SCREENING STUDY TO DETERMINE THE DISTRIBUTION OF COMMON BROMINATED FLAME RETARDANTS IN WATER

Report to the
Water Research Commission

by

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EXECUTIVE SUMMARY

BACKGROUND

Many organic compounds that are released either deliberately or accidentally into the environment often tend to cause contamination. One of the most recent environmental contaminants is brominated flame retardants (BFRs). Pentabromodiphenyl and octabromodiphenyl ethers have been listed in Annex A of the Stockholm Convention for elimination by Parties. They have been listed because of their persistence, bioaccumulative and endocrine disrupting characteristics.

AIM

The main aim of this study was to investigate the presence and concentrations of common BFRs in water systems in Gauteng province. Data on these emerging environmental contaminants is extremely important as South Africa is gearing towards the national implementation of the National Toxicology Monitoring Programme (NTMP). The aim was achieved via the following specific objectives:

- Determine the occurrence and concentrations of common BFRs in selected landfills, surface water, groundwater, wetlands and sediments in Gauteng, using established analytical methods;
- Characterize exposure to BFRs using common aquatic organisms found within the water systems;
- Investigate seasonal trend of those BFRs found within the water systems;
- Develop an environmental contamination profile of landfills, surface water, wetlands, groundwater, sediment and biota within the study area with respect to BFRs;
- Employ derivatization techniques to develop a sample pre-concentration extraction kit that can be used to analyse high molecular weight BFRs and
- Attempt to identify the sources of BFRs if found present in relation to land use.

METHODOLOGY

Samples were collected from different catchments: Jukskei (7 water and 7 sediment); Upperklip (1 water and 1 sediment); Rietklip (1 water); Lowerklip (1 water and 1 sediment); Vaal (2 water and 2 sediment) and Marco/Crocodile (1 water and 1 sediment). For groundwater, only 1 water sample per site was collected from the following sites Wonderboom Park, Eastling and Doorandjie. With respect to landfill samples; 1 leachate and 1 sediment sample was collected from Hatherly, Soshanguve, Garstkloof, Onderstepoort, Chloorkop and Robinson deep landfill sites. With respect to wetlands, samples; 1 water and 1 sediment sample were collected from Bullfrog, Klip River wetland, Rietvlei, Karlspruit for winter samples and Soshanguve. Fish samples were collected from the Vaal River. Samples were collected in both summer and winter seasons from the aforementioned sites.

RESULTS AND DISCUSSION

Results obtained indicated that the concentrations of common BFRs in the river water samples collected were below the instrument detection limit and, therefore, not presented in the current report. All the screened analytes were detected in river sediment samples except for BDE-154 that was below detection in Taaiboschspruit.

For the leachate samples BDE-47, 99, 100, 153, 154, 183 and 209 were all detected in the winter leachate samples. Levels of PBDEs in summer leachate samples were below detection. The values recorded for sediments samples were significantly higher than leachate samples. This is not surprising since sediments are known sinks for contaminants. Common PBDEs congeners were detected in sediment samples from all the sites in Jukskei River with the exception of BDE-183. The observed total PBDEs concentrations in summer sediment samples ranged from 0.07-2.41 ng g-1, with BDE-183 and BDE-99 exhibiting the lowest and highest total concentrations respectively. For winter samples, low levels of BDE were detected at six

sites. The concentrations of BDE detected in the winter sediment samples ranged from 0.06-10.40 ng g-1. This is significantly higher than the values for the summer sediments (0.07-2.41 ng g-1).

With respect to BDE in wetland water samples, the following BDE were detected: BDE-47, 153, 154 and 183 with Reitvlei wetland exhibiting the highest levels followed by Bullfrog and Karlspruit. No BDE was detected in Klip River wetland samples. With respect to BDE in wetland sediment samples, the following BDE were detected: BDE-47, 153, 154 and 183 with Rietvlei wetland exhibiting the highest levels followed by Bullfrog and Karlspruit. No BDE was detected in Klip River samples.

The detection of BDE-17, BDE-47, BDE-99 and BDE-153 in all the samples indicated that these were the most common BFRs in the samples analysed. One could, therefore, suggest that these congeners can be used as preliminary screening indicators of BFRs contamination in environmental matrices. The levels of BFRs obtained in the present study, however, were found to be lower than the values (23.7-2253 ng g-1 d.w) obtained from other parts of the world. The heavy rain experienced in late 2012 and early 2013 may have affected the levels of PBDEs observed in the current report.

A method for the analysis of APEs and BFRs in fish matrix was developed and applied to detect the levels of these compounds in bottom feeder type of fish. The method used silica gel and Aminopropyl cartridge for lipids removal. The recoveries ranged from 5.5± 10.9 (TBBPA) to 70.64 ± 3.94 (HBCD). The levels of these selected compounds ranged from 0.061 (BDE) to 6.49 (NP2E) ng g-1.

For the sample pre-concentration extraction kit, the feasibility of in situ derivatization of TBBPA with acetic anhydride as an extractive sample preparation procedure was demonstrated. In addition, the simultaneous extraction of the resulting TBBPA derivative together with other commonly investigated BFRs was also performed. The application of different extraction methods for the isolation of the target compounds indicated that the use of SPE techniques as pre-concentration tools was characterized with poor recoveries and pronounced matrix effect. However, the use of LLE employing separating funnel showed improved percent yield of the TBBPA derivative, although the extraction method was not employed for the simultaneous extraction of other BFRs. Consequently, the use of separating funnel extraction for the isolation of TBBPA derivative resulting from in situ derivatization is recommended in order to obtain acceptable analytical results.

CONCLUSIONS

The following conclusions were reached at the end of the project:

- BDEs were not detected in water samples, however, they were detected in sediment samples collected in winter and summer from Jukskei and Vaal Rivers and winter samples and these were significantly higher than the summer samples;
- Also the levels of BDE in landfill sediment samples were significantly higher than that of leachates and that unlined landfill sites showed higher BDE than lined landfill site, probably because of slow adsorption by soil in the former compared to a faster adsorption by geomembrane in the latter;
- The feasibility of in situ derivatization of TBBPA with acetic anhydride as an extractive sample preparation procedure was demonstrated; and the simultaneous extraction of the resulting TBBPA derivative together with other commonly investigated BFRs was also performed;
- The application of different extraction methods for the isolation of the target compounds indicated that the use of SPE techniques as pre-concentration tools was characterized with poor recoveries and pronounced matrix effect;
- The use of LLE employing separatory funnel showed improved percent yield of the TBBPA derivative, although the extraction method was not employed for the simultaneous extraction of other BFRs.

RECOMMENDATIONS

1. Chemical profile of water and sediment samples with respect to trace metals should be carried out in order to establish whether there is any relationship between the analytes of interest and other contaminants;
2. The developed sample pre-concentration extraction kit should be subjected to a mixture of other emerging contaminants to test its ruggedness;
3. Work should be done on the so called “novel flame retardants” that are currently used to replace the legacy flame retardants have been reported in water systems in developed countries, but not in any developing country;
4. Phosphorous flame retardants which have also replaced the BFRs should be monitored in water systems since information on these is still scarce in South Africa
5. The use of separating funnel extraction for the isolation of TBBPA derivative resulting from in situ derivatization is recommended in order to obtain acceptable analytical results.

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ACRONYMS & ABBREVIATIONS

APPA	Atmospheric Pollution Prevention Act 45 of 1965
ASE	Accelerate solvent extraction
AVCASA	Association of Veterinary and Crop Associations of South Africa
BFRs	Brominated flame retardantS
CAIA	Chemical and Allied Industries
DCM	Dichloromethane
DDT	Dichlorodiphenyltrichloroethane
DEA	Department of Environmental Affairs
DoA	Department of Agriculture
DoH	Department of Health
dw	Dry weight
DWS	Department of Water and Sanitation
ECA	Environmental Conservation Act
FFAS	Fertilizers, Farm feeds and Agricultural Remedies and Stock Remedies
ft	Feet
HBCD	Hexabromocyclododecane
HPLC	High pressure liquid chromatography
id	Internal diameter
mm	Millimetre
NEAQA	National Environmental Management Air Quality
NEMA	National Environmental Management Act and Regulations
NGO	None governmental organization
NTMP	National toxicology monitoring programme
PBBs	Polybrominated biphenyls
PBDEs	Polybrominated diphenyl ethers
PCBs	Polychlorinated biphenyls
RQS	Resource quality services
SABS	South African Bureau of Standards
SANS	South African National Standards
SARS	South African Revenue Services
SPE	Solid phase extraction
TBBPA	Tetrabromobisphenol
TUT	Tshwane University of Technology
UP	University of Pretoria
v/v	Volume per volume
wt	weight

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BACKGROUND

1.1 INTRODUCTION

Brominated flame retardants (BFRs) are technical flame retardants containing brominated organic compounds which are applied to combustible materials, such as plastics, wood, paper, electronics and textiles to meet fire safety regulations (Darnerud et al., 2001). Thus given the ubiquity of plastics in the modern world, it is not surprising that brominated flame retardants, particularly PBDEs are being detected in all environmental compartments, including aquatic ecosystem (Allchin et al., 1999; de Boer et al., 1998). The estimated global consumption of BRFs shows that their usage is on the increase (de Boer et al., 1998).

Being environmentally persistent compounds with high production volumes and lipophilic, polybrominated diphenyl ethers (PBDEs), polybrominated biphyenyls (PBBs), hexabromocyclododecane (HBCD) and tetrabromobisphenol A (TBBPA) are among the most abundant BFRs detected in the environment. Of the BFRs, pentabromodiphenyl ether and octabromodiphenyl ethers have been listed in Annex A of the Stockholm Convention; hence production and use have to be eliminated by Parties. The listing is as a result of their persistence, bioaccumulation and toxicity. Studies have indicated that it is possible for BFRs to leach from products into the environment if they are reactively added to those products (Darnerud et al., 2001). The widespread production and use of BFRs, and strong evidence of increasing contamination of the environment by these chemicals, heighten the importance of identifying data gaps.

1.2 PROJECT AIMS

The following were the aims of the project:

- Determine the occurrence and concentrations of common BFRs in selected landfills, surface water, groundwater, wetlands and sediments in Gauteng, using established analytical methods;
- Characterize exposure to BFRs using common aquatic organisms found within the water systems;
- Investigate seasonal trend of those BFRs found within the water systems;
- Develop an environmental contamination profile of landfills, surface water, wetlands, groundwater, sediment and biota within the study area with respect to BFRs;
- Employ derivatization techniques to develop a sample pre-concentration extraction kit that can be used to analyse high molecular weight BFRs and
- Attempt to identify the sources of BFRs if found present in relation to land use.

1.3 SCOPE AND LIMITATIONS

1.3.1 Scope

This project focused on water pollution with respect to BFRs in various parts of Gauteng Province in order to present an overview of the level of contamination by BFRs in the province. The water bodies monitored were classified under the following headings:

1. Rivers (surface water and sediment);
2. Groundwater (boreholes);
3. Landfill (leachates and sediments) and
4. Wetlands (surface water and sediment)

1.3.2 Limitations

Although rivers, landfill sites, wetlands and groundwater (boreholes) were identified using the National Toxicity Monitoring Programme, however, it was found out that some of the identified river and wetland sampling sites were dry. Furthermore, in the case of landfill sites, none existence of leachate collection ponds hampered sample collection. In the case of groundwater sampling locations identified, accessibility to the site where not possible since a number of the groundwater were in private properties. All the aforementioned obstacles with respect to sample collection from the rivers, landfill sites, wetland and groundwater were experienced during this study. << Describe how the report originated and what it is about. Give some historical background such as the relevant theory or previous work on which it is based. Summarise the Terms of Reference, if one exists.

CHAPTER 2: BROMINATED FLAME RETARDANTS

2.1 WHAT ARE FLAME RETARDANTS?

Flame retardants are chemicals used in a wide range of industrial and consumer polymer products such as paints, plastics, textiles, furniture, electronics, polyurethane foams and construction materials to retard ignition rate of fires (WHO/EHC 192, 1997(Daso et al., 2010; de Wit, 2002). They are used to protect the public from fires by reducing the flammability of materials in which they are incorporated or reacted with to provide additional protection from fires and to increase escape time when a fire occurs. Flame retardants are classified into two major categories; reactive and additive flame retardants. Reactive flame retardants are chemically bonded to the polymer during synthesis of the polymer through the formation of weak covalent bonds. Therefore, less likely to leach out of the matrix to the environment until the product is decomposed or burnt; typical examples are tetrabromophthallic anhydride and tetrabromobisphenol A (D'Silva et al., 2004). Additive flame retardants on the other hand, are incorporated into plastics often during or following polymerization when they are blended with the polymer constituents along with other additives like plasticizers. The blend is then applied to the substrate as a spray in a coating formulation. They are not permanently bonded to the polymer and as a result, they have the tendency to leach out of the polymer matrix prior to; during or after its operational life. Additive flame retardants include PBDEs, HBCDD and bis (tribromophenoxy) ethane as well as magnesium hydroxide and aluminium hydroxide (Hyotylainen & Hartonen, 2002).

2.1.1 History of flame retardants

Several chemical materials have been used since early times as fire retardants. For examples, the Romans (200 BC) applied alum and vinegar on wood. Similarly, the Egyptians (450 BC) prevented fire by soaking wood in alum (hydrated potassium aluminium sulphate), while the first patent on a flame retardant was the British patent 551(alum, ferrous sulphate and borax) by Obadiah Wilde in 1735, when he was able to flame retard canvas used in theatres and public buildings (Alaee et al., 2003; Blum et al., 2010). Increased use of flame retardants started in early 1970's when they were used in materials such as plastics in electrical equipment (televisions), synthetic fibres in sofa and curtains (La Guardia et al., 2010). This also, encouraged manufacturers to move away from traditional materials such as wood and metals. While these new materials provided many benefits, they had one problem; they were far more combustible than the materials they replaced.

2.1.2 Types of flame retardants

There are four main categories of flame retardant chemicals used in synthetic polymers namely; halogen containing, phosphorous containing, nitrogen containing and inorganic hydroxide flame retardants (WHO/EHC 192, 1997; Troitzsch, 1998).

- Halogen containing flame retardants: These include chlorinated and brominated organic compounds. Other elements of the same group (e.g. fluorine and iodine) are not used in practice because they do not interfere in the combustion process. Fluorine bonds too strongly and decomposes at much higher temperatures while iodine bonds too loosely to carbon and decomposes at slightly elevated temperatures (Alaee et al., 2003).
- Phosphorus containing flame retardants: primarily phosphate esters represent about 20% by volume of the worldwide production of FRs.
- Nitrogen containing flame retardants: These have limited applications in polymers e.g., melamine in polyurethane foams and melamine cyanurate in polyamides.
- Inorganic flame retardants: These are made up of mostly metal hydroxides, e.g., magnesium and aluminium hydroxides. Others include boron compounds and antimony trioxide which is used as a synergist with halogen containing flame retardants.

2.2 BROMINATED FLAME RETARDANTS

2.2.1 Overview

Brominated flame retardant (BFR) is the designated name for a group of brominated organic substances that have an inhibitory effect on the ignition of combustible organic materials (D'Silva et al., 2004). They belong to the group of organohalogenated flame retardants which include chlorinated and brominated flame retardants. Bromine-based flame retardants are highly brominated organic compounds with a relative molecular mass ranging from 200 to that of large molecule polymers. Bromine, added to organic compounds (brominated organic compounds) compared to elements of the same group such as fluorine, chlorine and iodine have shown to be the most effective in the inhibition of fires and are materials of choice because of the low cost and lower loading of substrate (D'Silva et al., 2004; Bientinesi & Petarca, 2009; Birnbaum & Staskal, 2004). BFRs are structurally diverse group of compounds which include aliphatic, cyclic and aromatic derivatives (Danerud, 2003). The bromine portion of the compound is responsible for the molecule's flame retardant activity and is unique in its ability to provide flame retarding function in the gas phase (Hardy, 2002). Most commonly used BFRs are; PBDEs, PBBs, HBCDD and TBBPA (Covaci & Dirtu, 2008). These are further categorised into additive and reactive flame retardants according to their uses. TBBPA is mostly used as a reactive flame retardant while PBB, PBDE, HBCDD and NBFRs are used as additives.

Brominated flame retardants are introduced into the aquatic environment through wet and dry deposition in similar pattern to other POPs such as PCBs (Ter Schure et al., 2004). There is increasing concern about the presence of these substances in the environment, animals and humans because of their persistence, bioaccumulation, toxicity (PBT) and long range atmospheric transport. They have been found in biotic and abiotic samples in the remote arctic environment (de Wit et al., 2006). They are known to have half-lives ranging from 2 to 10 years in the environment (D'Silva et al., 2004). Since PBDEs and NBFRs are used as additives in products, they have the potential to be released into the environment (de Wit, 2002), thereby

settling in dust, water, sediment and biota. Consequently, BFRs can bioaccumulate in fatty tissues thereby biomagnifying up in the food chain. Several studies have confirmed the presence of BFRs in fish (Bocio et al., 2003), birds (Polder et al., 2008), adipose tissue (Kunisue et al., 2007), breast milk (Sudaryanto et al., 2008) and serum of humans (Covaci et al., 2008; Jakobsson et al., 2002; Sudaryanto et al., 2008). The presence of BFRs in biotic and abiotic media has been reported by many researchers (Chen et al., 2009; Labadie et al., 2010; Li et al., 2010; Qiu et al., 2010; Lara et al., 2012; La Guardia et al., 2013; Li et al., 2013; Ryan et al., 2014; Shi et al.,2013).

2.2.2 Production trend of BFRs

Reports indicate that nothing less than 75 different types of brominated chemicals (Alaee et al., 2003: Covaci & Dirtu, 2008) have been produced on a commercial scale for use in products to meet wide range fire standards. Limited information is available on the current production volume of BFRs. The total world production of all brominated flame retardants in 1992 was estimated at approximately 150 000 metric tons. Forty percent of the distribution was to North America, 30% to the Far East and 25% to Europe. Brominated flame retardants are estimated to represent approximately 20 to 25% by volume of the global flame retardant production (de Wit, 2002). Estimated global BFR demand consumption per geographical distribution and consumption as per flame retardant are presented in Table 2.1 and Table 2.2, respectively. Table 2.2 shows the total global use of flame retardants in 2005, with a total use of brominated flame retardants of 311 000 metric tonnes, put at approximately 21% of the total consumption of flame retardants (Fink et al., 2008). The current total consumption of TBBPA produced worldwide is estimated to be 210 000 tonnes; the same study reported that world-wide demand for decabromodiphenyl ether was 54 800 tonnes/ year in 1999 (Alaee et al., 2003). It can, therefore, be concluded from the available information that there has been yearly increase in the production and use of BFRs; from a production of 150 000 in 1992 to 204 325 in 1999 and 311 000 metric tonnes in 2005. This is as a result of the effectiveness of BFRs in attaining high flame retarding capacity with lower loading at relatively low cost to meet stringent fire regulations as applicable in the UK and other developed countries (Blum et al., 2010). However, studies in the last decade have focused on the four common BFRs (PBDE, PBB, HBCDD and TBBPA) mainly because they are all high production volume (HPV) chemicals, while TBBPA remain the highest in production followed by deca-BDE and HBCDD. PBB, penta-BDE octa-BDE and HBCDD have since been banned in Europe and America leading to the their recent inclusion in the list of banned POPs (UNEP, 2009; UNEP, 2014).

