

POLYBROMINATED DIPHENYL ETHERS IN TREATMENT PLANT SLUDGES: METHOD VALIDATION AND FIRST RESULTS FOR TURKEY

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Introduction

Polybrominated diphenyl ethers (PBDEs) were widely used as additive flame retardants in upholstered furniture, electrical equipments, and textiles among others^{1,2}. PBDEs were commonly observed in different environmental matrices such as, air, dust, sediment, soil, sewage sludge³, and living organisms including humans⁴. Since they have potential health effects; such as endocrine disruption⁵, and pose risk to the environment due to their persistence, identification of their current situation in environmental compartments becomes a significant issue. PBDEs were included among the persistent organic pollutants (POPs), identified by the Stockholm Convention, in 2009⁶. Although current records of worldwide production was not clearly revealed, it is known that market demand for PBDEs had reached 70000 tons in 2001 before the start of their phase out⁷. Specifically, deca-BDE constituted 83% of this demand in the same year⁴. The most recent data about commercial deca-BDE demand in European countries indicated 2500-5000 tons per year sold in 2012⁸.

Due to the wide usage and persistency of PBDEs, studies focusing on these chemicals have increased in the last decade throughout the world. On the other hand, studies conducted on PBDE levels in Turkey have been relatively scarce, yet they indicate presence of use of PBDEs. Recent national studies showed that atmospheric and soil concentrations near industries in Izmir, Turkey, were comparable or higher than concentrations measured worldwide^{9,10,11,12}. Another industrial site in Kocaeli yielded comparable soil PBDE levels with Izmir¹³. Additional studies have evaluated the levels in human milk^{14,15}, fish¹⁶, and butter¹⁷ and all found that the levels were comparable with literature data. A very recent study revealed indoor PBDE concentrations in dust in Istanbul, and commented that they were lower than countries like China, USA and UK¹⁸. Currently, there is no information about the level of PBDEs in wastewater treatment plant (WWTP) sludges in Turkey. As a part of National Implementation Plan preparations, PBDE inventory study for Turkey was conducted and the data gathered was based on estimates of use in related sectors¹⁹. Since PBDE consumption is high in industries and they are globally found in commercial goods, industrial and domestic WWTPs can be sinks for these substances. Several analysis methods have been developed for the determination of PBDEs in different matrices, and summarized in comprehensive review studies^{20,21}. An analysis method is also developed for the identification of PBDEs in sludge samples for this study.

The objective of this study is two fold: (1) to compare the efficiency of different analytical methods and to optimize the steps involved for the determination of PBDEs in solid matrices and (2) use the developed method to investigate the PBDE levels of WWTP sludges for the first time in Turkey.

Materials and methods

All solvents (n-hexane, dichloromethane (DCM), acetone) used for analysis, anhydrous sodium sulfate (granular), copper fine powder (<63 µm), and aluminum oxide (0.063-0.200 mm) were purchased from Merck KGaA (Darmstadt, Germany). Individual standards of PBDE 209, surrogate standard PCB 141 (2,2',3,4,5,5'-CB) and internal standard PCB 209 (2,2',3,3',4,4',5,5',6,6'-CB) were supplied from Dr. Ehrenstorfer GmbH (Augsburg, Germany).

Sludge samples were collected from WWTPs located in four different geographical regions of Turkey: two being urban (U-1, U-2) and two being industrial (I-1, I-2) WWTPs. Industrial WWTPs serve organized industrial districts (OID) that consist mainly of textile factories, which is a leading sector in Turkey. Urban WWTPs are both high capacity plants that can also receive some industrial discharges. Dewatered sludge samples were collected during September to November 2014 and transported to the laboratory in a cooler, then stored at -18°C until analysis. Samples were dried in a lyophilizator (Christ Alpha 1-4 model) operating at a pressure of 0.006 mbar and -45°C condenser temperature. Dried samples were ground and sieved through a 2.0-mm sieve and stored in amber-glass bottles until extraction.

Three different extraction methods were tested in the scope of this study. These methods are summarized below. After the analysis of each method with laboratory control samples, the most efficient method was selected for the analysis of sludge samples. Within the scope of this study, BDE-209 levels were identified due to its being the most abundant congener in almost all types of environmental matrices.

Soxhlet extraction. U.S. EPA Method 3540C²² was followed. Ten grams of sample was placed into Soxhlet thimble and 300 mL of solvent mix (hexane:DCM:acetone, 7:7:1 v/v) was used for extraction. Sample was extracted with Soxhlet apparatus for 17 h after the addition of surrogate standard PCB 141. The extract was eluted from a column of anhydrous sodium sulfate, and concentrated to 10 mL with Kuderna-Danish (KD) evaporator with a 3-ball Snyder column or a rotary evaporator (RE).

