Environmental Science & Technology

Article

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Untargeted Screening and Distribution of Organo-Bromine Compounds in Sediments of Lake Michigan

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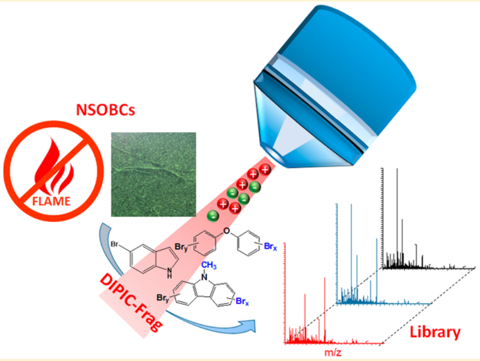
Supporting Information



ABSTRACT: Previously unreported natural and synthetic organo-bromine compounds (NSOBCs) have been found to contribute more than 99% of total organic bromine (TOB) in environmental matrices. We recently developed a novel untargeted method (data-independent precursor isolation and characteristic fragment, DIPIC-Frag) and identified ∼2000 NSOBCs in two sediments from Lake Michigan. In this study, this method was used to investigate the distributions of these NSOBCs in 23 surficial samples and 24 segments of a sediment core from Lake Michigan. NSOBCs were detected in all 23 surficial samples and exhibited 10- to 100-fold variations in peak abundance among locations. The pattern of distributions of NSOBCs was correlated with depth of the water column ($r^2 = 0.61$, $p < 0.001$). Hierarchical cluster analysis showed that sediments in close proximity exhibited similar profiles of NSOBCs. Distributions of NSOBCs in 24 segments of a sediment core dated from 1766 to 2008 were investigated, and samples from similar depths exhibited similar profiles of NSOBCs. NSOBCs were grouped into four clusters (soft-cluster analysis) with different temporal trends of abundances. 515 and 768 of the NSOBCs were grouped into cluster 1 and cluster 3 with increasing temporal trends, especially since 1950, indicating that abundances of these compounds might have been affected by human activities.

INTRODUCTION

Natural and synthetic organo-bromine compounds (NSOBCs) in the environment are of special concern due to their environmental persistence, bioaccumulation, and toxic potencies.[1,2] Well-known NSOBCs, such as hexabromocyclododecane (HBCD), polybrominated diphenyl ethers (PBDEs), hydroxylated PBDEs (OH-BDEs), and methoxylated PBDEs (MeO-BDEs). have been reported to be ubiquitous in abiotic environmental matrices,[3−6] wildlife,[7−10] and humans.[11−14] It has been found in both laboratory experiments and epidemiological investigations that synthetic brominated compounds can cause a variety of adverse effects.[1,12,15−17] Natural brominated compounds can exhibit toxicity exemplified by OH-BDEs, which bind to the thyroid receptor and cause greater neurotoxicity compared to synthetic PBDEs.[18,19] Identification and quantification of these brominated compounds in the environment is important for the assessment of potential health and ecological risks.

Received: September 25, 2015
Revised: November 23, 2015
Accepted: November 30, 2015
Published: November 30, 2015

DOI: 10.1021/acs.est.5b04709
Environ. Sci. Technol. 2016, 50, 321−330

Sediment is a primary depository of many environmental pollutants accumulated in natural waters, and the potential of releasing the chemicals from sediment to organisms in food webs is of special concern.[20,21] Various NSOBCs, such as PBDEs and HBCD, both of which are brominated flame retardants, have been detected in sediments with concentrations commonly in the ~ng/g dry mass (dm) range.[22−27] However, concentrations of total organic bromine (TOB) in samples of marine organisms and sediment have been found to be in the ~μg/g dm range,[28] which suggests that the currently known NSOBCs contribute <0.1% to TOB. In addition to synthetic brominated compounds and their byproducts, more than 2200 natural brominated compounds produced by marine organisms have been identified.[29] Thus, it was deemed useful to systematically investigate unknown NSOBCs in sediments.[28] Although several studies have made important progress in identifying novel NSOBCs in environmental samples using high-resolution mass spectrometry,[20,30−32] untargeted screening of NSOBCs in sediment is still challenging due to limitations on dynamic range, coverage, and specificity, as well as a lack of authentic standards and libraries of mass spectra. We recently developed a novel, untargeted, data-independent precursor isolation and characteristic fragment (DIPIC-Frag) method using liquid chromatography (LC) and atmospheric pressure chemical ionization (APCI) orbitrap (Q Exactive) ultrahigh resolution mass spectrometry.[33] This method greatly expanded the dynamic range and identified >2000 NSOBC peaks in two sediment samples from Lake Michigan.[33] In those samples, 1593 unique NSOBCs were identified for which accurate masses of precursor ions and predicted formulas were compiled. Preliminary results showed a great variation of concentrations and profiles of NSOBCs between two pilot samples of sediment from different locations,[33] which indicated different emission sources and behaviors of NSOBCs in the same lake.

Building upon the established library of 1593 NSOBCs determined by use of the DIPIC-Frag method, the study reported here further investigated the distribution of these NSOBCs in sediments from Lake Michigan. Distributions of NSOBCs were investigated in 23 surficial sediments and 24 dated sediment core sections with an approximately 250 year span. The objective was to investigate the spatial distribution and temporal trends of abundances of NSOBCs to identify potential natural and anthropogenic sources of emission.

■ MATERIALS AND METHODS

Chemicals and Reagents. Surrogate standard, 6-fluoro-2,2′,4,4′-tetrabromodiphenyl-ether (F-BDE47), was purchased from Wellington Laboratories Inc. (Guelph, ON, Canada). 5-bromoindole and 4-bromophenol were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). 1,3,6,8-tetrabromocarbazole was purchased from Toronto Research Chemicals Inc. (Toronto, ON, Canada). Florisil (6 cc, 1 g, 30 μm) solid-phase extraction (SPE) cartridges were purchased from Waters (Milford, MA). Methyl *tert*-butyl ether (MTBE), dichloromethane (DCM), hexane, methanol, and acetone were all of omni-Solv grade and were purchased from EMD Chemicals (Gibbstown, NJ).

Collection of Sediments. Samples of surficial sediments and cores of sediment were collected from Lake Michigan during September 2010, as described previously.[34] A total of 24 surface grab samples were collected using a PONAR sampler. Cores were collected by a box corer or an Ekman dredge and subsampled by polycarbonate tubes (10 cm i.d. × 58 cm). Each core was sectioned onboard into 1 or 2 cm segments using hydraulic extruders. Samples were separated into aliquots for analyses, and the portion for this work was placed in amber glass jars with aluminum foil liner caps. Samples were stored in the dark at −20 °C and shipped on ice in coolers to the laboratory. Each sample was lyophilized and homogenized by hand before being passed through a 1 mm sieve to remove stones and large organic matter. Radionuclide profiles in sediment cores were measured by gamma spectrometry in aliquots of lyophilized sediment, and calendar dates were obtained from the analysis of ^{210}Pb and ^{137}Cs profiles.

Sample Pretreatment and Analysis. Approximately 10 g of surficial sediments or 5 g of sediment from core segments were used for identification and quantification of NSOBCs. The method of extraction has been described previously.[28,33] Briefly, after spiking 20 ng/g of surrogate standard F-BDE47, extraction was conducted using an accelerated solvent extractor (Dionex ASE-200, Sunnyvale, CA). A total of two solvents were used for extraction: (1) *n*-hexane/DCM (1:1) at 100 °C and 1500 psi and (2) *n*-hexane/MTBE (1:1) at 60 °C and 1000 psi. A total of two cycles (10 min each) of extraction were performed with each solvent per sample (about 50 mL for each solvent), and all fractions were combined. The extract was reduced to 1 mL by rotary evaporation and was then loaded onto a Florisil cartridge preconditioned with 6 mL of DCM. NSOBCs were eluted from Florisil cartridges using 5 mL of DCM and 5 mL of methanol. Final extracts were blown to dryness under a gentle stream of nitrogen and then reconstituted with 400 μL of acetone for analysis. The use of Florisil cartridges allowed removal of most of the yellow interferences in extracts of sediment. Because only a limited number of NSOBCs were detected in the methanol fraction (data not shown), only the DCM fractions from the cartridges were collected for NSOBCs screening.[33] Such a simple one-step sample cleanup method was useful for untargeted screening of NSOBCs in sediments to avoid potential loss of compounds and reduce background contamination.

