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Water pollution

IDENTIFICATION OF POPs IN LAKE SHKODRA/SKADAR USING BIOMIMETIC SPMDs AND GC-MS ANALYSIS

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Abstract. Situated on the Balkan peninsula and straddling the Albanian-Montenegrian boarder the lake Shkodra/Skadar is the receiving body for several large rivers and numerous small streams draining the surrounding catchment area. Six sampling sites were selected (three each in the Albanian and Montenegrian sectors) on the basis of anthropogenic influence. Semipermeable membrane devices (SPMDs) were deployed consecutively at each site for 21 days. Following the dialytic recovery of target analytes and sample clean-up, samples from each site were divided and subjected to either GC-MS analysis for priority pollutant polycyclic aromatic hydrocarbons (PP-PAH) and organochlorine pesticides (OCPs). Together, our results show that waterborne organic pollutants including specific PP-PAHs, OCPs are readily available for uptake by the aquatic biota of the lake Shkodra/Skadar. Our results also show that combining SPMD-based biomimetic sampling with traditional chemical analysis is an environmentally relevant tool for the identification of toxic POPs in aquatic environments.

Keywords: bioavailable, PAH, SPMD, POPs.

AIMS AND BACKGROUND

In recent years, social and economic changes both in Albania and Montenegro have lead to unprecedented levels of urban and industrial effluent entering the lake. Over the last decade water flowing into the lake has become increasingly influenced by anthropogenic discharge containing organic pollutants from industrial, municipal and agricultural activities. A number of organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) have already been detected in the waters and tributaries of the lake Shkodra/Skadar using conventional sampling and extraction methodologies[1]. The increasing input of toxic persistent

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organic pollutants (POP)s into the lake and the degree to which these compounds are available for uptake by aquatic biota. Semi-permeable membrane devices (SPMDs) have been shown to sample the readily bioavailable fraction of waterborne POPs and in doing so provide relevant data for exposure assessment.

The aim of our study was to make an initial assessment of the range of readily bioavailable hydrophobic organic pollutants (HOPs) in the lake using SPMDs as biomimetic samplers in conjunction with chemical analysis.

EXPERIMENTAL

The SPMD consists of a high molecular weight lipid (triolein) sequestering phase sealed inside a section of 'layflat' low-density polyethylene (LD-PE) tubing. Because of the size limitation, imposed by the LD-PE membrane the uptake of compounds by SPMDs is limited to chemicals with molecular weights < 600 daltons[2].

The uptake of waterborne compounds by SPMDs is, therefore, analogous to the bioconcentration of freely-dissolved, readily bioavailable fraction by aquatic biota and has led to the description of SPMD-based sampling as 'biomimetic sampling'[3]. LD-PE membrane was cleaned by pre-extracting 85 cm section of 50 μm thick LD-PE layflat tubing (Merck) in *n*-hexane (Merck) for three 48-hour periods. Membranes were rolled and stored under argon at –27 °C until required for construction. The lipid sequestering phase 800 μl volumes of 95% pure 1,2,3-tri [*cis*-9-octadecanoyl]glycerol (triolein, Sigma-Aldrich) were cleaned with methanol (Merck) two times. SPMDs were prepeared by spreading and heat-sealing 750 μl of purified triolein into a 70 cm section of pre-cleaned LD-PE. Eight SPMDs were deployed in each of three sites both in the Albanian and Montenegrin sectors of the lake Shkodra/Skadar for 21 days throughout July and August of 2003 (Fig 1.) The deployed SPMDs and field controls were returned to