Ultrasonic extraction. U.S. EPA Method 3550C²³ was followed. Five grams sample was placed into 40 mL vials and mixed with five grams of anhydrous sodium sulfate. After addition of surrogate standard, sample was kept overnight in 30 mL solvent mix (hexane:DCM:acetone, 7:7:1 v/v) and extracted in ultrasonic bath (Cole Parmer 8892) for 30 min. Extract was centrifuged at 2500 rpm for 5 min and collected. This procedure was repeated twice and total 60 mL extract was concentrated down to 10 mL with RE.

Vial extraction. This method was developed in a previous study for PCB analysis²⁴. 0.5 g of samples were extracted by vigorous shaking on a horizontal shaker at 350 rpm for 16 h with 10 mL solvent mix (hexane:DCM:acetone, 4.5:4.5:1 v/v) in 15 mL amber vials with Teflon-lined caps. Separation of solvent from the sample was achieved by centrifuging the vials at 2500 rpm for 5 min.

Purification of extracts. The colored extract after concentration step was treated with concentrated sulfuric acid (U.S. EPA Method 3665A²⁵). The top clear extract was purified with 0.5 to 3 g of alumina (deactivated to 6%) or silica (deactivated to 4.5%) topped with anhydrous sodium sulfate. The collected extract was concentrated to 10 mL with KD apparatus or RE, and to 2 mL by high purity nitrogen stream.

Instrumental analysis. One mL of extract was spiked with internal standard and analyzed with gas chromatography coupled with micro-cell electron capture detector (Agilent 6890N GC- μ ECD) with DB-5 MS capillary column (15 m x 0.25 mm ID x 0.10 μ m). Instrumental conditions were as follows: Helium was used as the carrier gas with 2.5 mL/min flowrate using a constant flow mode. The make-up gas for the detector was nitrogen with a flowrate of 30 mL/min. The injector and detector temperatures were 250°C and 350°C, respectively. The sample injection was carried out at 1 μ L with splitless injection mode. Oven temperature program started at 100 °C, held there for 1 min, raised at 50°C/min to 150°C, and raised to 310°C at 12.5/min.

QA/QC. A six-point internal calibration was performed for BDE-209 and the surrogate standard, yielding relative standard deviations (RSD) lower than 20% and R² greater than 0.995. Method detection limit (MDL) and limit of quantitation (LOQ) were determined according to Chapter One of U.S. EPA Method SW-846²⁶. The MDLs were 4.85 ppb and 0.11 ppb for BDE-209 and PCB-141, respectively. The LOQs were 15.4 ppb for the analyte and 0.35 ppb for the surrogate standard. The recoveries of surrogate standard were 88.1% \pm 5.6% (range: 81.7 – 101%) for all sludge samples analyzed with the selected analytical method. Method blank was analyzed for every batch of environmental samples and no peaks were detected for the analyte. The relative percent difference of matrix spike/matrix spike duplicate samples was <14%.

Results and discussion:

Method validation studies. For extraction of PBDEs, several different methods have been used in the literature such as Soxhlet extraction, ultrasonic extraction, pressurized liquid extraction, and etc²⁰. Additionally, vial extraction method was previously developed for the analysis of PCBs from WWTP sludge samples with high precision²⁴. Among these methods, Soxhlet, ultrasonic and vial extraction methods were tested in this study with laboratory control samples (LCS). Several different solvents were used for these methods in the literature, but solvent types and volumetric ratios were kept similar within the tested methods. Furthermore, as a purification step of the extracts, silica gel and alumina, which are commonly used, were applied here. Together with the LCS, solvent blank were also analyzed. The results of the method validation study (Table 1) demonstrated that use of alumina in Soxhlet extraction gave higher precision than use of silica gel, while the recoveries were comparable with both adsorbents in ultrasonic extraction. Especially, the surrogate recoveries in LCS extracted with Soxhlet and purified with silica exceeded the acceptable range provided by U.S. EPA as 70 – 130%²⁷. Although vial extraction method yielded acceptable surrogate recoveries with alumina, this method showed varying recoveries for analyte recovery with some lower values. On the other hand, the recoveries of both surrogate and analyte were comparable and within acceptable range for Soxhlet and ultrasonic extractions with alumina cleanup. Hence, considering solvent use and time constraints, ultrasonic extraction with alumina cleanup was selected to be used for detection of PBDEs in sludge samples.

Table 1. Surrogate and BDE-209 recoveries for three extraction methods and two purification adsorbents.