LC-Q Exactive Data Acquisition. Aliquots of extracts were analyzed using a Q Exactive UHRMS (Thermo Fisher Scientific, San Jose, CA) equipped with a Dionex UltiMate 3000 UHPLC system (Thermo Fisher Scientific). Separation of NSOBCs was compared among several types of HPLC columns including HILIC (1.7 μm; 2.1 mm × 50 mm; Waters), HSS T3 (1.7 μm; 2.1 mm × 100 mm; Waters), Hypersil GOLD phenyl column (5 μm; 2.1 mm × 100 mm; Thermo Fisher Scientific), Betasil C18 column (5 μm; 2.1 mm × 100 mm; Thermo Fisher Scientific), and Hypersil GOLD C18 column (3 μm; 2.1 mm × 50 mm; Thermo Fisher Scientific), and the Hypersil GOLD C18 column was selected for the present method considering the separation ability and sensitivity achieved with its use.[33] Injection volume was 5 μL. Ultrapure water (A) and methanol (B) were used as mobile phases. Initially, 20% of B was increased to 80% in 3 min, then increased to 100% at 8 min and held static for 19.5 min, followed by a decrease to the initial conditions of 20% of B held for 2 min to allow for equilibration. Rate of flow was 0.20 mL/min. Temperatures of the column and sample compartment were maintained at 30 and 10 °C, respectively.

Data were acquired in data-independent acquisition (DIA) mode. Parameters for DIA were one full MS^1 scan (150−2000 m/z) recorded at resolution R = 70 000 (at m/z 200) with a maximum of 3×10^6 ions collected within 100 ms, followed by six DIA MS/MS scans recorded at a resolution R = 35 000 (at m/z 200) with maximum of 1×10^5 ions collected within 60 ms.

DIA data were collected using 5-m/z-wide isolation windows per MS/MS scan. Each DIA MS/MS scan was chosen for analysis from a list of all 5 m/z isolation windows. In these experiments, 180 5-m/z-wide windows between 100 and 1000 m/z, were grouped into nine separate methods, each of which contained 20 windows. Small overlaps with neighboring windows were used to reduce the likelihood of placing window edges on critical target peaks. APCI (−) mode was used for the DIPIC-Frag method to increase coverage of the method, especially for nonpolar chemicals.[33] Mass spectrometric settings for APCI (−) mode were: discharge current, 10 μA; capillary temperature, 225 °C; sheath Gas, 20 L/h; auxiliary gas, 5 L/h; and probe heater temperature, 350 °C.

Quality Control and Assurances. Identification of NSOBCs was accomplished by comparing retention time (within 2%) and exact m/z value in DIA window (10 ppm mass window) with those of NSOBCs in the library of 1593 NSOBCs established previously.[33] For isomers with similar retention times and m/z values, to avoid potential effects of shifts in retention time on alignments of peaks among samples, a local-regression fitting method, LOESS, was conducted to adjust for shifts between runs, as used in a previous metabolomics study.[35] The dynamic time-warping (DTW) algorithm was used to align peaks of isomers as documented in previous studies.[36,37] To avoid contamination of samples, we rinsed all equipment regularly with acetone. A single procedural blank was incorporated in the analytical procedure for every batch of samples. 113 NSOBC peaks were detected in the blank, partly due to instrument carry-over. The background contamination from the blanks was subtracted from sediment samples for downstream data analysis, and those NSOBC abundances in sediment samples that were lesser than three times the background abundances in blanks were considered to be not detected.

Because most NSOBCs that were identified were novel compounds for which no authentic standards available commercially, absolute peak intensities in the extract were used to semiquantify their abundances in samples of sediment. Such semiquantitative strategy has been widely used for previous comparative proteomics and metabolomics studies. As conducted in our previous study, F-BDE47 was used as a surrogate standard.[38] Peak abundances of NSOBCs were corrected by F-BDE47 in corresponding samples, and the mean recovery of F-BDE47 was 89.0 ± 23.2% in all the samples. The use of single surrogate standard for multiple compounds has been well-documented in previous untargeted screening studies,[32] mainly due to the lack of commercially available surrogate standards for novel compounds. To enhance comparability among samples and avoid potential shifts in sensitivity among compounds during multiple sample injections, we used the same method (nine methods in total for each sample) for all samples. Method detection limits (MDLs) could not be calculated for NSOBCs, but a peak intensity cutoff of 1000 was incorporated into the DIPIC-Frag method as described previously[33] and used as the MDL for the NSOBCs. For the 113 NSOBC peaks detected in blank samples, three times the peak abundance was used as MDLs.

Data Treatment and Statistical Analyses. All data analyses, such as Pearson correlation, linear regression, and cluster analysis, were performed with an in-house R program (software version 3.1.2; http://www.R-project.org; R Foundation for Statistical Computing, Vienna, Austria). Distributions of abundances of NSOBCs were assessed for normality by use of the Shapiro-Wilk test, and a log transformation was used to ensure the normality of the distribution of data. For those results that were less than the MDLs, half of the MDL (peak intensity of 500) was assigned to avoid missing values in the statistical analyses. Statistical significance was defined as $p < 0.05$.

RESULTS AND DISCUSSION

Performance of the DIPIC-Frag Method for Analysis of Sediment Samples. The framework of the DIPIC-Frag method is shown in Figure 1A and described in detail elsewhere.[33] By combining multiple techniques and chemometric strategies, the DIPIC-Frag method established a mass spectrometry library containing 1593 NSOBCs identified in sediments from Lake Michigan. Due to the lack of chemical standards, the focus of the present study was to investigate patterns of distribution of NSOBCs in sediments from Lake Michigan by use of comparative analysis rather than absolute quantitation. The application of this strategy to comparative sample analysis required great precision of performance of the method. To assess inter-run precision, we injected a single sediment extract (sed-32) twice. Good reproducibility was achieved and correlation coefficients (r^2) were >0.99 between injections (Figure S1). Although F-BDE47 was used as a surrogate standard to adjust for potential losses during the pretreatment of samples, some polar compounds with acidic or phenolic groups could have exhibited different behaviors and affected the precision of quantification by use of F-BDE47. To avoid potential bias, we assessed the precision of the whole method by extracting the same sample of sediment (sed-32) two times for analysis by use of the DIPIC-Frag method. As shown in Figure S1, good correlation ($r^2 > 0.99$) was observed between two batches of analysis. These results confirmed reproducibility of not only the instrument portion but also the DIPIC-Frag method in its entirety and justified its use for large-scale comparative investigations.

Consistent with results of a previous pilot study on two sediments,[33] thousands of NSOBCs from the established library were detected in all 23 samples of surficial sediments, which demonstrated a broad occurrence of these previously unknown NSOBCs in sediment from Lake Michigan. By averaging peak abundances of all NSOBCs among the 23 surficial sediments, we showed the scatter plot of all NSOBCs along with their retention times, m/z, and intensities (Figure 1B). Retention times of NSOBCs varied from 1.0 to 29.3 min, while m/z values ranged from 170.9438 to 997.5217, and peak intensities ranged from $<10^3$ to $\sim10^8$, demonstrating considerable diversity among chemicals. Most NSOBCs had retention times on the C18 HPLC column between 9 and 18 min, suggesting their relatively great hydrophobicity and preferential partitioning to sediment. The peak abundances of these NSOBCs were 100−1000-fold greater than those of OH-BDEs, which indicates their potentially significant contributions to TOB in sediments (Figure S2). Compounds with greatest peak intensities appeared at ∼13 min and were predicted to be polyhalogenated carbazoles (PHCZs) and their derivatives on the basis of their formulas and MS^2 information. Some of the native PHCZs have been reported previously in sediments from Lake Michigan.[38,39] Because previous studies have also found PHCZs in biotic samples,[30] and a very recent study reported that these compounds are relatively strong agonists of aryl hydrocarbon receptor (AhR),[40] future studies are warranted to investigate their potential toxicity and risk to wildlife. Among the 1593 novel NSOBCs, three of them (bromophenol, bromocarbazole, and bromoindole) have been validated by use of commercial standards

