

# A preliminary bioassessment of Lake Baikal sediment toxicity in the vicinity of a pulp and paper mill

M. Munawar\*, I.F. Munawar, D. Sergeant, C. Wenghofer

Department of Fisheries and Oceans, Canada Centre for Inland Waters, 867 Lakeshore Road, P.O. Box 5050, Burlington, ON, Canada L7R 4A6

## Abstract

The chemical and toxicological information given in this short communication is based on two sediment samples of limited amount taken from Lake Baikal. The samples received detailed chemical assessment since information about Lake Baikal sediments is scarce. The sediments were tested for sediment bound toxicity by liquid phase (Microtox) and solid phase (*Hyaella*) assays. The organic and inorganic chemical analyses were carried out with current technology followed in Canada, which may be useful for comparisons with other aquatic ecosystems. A wide range of organic compounds such as organo-chlorine pesticides, chlorinated diphenyl ethers, polychlorinated biphenyls, chlorinated dibenzo-*p*-dioxins and furans, coplanar polychlorinated biphenyls and polyaromatic hydrocarbons comprised the suite of compounds analysed for environmental levels. Generally, the full scan for high-resolution gas chromatography/high-resolution mass spectrometry electron impact analysis showed somewhat similar profiles for both the near-shore and off-shore stations. However, organic analysis indicated the near-shore station to be relatively more contaminated than the off-shore station. These chemical results were not supported by the Microtox and *Hyaella* bioassay experiments. The bioassays suggested that the near-shore and off-shore sediment samples were non-toxic. The necessity of performing both chemical as well as biological evaluations with a battery of assays is once again emphasized for ecosystem health assessment. Crown Copyright © 2000 Published by Elsevier Science Ltd and AEHMS. All rights reserved.

*Keywords:* Contaminants; Bioassays; Organic; Metals; Microtox; *Hyaella*

## 1. Introduction

Lake Baikal is the deepest and most pristine lake of the world and its conservation has attracted global attention. However, there are two pulp mills located in the watershed, one upstream on the Selenga River. The other is the Baikalsk Pulp and Paper Mill (BPPM) located on the lake. The Baikalsk Mill manufactures

chlorine bleached kraft cellulose, which involves the discharge of treated wastewater contributing particulates and other material into the lake. These materials eventually settle and mix with the sediments. Although considerable information about the toxicity of effluents originating from BPPM is available in Stepanova et al. (2000) and Kotelevtsev et al. (2000), very little is known about the sediment associated toxicity in the vicinity of the mill. In this communication, detailed results of chemical analyses as well as preliminary bioassessment are presented for two surface sediment samples. Both the chemical and

\* Corresponding author. Tel.: +1-905-336-4867; fax: +1-905-336-6437.

E-mail address: munawarm@dfo-mpo.gc.ca (M. Munawar).