Table 2.1: Estimated world market demand for PBDEs, TBBPA and HBCDD (metric tons) in 1999

	PeBDE	OcBDE	DeBDE	TBBPA	HBCDD	Total BFR
Americas	8 290	1 375	24 300	21 600	3 100	58 665
Europe	210	450	7 500	13 800	8 900	30 860
Asia	0	2 000	23 000	85 900	3 900	114 800
Total	8 500	3 825	54 800	121 300	15 900	204 325

Source: www.bsef.com

Table 2.2: Global consumption of flame retardants and their geographical distribution

Category	United States	Europe	Japan	Other Asia	Total volume [1000 metric tonnes]	Total Volume (%)	Value [million USD]
Aluminium hydroxide	315	235	47	48	645	43.55	424
Organo Phosphorus	65	95	30	14	205	13.84	645
Brominated FRs	**66**	**56**	**50**	**139**	**311**	**21**	**930**
Antimony trioxide	33	22	17	44	115	7.76	523
Chlorinated FRs	33	35	5	10	82	5.54	146
Other FRs	51	47	11	14	123	8.3	197
Total	**564**	**489**	**160**	**269**	**1481**	**100**	**2865**

Source: (Fink et al., 2008).

2.2.3 Common brominated flame retardants

2.2.3.1 Tetrabromobisphenol A (TBBPA)

Tetrabromobisphenol A (2, 2', 6, 6'-tetrabromo-4, 4', isopropylidenediphenol) is usually used in reactive mode which results in chemical binding of the substance to the material. Therefore, potential emission of TBBPA from products to the environment is likely to be limited in comparison to additive BFR compounds. The main application of TBBPA is in printed circuit boards of electrical electronic equipment such as televisions, vacuum cleaners, washing machines, copiers, computers, printers, fax machines and radios. Other uses include automotive, aviation and all entertainment equipment, it is used as additive in ABS plastic housing and as an intermediate in the production of other FR systems derivatives and brominated epoxy oligomers where it is integrated into the resin as well (BSEF, 2009a). TBBPA is the largest used BFR with an estimated production of 210 000 tonnes/year (Alaee *et al.,* 2003; BSEF, 2011). According to Covaci *et al*. (2011), the EU legislation defines a high production volume (HPV) chemical as a chemical produced above 1000 tons/year, while a low production chemical is manufactured below 1000 tons/year.

The photolytic degradation of TBBPA generates 2, 4, 6- tribromophenyl (TBP) which has been used as a pesticide for controlling insects, fungi and bacteria, is a key component of wood preservatives (Eriksson & Jakobsson, 1998). Furthermore, TBP has been used as a flame retardant, and is a synthetic intermediate of most of the important BFRs. TBBPA is not readily biodegradable and tend to persist in the environment (Nichkova *et al*., 2008). TBBPA is produced via bromination of bisphenol A in organic solvent and has been

found in the environment; sediment, sewage sludge, blood samples of humans (Jakobsson *et al.,* 2002) and is still been used globally with no restrictions (BSEF, 2009a).

2.2.3.2 Hexabromocyclododecane (HBCDD)

HBCDD is an aliphatic, brominated cyclic alkane. HBCDD is used primarily as an additive flame retardant. It has twelve carbon atoms with six bromine atoms tied to the ring and it is produced by bromination of cyclododecatri-1, 5, 9-ene, a process which leads theoretically to the formation of a mixture of 16 stereoisomers (six enantiometric pairs and four meso forms). The four main products in which HBCDD is used are:

- Expandable polystyrene (EPS);
- Extruded polystyrene (XPS);
- High Impact Polystyrene (HIPS); and
- Polymer dispersion for textiles (Arnot *et al.,* 2009).

The commercial HBCDD product consist of a mixture (t-HBCDD) of α-, β-, and γ-HBCDD diastereomers, with the γ-HBCDD isomer being the dominant (≥ 70%) (Heeb *et al*., 2005; Law *et al*., 2005). In biota, the α-HBCDD has been found to be the most prevalent (Janak *et al*., 2008). HBCDD is a high volume production chemical with worldwide production of 16 700 tons in 2001. It is a very effective flame retardant and low levels of between 0.7% and 2.5% are required to reach desired flame retardant levels in expanded (EPS) and extruded polystyrene (XPS), respectively (Covaci & Dirtu, 2008). HBCDD is also persistent and bioaccumulative and has been found in biota, especially the bird eggs of some South Africa species (Polder *et al.*, 2008). For α- β- and γ-HBCDD, respectively these three diastereomers have different physical-chemical properties and do, therefore, display distinct fate and transport characteristics in the environment. The stereochemistry of HBCDD is complex including 16 stereoisomers (Law *et al*., 2006). Half- lives in air, water, sediment and soil respectively range between 1.3 to 85 days (Arnot *et al*., 2009). Solubility of α- β- and γ-HBCDD in water is 48.8, 14.7, and 2.1 µg L^{-1} respectively (Hunziker *et al.,* 2004). HBCDD has currently no technically and commercially feasible alternative for EPS and XPS applications despite intensive research (BSEF, 2009b). Due to its persistence, bioaccumulative and toxicity character, the ban on production and usage of HBCDD came into effect when its amendment listing in Annex A to the Stockholm Convention entered into force on 26 November 2014 (UNEP 2014).

2.2.3.3 Polybrominated biphenyls (PBB)

PBBs are compounds that exhibit chemical and physical properties similar to their chlorinated counterpart, polychlorinated biphenyls (PCBs), except for the presence of bromine atom instead of chlorine. Just like PCBs, PBBs have been found to be persistent and bioaccumulative toxins (PBT) and are classified as possible carcinogens. The three different technical mixtures that were produced included technical hexabromobiphenyl (THBB), technical octabromobiphenyl (TOBB) and technical decabromobiphenyl (TDBB) with bromine contents of 76%, 81% and 85% respectively (Vetter *et al.*, 2008).

PBBs are similar to PBDEs in their use, manufacture, structure, contamination pathways, toxicological impacts and numbering of both PBBs and PBDEs are analogous to PCBs (D'Silva *et al*., 2004). Due to a toxic accident involving hexabromobiphenyl flame retardant in the US in 1973, the product (PBB mixture) was banned in 1974 (D'Silva *et al*., 2004; Herat *et al.*, 2008; Small *et al.*, 2007; Yard *et al*., 2011). A PBB mixture (Fire master) used as flame retardant was erroneously fed to dairy cows and other farm animals instead of a magnesium oxide food supplement (Nutrimaster). Synthesis of PBBs is similar to that of PBDEs and it is via the exhaustive bromination of biphenyl in the presence of a metal halide catalyst such as iron (II) bromide (D'Silva *et al.*, 2004). France ceased the production of deca-BB in 2000 (de Wit *et al*., 2010).

2.2.3.4 Polybrominated diphenyl ethers (PBDEs)

PBDEs are additive flame retardants with very wide application and as a result, are found in a variety of consumer products. They are closely related to PCBs in their chemical and physical properties. However, they exhibit fewer congeners than the latter. They are waxy solids with boiling points that range from 310 to 425^{o}C and log k_{ow} range of 5.74±0.22 to 8.27±0.26. This shows that they are extremely lipophilic, therefore, can accumulate in fatty tissue and oily components of biota (D'Silva *et al.*, 2004).

Theoretically, PBDEs like PCBs each has a total number of 209 possible congeners, divided into 10 homologous groups based on the degree of bromination (Ikonomou, 2002). Contrary to the theory, however, only a handful of these congener mixtures are found in the commercial blends. Three major commercial formulations of PBDEs were produced globally in the last decade; Penta-BDE, Octa-BDE and Deca-BDE. Penta mixture is mainly used for printed circuit boards, cable sheets, furniture and textiles. Octa mixture is used for television sets, computer housings, business machines, household appliances and other small electronics (e.g., remote controls). Deca mixture is used for television sets, computer housings, electronic components, printed circuit boards, furniture and textiles. It is also widely used in transportation, construction and building sector (Leisewitz *et al*., 2001; de Wit *et al.,* 2010). The penta and octa-BDE derivatives were banned by the European Union (EU) in August 2004 (BSEF, 2006). Since then, Penta and Octa-BDE derivatives have been recognised as persistent organic pollutants (POPs) and now included on the list of new POPs under the Stockholm Convention (UNEP, 2010). However, deca-BDE is still being used without any restrictions. Recent trend shows that the production of deca-BDE among brominated flame retardants is second after TBBPA (BSEF, 2011). PBDEs have been reported to be persistent, bio-accumulative and toxic (de Wit *et al*., 2006).

PBDEs are generally produced by addition of bromine to diphenyl ether in the presence of Lewis acid with aluminium bromide or iron catalyst (Teclechiel, 2008). The constituent PBDE congeners vary depending on the formulation. The Penta formulation is composed of penta congeners (50-62%), tetra (24-38%) and hexa congeners (4-12%), the octa formulation consist of hepta (45%), octa (33%), hexa (12%) and nona (10%) while the deca formulation is composed of essentially all BDE 209 (97-99%) with 1-3% nona congeners (USEPA, 2010). Since PBDEs are used as additives in products, they have the potential to be released into the environment thereby settling in dust, water, sediment and biota (de Wit, 2002). Consequently, PBDEs can bioaccumulate in fatty tissues thereby biomagnifying up in the food chain.

Several studies have confirmed the presence of PBDEs in biotic and abiotic environment (Covaci *et al.*, 2008; Jakobsson *et al.*, 2002; Sudaryanto *et al.*, 2008). Table 2.3 shows the summary of PBDE numbers and composition of the most commonly studied PBDEs. PBDEs can undergo photo catalytic degradation from higher congeners to lower molecular weight congeners. Examples of these were presented in studies by Li *et al*. (2010) and Christiansson, (2009), by the debromination of BDE-209 under visible light irradiation in the presence of BiOBr and in different solvents respectively.

Table 2.3: PBDE congeners in the different homologue groups

BDE congener number	**Chemical formula**	**BDE congener number**	**Chemical formula**
I. **MonoBDE**		BDE 118	2,3',4,4',5-BDE
BDE 3	4-BDE	BDE 119	2,3',4,4'6-BDE
II. **DiBDE**		BDE 126	3,3',4,4',5-BDE
BDE 7	2,4-BDE	BDE 138	2,2',3,4,4',5'-BDE
BDE 8	2,4'-BDE	BDE 140	2,2',3,4,4',6-BDE
BDE 11	3,3'-BDE	**VI. HexaBDE**	
BDE 12	2,6-BDE	BDE 153	2,2',4,4',5,5'-BDE
BDE 13	3,4'-BDE	BDE 154	2,2',4,4',5,6'-BDE
BDE 15	4,4'-BDE	BDE 155	2,2',4,4',6,6'-BDE
III. **TriBDE**		BDE 166	2,3,4,4',5,6-BDE
BDE 17	2,2',4-BDE	VII. **HeptaBDE**	
BDE 25	2,3',4-BDE	BDE 181	2,2',3,4,4',5,6-BDE
BDE 28	2,4,4'-BDE	BDE 183	2,2',3,4,4',5',6-BDE
BDE 30	2,4,6-BDE	BDE 190	2,3,3',4,4',5,6-BDE
BDE 32	2,4',6-BDE	VIII. OctaBDE	
BDE 33	2',3,4-BDE	BDE 196	2,2',3,3',4,4',5',6-BDE
BDE 35	3,3',4-BDE	BDE 197	2,2',3,3',4,4',6,6'-BDE
BDE 37	3,4,4'-BDE	BDE 203	2,2',3,4,4',5,5',6-BDE
IV. **TetraBDE**		**IX. NonaBDE**	
BDE 47	2,2',4,4'-BDE	BDE 206	2,2',3,3',4,4',5,5',6-BDE
BDE 49	2,2',4,5'-BDE	BDE 207	2,2',3,3',4,4',5,6,6'-BDE
BDE 66	2,3',4,4'-BDE	BDE 208	2,2',3,3',4,5,5',6,6'-BDE
BDE 71	2,3',4',6-BDE	X. **DecaBDE**	
BDE 75	2,4,4',6-BDE	BDE 209	2,2',3,3',4,4',5,5',6,6'-BDE
BDE 77	3,3',4,4'-BDE		
V. PentaBDE			
BDE 85	2,2',3,4,4'-BDE		
BDE 99	2,2',4,4',5-BDE		
BDE 100	2,2',4,4',6-BDE		
BDE 105	2,3,3',4,4'-BDE		
BDE 116	2,3,4,5,6-BDE		

Source: (USEPA, 2010)

Studies have reported the degradation of BDE 209, and other PBDE congeners in sediment by reductive debromination via anaerobic break down. This is believed to be potential source of more harmful and persistent lower congeners (with longer half-lives) that are of greater concern to human and environmental health (Tokarz I I I, 2008; Yen *et al*., 2009). PBDEs have also been demonstrated to break down by aerobic catabolism using different strains of bacteria. Five selected bromodiphenyl ethers (4-bromo-, 2,4-dibromo-, 4,4′-dibromo-, 2,4,6-tribromo-, and 2,4,4′-tribromodiphenyl ether) was verified in transformation experiments by using resting cells of *Sphingomonas* sp. PH-07. Thus, 4-bromodiphenyl ether was transformed to yield 4-bromophenol and 2-hydroxymuconic acid (Kim *et al.,* 2007).

The bioaccumulation and biomagnification of PBDEs have been described in a number of studies. In a food web comprising Chinese mystery snails, prawn, mud carp, crucian carp and water snake and northern snakehead, the TMF (trophic magnification factor) values for PBDEs ranged from 0.26 to 4.47, with BDE-47 (2.28), BDE-100 (2.64), BDE-154 (2.25) and ∑PBDEs (1.86) having TMF values significantly greater than one ($p < 0.05$) indicating their biomagnification in the food web (Wu *et al*., 2008). Study by Kuo *et al.* (2010) in the food web of Lake Michigan also gave evidence of bioaccumulation and biomagnification in different fish species with the dominance of BDE-47 and BDE-99 (BMFs for BDE -47 and -100 are all larger than one (1.07-2.25 and 1.09-1.52). Further evidence of bioaccumulation includes studies conducted in Pearl River estuary and Deep Bay in south China. Findings are consistent to the bioaccumulation of PBDEs in organisms with values for Pearl River estuary ranging from 6.2 to 208 ng g^{-1} lipid weight and trophic magnification factors (TMFs) for nine PBDE congeners were calculated with values ranging from 0.78 to 3.0. TMFs of BDE-47, -66, -100, -99, -154, and -153 were statistically greater than one, indicating a bio-magnification potential for these congeners (Yu *et al*., 2009), while PBDE concentrations in Deep Bay are lower, compared to that of Pearl River estuary which is adjudged to be more polluted (Qiu *et al.*, 2010).

2.2.4 Novel brominated flame retardants (NBFRs)

These are brominated flame retardants that are used as replacement for the banned BFRs. They have recently been detected in the environment (Covaci *et al.*, 2011). Common members of this group according to Covaci *et al*. (2011) and Papachlimitzou *et al.* (2011) include: DBDPE, BTBPE, TBB or EH-TBB, TBPH or BEH-TBEP, TBBPA-DBPE and HCDBCO. Similar to most BFRs, there is little information available on the current levels of production and usage. Harju (2009) gave two different estimates ranging between 100,000 and 180,000 tonne/year for total global NBFR production. Current state of knowledge concerning NBFRs has been reviewed recently (Covaci *et al.*, 2011; de Wit *et al*., 2010; Harju, 2009; Papachlimitzou *et al.*, 2011)

NBFRs are similar in their behaviour to BFRs and because they are mostly used as additives, they are emitted into the environment from the products in which they are used to retard combustion. Very few studies have reported on their presence in biotic and abiotic environment. Ali *et al.* (2011) reported on the high detection frequencies in indoor dust from homes and offices in Belgium and the UK, suggesting these compounds have become ubiquitous in indoor environments as a consequence of their use as alternatives to PBDEs. For the following compounds, they reported the concentration ranges; decabromodiphenyl ethane (DBDPE) (range; <20-2 470 ng g^{-1}), 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE) (range; <0.5-1 740 ng

g^{-1}), tetrabromobisphenol A-bis(2,3-dibromopropylether) (TBBPA-DBPE) (range; <20-9 960 ng g^{-1}), 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EH-TBB) (range; <2-436 ng g^{-1}) and bis(2-ethylhexyl)-3,4,5,6-tetrabromophthalate (BEH-TBEP) (range; <2-6 175 ng g^{-1}).

2.3 SOURCES OF BROMINATED FLAME RETARDANTS

The release into the environment of brominated flame retardants occurs through a number of ways. Examples include; process of manufacture, incorporation into polymers or related finished products, disposal or recycling, leaching from products in use and disposed of in landfills (WHO, 1997; de Wit et al., 2010). According to Alcock et al. (2003), six points have been identified at which PBDEs could be released to the environment. These include combustion of the waste containing PBDEs; accidental fire of products containing PBDEs; emission from products in use containing PBDEs; blending of PBDEs within polymers; recycling of plastic products containing PBDEs and formation during textile finishing with PBDEs.

Some of the possible indoor sources of BFRs are electronic appliances such as television sets, computers, and fabric products (Hirai et al., 2006; Takigami et al., 2008; Chen et al., 2008). Computer casing, circuit boards and certain laser printers emit brominated flame retardants (He et al., 2007). Research also verified dependence of PBDE emission levels on the environmental conditions; during warm months PBDEs concentration was higher than those recorded in colder months. The same study reported a decrease in PBDE concentrations when old computers were replaced by new ones (Hazrati & Harrad, 2006). Similarly, Cheng et al. (2009) found higher PBDE concentrations in indoor dust collected when computers are working than when they are switched off. These findings indicate that an increase in temperature facilitates the release of volatile PBDEs and that old computers are a major emission source for indoor environments. Shih & Wang (2009) showed that photodegradation is one of the means of conversion of higher PBDEs, for example, degradation of BDE-209 to lower congeners. In addition, leachates from waste disposal, ash and air emission from landfills and incineration of disposed products containing PBDEs have been considered possible sources of emission to the environment. On the other hand, after finding a positive relationship for the sum of PBDE concentrations in dust and blood plasma, it was concluded that inhalation of PBDEs from dust has been identified as a potentially significant source of PBDEs (Lorber, 2007; Karlsson et al., 2007). They also found high levels of PBDEs in an office with many computers and deduced that PBDE emission from computers play a very important role in the pollution of indoor environments.

Furthermore, studies have reported BFRs in the atmosphere, soil and water bodies. Available evidence showed that significant contributions stems from non-point contamination of the environment. This is as a result of emissions from products in use (Kim et al., 2006), illegal unmanaged waste dump, storm water run-off into water bodies (Olukunle et al., 2012a), landfills (Osako et al., 2004; Odusanya et al., 2009), waste recycling plants and the use of sewage on farmlands for agricultural purposes (Cincinelli, 2012), spray irrigation of treated municipal wastewater (Goel et al., 2006). Polybrominated diphenyl ethers (PBDEs) were measured in atmosphere and soil samples taken in winter and summer at a PBDE production area of Laizhou Bay in China. The same report suggested that a large amount of PBDEs emissions into the

environment could also come from automobiles, while research bases are the local sources of PBDEs in the Antarctic (Mandalakis et al., 2008; Hale et al., 2006).

HBCDD is an additive (physically mixed into polymeric products) BFR and is not covalently bonded to the products as some other BFRs. It is, therefore, expected to have higher leaching and emission rates during the product life cycle compared to other BFRs that are chemically integrated (Law, 2006). The materials used in the construction and renovation of urban areas as well as the treated consumer products utilized by the population are probable sources of HBCDD to the urban atmosphere. Watanabe and Sakai (2003) suggested that HBCDD may also be released into the environment through incineration of municipal wastes, hospital or hazardous waste incinerators, facilities recycling plastics and metals from electronic devices, final disposal sites and accidental fires.
Therefore, the primary emission sources of BFRs into the environment can be summarised under three major groups: Release during production, process of incorporation into the polymer and emission from brominated flame retarded products during use, burning, recycling, or disposal.