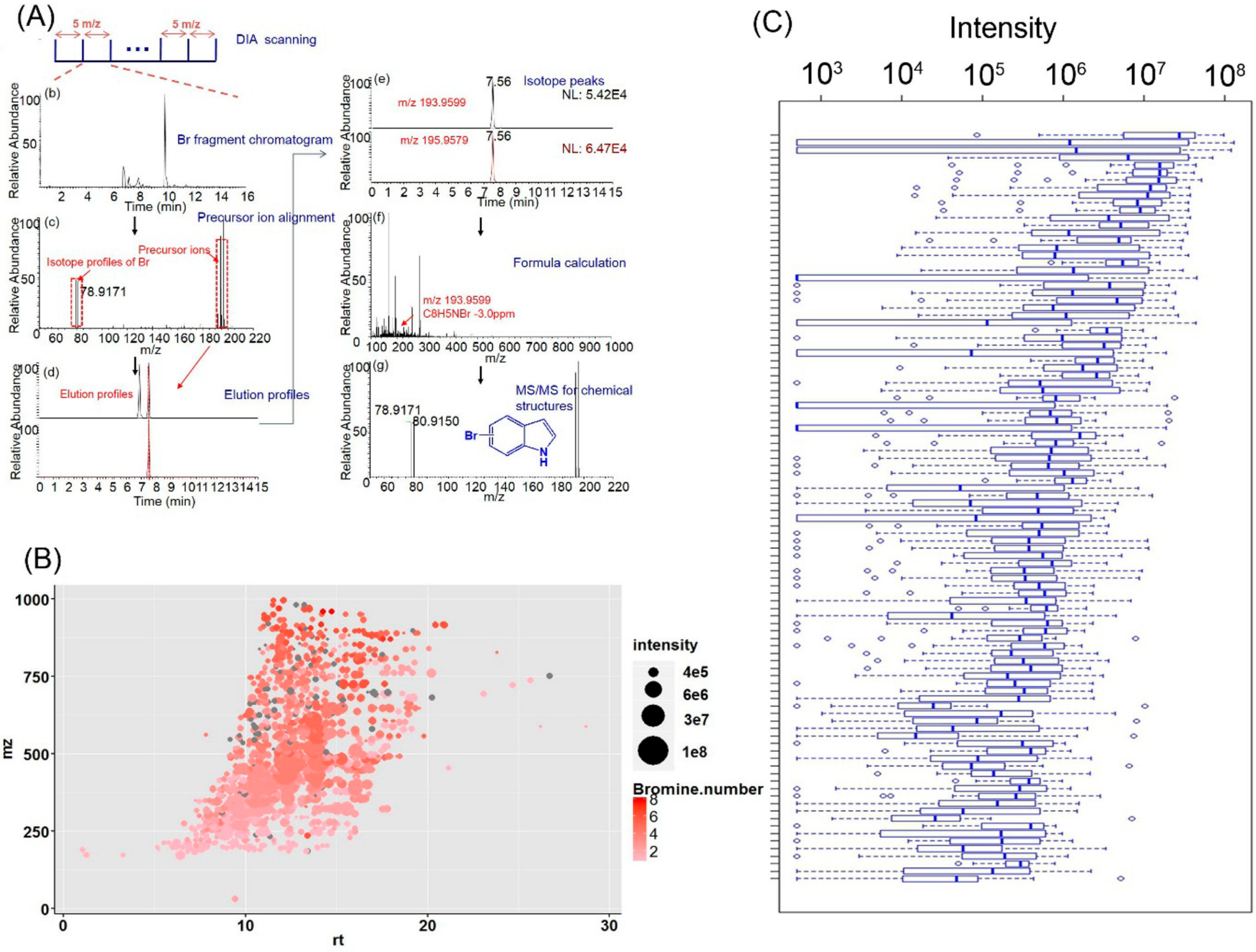


Figure 1. (A) Typical workflow of the DIPIC-Frag method used to identify brominated compounds (ref 33). (B) Distribution of the NSOBCs by retention time and *m*/*z* values. The size of the dots are proportional to the average abundance in 23 samples of surficial sediment from Lake Michigan. The colors of dots are proportional to bromine number. Red represents numbers of bromines, and gray represents those NSOBCs whose formulas could not be identified. (C) Boxplot of the abundances of the 100 most-abundant NSOBCs in 23 Lake Michigan surface sediment samples.

(chemical structures were shown in Figure S2). Compared to OH-BDEs with low detection frequencies in sediment samples due to their relatively low concentrations, these three NSOBCs exhibited high detection frequencies and were thus selected as model chemicals in subsequent spatial−temporal data analysis.

Spatial Distributions of NSOBCs in Lake Michigan Surface Sediment. Variations of peak abundances of individual NSOBCs were observed among 23 surficial sediments from different locations, and most compounds showed a range of 1 to 2 orders of magnitude difference in peak abundances (Figure 1C). Despite this variation, dominant compounds were similar in all samples from different locations (Figures 2A–D and 3A). PHCZs and their derivatives were abundant and had peak intensities greater than 1×10^7 in most extracts of surficial sediment. Similar profiles of dominant compounds contributed to strong correlations of abundance of NSOBCs among sediment samples, particularly for those collected in close proximity (Figure 2E,F). For example, significant correlations of abundance of NSOBC were observed between sed-93A, sed-93B, and sed-93C ($r^2 > 0.82$, $p < 0.001$) (Figure 2A,B). However, abundances of NSOBCs in sediments from different locations were frequently poorly correlated; e.g., sed-50 showed poor correlation with sed-93C ($r^2 = 0.49$), and sed-2 showed poor correlation with sed-48 ($r^2 = 0.33$).

Similarity heat maps and cluster analyses (Figure 2E) demonstrated that sediment samples were grouped into several clusters according to their Pearson correlation matrix: two sediment samples (sed-50 and sed-113) from the nearshore of the northern Lake Michigan showed similar profiles of NSOBCs ($r^2 = 0.67$, $p < 0.001$); three samples of sediment (sed-93A, sed-93B, and sed-93C) from the river estuary also showed similar profiles of NSOBCs and relatively strong correlation coefficients ($r^2 > 0.82$, $p < 0.001$); sediments from offshore of the northern lake (sed-120, sed-47, sed-103, sed-32, sed-41, and sed-83) were clustered into the largest group, which showed relatively similar profiles (r^2 values ranged from 0.64 to 0.86, $p < 0.001$). Such results indicated that NSOBCs in Lake Michigan have distinct emission profiles among locations and that the emission profiles are closely related to locations of samples where samples in close proximity may exhibit similar geochemical properties. Location- and geochemistry-related distribution patterns have been well-documented for other synthetic compounds and are mainly due to the transport trajectories of compounds.[34] For naturally occurring compounds, similar distributions in samples that were collected in close proximity could be partly attributed to the