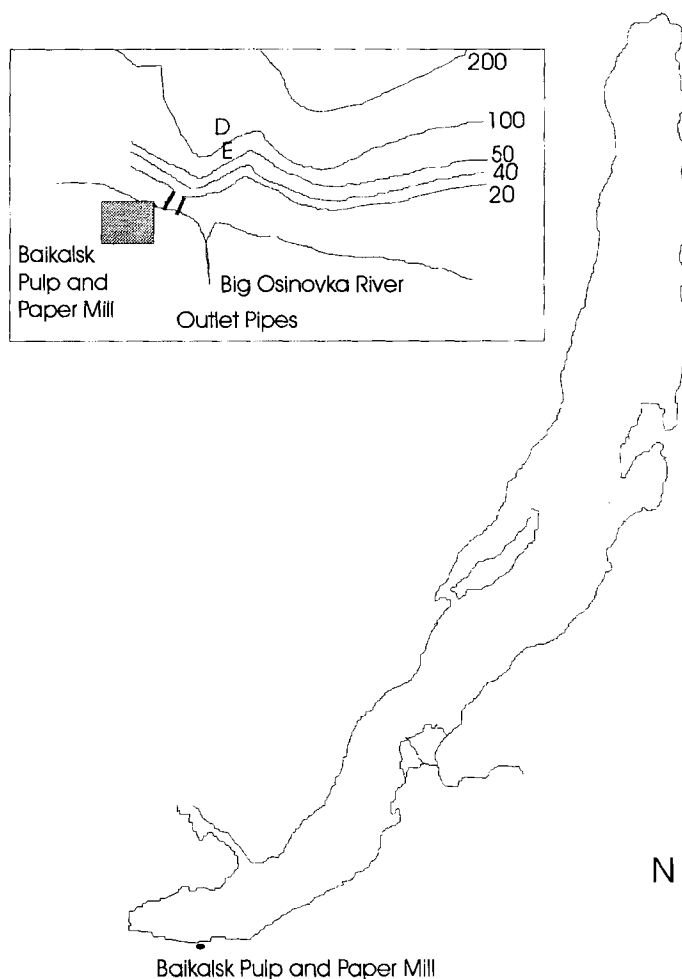


Fig. 1. Sampling stations in Lake Baikal, August 1993.

biological assessments were carried out in our laboratory in Canada.

## 2. Methods and materials

The surface sediment samples at a near-shore station E (180 m from shore; 40 m deep) and an off-shore station D (480 m from shore; 85 m deep) were collected in August 1993 with a grab sampler. The near-shore station is closer to the discharge of pulp and paper wastes than the off-shore station (Fig. 1).

### 2.1. Chemistry

Sediment samples were analysed for organo-

chlorine pesticides (OCs) and total polychlorinated biphenyls (PCBs) chlorinated diphenyl ethers (CDPEs) (Table 1), polyaromatic hydrocarbons (PAHs) (Table 2) and chlorinated dibenzo-*p*-dioxins and furans (PCDDs and PCDFs) (Table 3) using existing methodologies (Huestis et al., 1995). Organochlorine pesticides, PCBs and CDPEs were analysed from a single extraction, while PAHs, PCDDs and PCDFs were extracted separately. In the case of PAHs, deuterated PAHs were added to the samples and a separate extraction performed for these compounds with analysis on a Hewlett Packard 5780 GC/MSD on a 60 m Restek Rt<sub>x</sub>5 column with selected ion monitoring for the targeted compounds.

Sediment samples were also extracted and cleaned

Table 1  
Chlorinated organic contaminants analysed. Detection limit is 0.002  $\mu\text{g g}^{-1}$  for all analytes

Chlorinated diphenyl ethers	Organochlorine pesticides
22'44'-CDPE	a BHC
244'5-CDPE	HCB
33'44'-CDPE	b BHC
22'44'6-CDPE	g BHC
22'44'5-CDPE	d BHC
33'44'6'-CDPE	Heptachlor
22'44'56'-CDPE	Aldrin
22'344'-CDPE	Octachlorostyrene
22'44'55'-CDPE	Heptachlorepoxyde
22'344'6-CDPE	g Chlordane
22'344'6'-CDPE	a Chlordane
22'344'5-CDPE	pp' DDE
22'344'5'-CDPE	Dieldrin
22'344'66'-CDPE	Endrin
22'344'5'6-CDPE	b Endosulfan
22'344'56'-CDPE	pp' TDE
22'344'55'-CDPE	op' DDT
DECA-CDPE	pp' DDT
	Photomirex
	pp' Methoxychlor
	Mirex
	Total PCBs

up for high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) analysis to determine the unknown organic compounds. This was to gather preliminary information on non-target organics and to characterise chemically the near-mill and off-shore sediment samples. Each of the two sediments were bulk extracted (25 g), cleaned up by gel permeation chromatography and subjected to a modified silica gel clean up. A dry silica gel column was prepared (Huestis et al., 1995), the sample extract transferred to the top of the column with rinsings and eluted with 70 ml of benzene. Since the extract was coloured still, a further clean up was performed on a micro-alumina column, which was eluted with toluene. The final extracts were brought to 100  $\mu\text{l}$  final volume prior to transfer to an autosampler vial for HRGC/HRMS analysis.