2.4 ENVIRONMENTAL LEVELS OF BROMINATED FLAME RETARDANTS

2.4.1 Tetrabromobisphenol A

TBBPA is usually used in reactive mode that results in chemical binding of the substance to the material. Therefore, potential emission of TBBPA from products to the environment is likely to be limited in comparison to additive BFR compounds. This was also indicated by 1 to 2 orders of magnitude lower concentration of TBBPA detected in all the surface sediments ranging from 3.8 to 230 ng g-1 dry weight (d.w) compared to PBDEs (Zhang et al., 2009a). The concentrations of TBBPA detected from three sewage plants in Sweden ranged between 3.6 and 45 ng g-1 d.w (Sellstrom,1999) cited in Alaee et al., (2003). Tollback et al. (2006) reported a concentration of 1.2 to 20 ng g-1 d.w and 1.1to 9.4 ng g-1 d.w in bound and free surface sediments respectively and concentration of 470 ng g-1 d.w was reported in house dust from Germany (Abb et al., 2011). Zhang et al. (2009a) reported 3.8 to 230 ng g-1 d.w in sediment from the south of China and the highest concentration of 9 800 µg kg-1 d.w in sediment was reported by Morris et al. (2004) as cited in (Law, 2006). Johnson-Restrepo et al. (2008) reported a concentration of 0.048±0.102 ng g-1 lipid wt in human adipose tissue samples, 1.2±3 ng g-1 lipid wt in bottlenose dolphin blubber, 9.5±12 ng g-1 lipid wt in bull shark muscle and 0.872±0.5 ng g-1 lipid wt in Atlantic sharp nose shark muscle respectively. In addition Jakobsson et al. (2002) reported a range of 1 to 3.4 pmol g-1 lipid wt. in serum of computer technicians.

TBBPA was also found at concentrations ranging from 0.64 to 1.8 ng g-1 of lipid in the blood plasma samples from individuals who dismantle electronic components in Norway (Thomsen et al., 2001); in blood serum of four workers from an electronic dismantling plant in Sweden (Hagmar et al., 2000); in blood, ranging from 2.4 to 12.0 µg kg-1 lipid wt of individuals from Japan (Nagayama et al., 2001); in a sample of human hair taken from an individual living near TBBPA manufacturing facilities in Arkansas, USA (Decarlo, 1979). TBBPA was found in the interior of television sets (Tamade et al., 2002), sediment (Hakk & Letcher, 2003), and from sewage sludge (Morris et al., 2004). TBBPA was detected in 44% of the analysed breast

milk samples ranging from 0.06 to 37.34 ng g-1 lipid wt and also in 30% of serum samples ranging from 0.97 to 3.34 g ℓ-1 and 4.65 to 10.12 g ℓ-1 in cord and maternal serum respectively (Cariou et al., 2008). It was also detected in human tissue, dolphins and sharks in the USA (Johnson-Restrepo et al., 2008). It was also found in various industrial soil samples at a range of 3.4 to 32.2 ng g-1 and 0.3 ng g-1 in agricultural soil (Sanchez-Brunete et al., 2009). Generally, due to the rising of global market demand for TBBPA, its concentration levels in the environment is expected to increase in the future. This may heighten concern regarding the environmental fate and human exposure to TBBPA (Covaci et al., 2009). Furthermore, regardless of its major usage in the form of reactive flame retardant, TBBPA is widely distributed in the environment, human, animals and marine species.

2.4.2 Hexabromocyclododecane

HBCDD has been detected globally in different environmental compartments comprising air, sewage sludge, sediment and soils and biota. The result of HBCDD concentrations monitored in marine and river sediment in Japan gave values ranging from 0.02 to 0.09 mg kg-1 dry weight from three out of 69 sediment samples (OSPAR, 2004), river sediment in Sweden down-stream from a possible point source of HBCDD was given as 0.04-0.37 mg kg-1 and in pike fish muscle (0.02-0.06 mg kg-1 wet weight) by Sellstrom et al. (1998) cited in (OSPAR, 2004). In Germany, a concentration of 30-15 000 ng g-1 d.w was reported in household dust (Abb et al., 2011), 0.33-0.57 ng g-1 lipid wt in human adipose tissue samples, 7.38-18 ng g-1 lipid wt in bottlenose dolphin blubber, 77.7-128 ng g-1 lipid wt in bull shark muscle and 54.5-88 ng g-1 lipid wt in Atlantic sharp nose shark muscle (Johnson-Restrepo et al., 2008). The dominance of different di-astereoisomers of HBCDD was also, reported. In most sediment samples analysed, similar to the commercial formulations, γ-HBCDD isomer was found dominant (>90%); however, α-HBCDD isomer was more dominant in biota (>85%) while smaller amount of β-HBCDD was detected in sediment and biota. On the other hand, all were detected in sewage sludge in equal ratios (Morris et al., 2004). Enantioselective bioaccumulation of HBCDDs with an increase α-HBCDD and decrease γ- HBCDD have also been reported in marine species when passing through lower to upper trophic levels of the food web (Tomy et al., 2008; Wu et al., 2010).

Because of their high position in the food chain and the elevated exposure in the aquatic environment, fish often exhibit high residues of contaminants. Thus, HBCDD have been detected in many studies both in freshwater and marine biota (Budakowski & Tomy, 2003; Eljarrat & Barcela, 2004; Remberger et al., 2004; Janak et al., 2005). Some studies have investigated the occurrence of HBCDDs in birds (de Boer et al., 2004; Leonards et al., 2004; Morris et al., 2004; Lundstedt-Enkel et al., 2005; Verreault et al., 2005). HBCDD have also been detected in eggs of four bird species from South Africa, with the lowest concentration corresponding to 1.6 ng g-1 lipid wt in crowned plover bird eggs and the highest concentration in the eggs of African sacred ibis (71 ng g-1 lipid wt) (Polder et al., 2008). HBCDDs have also been detected in many components of fresh water aquatic species such as water snake, northern snakehead, crucian carp, mud carp, prawn and Chinese master snail with the average concentration ranging from 13.9-868 ng g-1 lipid wt (Wu et al., 2010) and in human breast milk (Thomsen et al., 2003; Kakimoto et al., 2007; Eljarrat et al., 2008).

Recently, 1-2 order of magnitude higher concentrations of HBCDDs in human breast milk was reported from Spain compared with that detected in other countries (Sweden, Norway, Mexico, Canada, USA, Japan, Russia or France). The reason for the higher concentration was suggested to be the increasing usage of HBCDDs in Spain (Eljarrat et al., 2009). Food-chain studies have shown that HBCDDs are bioaccumulative and can be transferred from sediment via invertebrates and predatory fish to fish-eating top predators, such as birds and seals (Leonards et al., 2004; Morris et al., 2004). The concentration of HBCDDs found was 14 ng g-1 d.w, in sediments, 186, 377 and 1 791 ng g-1 lipid wt in winkle, crucian carp and loach, respectively (Zhang et al., 2009b). A study conducted in Hong Kong, China observed a significant increasing trend of ΣHBCDDs was observed in dolphin samples from 1997 to 2007 whereas no significant temporal trends of ΣPBDEs appeared over the period (Lam et al., 2009). Furthermore, the reason for the increasing pattern in concentration was suggested to be the increasing usage of HBCDDs following the restriction or voluntary withdrawal of the production of PBDEs in several countries.

In addition, the bioaccumulative characteristics of HBCDDs have been studied through collection of sediments, winkle (Littorina littorea), crucian carp (Carassius carassius) and loach (Misgurnus anguillicaudatus) from two streams near an e-waste dismantling site in China, and HBCDD exposure test was then conducted on Chinese rare minnow. Generally, HBCDDs are widely distributed in the environment as a result of continuous production and usage without any restriction (Lam et al., 2009).

2.4.3 Polybromobiphenyls

Research findings show that PBBs exist in many different types of environmental samples. Recently, medium concentration of PBBs less than 182 ng g-1and 21.8 ng g-1 were detected in 94.9% and 33.3% of the house dust samples from e-waste recycling and urban areas of south China, respectively. In both areas, BB-153 was the dominant congener (73.6% on average), suggesting its persistence and continuous release from old products (Hale., 2010). Their presence have been observed in both soil and in the scalp hair of Chinese populations residing around electronic waste disassembly sites (Zhao et al., 2009); in indoor air at electronics recycling plant and other work environments (Sjodin et al., 2001).

Also BB-153 was found in various water bird species collected especially from e-waste recycling regions of south China. It was a major congener detected in 93% of the samples with concentrations ranging from 1 to 2 800 ng g-1. The levels of BB-153 were similar to those of the major PBDE congeners found in the samples, with the exception of the Chinese pond heron in which BB-153 was significantly less abundant than PBDE congeners (Luo et al., 2009). In another paper, the high concentration of BB-153 was also reported, among nineteen congeners of PBBs found in adipose tissue of women from Spain with BB-153 contributing 79% of all PBBs (Fernandez et al., 2009). The highest concentration of BB-209 (2 300 ng g-1) was also, detected in dust samples collected from an electronics waste room where discarded personal computers and printers are stored in Thailand (Muenhor et al., 2010).

Anaerobic conversion process of higher PBBs to the lower congeners was studied by incubating TOBB with super reduced cyanocobalamin. It was observed that after one day of incubation, the main components of TOBB were no longer detected. However, several penta and heptaBBs were detected, which confirms the predominant conversion process of higher to lower congeners to be reductive debromination (Von der Recke & Vetter, 2008). In summary, irrespective of the point source, PBBs are found everywhere in the environment.

2.4.4 Polybromodiphenyl ethers

PBDEs have been measured in various environmental compartments including air, water, dust, sewage sludge, soil and sediment and biota. A Swedish study conducted in 1999 sparked intense interest in the issue of PBDEs worldwide. The study showed that between 1972 and 1999, the concentration of PBDEs in the breast milk of mothers in Sweden increased exponentially, with concentrations of PBDEs doubling every five years. Similarly, concentration in marine mammals from Japan significantly increased during the last several decades and was 1-2 orders of magnitude higher in recent years than in the 1970s and 1980s (Tanabe, 2008). In addition, PBDE concentrations reported for the U.S. population were between 60-70 ng g-1 lipid wt whereas in Europe they were somewhere between 2.5 and 3.5 ng g-1 lipid wt. In another survey conducted on volunteers in Minnesota, 34 out of 35 individuals had BDE-209 detected in their blood serum, although the concentrations were too low to quantify in all but five of the volunteers with a range of 25-50 ng mℓ-1 (MPCA, 2008). Observed levels in sewage sludge from Switzerland ranged from 138-617 µg kg-1 dry matter for decaBDE (Kupper et al., 2008), In another study a range of 25-152 ng g-1 was detected in household dust from northwest of Spain (Regueiro, 2006). Isobe et al. (2010) reported 0.9-110 (BDE-209) and 0.10-18 ng g-1 d.w ∑PBDEs from Manila bay, Philippines.

Jin et al. (2011) detected concentration levels of 0.017-1.17 ng m-3 for ∑11PBDE in gaseous phase, 0.5-161.1 ng m-3 in particulate phase of air samples and 73-2 629 ng g-1 d.w in soil samples, however, the PBDE congener pattern in the gaseous phase differed from that in the particulate phase and the PBDE congener pattern in the particulate phase was similar with that found in soil. The presence of PBDEs in river sediments has been reported by many researchers (Chen et al., 2009; Labadie et al., 2010; Li et al., 2010; Qiu et al., 2010). A congener preference has also been observed. For example, BDE-209 increases in soils, sediments and sludges (de Wit et al., 2006; Law et al., 2006), while Penta-BDE congeners tend to dominate in the atmosphere and aqueous media (Hale et al., 2003). High concentrations of PBDEs were found in landfill leachates collected from landfill sites of South Africa (Odusanya et al., 2009). PBDEs were found in sediment samples at concentrations ranging from 22 to 136 ng g-1 d.w in the Llobregat River basin of Spain (Guerra et al., 2010); in soils amended with sewage sludge having BDE-209 as the predominant congener in all soil samples analysed, in addition, high levels of PBDE including BDE-209 were found in soils four years after the last sludge application; this indicates the persistence of PBDEs in soils (Eljarrat et al., 2008).

PBDEs were found in serum samples collected from three populations in Tianjin, China, including office cleaners, university students, and policemen. Findings showed that total PBDEs levels in office cleaners were significantly higher than in university students and policemen. This trend was related to higher

frequency of exposure of cleaners to office dust and the level of BDE-47 in serum (<30%) was found to be lower than in human milk, human blood and adipose tissue in North America and in some European countries (33-51%) (Zhu et al., 2009). PBDEs detected in wildlife tissues of a captive giant panda and a red panda from China ranging from 16.4 to 2 158 ng g-1 lipid wt were reported (Hu et al., 2008).

PBDEs have also been found in adipose tissue, blood, brain and liver of polar bears from East Greenland with the ∑PBDEs concentration in the order; adipose tissue > liver > blood > brain. Furthermore, localization preference of congener, for example, BDE-47 in brain, BDE-99 in liver and BDE-153 in adipose tissue were also reported (Gibbink et al., 2008). Similarly, 14 congeners of PBDEs were found in adipose tissue of women living in south eastern Spain with 96% contribution for all ∑PBDEs from BDE-153, BDE-47, BDE-183, BDE-99 and BDE-100 (Fernandez et al., 2007). A study conducted on juvenile carp whose food was spiked with PBDEs containing BDE-47, BDE-99, BDE-100, BDE-153, BDE-154 and BDE-183 found relatively high uptake of BDE-47 within whole body tissues and congener preference of liver also observed by uptake of elevated BDE-154 (Stapleton et al., 2002). A rationale behind the preference was suggested to be a kinetic limitation in the equilibrium partitioning of these congeners among fish tissues.

Birds are very important species for monitoring the levels and effects of persistent halogenated compounds in the environment because they are widespread, sensitive to environmental changes, and occupy the top position in the food chain (Sakellarides et al., 2006; Voorspoels et al., 2006). Consequently, many research findings have demonstrated the presence of PBDEs in bird eggs and tissues. For example, in eggs of water birds from the coastal area of south China and in tissues of birds of prey from north China (Chen et al., 2008; Lam et al., 2007); in bird eggs of South Africa (Polder et al., 2008) and in wild bird eggs from the Yellow River Delta, north China ranging from 4.6 to 146 ng g-1 lipid wt (Gao et al., 2009). In addition, comparable concentrations of PBDEs were found in eggs of peregrine falcons (Falco peregrinus) in northern USA and in North American seabird eggs. However, these North American concentrations were higher than levels observed in European peregrine eggs (Chen et al., 2008).

Generally, it is pertinent to note that PBDEs are most widely distributed in air, dust, sediment, water, fish, birds, marine mammals, and people, and in many cases, with variable concentrations and different congener types. Studies on BFRs carried out so far in the South African environment have shown varying degrees of contamination in a number of matrices (Darnerud et al. 2011; Daso et al. 2012; Kefeni & Okonkwo, 2012; Polder et al. 2008). In order to establish the concentration pattern of PBDEs in South African environmental, particularly within the industrial hub of the country, Gauteng Province, there was a need to embark on this project. This is in line with the Water Research Commission together with the Department of Water and Sanitation initiative to establish data base of common organic contaminants, particularly those that are emerging and endocrine disrupting. Data on these emerging environmental contaminants is extremely important as South Africa is geared towards the national implementation of the National Toxicology Monitoring Programme (NTMP).

CHAPTER 3: MATERIALS AND METHODS

3.1 INTRODUCTION

This chapter describes the study area with respect to the metro, district and local municipalities, land cover map and the identified sampling sites according to river catchments. In addition, the materials, apparatus and methods developed for the determination of selected polybromodiphenyl ethers and hexabromobiphenyl in sediment samples are described.

3.2 DESCRIPTION OF STUDY AREA

This project focused on water pollution hotspots (with respect to BFRs) in various parts of Gauteng Province in order to present an overview of the level of contamination by BFRs in the province. Therefore, a brief geographical description of the province is given as well as the map which is shown in Figure 3.1. Gauteng Province is situated in the Highveld, with high altitude grassland (circa 1,500 m/4,921 ft above sea-level). It is the smallest province in South Africa, with only 1.4% of the land area. However, it is highly urbanised and comprises the cities of Johannesburg and Pretoria. Gauteng is bordered by the Vaal River, North West, Limpopo and Mpumalanga to the south, west, north and east respectively. Gauteng is the only landlocked province of South Africa without a foreign border.

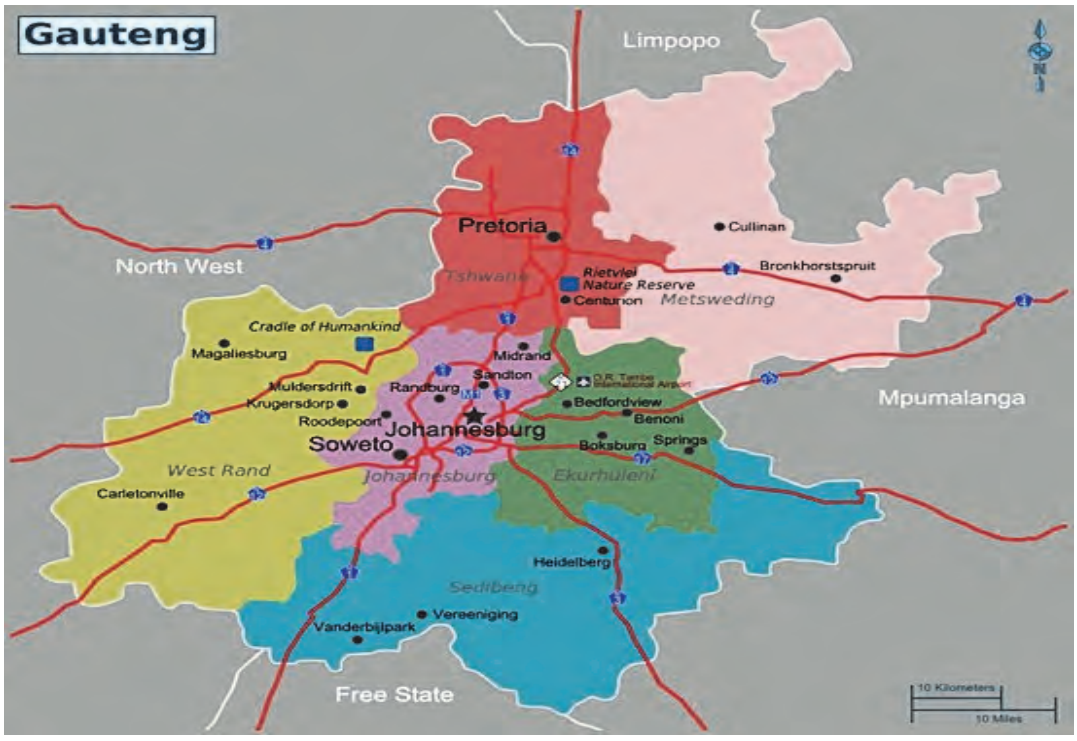


Figure 3.1: Map of Gauteng province showing the metropolitan, district and local municipalities (Source: Online)

Between Johannesburg and Pretoria, there are low parallel ridges and undulating hills, some part of the Magaliesberg Mountains and the Witwatersrand. The north of the province is more subtropical, due to its lower altitude and is mostly dry savannah habitat. The climate is mostly subtropical but cooler, especially in Johannesburg, at 1,700 m (5,577 ft) above sea level (Pretoria is at 1,330 m/4,364 ft). Most precipitation occurs as brief afternoon thunderstorms. However, relative humidity is moderate. Winters are crisp and dry with frost occurring often in the southern areas. Snow is rare, but it occurred in 2012 even in Pretoria. Gauteng Province is divided into three metropolitan municipalities and two district municipalities which are further divided into seven local municipalities. The three metropolitan municipalities include: the cities of Ekurhuleni, Johannesburg and Tshwane. Sedibeng and West Rand make up the two district municipalities and the seven local municipalities consists of Emfuleni, Midvaal, Lesedi all in Sedibeng district municipalities; and Mogale city, Randfontein, Westonaria and Merafong city all in the West Rand local municipality.