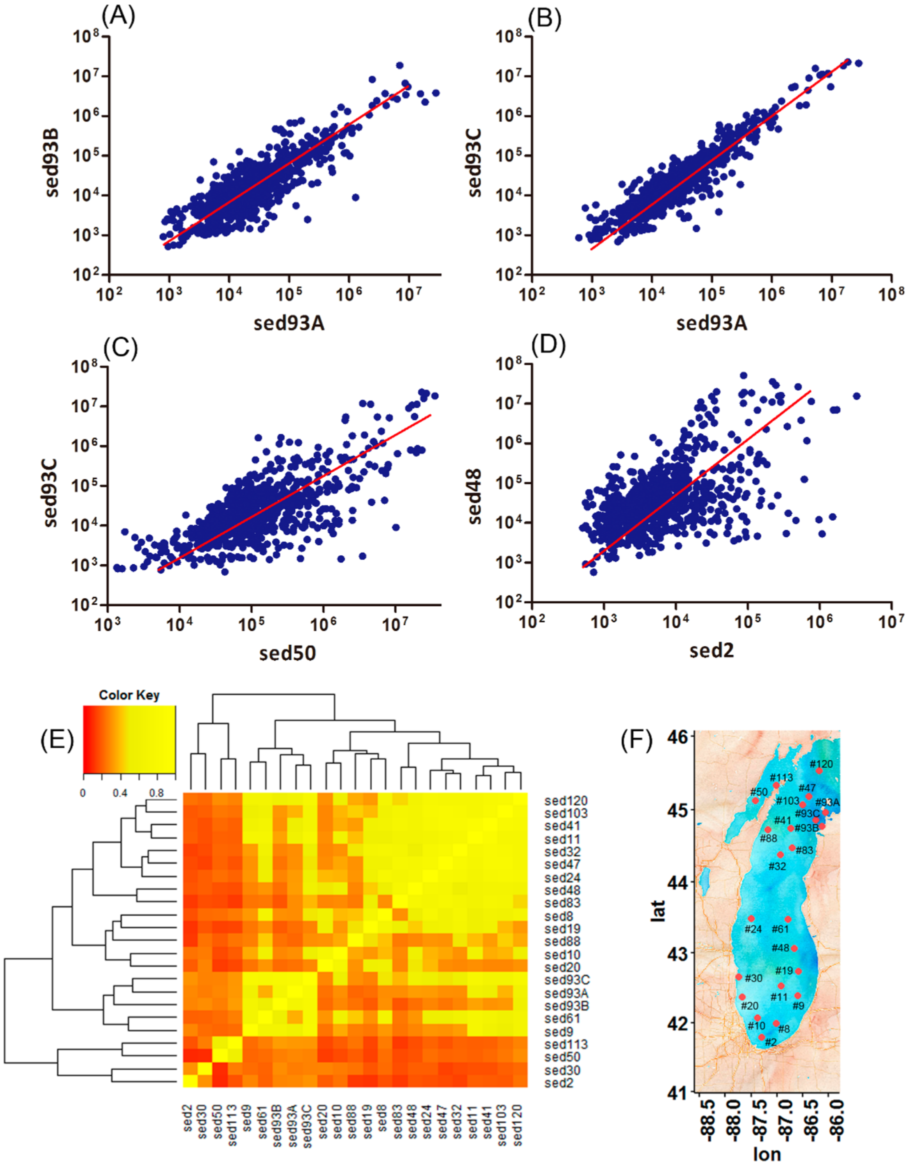


Figure 2. (A−D) Correlations of abundances of NSOBCs among surficial sediments. Stronger correlations were observed for three samples of sed-93 within proximities, but poorer correlations were observed for samples of sediments not in proximity. (E) Correlation heatmap and hierarchical clustering of samples of sediments based on their Pearson correlation coefficients. (F) Map of locations from which samples of surficial sediments were collected.

presence of similar species of bacteria that are known to produce brominated compounds.[41]

To further investigate potential emission profiles of NSOBCs, we investigated the correlation similarity heat map of all NSOBCs among samples of surficial sediment (Figure 3B). Distinct distributions in these samples were observed for different NSOBCs (r^2 values ranged from <0.1 to 0.95), which indicated that there were differences in sources or environmental behaviors of these compounds. On the basis of analyses of hierarchical trees, three major groups of NSOBCs were observed. The first group of compounds (bottom left of Figure 3B) showed particularly strong intrinsic correlations in abundance within the group. Although structures of chemicals could not be precisely determined, all of the more abundant compounds were predicted to be analogues of PHCZ compounds (with different numbers of iodine, chlorine, or bromine) were grouped into the same cluster, which indicated that these compounds might have originated from similar sources. Results of previous studies have suggested that PHCZs could be produced from natural sources,[38,42] a hypothesis that is supported by similarities in structural backbone of these compounds, which might indicate a common synthetic pathway and source. In fact, a recent study identified a gene cluster in the marine bacteria *Pseudoalteromonas* spp. that synthesizes bromophenols and bromopyrroles.[41] Future studies are warranted to determine if the gene cluster also produces PHCZs and other NSOBCs.

Distributions of abundances of NSOBCs among all 23 surficial sediments were further investigated (Figure 4). Distributions of the total abundances of NSOBCs varied among locations, such that sites with the greatest abundances of NSOBCs were located offshore of Lake Michigan's northern basin (Figure 4A). In contrast, previous studies of synthetic compounds, such as PFCs and PBDEs,[34,43] have reported that concentrations were greatest at sites with greatest population densities and industrial activity, such as in the southern basin of Lake Michigan. These differences in distributions indicated that anthropogenic emission sources were likely not the primary sources of most NSOBCs identified in this study.[34] Peak abundances of total

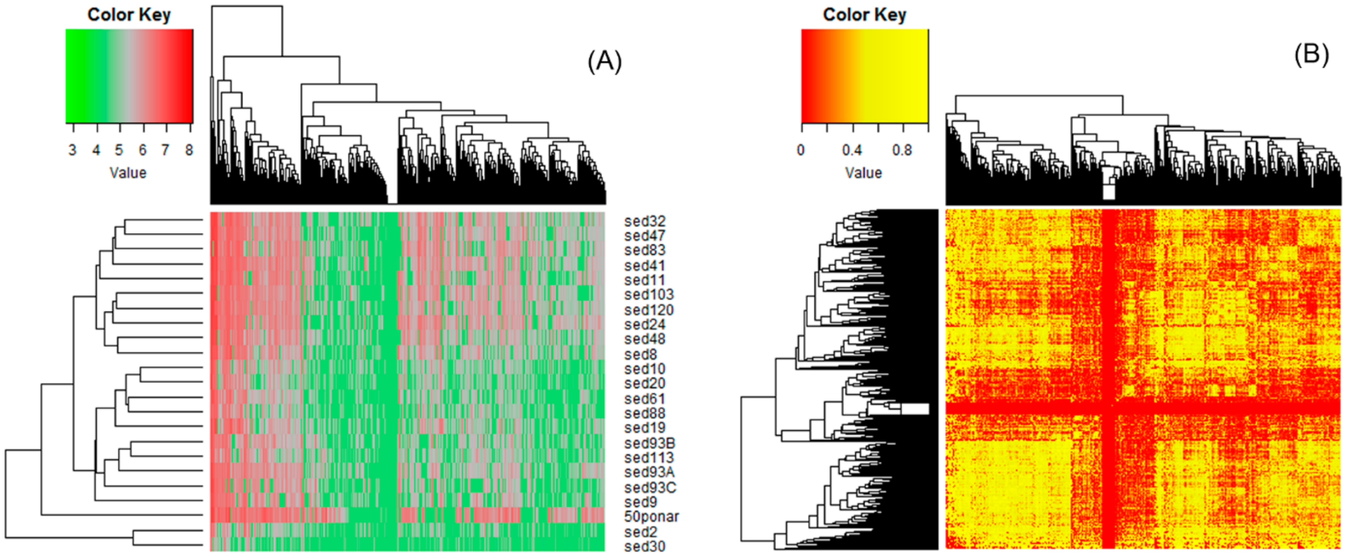


Figure 3. (A) Heatmap and hierarchical clustering of NSOBCs in 23 samples of surficial sediment from Lake Michigan. Color indicates log-transformed peak abundances, and the most abundant NSOBCs (indicated by red color) were always dominated in most sediments. (B) Similarity heatmap and hierarchical clustering of NSOBCs. Similarity between NSOBCs are defined as Pearson correlation coefficients among the 23 samples of surficial sedments.

NSOBCs among locations were significantly correlated with the depth of the water column ($r^2 = 0.61$, $p < 0.001$) (Figure S3), which might be due to greater abundances of micro-organisms that synthesize NSOBCs in areas of deeper water. Previous studies have reported production of natural, bromine-containing, products by different organisms, such as the sponge *Discodermia sp.* that inhabits deep water and a common freshwater green alga (*Scenedesmus subspicatus*).[44,45] Therefore, studies are warranted to investigate whether organisms that are capable of synthesizing brominated compounds inhabit areas of deeper water in Lake Michigan.