The HRGC/HRMS analyses were performed on an AutoSpec-Q mass spectrometer (Fisons, VG Analytical, Manchester, UK) coupled to a Hewlett Packard 5890 Series II (Hewlett Packard, Palo Alto, California, USA) gas chromatograph equipped with a CTC A200s autosampler (Leap Technologies, Chapel Hill, North Carolina, USA). Injections of 1  $\mu\text{l}$  were made on-column and the injection port programmed from 80 to 280 at 100°C min<sup>-1</sup>. The column was a

Table 2  
Polynuclear aromatic hydrocarbons (PAH) concentrations in near-shore and off-shore sediment samples, ng g<sup>-1</sup> (wet weight). Detection limit is 10 ng g<sup>-1</sup> for all analyses

Compound	Off-shore		Near-shore	
	Sample 1	Sample 2	Sample 1	Sample 2
Acenaphthylene	ND	ND	3.477	ND
Acenaphthene	ND	ND	ND	ND
Fluorene	25.39	ND	4.704	ND
Phenanthrene	33.08	25.07	48.82	30.81
Anthracene	3.436	2.224	4.516	6.358
Fluoranthene	33.87	15.95	54.33	43.03
Pyrene	22.82	9.086	39.85	28.94
Benzo (a) anthracene	24.5	15.26	37.88	29.49
Chrysene	35.69	27.4	51.06	40.13
Benzo (b) fluoranthene	46.03	26.92	48.65	49.79
Benzo (k) fluoroanthene	36.05	18.77	44.23	41.02
Benzo (a) pyrene	22.75	7.937	26.7	24.12
Dibenzo (a,h) anthracene	ND	ND	ND	ND
Indeno (1,2,3-c,d) pyrene	22.2	13.48	24.98	26.93
Benzo (g,h,i) perylene	25.02	13.97	28.49	25.03

Table 3  
PCDDs, PCDFs and coplanar PCBs in off-shore and near-shore sediment samples

Analytes	Off-shore	Near-shore
2378-TCDD	ND	ND
TCDD Total	ND	ND
12378-PCDD	0.5	ND
PCDD Total	0.5	ND
123478-H6CDD	0.63	ND
123678-H6CDD	0.87	0.3
123789-H6CDD	0.96	0.39
H6CDD Total	2.56	0.69
1234678-H7CDD	3.13	1.74
H7CDD Total	5.23	2.9
OCDD	ND	15.2
2378-TCDF	1.28	ND
TCDF Total	1.28	ND
12378-PCDF	0.83	0.27
23478-PCDF	ND	ND
PCDF Total	0.83	0.27
123478-H6CDF	1.55	1.16
234678-H6CDF	0.92	ND
123678-H6CDF	0.87	ND
123789-H6CDF	0.95	ND
H6CDF Total	4.64	1.16
1234678-H7CDF	2.43	1.73
H7CDF Total	2.43	1.73
OCDF	ND	3.06
PCB 77	8.62	9.13
PCB 81	0.57	0.41
PCB 126	4.34	3.27
PCB 169	0.33	0.32

60 m Restek Rt<sub>x</sub>5 (Restek Corporation, Bellefonte, PA, USA) with retention gaps to facilitate on-column injections (Huestis et al., 1995). The connection to the source of the AutoSpec was through a heated transfer line. Temperature program was a 1 min hold post injection followed by a 2°C min<sup>-1</sup> temperature ramp to 280°C final temperature. The mass spectrometer was operated in the electron impact mode at resolution 10,000 and programmed to scan from 50–450amu at a scan time of 1 s per decade. Calibration was with perfluorokerosene H. Spectra of chromatographic peaks were searched against the NIST library resident in the AutoSpec data system.

The inorganic analysis of the sediment for metals was carried out according to the methodology of Inland Waters Directorate (1979).

## 2.2. Sediment toxicity

### 2.2.1. Liquid-phase assay: pore water-Microtox test

Pore water samples were extracted from the sediment using a combination of centrifugation and filtration. For each station 150 g of the sample was placed in 250 ml polyethylene containers and centrifuged for 1 h at 8000 rpm at a temperature of 4°C. The supernatant liquid was then filtered through a Whatman glass fibre filter (4.25 cm diameter, 1.2 µm nominal pore size) under 20 psi pressure. Pore water was maintained in glass test tubes at 4°C and was tested by means of Microtox™ test within 4 days of preparation. This procedure is similar to that described by Giesy et al. (1988) with slight modifications.

The Microtox assay with *Photobacterium phosphoreum* as the test organism was conducted to measure the toxic effects of sediment pore water extracted as described above (Giesy et al., 1988). A suspension of bacteria was mixed with pore water and incubated concurrently for 5 and 15 min periods at 15°C. The bioluminescence of bacteria was then measured in the Microtox 500 analyser and an effective concentration (EC) value was calculated if the test sample showed a response.