3.2.1 Gauteng land cover map

In order to get a clear picture of the water bodies that should be considered for sampling in this study, land cover map of the province is shown in Figure 3.2. The map of the study area was developed by making use of the internet as well as information available from the National Toxicology Monitoring Programme (NTMP). Map of suspected hotspots are shown in Figure 3.3.

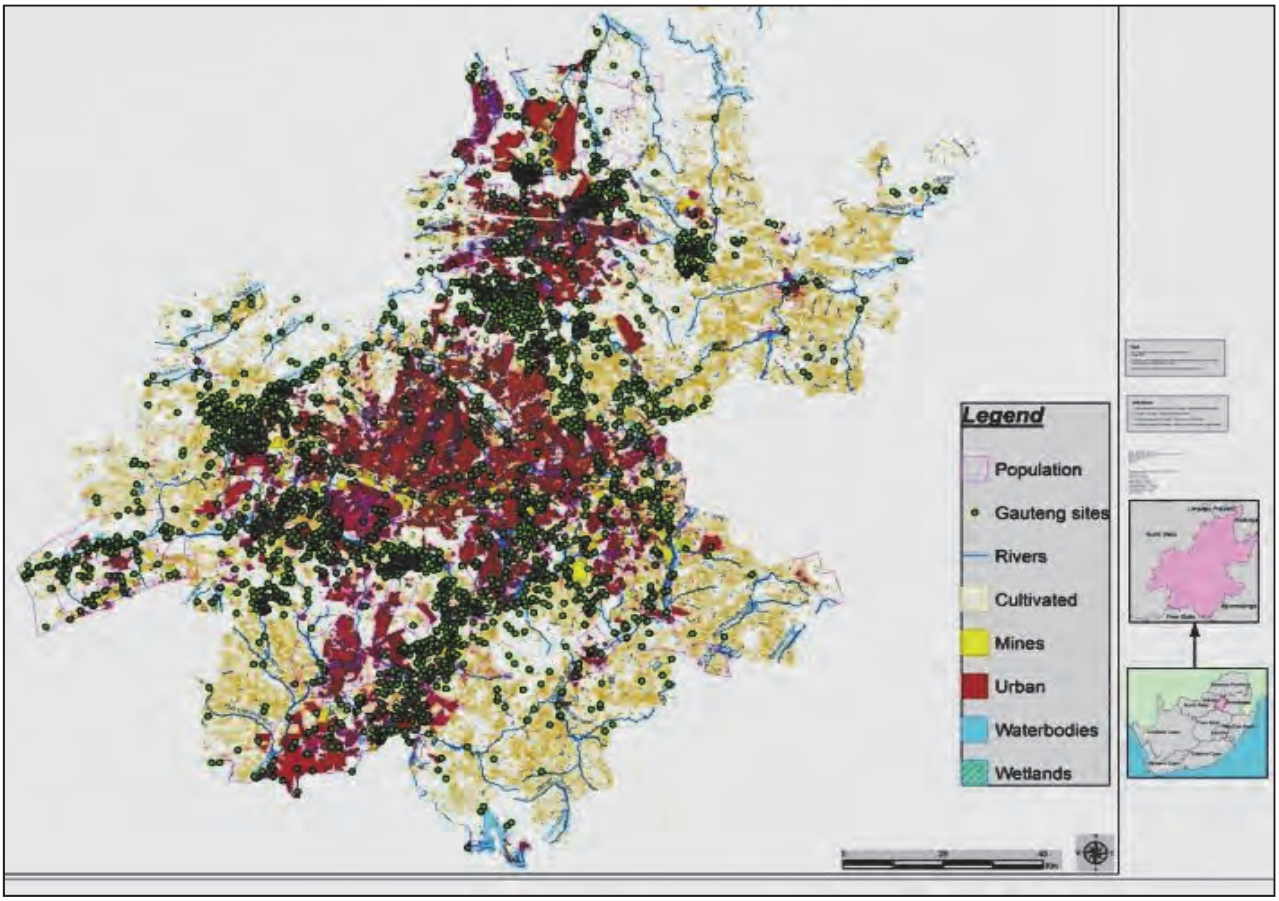


Figure 3.2: Land cover map of Gauteng and existing monitoring points (hot spots) on Water Monitoring Stations (WMS) (source: NTMP)

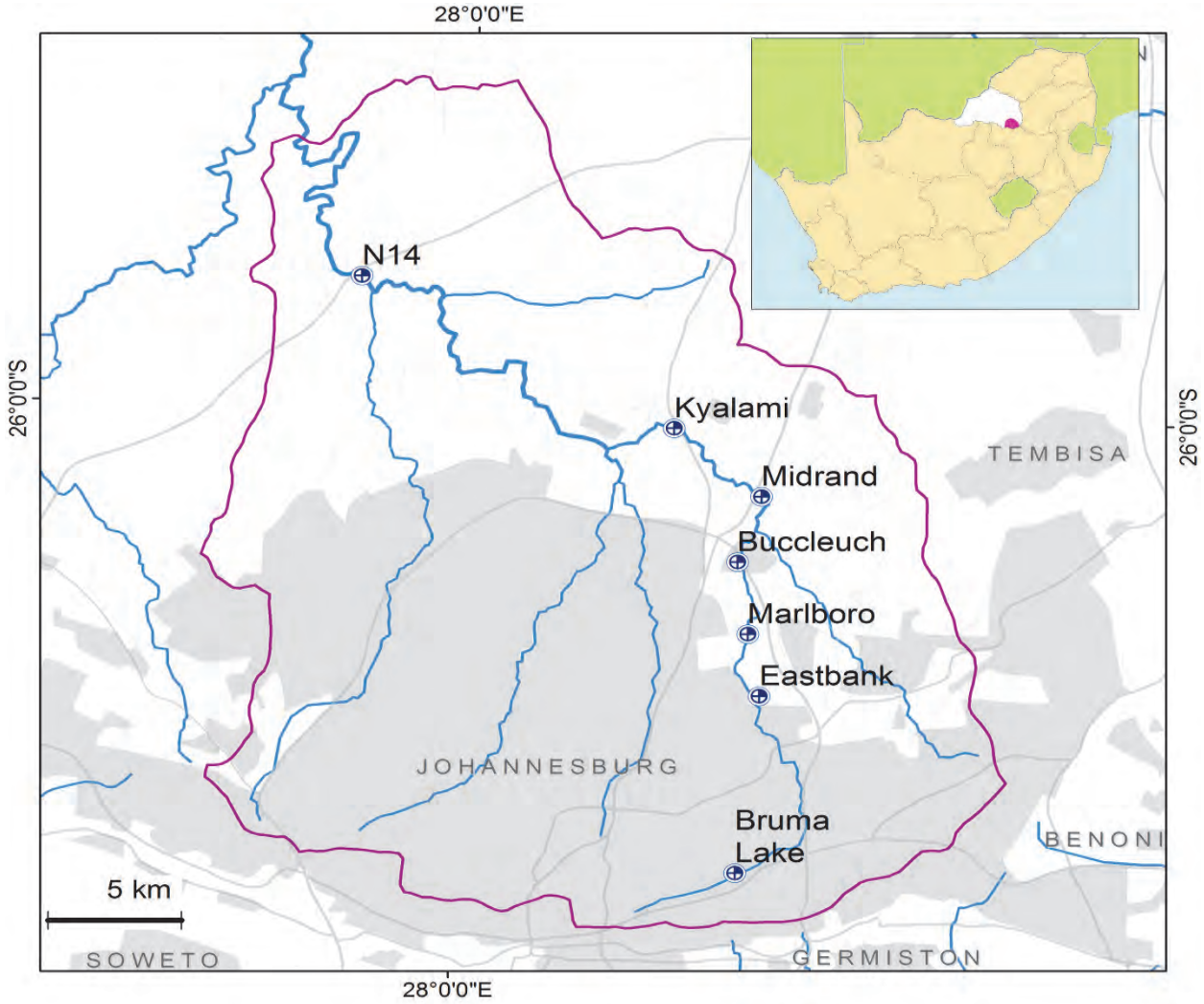


Figure 3.3: Identified sampling points for surface water, boreholes and wetlands in A21C Quaternary catchment on WMS (Jukskei River) (source: NTMP)

3.3 IDENTIFICATION OF SAMPLING SITES

3.3.1 Surface and groundwater

The details of the identified sites for surface water and borehole samples are shown in Tables 3.1 and 3.2. The identified catchments are surrounded by industrial, sewage treatment plants and agricultural activities in most cases. These activities present sources of pollution into the water system. With the help of a statistician at Tshwane University of Technology, sampling was carried out in summer and winter periods in order to ascertain seasonal variation. Samples (water and sediments) were collected for six months (January-March and June-August) per year and between 4 to 12 sampling points/sampling sites were identified in the identified catchments depending on the accessibility. Groundwater samples were collected within the neighbourhood of the rivers, where possible.

Table 3.1: Names and information on suspected polluted surface water in Gauteng obtained from the NTMP

Names/information on suspected polluted surface water (hotspot) Uppervaal water management						
SITE NAME / RIVERS	**MIDa**	**CIDb**	**GIS**	**WATER**	**SEDIMENT**	**REMARK**
			Jukskei River			
*Marlboro (Eastgate)	188571	A21C	S26.084917, E28.108806	1	1	
*Midrand (Waterval)	90186	A21C	S26.031389, E28.112194	1	1	
*N14 (Knoppieslaagte)	188572	A21C	S25.949333, E27.958778	1	1	
*Kyalami	n/a	A21C	S26.0056, E28.07836	1	1	
*Buccleuch	n/a	A21C	S26.0570, E28.1040	1	1	
*Eastbank	n/a	A21C	S26.1090, E28.1140	1	1	
*Bruma Lake	n/a	A21C	S26.178, E28.107	1	1	
			UpperKlip Catchment			
Russel Stream	90728	C22A	S26.39293, E27.99076	Nil	Nil	Dried up
*Klipspruit (Clarington)	100000517	C22A	S26.30167, E27.90083	1	1	
Tributary to KlipRiver	100000518	C22A	S26.31833, E27.85722			
			RietKlip Catchment			
*Wadeville (Rietklip)	100000502	C22B	S26 .277, E28.202	1	Nil	
Elsburgspruit on R554 U/S of confluence with Natalspruit	100000528	C22B	S26.28833, E28.16833	Nil	Nil	Dried
Klip River on Kareekloof RD D/S Waterval STW	100000520	C22D	S26.35194, E28.08694	Nil	Nil	Dried
			LowerKlip Catchment			
*Kookfontein 545 IQ Meyerton WWTW Final Effluent Discharging into Fouriespruit	100000522	C22E	S26.36985, E27 .99904	1	1	

Table 3.1 (continued)

Blesbokspruit/Suikerbosrand Catchment						
Blesbokspruit @ Welgedacht	177831	C21E	S26.19861, E28.47972	Nil	Nil	Not accessible
R555 Road Bridge on Blesbokspruit	90698	C21E	S26.2133, E28.48143	Nil	Nil	Not accessible
Wonderfontein Catchment						
Goed 402 IQ at Randfontein Azaadville Bridge on Wonderfonteinspruit (C2H153Q01)	90701	C23D	S26.33854, E27.74826	Nil	Nil	Dried
Gemspost 288 IQ on Wonderfonteinspruit (C2H060Q01)	90631	C23D	S26.2888, E27.6692	Nil	Nil	Dried
Wonderfonteinspruit @ end 1m pipeline from Venterpost gold mine	90663	C23E	S26.3264, E27.4106	Nil	Nil	Dried
Mooirivierloop (River) at Blaauwbank (C2H069Q01)	90652	C23E	S26.3756, E27.2308	Nil	Nil	Dried
*****Turffontein 126 IQ @ Gravel RD bridge to Muiskraal on Mooirivierloop (C2H161Q01**)**	90704	C23G	S26.35861, E27.43329	1	Nil	Concrete channel, no sediment
Carletonville						
Grootdraai Catchment						
*Witpuntspruit @R29/N2 camden bridge (gddc09)	177947	C11B	S26.59277, E30.09687	1	1	
Douglas dam flow at N11 bridge	177940	C11F	S26.4644, E29.9556	1	1	

Table 3.1 cont'd

Brummerspruit at N17 d/s Ermelo S/W	100001042	C11F	S26.5128, E29.9081		
Gddc06 spruit at old Spitzkop	177943	C11F	S26.9081, E29.9733		
*Gddc21 Amersfoort - Final effluent at Amersfoort WWTW	177963	C11E	S27.15062, E29.88609	1	1
Blesbokspruit d/s of Bethal sewage works at pieksdal (GDDC16)	177958	C11H	S26.68174, E29.40723		
		Vaal River and Vaal Barrage Catchment			
*****Lts 24 Vaal barrage on Vaal River near barrage wall	90780	C22K	S26.93911, E27.66695	1	1
Lts 13 Leeuspruit at R59 bridge	100000949	C22K	S26.56505, E28.10078		
Lts **21** Taaiboschspruit downstream of webbs dam at rail line	100001005	C22K	S26.54534, E27.50276	1	1
		MARICO/CROCODILE CATCHMENT			
Alberton			S26.264, E28.1242	1	1

[a]**Monitoring ID** [b]**Catchment ID**

Table 3.2: List and location of groundwater (boreholes) sampling points

LOCATION NAME	POINT ID	LATITUDE	LONGITUDE
*WONDERBOOM PARK	-	-25.7.6708	281241.3
*EASTLING	-	-25.71016	28.211397
*DOORNRANDJIE	-	-26.33073	27.97099
2628AA01100 JOHANNESBURG CNR ESSELEN AND DQUARTE HILLBROW	184149	-26.1917	28.0500
MAYFAIR (DUPL NAME 2)	148284	-26.1667	28.0000
PARKHURST JOHANNESBURG	176352	-26.1383	28.0197
BRYANSTON – BN1	1000013836	-26.0500	28.0278
2628AA01101 JOHANNESBURG IDLEWILD BUILDING	184169	-26.0250	28.0500
ZQMMRN1 MIDRAND – SMUTSSTRAAT 14	90118	-25.9925	28.1175
DIEPSLOOT (DUPL NAME 2)	170069	-25.9333	28.0472
DIEPSLOOT (DUPL NAME 3)	170070	-25.9306	28.0000
DIEPSLOOT (DUPL NAME 4)	170071	-25.9194	28.0028
2527DD00069 DOORNRANDJIE – GP00247	1000015986	-25.8965	27.9964
LEITRIM (DUP NAME 19507)	160113	-26.8667	27.8667
LEITRIM (DUP NAME 5171)	96864	-26.8558	27.8667
SASOLBURG	96867	-26.8183	27.8211
RIETFONTEIN (DUP NAME 4997)	96575	-26.8006	27.7272
VEREST STASIE	148185	-26.7833	27.9167
FRAAIUITZICHT	96574	-26.7792	27.7050
BERSHEBA	96866	-26.7767	27.7769
DRIEFONTEIN (DUP NAME 5063)	96654	-26.7431	27.7653
ZUURFONTEIN (DUP NAME 5061)	96652	-26.7150	27.7856

*Groundwater sampling sites.

Figures 3.4 and 3.5 shows those rivers suspected to be polluted (obtained from NTMP) and sampling spots in the Vaal River catchment respectively.

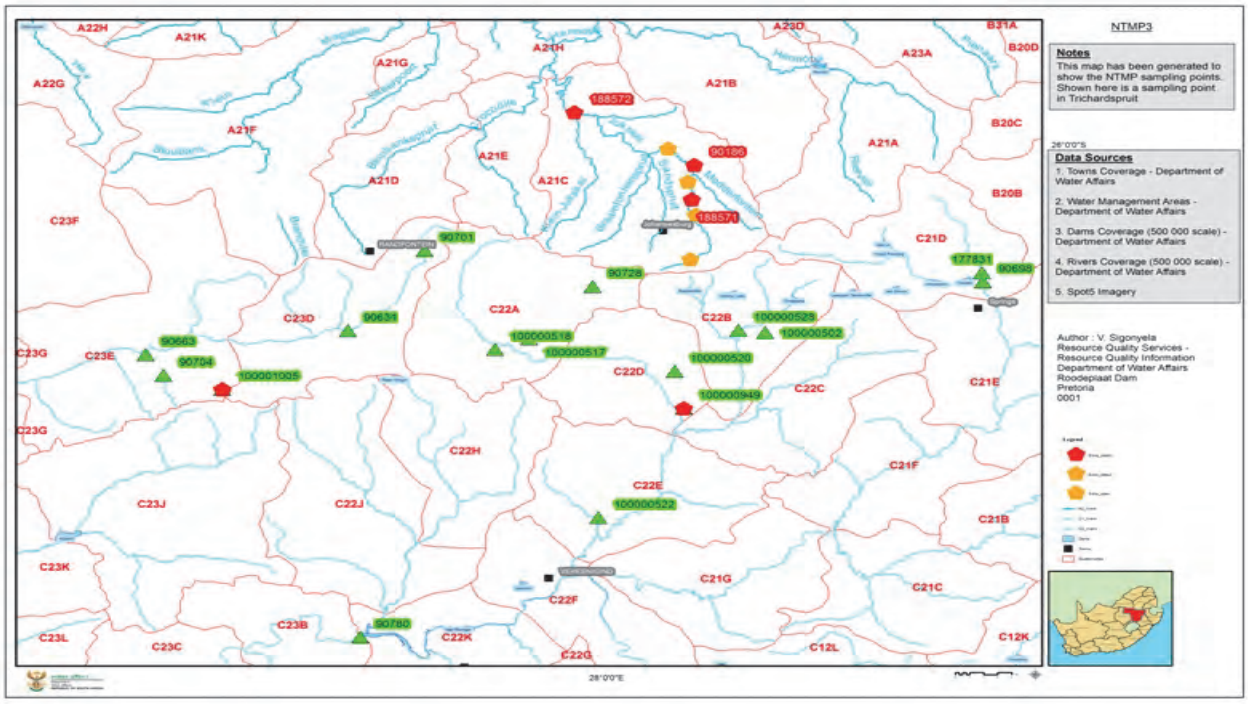


Figure 3.4: Suspected polluted surface water (hotspots) in Gauteng obtained from the NTMP

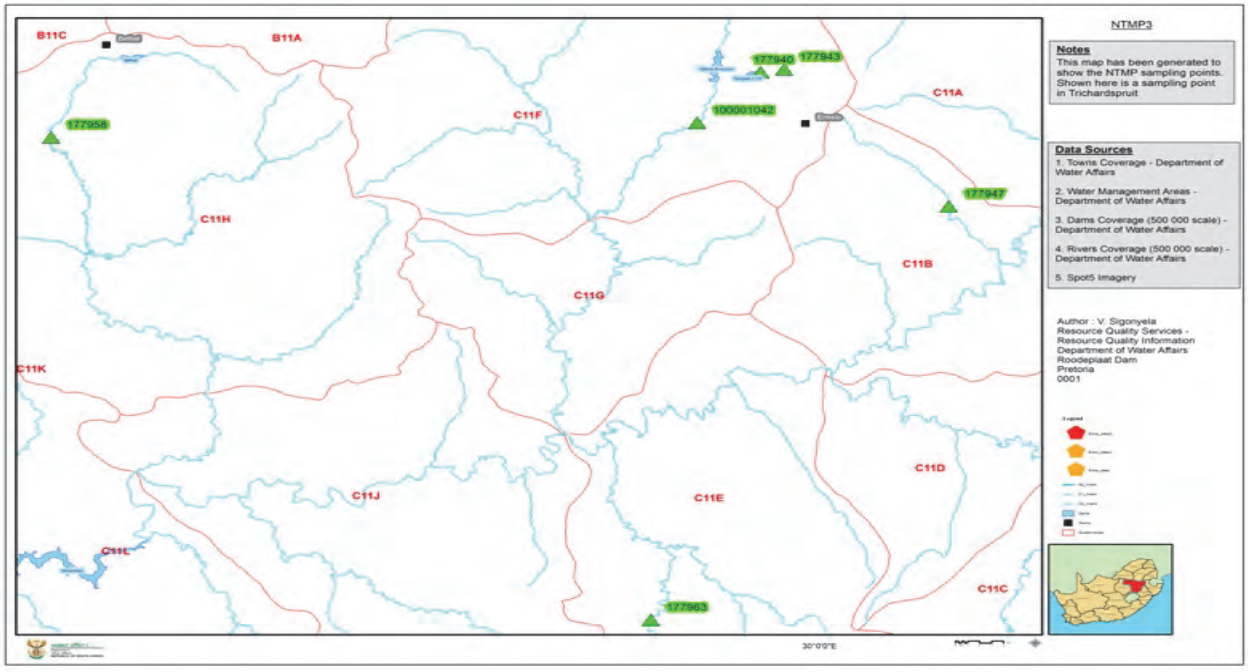


Figure 3.5: Selected sampling points in the Vaal River Catchment and Vaal Barrage Catchment (source: NTMP)

As mentioned earlier, samples could not be obtained from some rivers and wetlands because they were dry. Figure 3.6 shows sampling sites where samples were collected. It is also worth mentioning that a number of the boreholes identified in Table 3.2 could not be accessed because they are within private properties.