In addition to the analysis of total NSOBCs, three distinct groups of compounds with similar distribution profiles, which emerged from the cluster analysis, were investigated separately. Similar to total abundance of NSOBCs, all three groups of NSOBCs exhibited relatively great abundances in the northern basin of Lake Michigan (Figures 4 B–D and S4), which indicated a possible common source. Despite the trend toward greater abundances of NSOBCs in the northern basin, NSOBCs from cluster I and cluster II showed a specific distribution pattern, in which relatively great abundances were detected in sed-50 in Green Bay. Among the three model NSOBCs, bromophenol was grouped into cluster I and exhibited a relatively high peak abundance in sed-50 (Figure S4C). Considering that several industries, including paper production, discharge into this location and that there is evidence that halogenated compounds are produced during the paper-bleaching process,[46] the distribution of these two clusters of NSOBCs could be affected. Specifically, during chlorination or other oxidative processes, the bromide ion (Br^-) can be oxidized to hypobromous acid, which can then react with organic compounds to produce a range of brominated organic compounds (e.g., bromophenol).[47,48] This may explain the relatively high concentration of bromophenol at sed-50 because previous studies have reported the high concentrations of bromophenols produced in disinfection processes.[49]

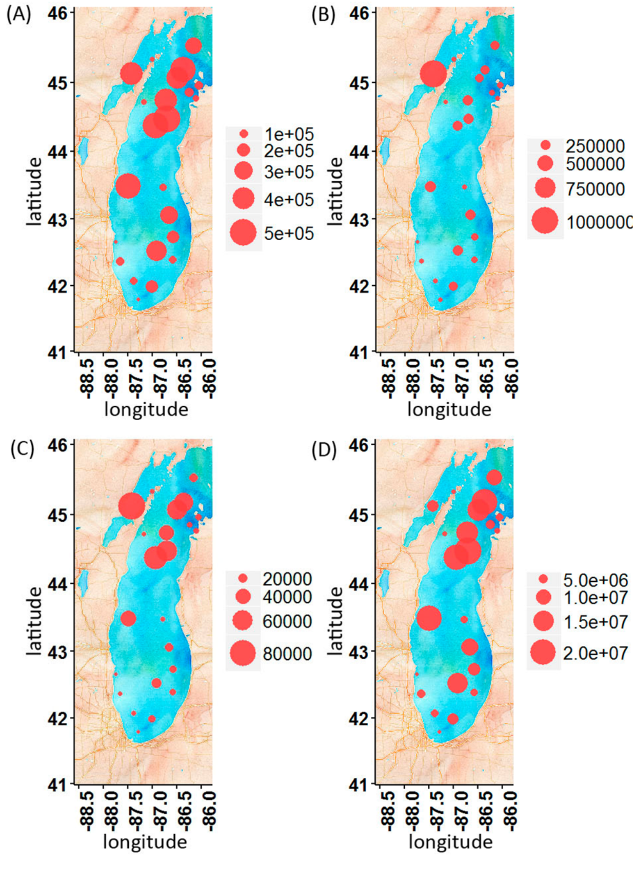


Figure 4. Distribution of total abundances of NSOBCs (a), NSOBCs in cluster 1 (b), NSOBCs in cluster 2 (c), and NSOBCs in cluster 3 (d) in surficial sediments from Lake Michigan. Sizes of circles are proportional to log-transformed abundances of NSOBCs.

Temporal Trends of NSOBCs in Core Sediment. To further investigate the potential natural or anthropogenic sources of NSOBCs, we examined temporal trends in 24 segments of a sediment core from the location with the greatest abundance of total NSOBCs (sed-32 from northern Lake). This location is remote from industrial areas but exhibits greater abundances of NSOBCs, which is representative of temporal trends of

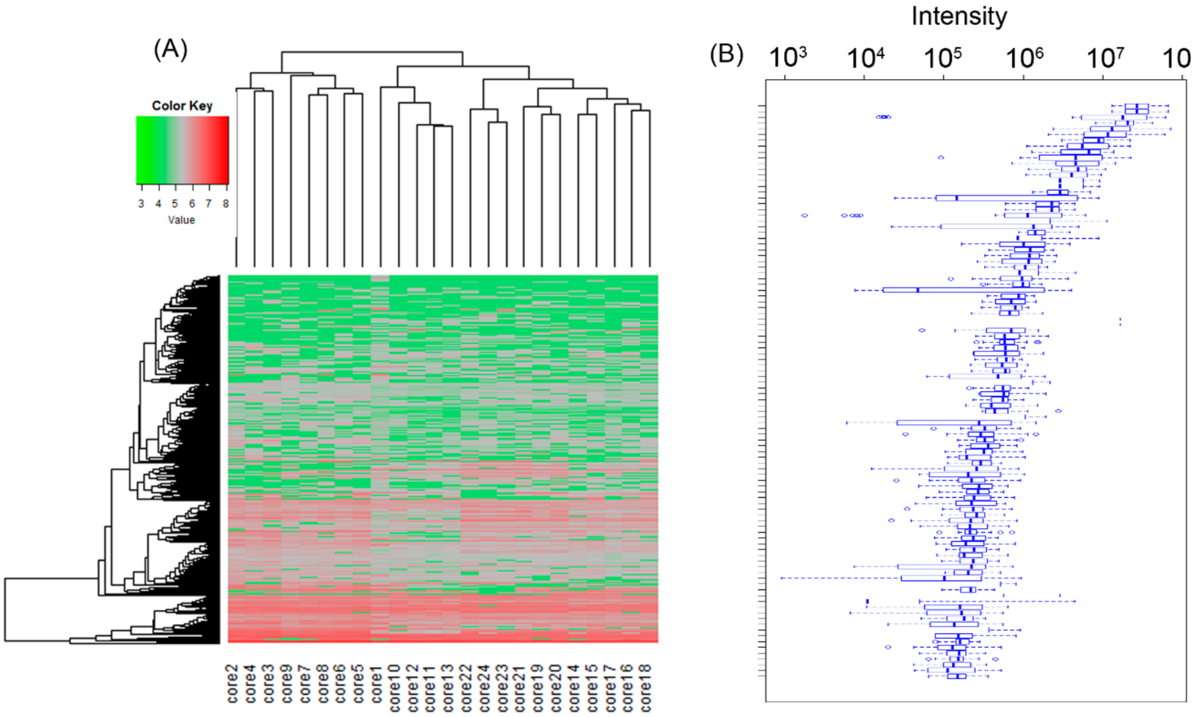


Figure 5. (A) Heatmap and hierarchical clustering of NSOBCs in 24 segments of a sediment core from Lake Michigan. Core number indicates the segment number, e.g., core 2 indicates segment 2 from the top. Segments from similar time periods (e.g, cores 14−18) were grouped into the same clusters. (B) Box plot of the abundances of the 100 most abundant NSOBCs in 24 sections of sediment cores from Lake Michigan.