The following criteria were applied for the interpretation of Microtox results:

1. A site was considered non-toxic if the Microtox program could not calculate an EC<sub>10</sub> or greater.
2. Any EC values with a confidence limit exceeding a factor of five (i.e. 5–25) were considered invalid and the sample non-toxic.
3. The correlation values (*R*) were analysed and any site that had values below 0.8 was considered non-toxic.
4. Any EC value greater than 100% was considered non-toxic.

The EC<sub>50</sub> value is defined as the concentration of pore water that reduced bioluminescence of bacteria by 50%. The data are expressed as percentage of pore water required to inhibit the bioluminescence by 50%, that is, the EC<sub>50</sub>.

### 2.2.2. Solid-phase assay: *Hyalella azteca* test

The chronic toxicity test using *H. azteca* was conducted for 4 weeks according to the procedure of

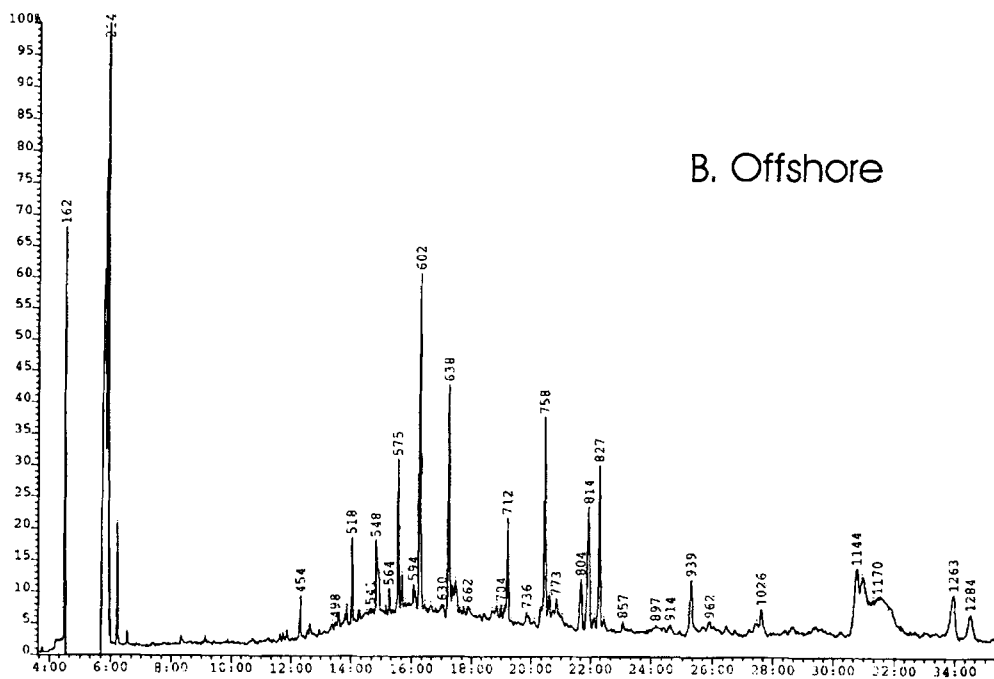
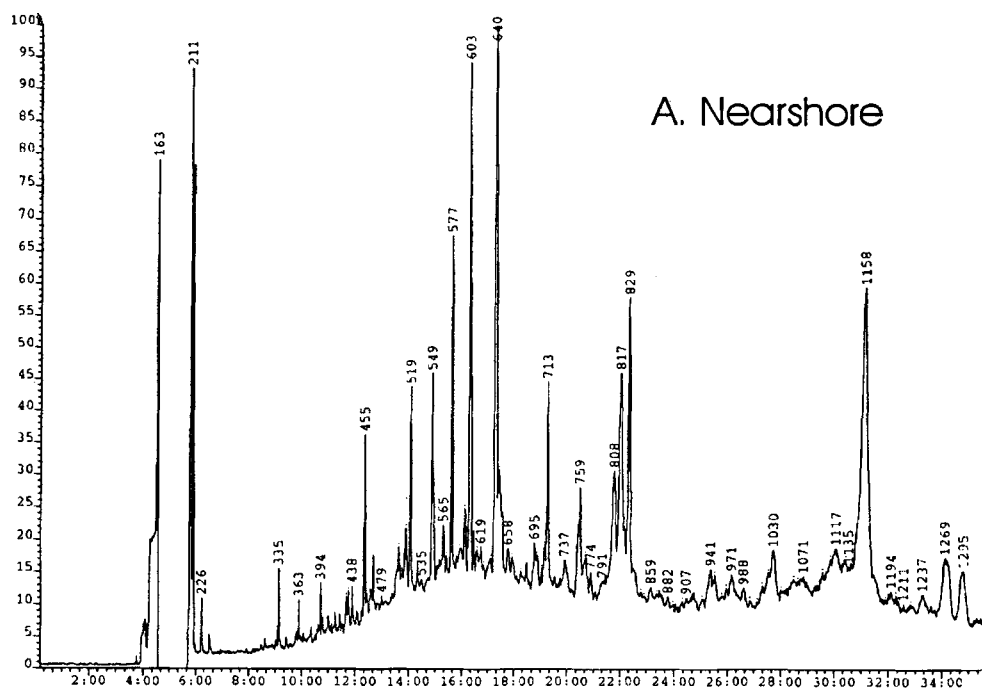