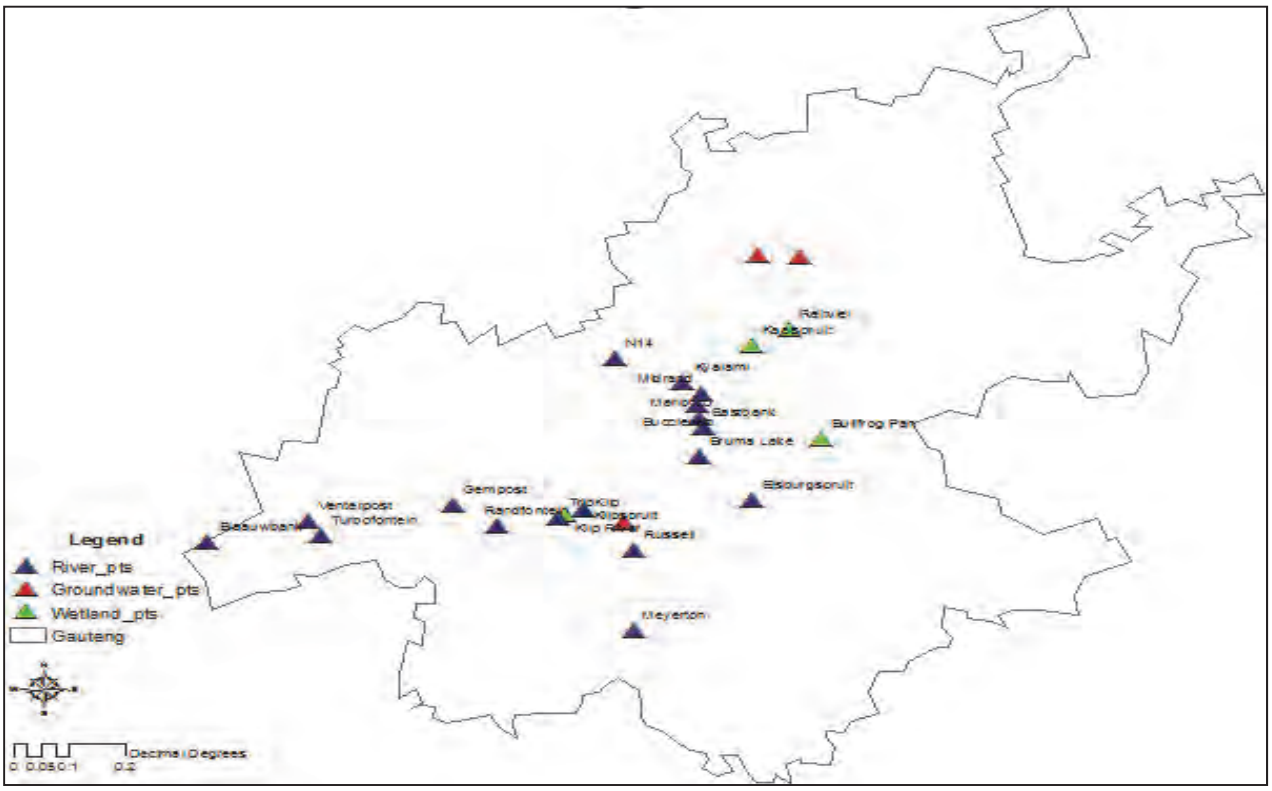


Figure 3.6: Map of Gauteng showing sample collection sites accessed

3.3.2 Landfill sites

With respect to landfill sites, the identified landfill sites included those shown in Table 3.3. The following criteria were used to consider the selection of landfill sites:

- Type and volume of waste;
- Possible leachate generation;
- Possible polyethylene geomembrane Liner/no liner and
- Possible accessibility to landfill site

In Table 3.3, leachate samples were only collected from six landfill sites (in bold) during sampling. Furthermore, full general information about the landfill sites was collected for only nine landfill sites and scanty information for three landfill sites. The GPS coordinates of some of the sampling sites are shown in Table 3.4.

Table 3.3: General information on identified landfill sites in Gauteng Province

Name	Age (yrs)	Avg. ton/mth	Size (Ha)	% monthly waste classification in tonnage			
				Building	Garden	Household	Industrial
+Kwaggasrand	40	36,980	27.2	10	15	70	5
*Valhalla	30	32,766	11.7	5	15	75	5
***Garstkloof**	25	45,090	43.6	30	60	5	5
***Onderstepoort**	8	38,033	51.82	10	20	50	20
***Hatherley**	7	18,637	96	10	10	75	5
***Soshanguve**	10	12,200	19.5	5	10	80	5
#**Chloorkop**	17	500	20				
Robinson deep	14						
Derdepoort	8	24,545	12.4	20	70	5	5
*Garankuwa	10	15,637	20	18	30	42	10
**Temba*	10	9,363	3.7	10	10	70	10
+*Boitshepi*	40	22,000		5	5	70	20

*Tshwane, +Johannesburg (Vereeniging-Vanderbijlpark); #Ekurhuleni; αWest Rand (Merafong)

Table 3.4: GPS coordinates of the landfill sites sampled

Site name	GPS co ordinates
Onderstepoort	S 25° 39' 2", E 28° 11' 3"
Garstkloof	S 25° 49' 54.12", E 28° 16' 7.68"
Hatherly	S 26° 0' 28.8", E 27° 51' 52.56"
Soshanguve	S 25°31′37″, E 28°6′32″
Robinson Deep	S 26° 13' 59.03", E28° 02' 14.77"
Chloorkop (Spring)	S 26° 02' 30.35", E28° 10' 04.58"

3.3.3 Wetlands in Gauteng Province

Table 3.5 shows the list of wetlands in Gauteng province that were identified to be sampled for the presence of BFRs. Of the seven wetlands, samples were collected from five wetlands (in bold). The remaining four wetlands were found to be dry during sampling. Table 3.6 shows the type of samples collected and the method of collection.

Table 3.5: List of wetlands in Gauteng Province identified for the current study

WETLAND	AREA	RIVERS & STREAMS
Blesbokspruit	Near Springs in Gauteng Province and it is surrounded by Boksburg, Benoni and Brakpan in the northwest, while Nigel is located south of the site.	Blesbokspruit
Bullfrog Pan	Ekurhuleni Municipality	
Karlspruit	Ekurhuleni Municipality	
Klip River	City of Johannesburg	Klip River
Rietvlei	City of Tshwane	
Soshanguve	City of Tshwane	
Wonderfonteinspruit	Originates between the towns of Randfontein and Krugersdorp	

Table 3.6: List of sample type, sources and method of sampling

Sample type	Sample sources	Method of sampling
Water	River, groundwater (boreholes) and wetland	Dip sampling method: Grab water sample was collected just below the water surface with a narrow-mouthed dark glass bottle. For boreholes and monitoring wells, pump sampler or bailer or other point sampler was used.
Sediment	River, groundwater (boreholes), landfills and wetland	Grab sediment sample were taken by dragging the wide open mouth glass bottle at the bottom of the water body same spot where water sample was taken.
Leachate	Landfills i.e., leachate pond	Same as surface water sampling.
Biota	Tissue from fish	For fish, gill net was used to capture the required sample size. Thereafter, it was transported alive to the lab for further processing

3.4 SAMPLING STRATEGY

A statistician at TUT was consulted on sample quantity and frequency and the following was suggested:

- That samples should be collected in winter and summer periods in order to look at seasonal variation;
- That sampling points should constitute samples from various distances down of a water system to have a representative sample;
- That water and sediment samples should be collected from the same spot and day;
- That grab sampling method should be applied and
- Basic physiochemical water parameters which may influence the results should be determined as well.
- Three samples to be collected at each sampling point for water and sediment respectively to make them representative.

3.5 SAMPLE COLLECTION

Water and sediment samples were collected from different catchments as shown in Table 3.7. Summer and winter samples were collected from Jukskei, Lowerklip and Vaal catchments as well as other rivers. For groundwater, water samples only were collected from Wonderboom Park, Eastling and Doorandjie. With respect to landfill sites, summer and winter samples (leachate and sediment) were collected from Hatherly and Soshanguve; while only winter samples were collected from Garskloof, Onderstepoort, Soshanguve, Chloorklop and Robinson deep landfill sites. For wetlands, water and sediment samples were collected from Bullfrog, Klip River wetland, Reitvlei, Karlspruit for winter samples and Soshanguve for summer period. Fish samples were collected from Rand Water canal.

Table 3.7: Description and number of samples collected per season at each of the sampling sites

	Type of sample				
Sampling Sites	**Fish**	**Surface water**	**Sediment**	**Groundwater**	**Landfill leachate**
Jukskei River	-	7	7	-	-
Upper Klip	-	1	1	-	-
Rietklip	-	1	-	-	-
Lowerklip	-	1	1	-	-
Vaal River	-	2	2	-	-
Marico/Crocodile	-	1	1	-	-
Wonderboom park	-	-	-	1	-
Eastling	-	-	-	1	-
Doorandjie	-	-	-	1	-
Hatherly	-	-	1	-	1
Soshanguve	-	-	1	-	1
Garstkloof	-	-	1	-	1
Onderstepoort	-	-	1	-	1
Chloorkop	-	-	1	-	1
Robinson deep	-	-	1	-	1
Bullfrog	-	1	1	-	-
Klip River	-	1	1	-	-
Rietvlei	-	1	1	-	-
Karlspruit	-	1	1	-	-
Soshanguve wetland	-	1	1	-	-
Rand Water canal	3	-	-	-	-

3.5.2 Water samples collection

One litre of river water sample was collected from seventeen catchment sites, groundwater samples from three sites, landfill leachate and sediment samples from six sites, and water and sediment samples from four wetland sites (Table 3.7). Samples were collected in thoroughly cleaned Winchester brown bottles by grab sample method, acidified with H_2SO_4, placed in a cooler bag and transported to the laboratory where they were stored in a cold room until analysis. After collection, samples were transported to the laboratory in cooler boxes and were kept frozen at -4^{0}C until analysis. Water samples were first filtered and later extracted.

3.5.3 Sediment samples collection

River sediment samples were collected from fifteen catchment sites, landfill sediment samples from six sites and sediment samples from four wetland sites (Table 3.7). Sediment samples were collected at the same spot where water samples were collected, at a depth of 0-5 cm below the surface with stainless grab into previously cleaned wide mouth 500 mℓ bottles wrapped with aluminium foil. After collection, samples were transported to the laboratory in cooler boxes and were kept frozen at -4 ^{0}C until analysis. Sediment samples were dried under fume hood at room temperature for 3-4 days. Dried and caked sediment were broken and foreign bodies such as wood splinters, glass and stone were removed. Thereafter, the samples were ground and homogenised with mortar and pestle and then sieved (250 µm).

3.5.4 Fish collection

Four bottom feeders, *Labeo umbratus* (lipid~ 2.4%; n=3), were collected from the Vaal Barrage using fishing net (Table 3.7).

3.6 METHOD DEVELOPMENT, INSTRUMENT CALIBRATION AND OPTIMIZATION AND RECOVERY TESTS

3.6.1 Reagents

The standard reagents used are shown in Table 3.8. These were purchased from Wellington Laboratories (Guelph, Ontario, Canada); copper powder (purity 99.98% from Saarchem (Pty) Ltd., Muldersdrift, South Africa), silicagel (100-200 mesh), sodium sulphate (purity 99.9%), glass wool and HPLC grade solvents: acetone, hexane, dichloromethane, methanol and toluene (products of Sigma Aldrich (Chemie GmbH, Steinheim, Germany), 50 mℓ of nonane (Purity 99.8%, Sigma Aldrich, product of Switzerland) were purchased from Industrial Analytical Pty. Ultra-pure water was dispensed from Purite water equipment (Purite Ltd, Thame, England) supplied by Lasec South Africa. Derivatizing agents (heptafluorobutyric anhydride (HFBA) was of analytical grade purchased from Sigma-Aldrich, South Africa. The solvents acetone and

hexane used in the study were of GC grade and were used without further purification. The alkylphenol ethoxylates (APEs) and polybromobiphenyl (PBBs) were purchased from Dr Ehrenstorfer-Schäfers laboratory, Augsburg, Germany. Only the nonylphenol ethoxylates (NPE), nonylphenol penta ethoxylates (NPPE) and octylphenol penta ethoxylates (OPPE) were of technical grade and the remaining APEs, PBBs and PBDEs were of analytical grade. Tetrabromobisphenol A and hexabromocyclododecane, all technical grades, were purchased from AccuStandard, USA.

Table 3.8: List of PBDEs evaluated including analysed congeners in environmental samples

Code	International Union of Pure and Applied Chemistry name	Mol wt	No of Bromine
BDE-17	2,2',4-TriBDE	406.9	3
BDE-47	2, 2', 4, 4'-TetraBDE	485.8	4
BDE-77	3,3',4,4'-TetraBDE	485.8	4
BDE-100	2,2',4,4',6-PentaBDE	564.7	5
BDE-99	2, 2', 4, 4', 5-PentaBDE	564.7	5
BDE-118	2,3',4,4',5-PentaBDE	564.7	5
BDE-154	2, 2', 4, 4', 5, 6'-HexaBDE	643.6	6
BDE-153	2, 2', 4, 4', 5, 5'-HexaBDE	643.6	6
BDE-139	$^{13}C_{12}$-3,3',4,4'-PentaBDE	656	5
BDE-128	2, 2', 3, 3',4,4'-HexaBDE	643.6	6
BDE-183	2, 2', 3, 4, 4', 5', 6-HeptaBDE	722.5	7
BDE-209	$^{13}C_{12}$-DecaBDE	971.1	10
BDE-209	Deca-BDE	959.2	10

3.6.2 Reagents and their purification

All solvents used were of analytical grade and kept away from contamination. Other materials such as silica gel, sodium sulphate and copper powder were activated before use at the recommended conditions. Anhydrous sodium sulphate and silica gel were first heated in a muffle furnace at 450^{o}C for 16 h before use. For acidic silica; 44 g of concentrated H_2SO_4 was added to 100 g of previously activated silica gel and stirred until fine powder was achieved; basic silica was prepared by mixing 30 g of 1M NaOH with 100 g of previously activated silica as stated above and stirred into fine powder. Copper powder was activated by soaking in 6M HCl for 3 min then rinsed thoroughly with ultrapure water followed by methanol and kept under toluene until use. Preparation of standards and serial dilution of working standards were done under fume hood. Further details of PBDEs standards used are presented in Table 3.8.

3.6.3 Development of gas chromatographic conditions (GC-MS)

The following parameters for optimisation were varied for GC-MS: carrier gas flow rate (high purity helium (99.999%), injection temperature, oven temperature and detector temperature. Inertcap 5MS/NP capillary column (30 m, 0.25 mm I.D., 0.25 µm d_f) with temperature programming and splitless time of 1 min was used. However, other columns ZB-5 (30 m, 0.25 mm I.D., 0.1 µm d_f) and (15 m, 0.25 mm I.D., 0.1 µm d_f) were tested for comparison in the course of the project. The GC was coupled to a Shimadzu MS QP2010 ultra quadrupole detector, operated in electron ionisation (EI) mode. Operating conditions were set as follows: ion source; 250^{o}C, and inter-phase at 300^{o}C. Identification of analyte peaks were carried out using selected ion monitoring (SIM), by monitoring the presence of the mass spectra of molecular ion and two qualifier ions of each congener at the elution retention time. Each congener was quantified against five level external standard calibration curves. A summary of the optimised condition applied for the GC-MS is presented in Table 3.9. ZB-5 capillary column 15 m, (0.25 mm I.D., 0.25 µm) and 15 m, (0.25 mm I.D., 0.1 µm) was found to give the best peak resolution and, therefore, was used in subsequent analysis. Other chromatographic parameters mentioned earlier were maintained. Each congener was quantified against five level external standard calibration curves. A summary of the optimised condition applied for the GC-MS is presented in Table 3.9.

Table 3.9: Summary of optimized instrument conditions for GC-MS

Oven Temp. Prog.	Injector Temp (^{o}C)	Detector Temp (^{o}C)	Carrier gas flow rate (ml)min^{-1})	Analysis time (min)
90^{o}C (1min) to 300^{o}C @ 30^{o}C m^{-1}(5min) to 310^{o}C @ 10^{o}C m^{-1} (2min)	290^{o}C	300^{o}C	1.5	16.00

3.6.4 Retention time determination

Lower concentrations (0.3-3.0 ng µℓ$^{-1}$) of individual and mixture of standards were prepared from stock solutions of 50 µg mℓ$^{-1}$ by serial dilution. One micro litre of 0.3-3.0 ng µℓ$^{-1}$ of the individual and mixed standards was, thereafter, injected into the GC to determine their retention times and this was reported as mean of triplicate injections.

3.6.5 Determination of instrumental limit of detection and calibration

The statistical and empirical methods of determining LOD were used. The statistical procedure involved running a series of blank samples; the mean blank value and the standard deviation (SD) were calculated and LOD determined as the mean blank value plus 3 times SD. The empirical method for LOD was

determined by measuring the lowest (increasingly lowering concentration of analytes) concentration the instrument can measure under optimized condition. However, LOD was calculated using the S/N > 3:1 and LOQ S/N > 10:1. LOD and LOQ values range between 0.01-0.02 ng $\mu\ell^{-1}$ and 0.02-0.06 ng $\mu\ell^{-1}$ respectively.

A minimum of 8 level external calibration of standard mixture were used for calibration. For all the congeners good linear regression of 0.999 was obtained. Figure 3.7 shows an example of chromatograms obtained from the mixture of standards used for calibration of the instrument. Figure 3.8, 3.9 and 4.3 show the mass spectrum and the molecular ions of BDE-47, BDE-99 and calibration curves, respectively. Similar information obtained for the rest of the congeners are contained in Appendix A. The values for mean retention time, limit of detection and limit of quantification are given in Table 3.10.