NSOBCs. NSOBCs were detected in all 24 segments. Compared to samples of surficial sediment, concentrations, and profiles of NSOBCs in sediment core sections were relatively uniform (Figure 5A). A box plot analysis showed that variations in the abundances of the top 100 most abundant NSOBCs in the core segments was relatively minor, typically less than 5-fold (Figure 5B), compared to the 10 to 100-fold differences between samples of surficial sediments. This result was unexpected because an approximate 250 year span (1766−2008) was covered by the core. These results imply that most of the compounds were from natural sources, and distributions of NSOBCs were mainly driven by geochemistry at each location that was relatively stable during the investigated time period. Despite the small variations in abundance of NSOBCs in sediment core segments, hierarchical cluster analysis showed that samples dated to similar years were always grouped into the same cluster (except for the top segment), with segments 2−4, 6−9, 10−13, and 14−24, which were grouped into four different clusters (Figure 5A). Generally, sediment sections dated to similar time periods had similar profiles of NSOBCs. Such distributions were likely determined by geochemistry or communities of micro-organisms in the sed-32 location over the past ∼250 years, and a previous study has documented well the shift of microorganism communities in segments of sediment cores from different time periods.[50]

To further investigate temporal trends of abundances of NSOBCs in segments of sediment from cores, we conducted soft-cluster analysis because of its ability to generate internal cluster structures.[51] This approach has been widely used to investigate temporal changes in expression of genes or proteins.[52] The number of clusters was optimized to four for all NSOBCs (Figure 6) by decreasing the values from 10 until the minimum cluster distance became stable. The most abundant PHCZs were clustered into the same group, indicating the chemical relevance of the optimized soft-cluster parameter (Figure S5). No clear temporal trends were observed for the abundances of NSOBCs included in cluster 2 (Figure 6B), but a trend of decreasing abundances of peaks with time was observed for NSOBCs included in cluster 4 (Figure 6D). Such temporal trends for abundances of NSOBCs from cluster 2 and 4 were different from the increasing abundances of anthropogenic compounds including PFCs and PBDEs.[34,38] The synthesis of NSOBCs by bacteria, algae, and invertebrates has been reported previously.[41,53,54] Together, these results indicate that NSOBCs in clusters 2 and 4 were likely produced naturally. Another 515 and 768 NSOBCs were grouped into cluster 1 and cluster 3, respectively (Figure 6A,C). Abundances of NSOBCs included in cluster 1 showed an increasing trend since ∼1900, whereas abundances of NSOBCs included in cluster 3 have increased since ∼1950. Specifically, all three model NSOBCs (bromophenol, bromoindole, and tetrabromocarbazole) were grouped into these two clusters and showed increasing temporal trends of their abundance in recent decades (Figure S6), although bromophenol exhibited a second local maximal peak around ∼1800. NSOBCs have been reported to be formed during the disinfection processes and production of flame retardants.[31,47] Therefore, considering the relatively great abundances of NSOBCs in sed-50, direct emission from these human activities might also contribute to the increasing temporal trends of peak abundances of NSOBCs from clusters 1 and 3. Alternatively, such an increasing temporal trend might be due to indirect impact by human activities, e.g., the emission of total phosphorus to Lake Michigan lead to the increase of microorganisms to produce more NSOBC compounds.[55] This study of temporal trends of NSOBCs is limited because a single sediment core from the northern lake site was used in this analysis. This is partly due to the substantial time investment of the DIPIC-Frag method (9 analyses per sample; 126 sample runs from a single core,

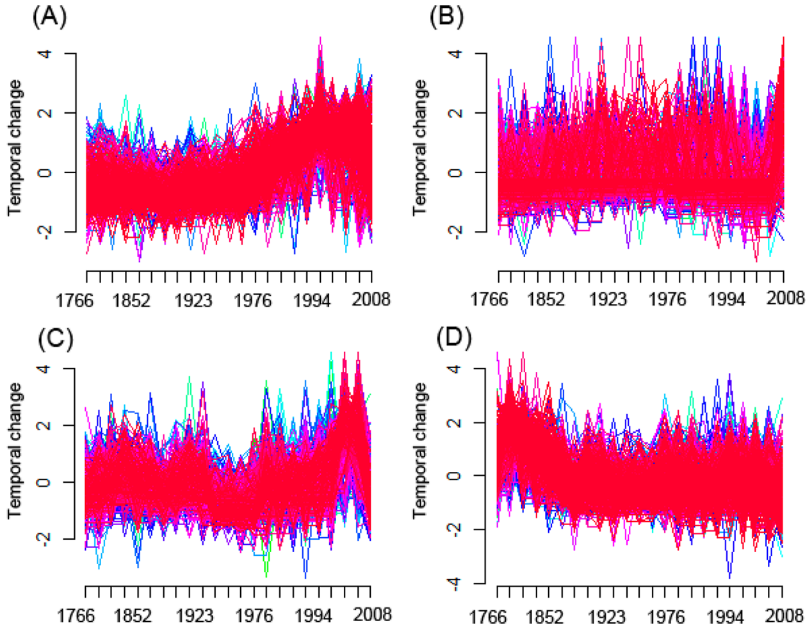


Figure 6. Soft-cluster analysis of temporal trends of abundance of NSOBCs in segments of sediment core from Lake Michigan. NSOBCs were grouped into one of four clusters according to changes in the abundance of NSOBCs as a function of time (segment depth). Fold-change on the *y*-axis was relative to the mean abundance of NSOBCs and was log-transformed. The number of clusters was fixed at 4, and panels A−D represent clusters 1−4, respectively.

~21 GB raw file size). Analyses of multiple cores is warranted to better identify sources of NSOBCs in future studies.

Implications. The discovery of unknown NSOBCs in environmental samples is of importance because previously identified NSOBCs contributed <0.1% of TOB. The detection of 1593 compounds in the sediments of Lake Michigan demonstrated the existence of numerous previously unknown NSOBCs in the environment. The distribution of NSOBCs in sediment core samples provided evidence that at least half of the compounds were from natural sources. Also, the strong correlations between abundances of NSOBCs and depth of water among locations indicated that geochemistry-related properties (i.e., communities of micro-organisms) might be responsible for the variation of abundances of NSOBCs. Another result from the present study was that some of the NSOBCs, especially the most abundant compounds from cluster 1 and 3 (e.g., PHCZs), showed increasing temporal trends of peak abundances in recent decades, suggesting that their input might be affected by human activities. Toxic potencies of synthetic and natural brominated compounds, such as OH-BDEs and PHCZs, have been widely reported previously,[18,40,56] indicating the potential health and ecological risk of NSOBCs, especially considering the trends of increasing concentrations of NSOBCs of greater abundance.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b04709.

> Figures addressing (1) method precision evaluation; (2) structures and distribution of NSOBC peak abundances; (3) correlations between NSOBC peak abundances and water depth; (4) spatial distribution of three model NSOBCs; (5) distribution of NSOBCs in four groups from soft-cluster analyses; (6) temporal trends of three model NSOBCs in sediment core samples. (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was part of the Great Lakes Sediment Surveillance Program funded by a Cooperative Agreement from the U.S. EPA Great Lakes Restoration Initiative with Assistance no. GL-00E00538 (U.S. EPA Program Officer Todd Nettesheim), a Discovery Grant from the Natural Science and Engineering Research Council of Canada (project no. 326415-07), and a grant from the Western Economic Diversification Canada (projects no. 6578, 6807, and 000012711). C.C.L. acknowledges financial support from the China Scholarship Council. The authors acknowledge the support of an instrumentation grant from the Canada Foundation for Infrastructure. Prof. Giesy was supported by the Canada Research Chair program, and the 2014 "High-Level Foreign Experts" (no. GDT20143200016) program, funded by the State Administration of Foreign Experts Affairs, the P.R. China to Nanjing University, and the Einstein Professor Program of the Chinese Academy of Sciences.

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Untargeted Screening and Distribution of Organo-Bromine Compounds in Sediments of Lake Michigan

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Pages	7
Figures	6
Tables	0

This supporting information provides figures addressing (1) Precision of the methods; (2) Distribution of abundances of molecules associated with each NSOBCs peak; (3) Correlations between abundances of molecules in NSOBC peaks and water depth; (4) Spatial distributions of three model NSOBCs; (5) Distribution of NSOBCs in four groups from soft cluster analyses; (6) Temporal trends of three model NSOBCs in sediment cores.