Fig. 2. Full scan total ion chromatogram of HRGC/HRMS analysis of the Lake Baikal: (A) near-shore and (B) off-shore sediment samples. Tentative identification of the compounds are given in Table 4.

Table 4

Compounds tentatively identified by HRGC/HRMS in a Lake Baikal near-shore sediment sample. Scan numbers indicate elution order (Fig. 2) and purity indicates the degree of fit found by the NIST library search

Chemical name	Scan number	Purity
Toluene	163	977
Cyclohexane, ethyl-	211	949
Benzaldehyde	226	905
Pentadecane	335	987
Tetratetracontane	363	963
Nonadecane	394	935
Docosane	394	935
Dotriacontane	429	744
Phenol, 2,6-bis (1,1-dimethylethyl)-4-ethyl-	433	694
Phenanthrene	455	998
Anthracene	455	998
1-Nonadecene	463	751
Hexadecanoic acid	519	665
1-Nonadecanol	549	960
Stearic acid, 2-(9-octadecenyloxy)ethyl ester	565	613
Fluoranthene	577	995
Benzo [b] naphtho [2,3-d] furan	619	648
Phenanthrene, 1-methyl-7-(1-methylethyl)-	645	760
Dotriacontane	705	811
9-Octadecenamamide,(Z)-	709	703
Acetic acid, octadecylester	713	971
Benzo [g,h,i] fluoranthene	767	941
Tetratetracontane	774	640
Triphenylene/Chrysene	808	968
Chrysene/Triphenylene	817	990
Bis (2-ethylhexyl) phthalate	829	994
Octadecane, 1-chloro-	859	636
Benz [a] anthracene,-1-methyl-	929	600
Benzene, 1,3,5-tris (2,2-dimethylpropyl)-2-methyl-4-nitro-	971	572
4H-1-Benzopyran-4-one,2-(3,4-dihydroxyphenyl)-6,8-di-β-D-glucopyranosyl-5,7-dihydrox	997	677
2-Dodecen-1-yl(-)succinic anhydride	1007	522
3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthyl-1,1',4,4'-tetrone	1014	680
1,2-Benzenedicarboxylic acid, diisononyl ester	1023	831
Didecyl phthalate	1030	935
3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthyl-1,1',4,4'-tetrone	1063	470
1,2-Benzenedicarboxylic acid, ditridecyl ester	1098	766
Benzo [g,h,i] perylene	1131	784
Dibenzo [def,mno] chrysene	1135	818
ψ,ψ-Carotene,7,7',8,8',11,11',12,12',15,15'-decahydro-	1158	518
1,2-Benzenedicarboxylic acid, diisodecyl ester	1201	583
Acetamide, N-methyl-N-[4-[4-methoxy-1-hexahydropyridyl]-2-butyryl]-	1237	437
Perylene	1269	977

Borgmann and Munawar (1989). Young (0–1 week) *Hyalella* were taken from the laboratory grown cultures maintained in 1.5 l jars in an incubator at 25°C with a 16 h light: 8 h dark photo period (Borgmann et al., 1989). The rearing culture jars

contained cotton gauze strips instead of sediments. Cultures received Tetra-Min fish food flakes. Water was changed on a weekly basis. For the toxicity test, sediment was placed in 250 ml beakers and dechlorinated water added at a ratio of 4:1. Twenty animals

were added to each beaker 24 h later. Eight replicates were run per station. Each jar received 20 mg of Tetra-Min every second day. The experimental period was 4 weeks. The water was aerated and oxygen and ammonia levels monitored. At the end of the test, animals were counted and classified as dead or live (Borgmann and Munawar, 1989).