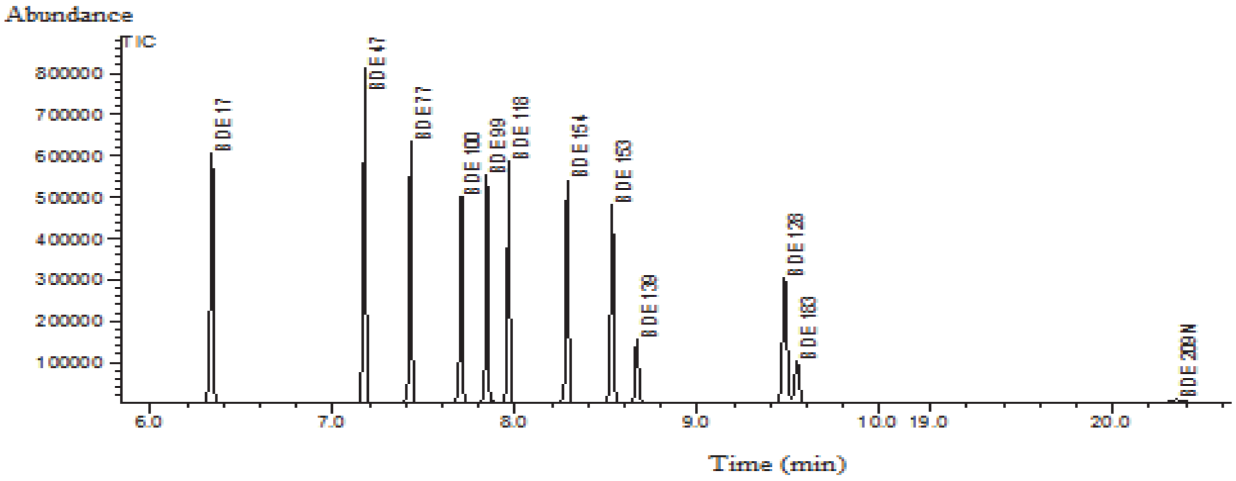


Figure 3.7: GC-MS chromatograms of PBDE standards for the six level calibrations

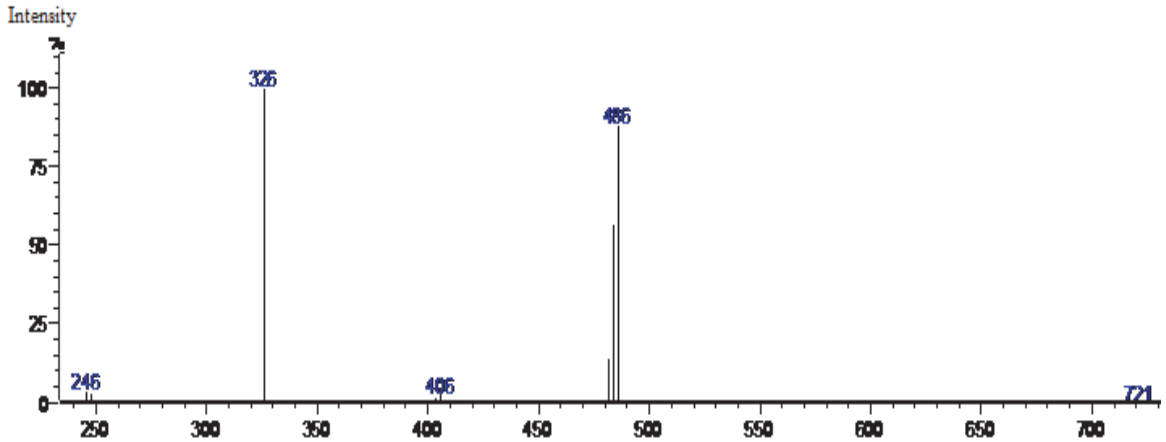


Figure 3.8: Molecular ion and fragment pattern of BDE-47

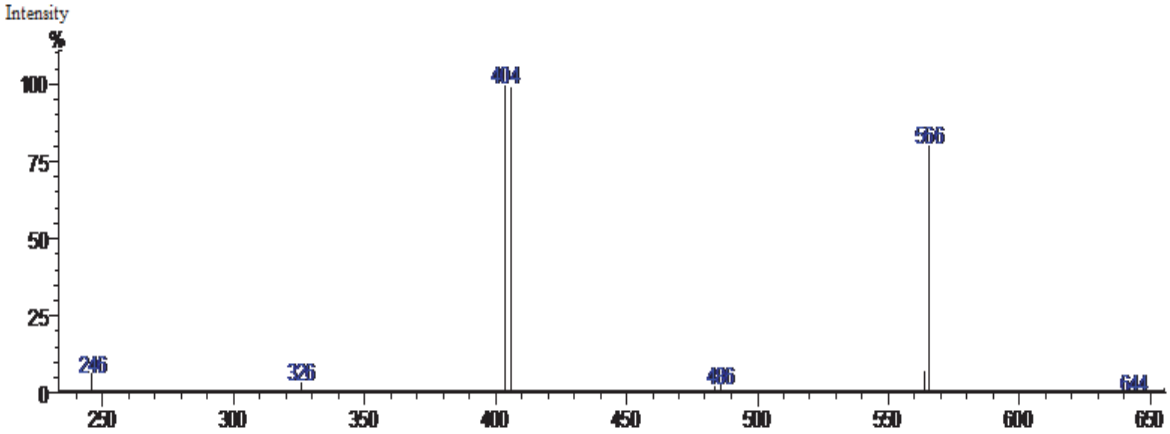
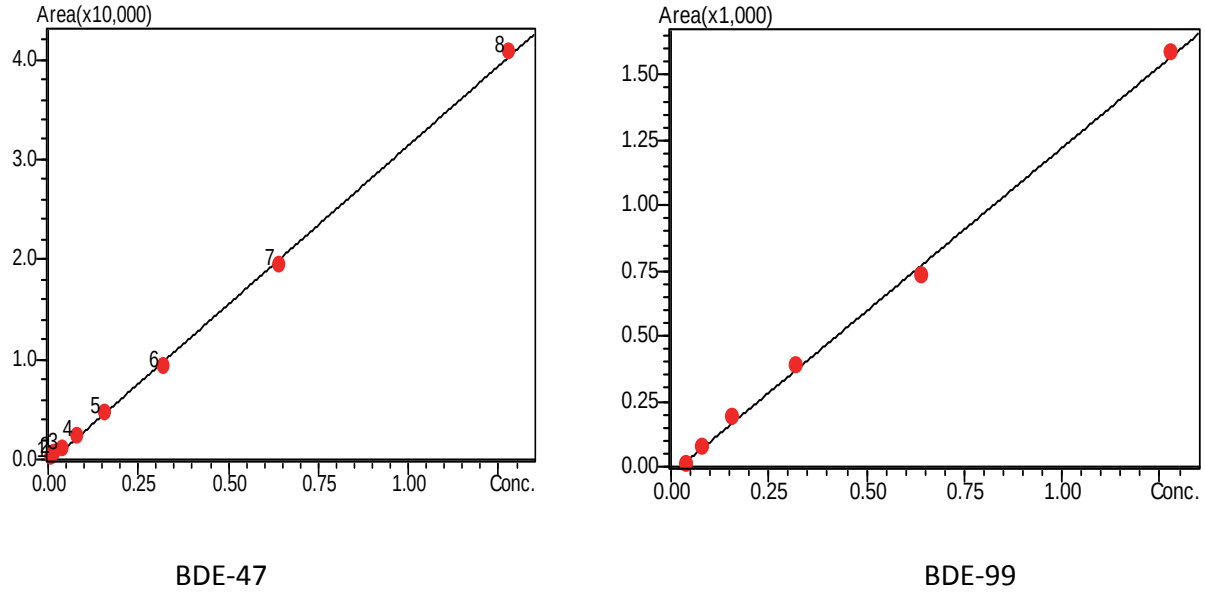


Figure 3.9: Molecular ion and fragment pattern of BDE-99



BDE-47 BDE-99

Figure 3.10: Method calibration curves of BDE-47 and BDE-99

Table 3.10: Mean retention times (15 m column), standard deviation and relative standard deviation of PBDE standards

	Retention times (min)							
Congeners	A	B	C	Mean	SD	RSD (%)	LOD*	LOQ*
BDE-17	5.455	5.461	5.45	5.46	0.01	0.10	0.019	0.12
BDE-47	6.124	6.125	6.126	6.13	0.00	0.02	0.02	0.09
BDE-77	6.523	6.529	6.509	6.52	0.01	0.16	0.02	0.11
BDE-100	6.761	6.765	6.759	6.76	0.00	0.05	0.03	0.19
BDE-99	6.909	6.912	6.905	6.91	0.00	0.05	0.03	0.32
BDE-118	7.031	7.035	7.026	7.03	0.00	0.06	0.018	0.15
BDE-154	7.498	7.5	7.494	7.50	0.00	0.04	0.01	0.33
BDE-153	7.686	7.688	7.682	7.69	0.00	0.04	0.01	0.32
BDE-139L	7.581	7.583	7.578	7.58	0.00	0.03	0.038	0.41
BDE-128	8.047	8.053	8.038	8.05	0.01	0.09	0.035	0.31
BDE-183	8.06	8.061	8.057	8.06	0.00	0.03	0.01	0.33
BDE-209	13.61	13.625	13.605	13.61	0.01	0.08	0.24	0.95

*Concentration in ng $u\ell^{-1}$

3.6.6 Method validation

3.6.6.1 Mean percentage recoveries of PBDE in water and sediment

About 100 µℓ of 3.3 ng $\mu\ell^{-1}$ PBDE standards was dissolved in 5 mℓ acetone then spiked into 500 mℓ Ultra-pure water and left for 24 h for equilibration. The mixture was, thereafter, extracted by liquid-liquid extraction. To test the yield and extraction efficiencies of some selected solvents found in the literature, *n*-hexane, toluene, and dichloromethane were used individually and mixed. Dichloromethane gave the best recovery for most of the target analytes than others. Observed values for a mixture of *n*-hexane/toluene (1:1, *v/v*) ranged from 23-103%, *n*-hexane/toluene (2:1, *v/v*) (28-130%), DCM/toluene (25-96%), DCM/hexane (39-130%) and DCM (75-101%). Similarly, test of solvents as used with LLE was also applied to the SPE; different solvents were used for elution and their respective recoveries compared. However, LLE gave satisfactory results for all analytes and was, therefore, reported in this work. Three times extraction using LLE with 50 mℓ DCM was found adequate for recovery of all analytes. The mean percentage recoveries are shown in Table 3.11.

Similarly for solids, 1 ng $\mu\ell^{-1}$ x 120 µℓ (BDE-77 and $^{13}C_{12}$-labelled BDE-139) was dissolved in acetone before spiking into the pre-extracted sediment samples. The impregnated samples was transferred into a glass fibre thimble, 2 g activated copper was added and the mixture Soxhlet extracted with 180 mL hexane/acetone (1:1, *v/v*) for 16 h. Solvent containing BFRs was collected and concentrated to 1 mℓ by rotary evaporator. Clean up followed the procedure earlier reported by (Olukunle *et al.*, 2011) using hexane/DCM (1:1, *v/v*) for elution. The eluate was concentrated to near dryness under N_2 to 400 µℓ and 1 µℓ was injected into the GC-MS.

The clean extracts was analysed by Shimadzu model 2010 plus gas chromatograph coupled with a model QP2 010 ultra-mass spectrometer (Shimadzu, Japan) using electron ionisation and injected automatically by a Shimadzu A0C-20i auto sampler. Operation mode was in the selected ion-monitoring (SIM) mode. The method was validated with the recovery of surrogate standards (BDE-77 and $^{13}C_{12}$-labelled BDE-139) and were both observed to be 90% respectively.

Table 3.11: Mean percentage recovery of PBDE standards from spiked ultrapure water

S/N	CONGENER	RT	C 1	C 2	C3	MEAN	SD	RSD	REC (%)
1	BDE-17	7.89	0.51	0.53	0.55	0.53	0.02	3.8	88
2	BDE-47	8.84	0.5	0.50	0.53	0.51	0.02	3.4	85
3	BDE-77	9.20	0.52	0.50	0.56	0.53	0.03	5.8	88
4	BDE-100	9.66	0.56	0.58	0.64	0.59	0.04	7	99
5	BDE-99	9.92	0.44	0.46	0.50	0.47	0.03	6.5	78
6	BDE154	10.89	0.47	0.48	0.52	0.49	0.03	5.4	82
7	BDE-153	11.43	0.41	0.44	0.47	0.44	0.03	6.8	73
8	BDE-139	11.76	0.45	0.47	0.50	0.47	0.03	5.3	79
9	BDE-183	13.77	0.44	0.45	0.47	0.45	0.02	3.4	76

*Concentration in ng $\mu\ell^{-1}$

3.6.6.2 Derivatization using HFBA

Into a vial, 0.15 mℓ of; APs (1 mg ℓ^{-1}), APEs (5 mg ℓ^{-1}), PBBs (1 mg ℓ^{-1}), PBDEs (1 mg ℓ^{-1}), HBCD (5 mg ℓ^{-1}) and TBBPA (4 mg ℓ^{-1}); 0.1mℓ hexane; 40 µℓ of 0.1 M triethylamine and 4 µℓ HFBA were added and the content heated to 50°C for 30 min. Thereafter, the contents were cooled, washed with 0.5 mℓ aqueous K_2CO_3 (3%). The organic phase was then drawn off, internal standards (Chrysene and PBB80) added and the volume made up to 200 µℓ. Thereafter, 1 µℓ was injected into the gas chromatography-mass spectrometry analysis.

3.6.7 Quality assurance

At the beginning of every analysis prior to sample analysis and after, an initial solvent blank, a laboratory performance standard check (linearity of the calibration curve) was performed using individual and mixtures of the most common brominated flame retardants. This is to ensure proper performance of the GC-MS. The use of surrogate standards during extraction and after clean-up was done to ensure accuracy. Retention times matched those of the standards and quantification was done by monitoring the molecular and reference ions using both internal and external methods. The limit of detection was taken as 3 times the signal to noise ratio and limit of quantification as 10 times signal to noise ratio. The spiking method was used in the quality assurance process of analytical method due to unavailability of certified reference material for

target compounds. Fish muscle (mean lipid ~1.9%; n= 3) from Rand Water canal was spiked with 150 µℓ of standard mixture of 1.0 mg $ℓ^{-1}$ APs, PBBs and PBDEs; 5.0 mg $ℓ^{-1}$ HBCD, TBBPA and APEs and was taken through the same extraction and derivatization procedure mentioned above prior to GC analysis. Several quality assurance measures were also routinely used in this study and included running fish muscle without spiking (i.e. fish blanks) in between samples and analyzing samples in triplicates.

3.7 SAMPLE EXTRACTION PROCEDURE

Initially, several extraction methods were tested for both water and sediment samples. However, liquid-liquid and Soxhlet extraction methods were found to yield high percentage recoveries and, therefore, were used in subsequently sample extractions throughout the study. However, the other extraction methods are still described in this report.

3.7.1 Liquid-liquid extraction (LLE) of water sample

About 500 mℓ of water samples was extracted thrice using liquid-liquid (LLE). Dichloromethane and dichloromethane/hexane were used for LLE and SPE respectively for extraction. These methods have been chosen since they have been tested to yield acceptable amount of organic analytes from liquid samples with the right solvent (Olukunle *et al*., 2012).

3.7.2 Solid phase extraction (SPE) of water samples

About 500 mℓ of water samples was extracted thrice using solid phase extraction (SPE). Dichloromethane and dichloromethane/hexane were used for LLE and SPE respectively for extraction. These methods have been chosen since they have been tested to yield reasonable amount of organic analytes from liquid samples with the right solvent.

3.7.3 Soxhlet extraction (SE) of sediment

About 10 g of dried and sieved (250 µm) sediment samples were weighed into glass fibre thimble in a Soxhlet apparatus and extracted with 170 mℓ (dichloromethane: hexane 1:1,*v/v*) for 10 h. Extracts were reduced to 1 mℓ by rotary evaporation, before subjecting to column cleaning. The recovery yield was by far the best compared to ultra-sonication and accelerated solvent extraction described below. Consequently, Soxhlet extraction was used throughout the experiment.

3.7.4 Ultrasonic Assisted Extraction (UAE) of sediment

For UAE method, about 10 g of sediment was weighed into 100 mℓ beaker and extracted with 40 mL (hexane : acetone, 2:1, *v/v*) for 10-25 min and at 35 & 45^{o}C using ultrasonic bath (Elmasonic S 40H, Germany) with maximum power of 340 watts . Boiling chips were added into the beaker to maximise mixing. This procedure was repeated two more times and all three extracts were collected in round bottom flask for rotary evaporation to 1 mℓ and then subjected to cleaning. The results of this extraction method were discouraging and, therefore, this method was not used in subsequent extraction protocol.

3.7.5 Accelerated Solvent Extraction (ASE) of sediment

Sediment sample extraction was carried out with Accelerated Solvent Extractor (ASE 350-Dionex, Sunnyvale, CA, USA) equipped with 34 mℓ stainless-steel cells. The instrument conditions used were at the following conditions; Pre cleaned (with *n*-hexane and heated in an oven at 200^{o}C for 1 h); stainless steel cells loaded by placing a cellulose filter at the bottom of a tightly screwed cell and followed in the order; 5 g washed sand, 4.5 g silica gel, 0.6 g pesticarb (clean up material), mixture of 10 g sample, 10 g washed sand and 5 g sodium sulphate and another cellulose filter was placed at the top. The cells were tightly screwed and loaded into the instrument at 120^{o}C, pressure; 11 MPa, heating time; 5 min, cycles; 2, flush volume; 90% and purged for 1.5 min with nitrogen. The same low recovery result as mentioned above was experienced with ASE and consequently, the method was not in subsequent experiments.

3.8 SAMPLE ANALYSIS BY COLUMN CHROMATOGRAPHY

All the crude extracts obtained from liquid-liquid extraction (LLE), solid phase (SPE) and Soxhlet (SE) was subjected to column clean up before injection into the GC-MS. The clean-up column (Figure 3.1) was achieved by packing in layers from bottom into Pasteur pipettes (230 mm) about 0.16 g of silica, 0.06 g pesticarb, and 0.16 g silica and finally topped with 0.5 g sodium sulphate. Before introduction of 1 mℓ reduced extract the packed column was eluted to saturation with (4 mℓ) 12 mℓ hexane. Sample was introduced into the column before the solvent reaches the bed of the sodium sulphate and was further eluted with (4 mℓ) 15 mℓ hexane. Thereafter, nitrogen gas was bubbled into the combined elute to concentrate it to 200 µℓ. About 20 µℓ of internal standard was added and then, 1.0 µℓ of the extract injected into the GC-MS under the optimized instrumental conditions.

3.8.1 Muscle tissue sample preparation: homogenization, extraction and clean-up

About 12.5 g of the muscle tissue from *Labeo umbratus* was weighed and mixed with 50 g anhydrous sodium sulphate. The contents were extracted with 50 mℓ of hexane/acetone mixture (4:1) amended with 0.25% acetic acid at 55°C for 30 min. After the ultrasonic extraction, the extracts were concentrated to about 3 mℓ

using TurboVap II instrument. Aminopropyl cartridges (APS, 500 mg, 3 mℓ LC-NH_2) and silica gel were used for lipid removal. The concentrated extract was first passed through pre-conditioned (Aminopropyl) cartridges at a rate of 5 mℓ min^{-1}. The cartridges were conditioned with 3x3 mℓ acetone then 1x3 mℓ of DCM and finally 3x3 mℓ of hexane. The BFRs were collected first while the APEs were eluted from the cartridges with 7 mℓ of hexane: 2-propanol (9:1) solution. The combined extracts were then treated with acidic silica column clean-up (Figure 3.7).