Sample	Extraction method	Clean up ^a	n	Surrogate Recovery (%)		BDE-209 Recovery (%)	
				Average ± St.dev. (range)	RSD	Average ± St.dev. (range)	RSD
Solvent Blank	Soxhlet	S	4	113.9±9.11 (106-127)	8.00		
	Soxhlet	A	2	98.2±5.44 (94.3-102)	5.55		
	Ultrasonic	S	2	84.5±3.46 (82-86.9)	4.10		
	Ultrasonic	A	4	89.4±4.68 (84.6-94.1)	5.23		
	Vial	S	4	89.5±1.65 (88.2-91.8)	1.85		
	Vial	A	4	88.4±3.22 (84.3-91.8)	3.65		
Laboratory Control Sample	Soxhlet	S	4	131.1±36.9 (81.9-171.5)	28.2	108.5±24.9 (73-130.5)	22.9
	Soxhlet	A	4	101.9±2.21 (99.6-104.8)	2.17	86.3±7.02 (78.3-95.4)	8.13
	Ultrasonic	S	2	95.7±2.69 (93.8-97.6)	2.81	96.2±7.42 (90.9-101.4)	7.72
	Ultrasonic	A	4	88.4±1.77 (86.4-90.6)	2.00	96.8±8.35 (86-105.7)	8.63
	Vial	S	4	74.6±3.96 (69.2-77.8)	5.30	52.9±11.2 (43.6-67.7)	21.2
	Vial	A	6	90.3±3.72 (83.9-95.3)	4.12	78.0±13.6 (62.3-96.1)	17.5

^a S: silica gel, A: alumina cleanup

Levels of PBDEs in sludges. Urban WWTP serving a metropolitan city (more than one million population equivalent) in Turkey (U-1) exhibited 193.3 ± 55.9 ng/g dw of BDE-209 concentration. Same sludge was also analyzed with Soxhlet extraction to observe the variation between methods. Soxhlet extraction yielded 209.1 ± 0.04 ng/g dw, resulting in 5% relative percent difference between methods. The second urban WWTP sludge sample (U-2) found to contain 353.3 ± 10.9 ng/g dw. The higher level found in this sample is attributed to the industrial inputs to this WWTP, which was known to receive some discharges from chemical, food and textile industries. One of the industrial WWTPs (I-1) serves the textile sector, mainly manufacturing towels and bed sheets. This sludge sample showed the lowest level of contamination among other samples in this study, with a concentration of 58.3 ± 6.3 ng/g dw. The other industrial WWTP (I-2) had much greater BDE-209 levels with $52.2 \times 10^5 \pm 18.6 \times 10^5$ ng/g dw. This huge amount of BDE-209 accumulated in sludge is attributable to the products manufactured within the OID that the WWTP serves for. The main production for this district is based on automotive industry, its accessories, upholstery for car cushions and insulation materials. These are among the major usage areas of PBDEs. Although the information on the inventory of PBDEs in Turkey is based on estimates for sectors, it was revealed that 200 tons of PBDEs are expected annually due to transport sector¹⁹. The significantly high level of BDE-209 found in this sludge sample is crucial since it demonstrates the direct usage of PBDEs in Turkey.

The recent sludge sampling studies from different parts of the world showed comparable values with this study except for I-2 (Table 2). All studies identified concentration ranges for different number and type of WWTPs. Studies did not particularly indicate whether WWTPs were industrial, except the South Korean study³⁴. Although a very high level was observed in USA, I-2 WWTP of this study yielded hundred times greater level. This finding has also an implication in terms of worldwide PBDE levels.

Table 2. Comparison of BDE-209 levels in dewatered sludge samples of this study with studies worldwide.

Country (Sampling year)	BDE-209 level (ng/g dw)	No. of WWTPs	Ref.
Turkey (2014)	$58.3 - 52.2 \times 10^5$	4	<i>This study</i>
Spain (2009)	ND – 2303 (Median: 285)	17	28
Spain (2010)	ND – 319	6	29
United States of America (2001)	1420 – 14200	94	30
Switzerland (2003–2005)	138 – 617	16	31
Sweden (2010)	Median: 513	9	32
United States of America (2006–2010)	636 – 2933	4	33
Korea (2006)	6.18 – 9740	8 (industrial)	34
South Africa (2010–2011)	ND – 297	1 ^a	35

^a The same WWTP was monthly sampled for 6 months.

To conclude, ultrasonic extraction served well for the purposes of this study, as a relatively quick and efficient extraction method for PBDE analysis in solid matrices. Secondly, this preliminary study for the identification of

PBDE levels in WWTP sludge samples in Turkey revealed the significance of the determination of POPs inventory throughout the country. Specifically, very high levels found in one of the industrial WWTPs demonstrated the potential severity of the problem. Further studies should be conducted for all major organized industrial district WWTPs and various time intervals, to reveal use and release of PBDEs. As regulations come into force for control of POPs, these industries require use of alternative chemicals. Management of any environmental contamination should also follow.

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