### 3. Results and discussion

This was a preliminary study of Lake Baikal sediments since only two samples (near-shore and off-shore) were collected during a scientific Lake Baikal expedition in 1993. This study was undertaken as a first step in determining the chemical and biological effects of pulp and paper discharges on sediments. Consequently, in order to gain as much information on sediment contaminants as possible a wide range of different known contaminants were selected for analysis. Thus OCs, CDPEs, PCBs, PCDDs, PCDFs, coplanar PCBs and PAHs comprised the suite of compounds selected for the evaluation of environmental concentration.

Other unidentified contaminants were expected to be present in the sediments. To obtain a profile of these contaminants and to determine whether the near-shore and off-shore sites had different profiles, a larger sub-sample of each sediment was worked up and the extract subjected to a full scan HRGC/HRMS analysis. The results of these tests are discussed below.

Analysis of the near-shore and off-shore sediment samples for OCs and CDPEs found no compounds (Table 1) above the detection limit of the method,  $0.002 \mu\text{g g}^{-1}$ . In comparison, Iwata et al. (1995), using similar detection limits, did find many of these compounds at about two to three times more than the detection limits in sediment. Total PCBs were found to be 0.129 and  $0.150 \mu\text{g g}^{-1}$  wet weight for the near-shore and off-shore sediment samples, respectively.

Table 2 presents the PAH results for duplicate analyses of sediments at each of the sampling sites. The data indicated higher values at both the stations than the detection limits,  $10 \text{ ng g}^{-1}$ . Naphthalene was also observed in the samples, but the values are considered low for sediment samples.

Analyses of dioxin, furan and coplanar PCB (Table

3) also yielded low to less concentrations than the detection limit for congeners analysed. No 2,3,7,8-tetradoxin was found; however 2,3,7,8-tetrafurin was found at a very low level in the off-shore sample. Levels of the coplanar PCBs 77, 81, 126 and 169 were also very low. This indicates minimal environmental contamination by the mill processes, especially for PCDDs and PCDFs.

The full scan HRGC/HRMS electron impact analysis of the two sediment extracts showed somewhat similar profiles of compounds (Fig. 2A and B) for near-shore and off-shore samples, respectively. The data shows that the near-shore sample to be more contaminated than the off-shore. Some of the compounds identified by a search of the NIST Library are given in Tables 4 and 5 for the near-shore and off-shore stations, respectively. The purity numbers are an indication of the degree of spectral match between the sample mass spectrum and that of the library entry, with 1000 being the highest purity. Lower purity numbers indicate a decreased probability that the compound identified is the compound in the sample. Some of the compounds found are of biological origin and others such as the PAHs, which seem to predominate at the two sites, are more likely anthropogenic in origin. Since no attempt was made to obtain authentic standards of identified compounds and analyse them under identical conditions, the results of the spectral search were considered to be tentative identifications.

Table 6 shows the results of the metal analysis for sediments. Most of the metals examined showed relatively high concentrations in the sediments. These values are higher than the Canadian guidelines for the protection and management of aquatic sediment quality in Ontario (OMOE, 1993).

In accordance with the multi-trophic battery of tests approach suggested by Munawar et al., 1989; 1992 two types of bioassays were conducted: the liquid-phase pore water test using the standard Microtox procedure; and the solid phase-whole sediments test with *H. azteca* (Table 7). It was not possible to conduct additional assays due to the limited quantity of sediments available.

On an overall basis, the Microtox results indicated that the sediments of both the stations were non-toxic showing no significant impact of the pulp and paper mill. An extrapolated  $\text{EC}_{50}$  value of 136.1% was

Table 5

Compounds tentatively identified by HRGC/HRMS in a Lake Baikal off-shore sediment sample. Scan numbers indicate elution order (Fig. 1) and purity indicates the degree of fit found by the NIST library search