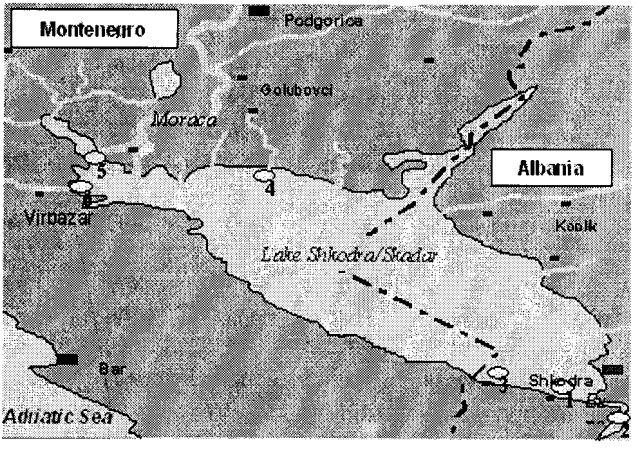

Fig. 1. Map of the lake Shkodra/Skadar showing sampling site

the laboratory and any particulate matter to the external surfaces of the LD-PE membrane was then removed by washing in distilled water. The individual membranes were dried in acetone (Merck), dialysed in 100 ml analytical grade *n*-hexane at 18°C for 24 h. The *n*-hexane was then exchanged and the SPMDs dialysed for a further 12 h. Potential interferants including co-dialysed triolein, methyl oleate and LD-PE oligomers were removed from the SPMD dialysates using a low pressure size exclusion chromatographic (SEC) system. This consisted of 30 cm × 1.2 cm i.d. glass column and compatible injection filled with SX-3 Bio-Beads (Biorad Laboratories, Munich, Germany) and eluted with 50:50 v:v HPLC grade dichloromethane: *n*-hexane (Merck) at a flow rate of 2.0 ml/min and UV detector at 254 nm. A single fraction potentially containing target analytes was collected between 13.5 and 30.0 min from each chromatographed SPMD dialysate.

GC-MS analysis of samples was carried out using a Hewlett-Packard P 5890 series II gas chromatograph fitted with a 30 m × 0.025 mm i.d. HP-5 (5%) PH ME siloxane GC column eluting into a Hewlett-Packard 5971 A mass selective detector (all Hewlett-Packard). Helium was used as a carrier gas at 1.2 ml/min, injector temperature was set to 290°C and 1.0 µl of the respective sample was injected per analysis using a Hewlett-Packard 7673 auto-injection system. Oven temperature programme starting from an initial value 50°C and ramping by 5°C/min up to 290 °C and holding for 5 min. Total ion chromatograms (TICs) were recorded by monitoring total ion current for m/z range 50 .550. Whole dialysates were also subjected to quantitative selected ion monitoring (SIM) analysis for 15 priority pollutant PAHs (Table 1).

Table 1. Priority pollutant polycyclic hydrocarbons (PP-PAHs). ng/SAMD at samplig sites 1-6

Compound	Sampling site					
	1	2	3	4	5	6
Naphthalene	2079	362	5.7	470	16	35
Acenaphthylene	63	116	26	40	23	132
Acenaphthene	92	110	39	76	n.d.	313
Fluorene	328	394	236	192	197	920
Phenanthrene	3405	1941	3654	1072	899	2573
Anthracene	n.d.	83	53	n.d.	n.d.	763
Fluoranthene	1866	36	4290	1492	2380	218
Pyrene	343	12	92	n.d.	n.d.	694
Benzo(a) anthracene	506	312	1675	472	971	983
Benzo(b)fluoranthene	109	n.d.	453	146	n.d.	367
Benzo(a)pyrene	450	697	75	19	n.d.	57
Indeno(1,2,3-cd)pyrene	n.d.	n.d.	88	n.d.	n.d.	79
Dibenzo(a,h)anthracene	14	71	7.9	12	n.d.	44
Benzo(ghi)perylene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

RESULTS AND DISCUSSION

GC-MS fullscan analysis of deployed SPMD dialysates from the various sampling sites indicated the presence of numerous compounds sequestered at each site (Fig. 2). Examination of the mass spectra produced by the various peaks on

