + DahA). Abbreviations in Table 1.

Figure 2. Total TU1 values from European high mountain lake sediments. 1, Maam; 2, Escura; 3, Cimera; 4, La Caldera; 5, Redon; 6, Noir; 7, Schwarsee ob Sölden; 8, Gossenköllesee; 9, Øvre Neadalsvatn; 10, Arresjøen; 11, Bubreka; 12, Negru; 13, Dlugi; 14, Starolesnianske Pleso.

Figure 3. Total TU2 values from European high mountain lake sediments. 1, Maam; 2, Escura; 3, Cimera; 4, La Caldera; 5, Redon; 6, Noir; 7, Schwarsee ob Sölden; 8, Gossenköllesee; 9, Øvre Neadalsvatn; 10, Arresjøen; 11, Bubreka; 12, Negru; 13, Dlugi; 14, Starolesnianske Pleso.

Figure 4. Toxic Equivalent values (TEQs-PAHs) from the European high mountain lake sediments. 1, Maam; 2, Escura; 3, Cimera; 4, La Caldera; 5, Redon; 6, Noir; 7, Schwarsee ob Sölden; 8, Gossenköllesee; 9, Øvre Neadalsvatn; 10, Arresjøen; 11, Bubreka; 12, Negru; 13, Dlugi; 14, Starolesnianske Pleso.

Figure 5. Triangular diagram of percentage of contribution for concentration and TU1 values for PAHs in high mountain lake sediment

Figure 6. Triangular diagram of percentage of contribution for concentration and TU2 values for PAHs in high mountain lake sediment