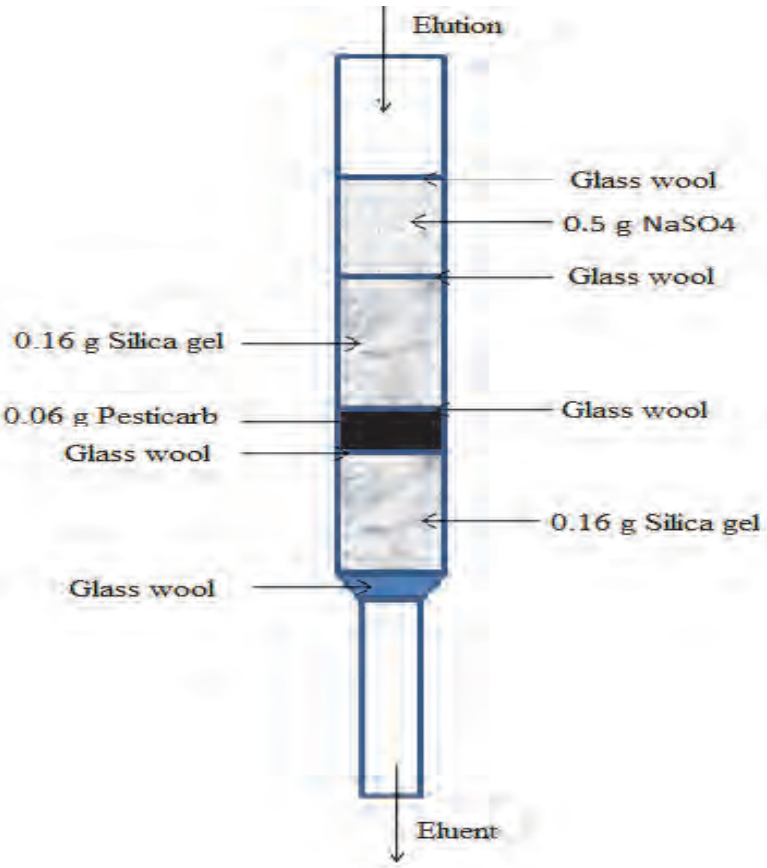


Figure 3.11: Diagrammatic illustration of column chromatography clean up

This column contained 2.5 g of silica gel and 1.5 g of anhydrous sodium sulphate. The column was pre-eluted with hexane, and the extract was placed on the column and eluted with 40 mℓ of hexane: 2-propanol mixture. The eluates were concentrated under a gentle stream of nitrogen to dryness and placed under derivatization conditions as mentioned above. The cleaned water and sediment extracts were concentrated to 200 µℓ under a gentle stream of nitrogen and solvent exchanged to hexane before injecting an aliquot of 1µℓ into the GC-MS for analysis.

3.9 SAMPLE ANALYSIS BY GC-MS

3.9.1 Concentration of extracts

After clean-up, all samples were concentrated to 200 µL under a gentle flow of N2 by placing the vials in the heating block of the controllable temperature heating module (#TS-18822, model no: TS-18802) using the Reacti-Vap (#TS-18828) from Thermo Fisher scientific, Bellefonte P.A, USA and supplied by Anatech Pty, South Africa) and solvent exchanged to hexane before injecting an aliquot of 1µℓ into the GC-MS for analysis. Triplicate extract of the sediment was carried out. In this present report, ZB-5 capillary column 15 m, 0.25 mm i.d., 0.25 µm and 15 m, 0.25 mm i.d., 0.1 µm were tested for peak resolution. Both columns (ZB-5 MS) were of same length and internal diameter but with different film thickness.

3.9.2 Analysis of fish samples

An Agilent 6890 GC equipped with 5973 mass selective detector (MSD) was used for GC/MS analysis. The GC was equipped with a Gerstel autosampler. The GC separation was performed on a capillary column (Restek RTx-1614, film thickness 0.10 µm, 15 m x 0.25mm I.D., (Chromspec cc South Africa)). The GC-MS conditions used for analysis were as follows: carrier gas He; linear velocity, 40 cm s^{-1}; injector temperature, 275°C; transfer line temperature, 280°C; ion source 150°C. For analysis 1µℓ splitless injection were carried out by autosampler. The GC temperature program conditions were as follows: initial temperature 50°C, heated to 120°C by a temperature ramp of 7.5°C min^{-1} then 275°C by a temperature ramp of 15°C min^{-1} then finally heated to 280°C (held for 1 min) by a temperature ramp of 25°C min^{-1}.

CHAPTER 4: BDE CONCENTRATIONS IN ENVIRONMENTAL SAMPLES

4.1 INTRODUCTION

The results of the analyses of leachate and sediment samples collected are presented and discussed in this chapter. BDE levels in the water samples (rivers, wetland and groundwater) were below the detection limit and, therefore, not included in the present report.

4.2 GC-MS CHROMATOGRAM (TIC) OF PBDE STANDARDS

The GC-MS chromatogram obtained is shown in Figure 4.1. From Figure 4.1, the congeners are fairly separated using ZB-5 capillary column. Other capillary columns which were tested exhibited lower peak resolution and did not detect BDE-209. To identify the ZB-5 column with a better peak resolution, two ZB-5 columns were tested and the comparative plot is shown in Figure 4.2. As can be seen in Figure 4.2, ZB-5 capillary column 15 m, 0.25 mm i.d., 0.25 µm, compared very well with the 15 m, 0.25 mm i.d., 0.1 µm, but with a slight increase in retention times for most of the congeners except for BDE-209 which can be attributed to increased interaction of the analytes with the stationary phase in a thicker film. Consequently, the 0.1 µm column was used in most of the analysis in the present report for faster analysis time.

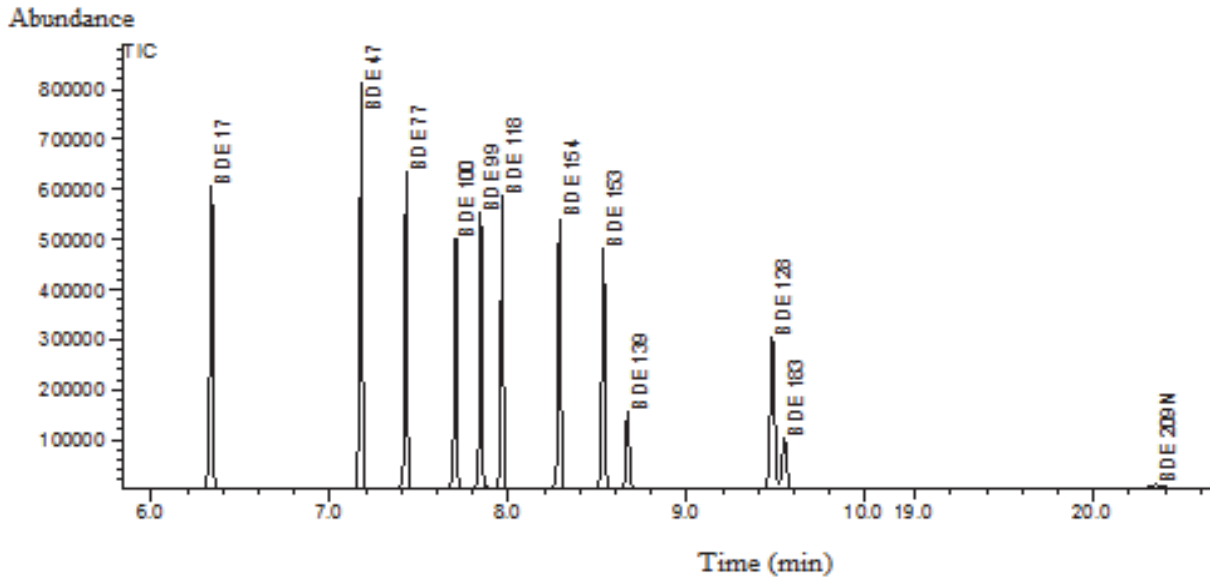


Figure 4.1: GC-MS chromatogram (TIC) of PBDE standards

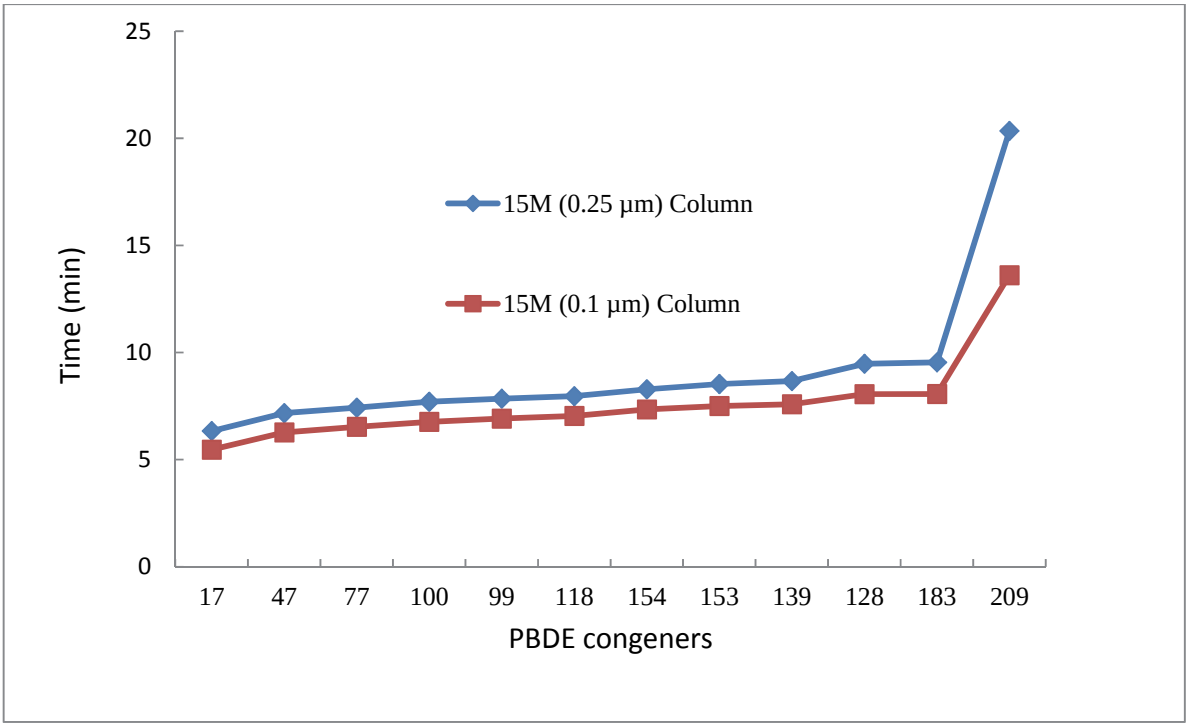


Figure 4.2: Retention time difference of two columns of varying film thickness (ZB-5 capillary column 15 m, 0.25 mm i.d., 0.25 µm & 15 m, 0.25 mm i.d., 0.1 µm columns)

4.3 PHYSICO-CHEMICAL WATER QUALITY PARAMETERS OF WATER AND LEACHATE SAMPLES

The physico-chemical water quality parameters recorded for the sites in the two seasons are shown in Appendix D, the following values 6.46-9.17, 12.8-1423 (µs), 0.104-3.531 mg ℓ^{-1}, 25.591-4597.37 mg ℓ^{-1}, 0-0.494 mg ℓ^{-1}, 0-2.038 mg ℓ^{-1}, 0-37.83 mg ℓ^{-1}, 0-10.194 mg ℓ^{-1} and 0.82-123.395 mg ℓ^{-1} for pH, electrical conductivity, fluoride, chloride, nitrite, bromide, nitrate, phosphate and sulphate respectively were observed for winter. The summer results when compared to winter indicates a significant change in water quality of the rivers , especially for Jukskei River that recorded very lower pH values at all the sampling points of about 4.82 on average, high electrical conductivity and high sulphate levels. This confirms the huge impact of vast run-offs into the river from agricultural farmlands into the river.

4.4 BDE CONCENTRATIONS IN RIVER SAMPLES

4.4.1 BDE concentration in river water samples

The concentrations of the BDE in water samples from the seventeen catchments were below the instrument detection limit and, therefore, not presented in this report.

4.4.2 BDE concentration in river sediment samples

4.4.2.1 Jukskei catchment sediment samples

The mean concentrations (obtained from triplicate measurements) of common PBDEs congeners are shown in Tables 7.1. As can be seen in Table 7.1, all target BDEs were detected in the sediment samples from all the sites in Jukskei River with the exception of BDE-183 at Eastgate, Eastbank, Kyalami and Bruma Lake sites respectively. The observed BDE concentrations ranged from 0.07-2.41 ng g^{-1}, with BDE-183 and BDE-99 exhibiting the lowest and highest total concentrations respectively. The second highest concentration was shown by BDE-47. It can also be observed that Bruma site accounted for the highest ΣBDEs with values of 3.18 ng g^{-1} and 3.46 ng g^{-1} for Soxhlet and accelerated solvent extraction methods respectively from summer samples.

The seven sampling sites identified on Jukskei River included, Eastgate (Marlboro), Midrand (Eastgate), N14 (KNP), Bruma Lake, Eastbank, Kyalami and Buccleuch. Seven sediment samples were collected from these points and screened for BFRs. Figure 4.3 shows the mean concentrations of BDE obtained in the seven different sites. Low levels of BDE were detected at six sites while Bruma Lake had the highest concentration with more than two orders of magnitude greater than the rest. The concentrations of BDE detected in the winter sediment samples ranged from 0.06-10.40 ng g^{-1}. This is significantly higher than the values for the summer sediments (0.07-2.4 ng g^{-1}). It is probable that dilution effect as a result of precipitation may have contributed to the observed low levels of BDE in the sediment in summer samples. The sum BDE in summer samples ranged from 1.01 to 3.18 ng g^{-1} (Figure 4.4) while for winter; 0.35 to 40.27 ng g^{-1} d.w with the latter corresponding to levels in Bruma Lake as can be seen in Figure 4.5. Lowest concentration of 0.34 ng g^{-1} d.w was detected at Marlboro (Eastgate). The highest sum BDE per site was observed at Bruma site. The observed high levels at this site could be attributed to various land use activities (sewage treatment plant and agricultural practice) around this area.

Table 4.1: Jukskei River mean concentration (ng g-1 d.w) and standard deviation of PBDEs congeners in sediment samples (summer)

BFR	N14(KNP)	ESG(MR)	EB	MB(WAT)	KYA	BUC	BRUMA	TOTAL
BDE-17	0.22±0.25[a]	0.25±0.20[a]	0.20±0.10[a]	0.11±0.21[a]	0.14±0.23[a]	0.12±0.26[a]	0.12±0.19[a]	1.16[a]
	0.26±0.15[b]	0.20±0.12	0.12±0.12[b]	0.14±0.12	0.20±0.18	0.17±0.11	0.14±0.12	1.23
BDE-47	0.35±0.31[a]	0.18±0.16[a]	0.28±0.12[a]	0.28±0.11[a]	0.28±0.22[a]	0.23±0.16[a]	0.55±0.09[a]	2.15[a]
	0.30±0.23[b]	0.20±0.15[b]	0.20±0.14[b]	0.27±0.18[b]	0.30±0.25[b]	0.20±0.12[b]	0.45±0.30[b]	1.92[b]
BDE-99	0.16±0.12[a]	0.35±0.21[a]	0.30±0.15[a]	0.56±0.21[a]	0.54±0.33[a]	0.20±0.12[a]	2.2±0.23[a]	4.31[a]
	0.13±0.10	0.30±0.25	0.25±0.17[b]	0.61±0.25	0.48±0.24	0.24±0.20	2.4±0.19	4.41
BDE-100	0.24±0.19[a]	0.21±0.11[a]	0.19±0.13[a]	0.26±0.19[a]	0.23±0.20[a]	0.14±0.11[a]	0.05±0.02[a]	1.32[a]
	0.30±0.22[b]	0.18±0.14[b]	0.15±0.12[b]	0.22±0.13[b]	0.19±0.15[b]	0.20±0.16[b]	0.10±0.08[b]	1.34[b]
BDE-153	0.25±0.20[a]	0.09±0.03[a]	0.14±0,09[a]	0.43±0.12[a]	0.14±0.12[a]	0.11±0.08[a]	0.19±0.13[a]	1.35[a]
	0.18±0.14[b]	0.12±0.10[b]	0.08±0.05[b]	0.30±0.23[b]	0.19±-.10[b]	0.07±0.03[b]	0.23±0.12[b]	1.17[b]
BDE-154[a]	0.18±0.11[a]	0.13±0.09[a]	0.20±0.12[a]	0.14±0.09[a]	0.21±0.11[a]	0.12±0.08[a]	0.07±0.04[a]	1.05[a]
	0.25±0.14[b]	0.10±0.06[b]	0.08±0.10[b]	0.20±0.10[b]	0.25±0.13[b]	0.17±0.13[b]	0.14±0.10[b]	0.97[b]
BDE-183[a]	0.11±0.10[a]	<dl	<dl	0.08±0.02[a]	<dl	0.09±0.06[a]	<dl	0.28[a]
	0.07±0.02[b]	<dl	<dl	0.16±0.10[b]	<dl	0.10±0.05	<dl	0.31[b]
ΣPBDE	1.51[a]	1.21[a]	1.31[a]	1.86[a]	1.54[a]	1.01[a]	3.18[a]	11.62[a]/11.62
	1.49[b]	1.10[b]	0.88[b]	1.90[b]	1.61[b]	1.15[b]	3.46[b]	11.59[b]/11.35

N14 = KNP =Knoppieslaagte; ESG = Eastgate =MR =Midrand; EB =Eastbank; MB =Marlboro =WAT =Waterval ; KYA = Kyalami; BUC = Buccleuch; Bruma = Bruma. [a]Values obtained using Soxhlet extraction method and [b]Values obtained using Accelerated solvent extraction

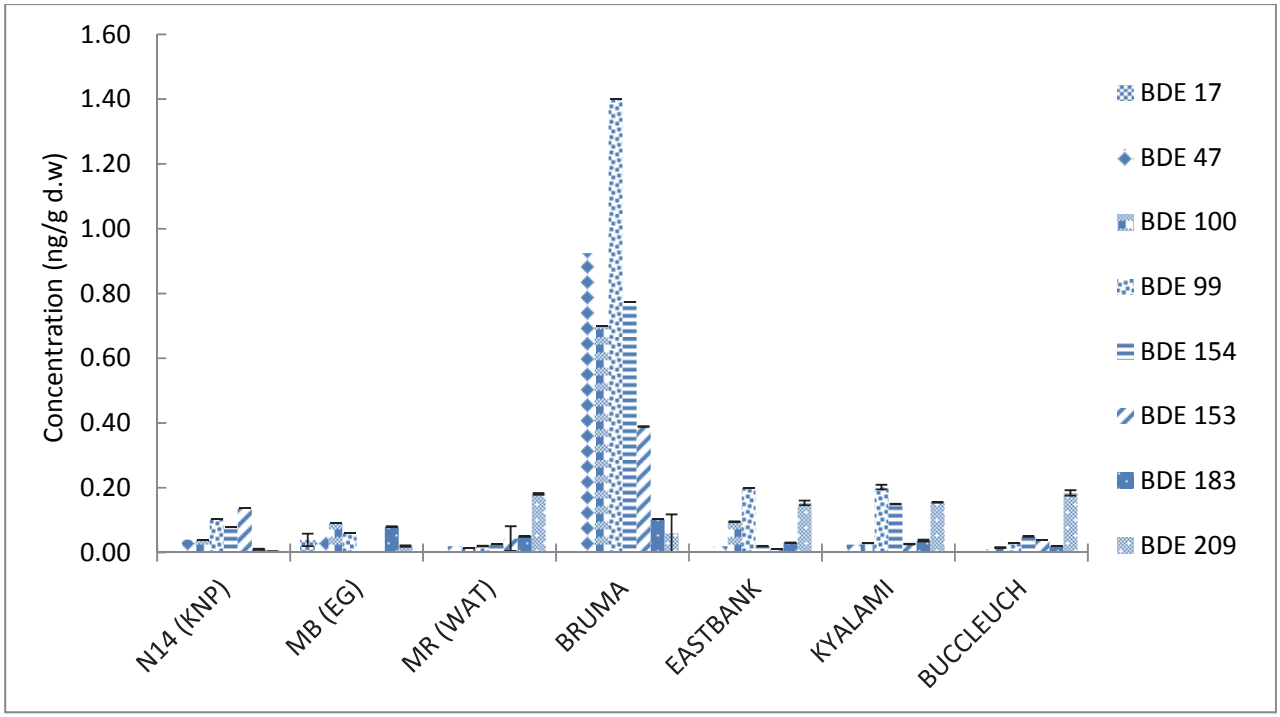


Figure 4.3: Mean concentration of BDE per site in Jukskei River sediment (winter)