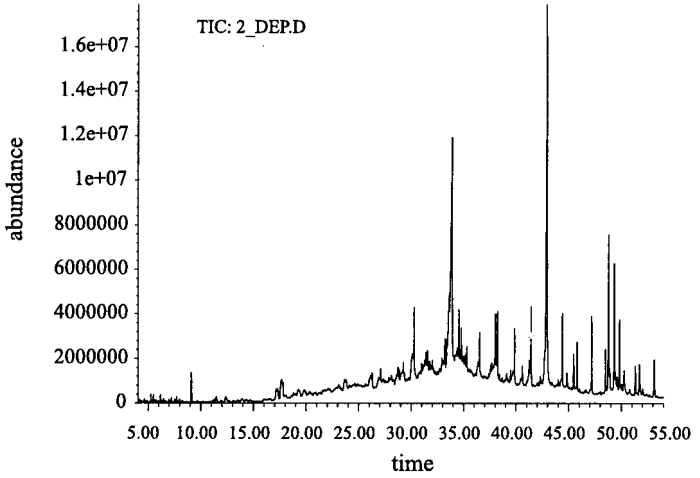

Fig. 2. Typical GC-MS fullscan chromatogram produced by a deployed SPMD dialysate

deployed SPMD TICs and comparison of these to reference spectra suggested the presence of toxicologically relevant POPs at the various sampling sites. Compounds tentatively identified in dialysates from the six sampling sites are detailed in Table 2. Some compounds, particularly the alkylated phenanthrenes, alkylated naphthalenes and cholesterol, were present in dialysates from most or all sampling sites indicating their widespread distribution throughout the lake.

Results of GC-MS selected ion monitoring (SIM) analysis for priority pollutant polycyclic aromatic hydrocarbons (PP-PAHs) showed levels of these compounds in SPMDs between 5.7 and 4290 ng /SPMD. Total PP-PAH concentrations were generally higher at sites in the Albanian sector with dialysates from sites 1 and 3, immediately adjacent to the villages of Shiroka and Zogaj, containing almost twice the total mass found in other samples (Table 1 and Fig. 3).

Table 2. Compounds tentatively identified ini deployed SPMD dialysates from sites 1-6

1. Cholesterol
2. Aristolene
3. Epicholesterol
3. Demosterol
5. Compesterol
6. β-Sitosterol
7. Dibuthylphtalate
8. 2,5-Dimethylphenanthrene
9. 2,3,4-Trimethylphenanthrene
10. Benzo(b)naptho(2,3)furan
11. 7-Methyl benzo(o)anthracene
12. Stigmasterol
13. 2,3-Dimethylfluorene
14. Triphenilene
15. HCH isomers (α, β and γ)
16. Heptachlor
17. Bromacil
18. α-Methyl stilbene
19. Methyl dibenzothiophene
20. 1,13-Tetradecadiene
21. Dihydrocholesterol
22. Ergost 2,2-en 3-one

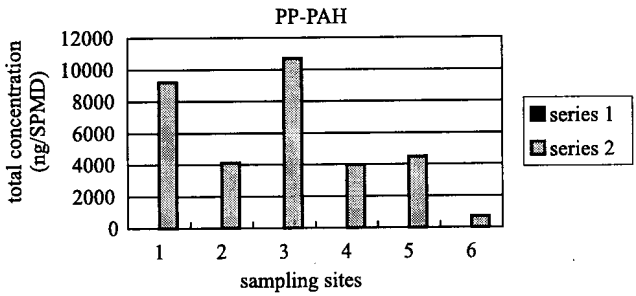

Fig. 3. Total concentrations (ng/SPMD) of the targeted PP-PAHs present in the dialysates of SPMDs from sampling sites 1-6

CONCLUSIONS

The combination of SPMD-based sampling with chemical analysis provided an environmentally relevant tool for the identification of waterborne pollutants in the lake Shkodra/Skadar. Our results show that toxicologically relevant POPs

are widely distributed in the lake and readily available for uptake by aquatic biota. As anthropogenic influences continue to increase, SPMD-based sampling is expected to play a central role in future research concerned with the identification, monitoring and assessment of the risk posed by POPs to the lake Shkodra/Skadar's aquatic biota.

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