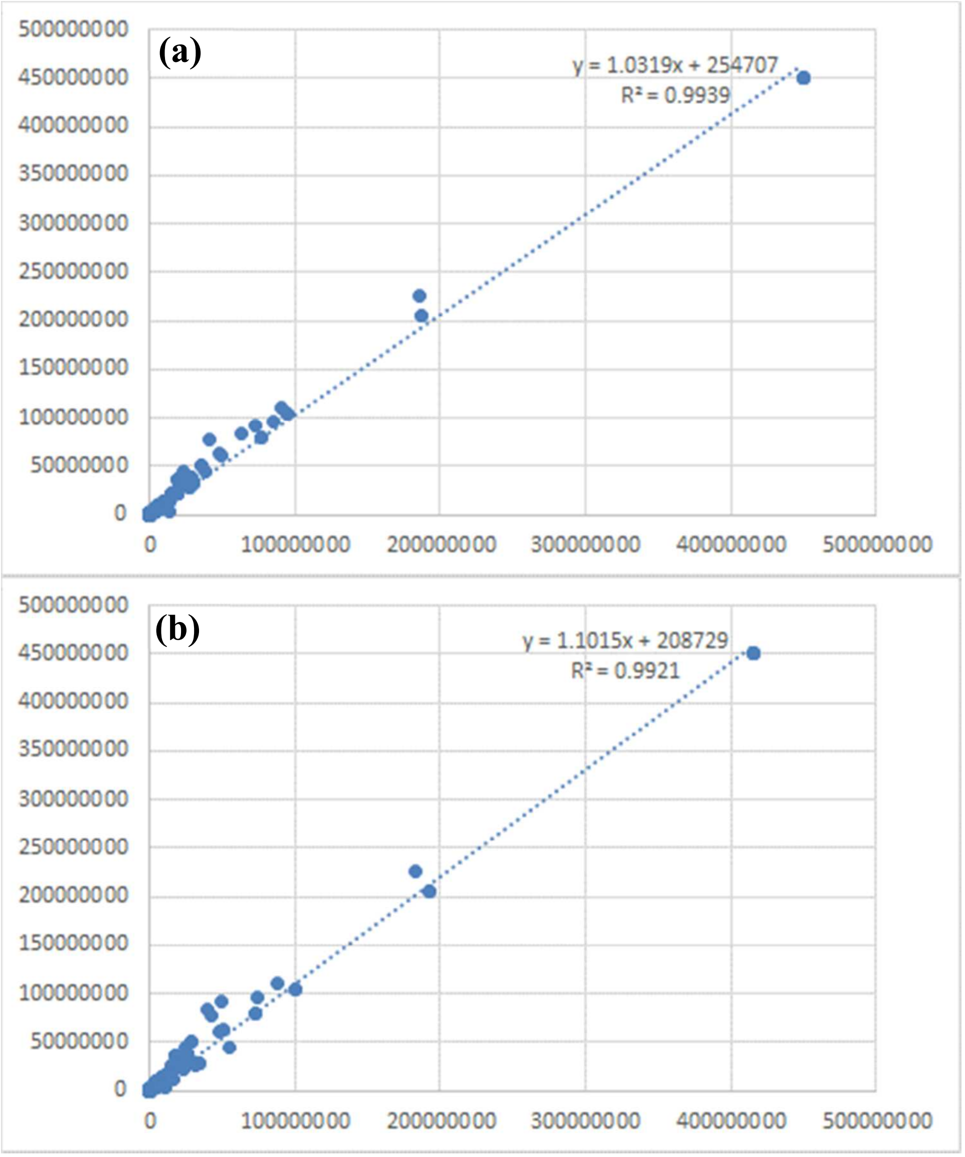

Figure S1. Evaluation of precision of the method. (a) Correlations of abundances of NSOBC in peaks between two injections of the same sediment extract (Sed-32); (b) Correlations of abundances of NSOBC peaks between two extracts of the same sediment sample (Sed-32).

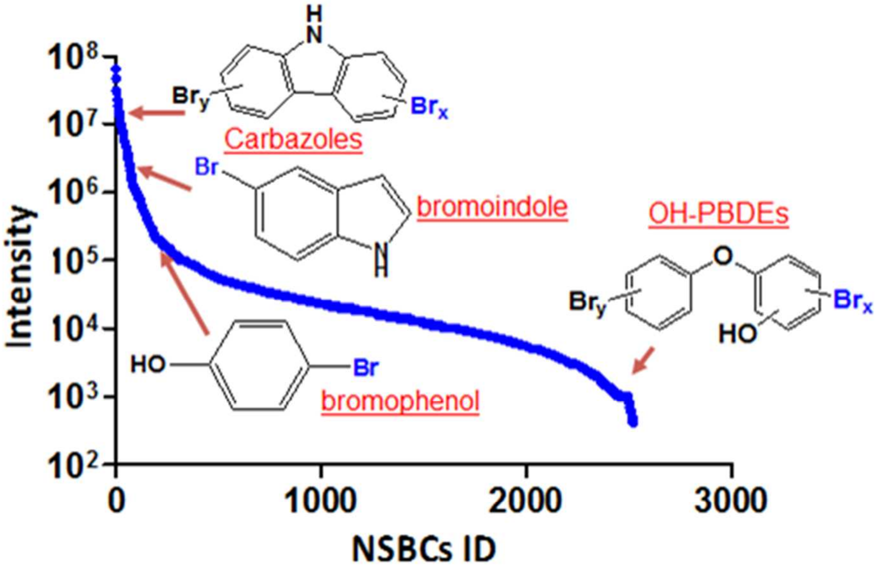

Figure S2. Structures of three model NSOBC chemicals and abundances of their peaks compared to other NSOBCs and OH-PBDEs.

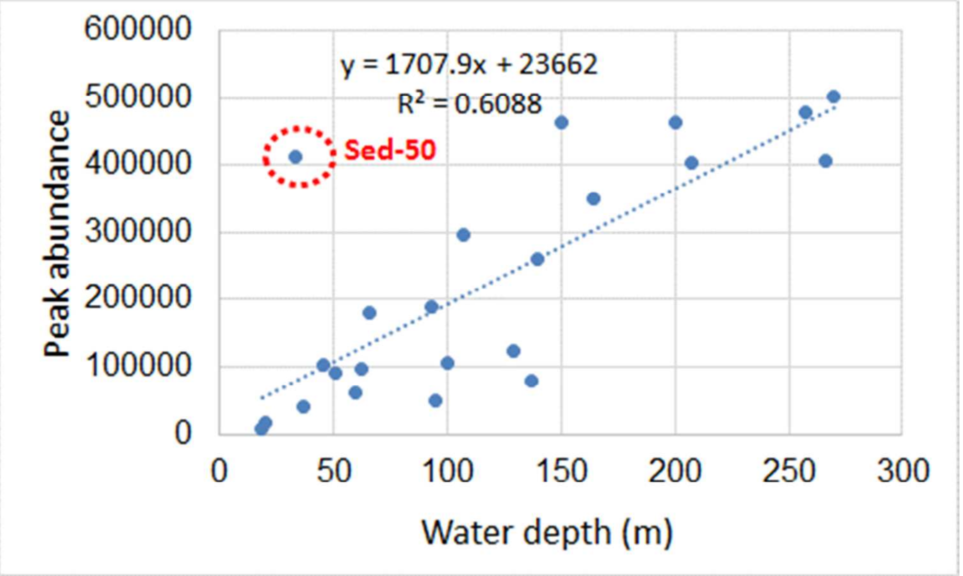

Figure S3. Relationship of peak abundance of total NSOBCs with water depth. Sed-50 (red circle) was also included in the correlation analysis, but this sample may exhibit different emission source from other sediments.