Chemical name	Scan number	Purity
Toluene	162	972
<i>p</i> -Xylene	214	920
Benzene, 1,3-dimethyl-	217	995
Benzaldehyde	226	993
9-Octadecenoic acid	305	635
Anthracene	453	992
Octadecane,	466	853
1-chloro-		
2-Phenyl naphthalene	527	740
1-Nonadecanol	548	920
Stearic acid, 2-(1-octadecenyloxy) ethyl ester	564	765
Fluoranthene	575	997
1,8-Anthracenediamine	594	619
1,2-Dithiol-1-ium,3,5-diphenyl-,bromide	638	910
1-Pentacontanol	646	773
Pentalene, octahydro-1-(2-octyldecyl)-	693	611
Acetamide, <i>N</i> -methyl- <i>N</i> -[4-[4-methoxy-1-hexahydropyridyl]-2-butynyl]-	704	850
Acetic acid, octadecyl ester	712	969
Benzo [b] naphtho [2,1-d] thiophene	753	941
Benzo [g,h,i] fluoranthene	764	939
1,12-Benzperylene	769	612
Dibenzo [def,mno] chrysene	773	783
Triphenylene	814	997
Didecyl phthalate	821	878
Bis (2-ethylhexyl) phthalate	827	997
Dotriacontane	857	782
Chrysene, 4-methyl-	914	563
Octadecanoic acid, 2-oxo-, methyl ester	962	593
1,2-Benzenedicarboxylic acid, diisononyl ester	1021	830
1,2-Benzenedicarboxylic acid, isodecyl octyl ester	1026	965
1,2-Benzenedicarboxylic acid, ditridecyl ester	1065	726
Benz [e] acephenanthrylene	1144	988
$\gamma$ -Sitosterol	1164	645
Perylene	1263	986
Benzo [j] fluoranthene	1284	973

calculated for the near-shore station. On the other hand, an EC<sub>50</sub> value of 96.2% was observed for the off-shore station with a non-significant *R*-value (Table 7). These results were also confirmed by the *H. azteca* assay showing 88% (near-shore) and 79% (off-shore)

Table 6

Metal concentrations in near-shore and off-shore sediment samples in  $\mu\text{g g}^{-1}$  (wet weight)

Metal	Near-shore	Off-shore
Cd	<1	<1
Co	22.3	23.4
Cu	42.7	39.2
Fe	49,200	50,200
Pb	8.99	5.63
Mn	812	993
Ni	57.8	60
Zn	112	109
Cr	116	123
Hg	0.031	0.028

survival at the end of 4 weeks. The numbers of survivors in experiments were not significantly different from the control. It is interesting to compare these results with those of Stepanova et al. (2000) who reported some mutagenicity in sediments collected near the mill.

Although the study is limited in scope, both chemical and toxicological assessments provide interesting information. For example, the chemical data show a general occurrence of a variety of contaminants in the sediments. The PAHs showed high concentrations indicating anthropogenic impact. The data obtained using HRGC/HRMS electron impact analysis indicated the near-shore station to be relatively more contaminated than the off-shore. However, this observation, based on chemical assessment, is not supported by the bioassay results, which indicated that the station was non-toxic (Table 7). A toxicity evaluation should take into consideration other sediment bound contaminants such as metals, which might be playing a synergistic/antagonistic role in determining the over all toxicity resulting from interacting organic and inorganic contaminants.

Table 7

Summary of liquid- and solid-phase bioassays, August, 1993

Station	Liquid-phase: Microtox, EC50	Solid-phase: <i>Hyalella</i> , % survival
Near-shore (40 m)	136.1% pore water ( <i>R</i> -value: 0.927)	88.3 (std:2.07)
Off-shore (85 m)	96.2% pore water ( <i>R</i> -value: 0.744)	78.7 (std: 2.5)



The toxicity resulting from such synergistic interactions of a mixture of metals is well known (Wong et al., 1978; Munawar et al., 1987). However, in this study the metal concentrations were similar at both the stations with the exception of Mn and Cr, which showed relatively higher concentrations at the off-shore station (Table 6). Since the bioassay data indicated both the stations to be non-toxic and the metal concentrations were similar, no attempt was made to separate further organic and inorganic contaminants.

Although both the chemical and toxicological data presented in this brief communication is preliminary due to the limited number and amount of samples collected, it does provide new information using the latest technology not previously available for the Lake Baikal sediments. Such data may be useful for future comparisons with other aquatic ecosystems. The study re-emphasizes the necessity of an integrated approach for ecosystem health assessment including chemical and toxicological evaluation by applying a battery of tests.

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