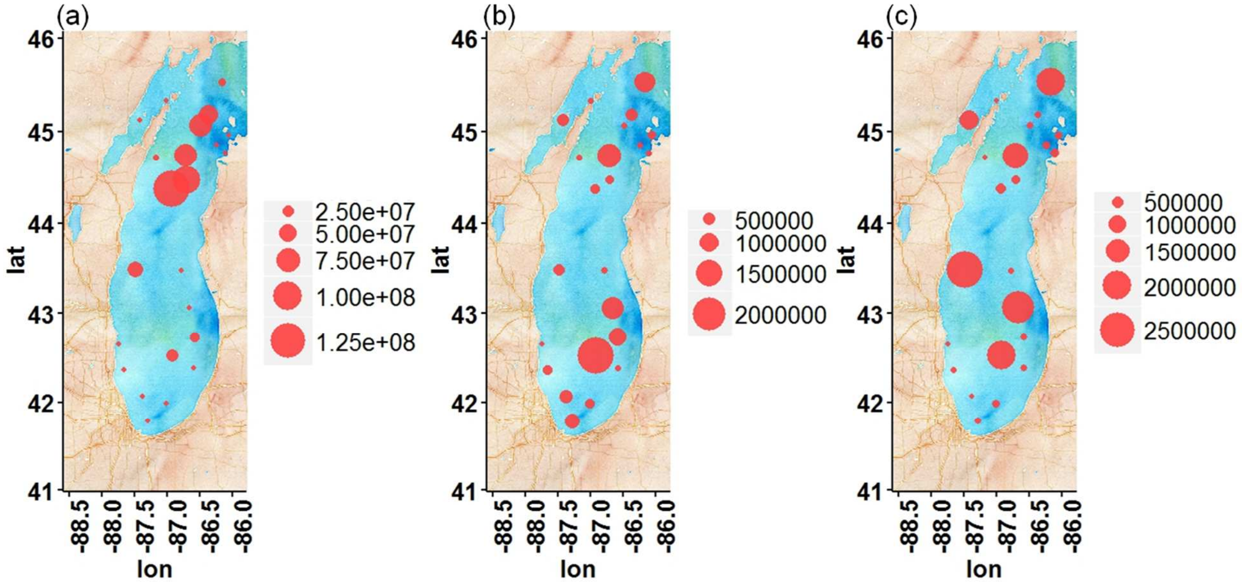

Figure S4. Spatial distribution of three model NSOBCs (a) tetrabromocarbazole, (b) bromoindole and (c) bromophenol among 23 surficial sediments from Lake Michigan.

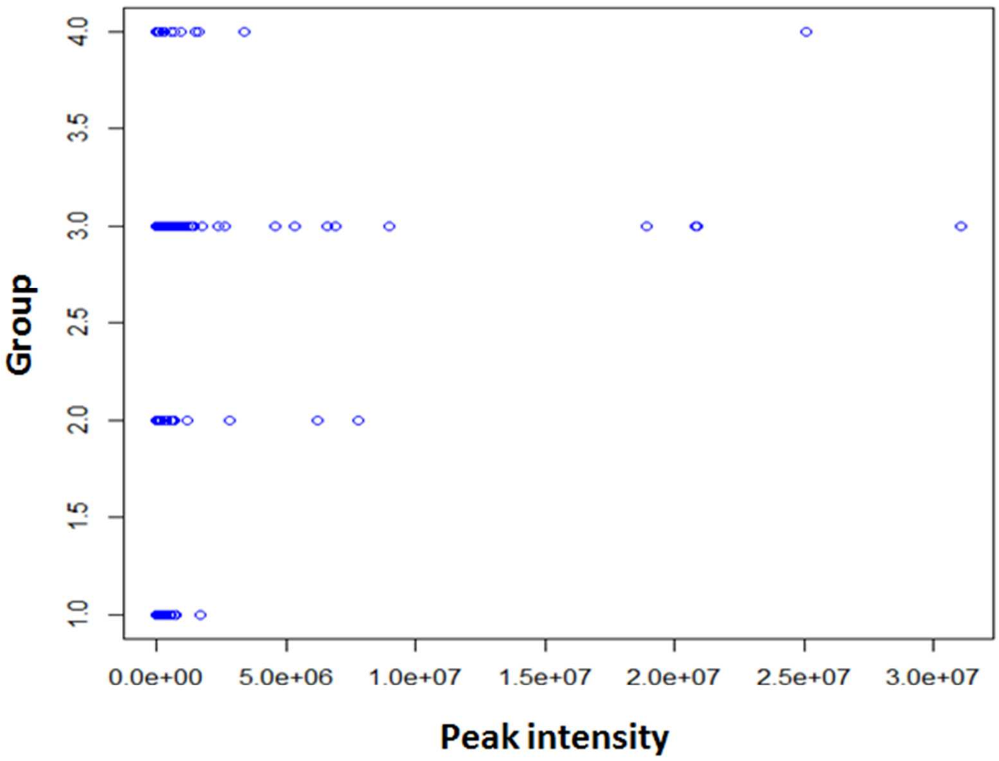

Figure S5. Distributions of abundances in peaks associated with NSOBCs placed into four groups developed by use of soft cluster analyses. The several high abundant peaks in group 3 were brominated carbazoles.

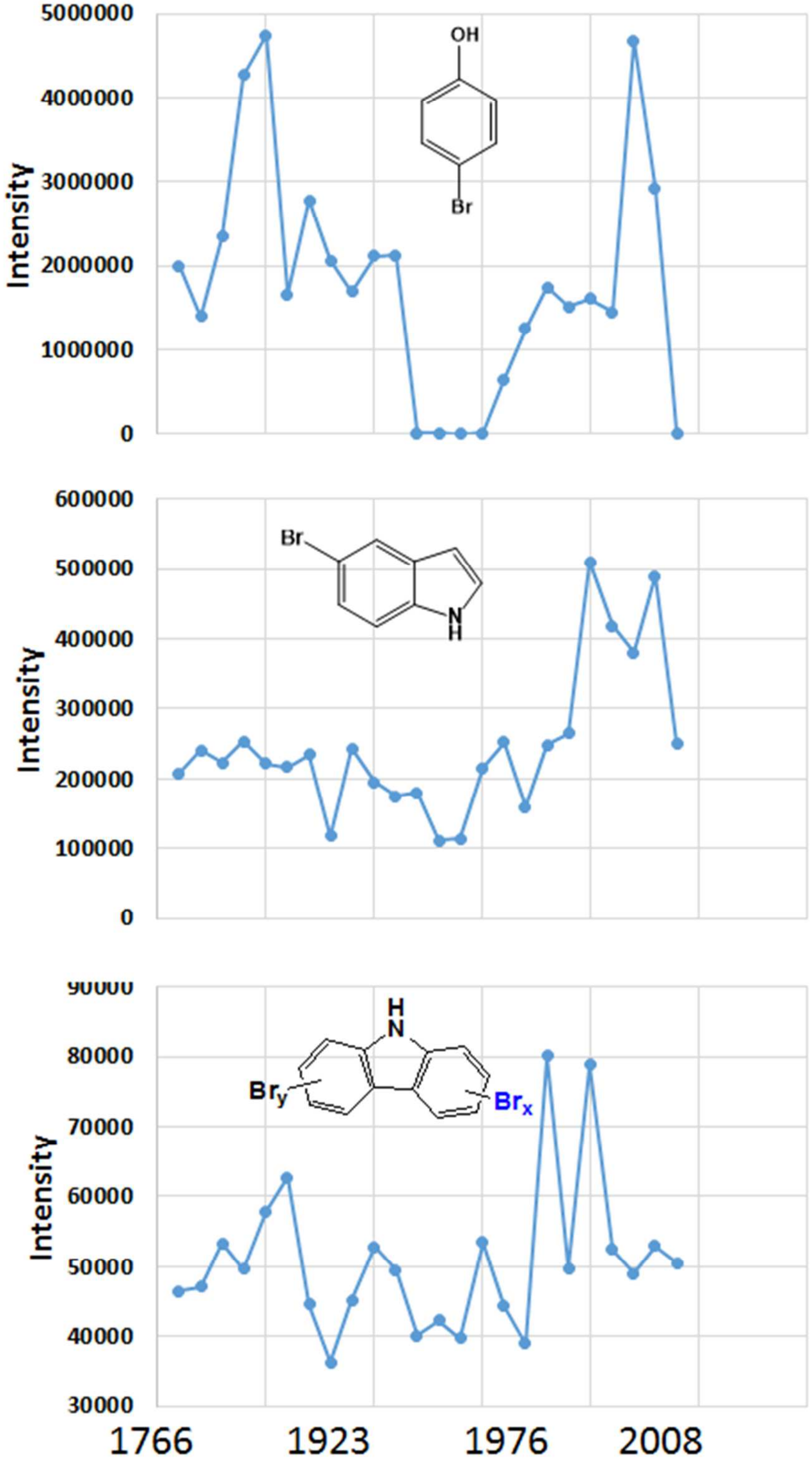

Figure S6. Temporal trends of abundances of three model NSOBCs bromophenol, bromoindole and tetrabromocarbazole.