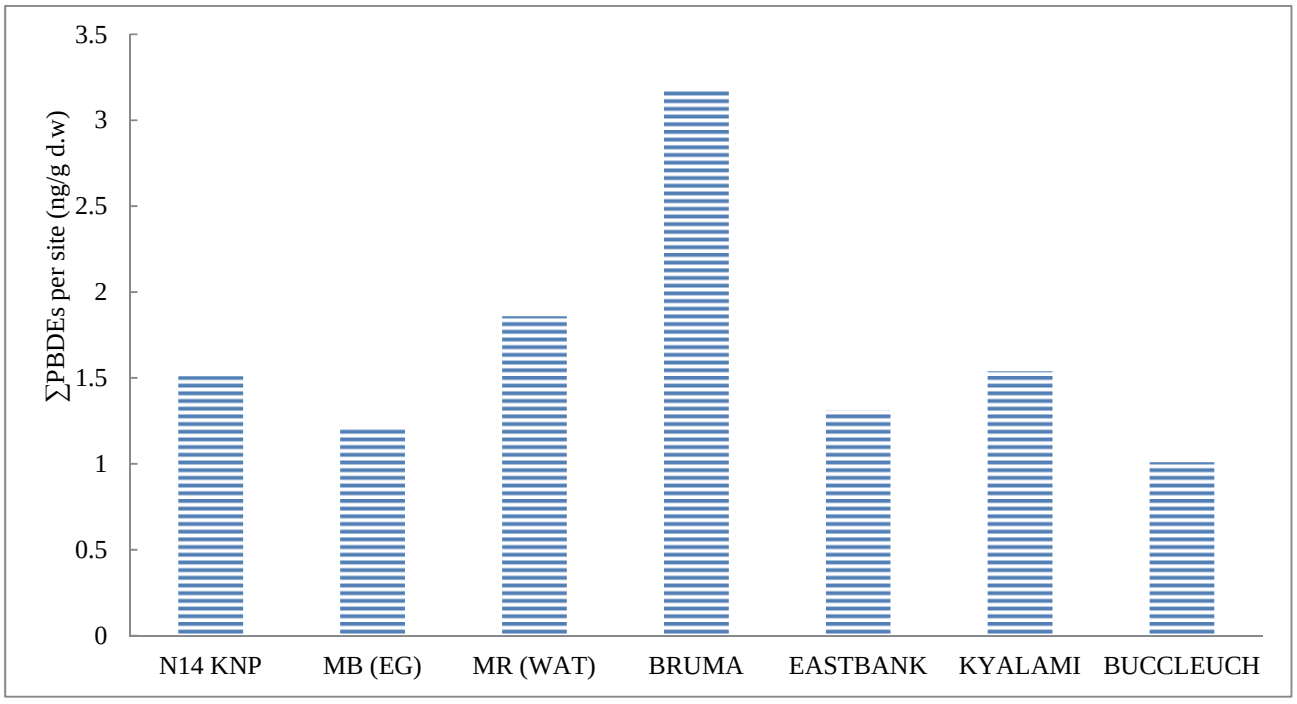


Figure 4.4: Sum BDE per site in Jukskei River sediment (summer)

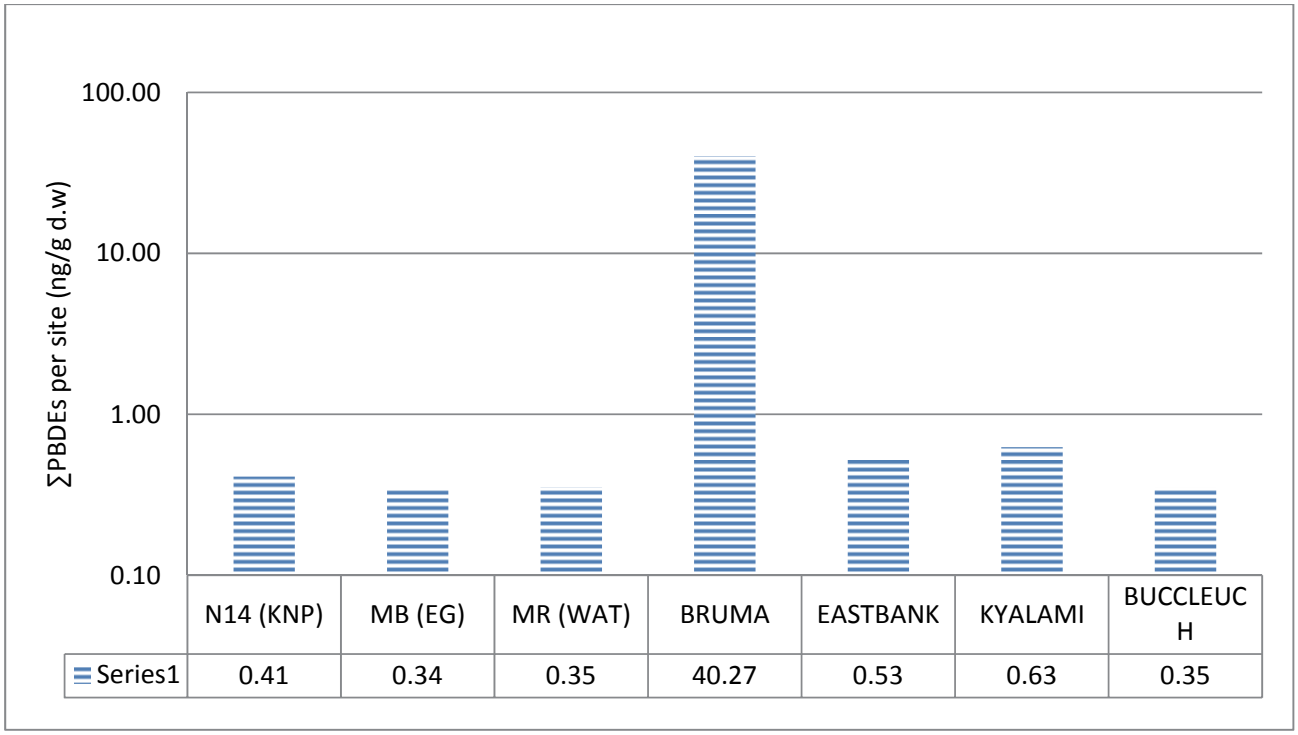


Figure 4.5: Sum BDE for Jukskei River sediment sites (winter) expressed in logarithmic scale base 10

Figure 4.6 shows the congener specific contribution of the pollutants in Jukskei River winter sediment samples. BDE-47, -99, -100, -153 and BDE-154 are the dominant congeners in all sediment samples with mean concentrations of 9.41, 11.02, 7.66, 8.07 and 5.52 ng g^{-1} d.w and percentage contribution to total PBDEs of 21.9%, 25.7%, 17.86%, 18.8% and <10% respectively. Similarly, BDE-99 was found to be the dominant congener in summer samples at a total concentration of about 4.5 ng g^{-1} as shown in Figure 4.7.

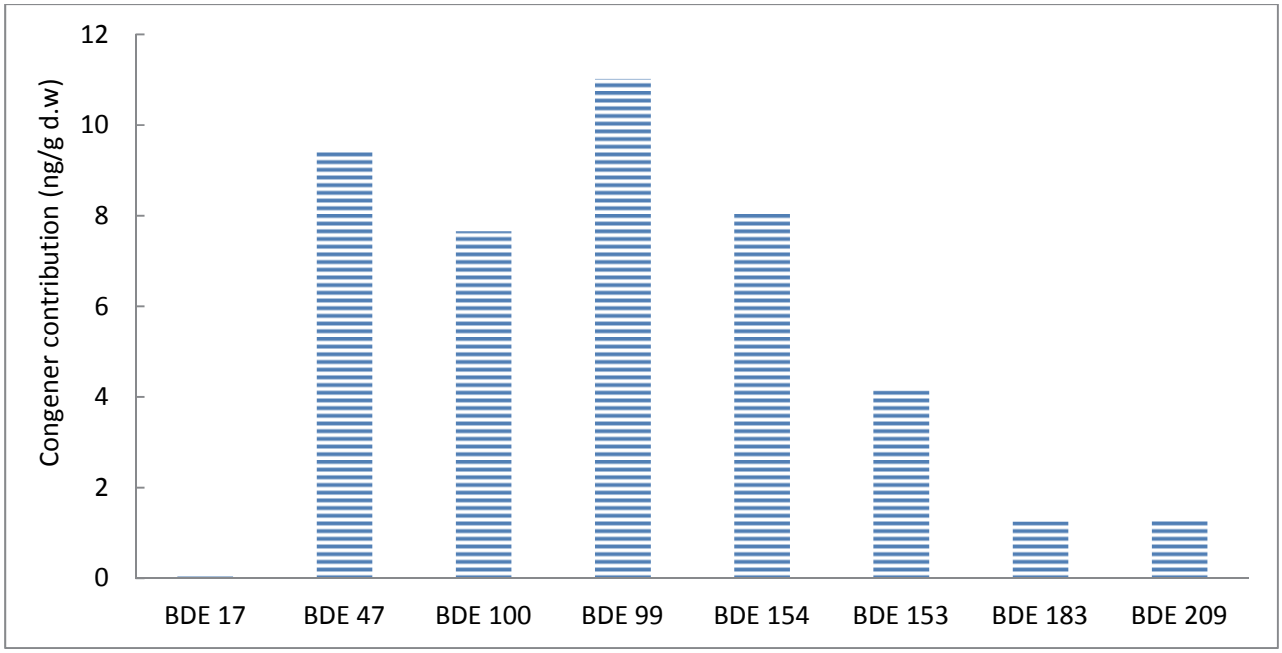


Figure 4.6: Congener specific contribution of common PBDEs in Jukskei River sediment (winter)

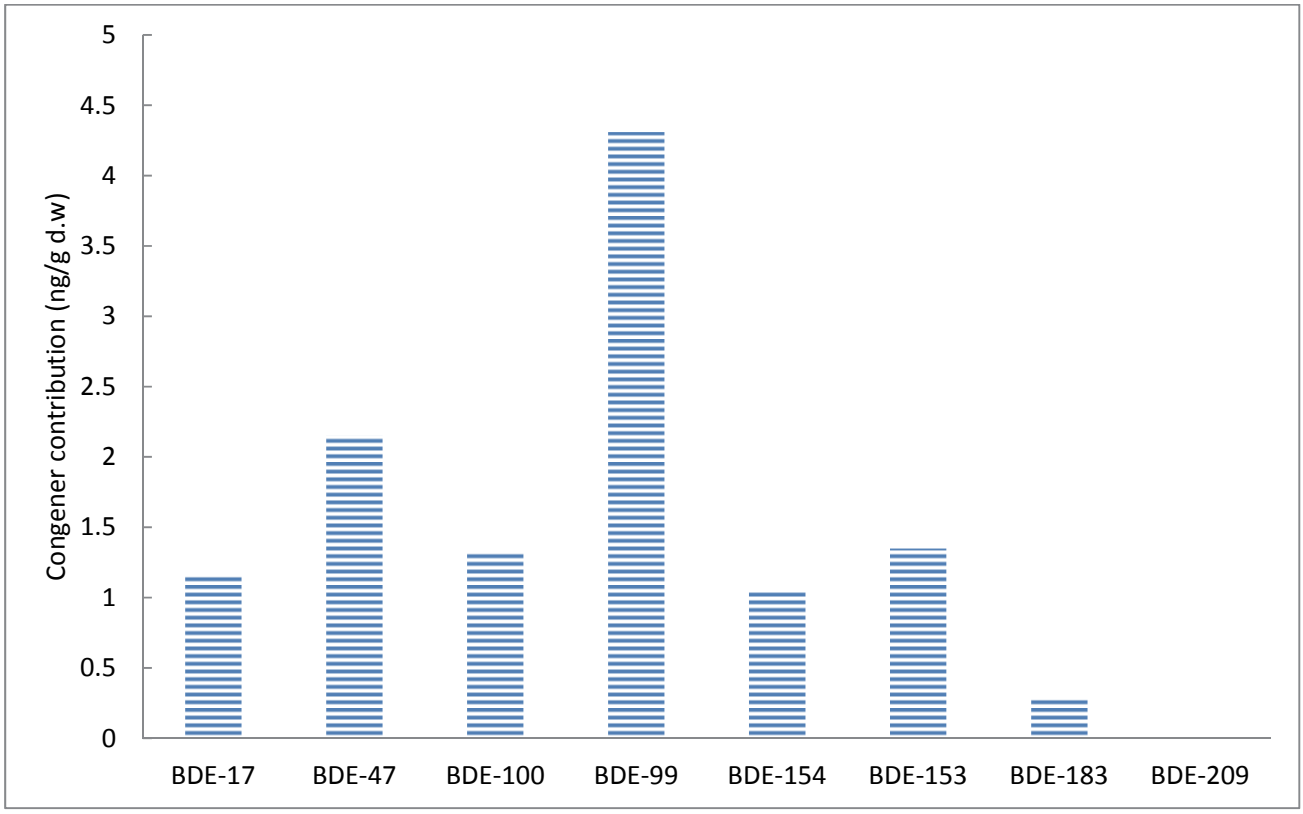


Figure 4.7: Congener specific contribution of common PBDEs in Jukskei River sediment (summer)

A scatter plot (Appendix C) of dissolved oxygen, temperature and electrical conductivity against PBDE concentrations was performed to determine a possible relationship (Pearson correlation (r) was applied). For dissolved oxygen (r = -0.79) a negative correlation was observed, i.e., the higher the dissolved oxygen content the lower the PBDE concentrations. It is expected that lower temperatures will be favourable to PBDE congeners when there is less likelihood of leaching and debromination, however, a negative correlation (r = -0.15) was observed. On the other hand, the relationship between electrical conductivity and PBDE concentrations also gave a negative correlation (r = -0.48) in winter samples which showed better results than summer.

4.4.2.2 Vaal (Taaiboschspruit and Vaal barrage) and Lowerklip (Meyerton) sediment

With respect to the concentrations of BDEs in Lowerklip and Vaal Rivers (Table 4.2), the highest concentration of 0.32 ng g^{-1} was observed at Lowerklip River compared to concentration of 0.30 ng g^{-1} observed in Vaal River. As shown in Table 4.2, the following PBDEs were present in both rivers: BDE-17, BDE-47, BDE-99 and BDE-153. BDE-100 was not detected in the Vaal River; BDE-154 not detected in Lowerklip River and BDE-183 not detected in both rivers. That the concentrations of BDE in water were observed below the detection limit was not surprising as these contaminants are very hydrophobic and detection is often at extremely very low levels. Moller et al. (2011) reported a very low concentration range of 0.005-0.64 pg ℓ^{-1} for BDE-47 and BDE-99 in seawater of the European Arctic. In another study, Booij, Zegers & Boon (2002) reported a concentration of 0.1-4 pg ℓ^{-1} for BDE-209 and 1, 0.5 and 0.2 pg ℓ^{-1} for BDE-47,-99 and BDE-153 respectively. Streets et al. (2006) reported a concentration of 18 pg ℓ^{-1} for $\sum_4$BDE (BDE-47, -66, -99 and BDE-100) in Lake Michigan. However, Oros et al. (2004) reported the highest concentration of ∑BDE in water in the San Francisco estuary. They reported values ranging between 3-513 pg ℓ^{-1}, with the highest concentration corresponding to samples taken from a section of the estuary that receives 26% of wastewater effluent from publicly owned treatment works.

Table 4.2: Mean concentration (ng g-1 d.w) and standard deviation (±) of PBDEs in sediment samples (summer) from Lowerklip and Vaal River

Congener	Lower Klip River	Vaal River
BDE-17	0.12±0.11	0.11 ±0.08
BDE-47	0.28±0.18	0.30±0.1
BDE-99	0.17±013	0.19±0.12
BDE-100	0.17±0.12	<dl
BDE-153	0.19±0.14	0.14±0.10
BDE-154	<dl	0.08±0.05
BDE-183	<dl	<dl
ΣPBDEs	0.93	0.82

Sediment samples taken from two sampling sites (Taaiboschspruit and Vaal barrage) from the Vaal River, in winter and summer were screened for the common BDE. The concentrations of BDE in winter sediment samples from Taaiboschspruit and Vaal barrage, on Vaal River are shown in Figure 4.8 and the concentrations range from 1.62-48.53 ng g^{-1} and 4.42-83 ng g^{-1} dry weight respectively. As shown in Figure 4.8, all screened analytes were detected in sediment samples except for BDE-154 that was below detection in sample from Taaiboschspruit. BDE-209 was observed to be the dominant congener from the two sites, with concentrations from Vaal barrage (83 ng g^{-1} d.w) about two times Taaiboschspruit (48.53 ng g^{-1} d.w), and total BDE per site $\sum_8$ BDE 159.62 ng g^{-1} d.w and $\sum_8$ BDE 111.62 ng g^{-1} d.w respectively. Figure 4.9 shows observed BDE concentrations from the two sampling points in summer samples. Concentrations were significantly lower than was observed in winter samples. BDE-99 and 153 were the most common congeners found in both samples while BDE-28 and 209 were found below detection.

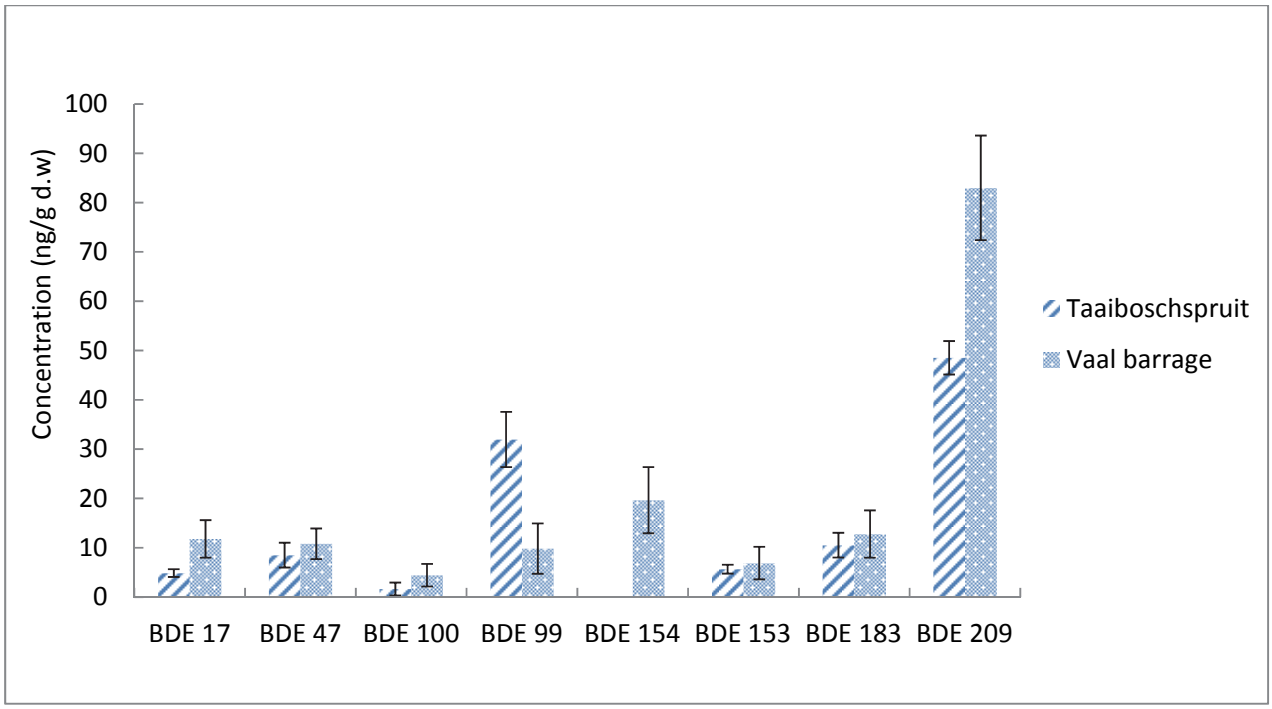


Figure 4.8: Mean concentration of PBDE congeners in sediment samples (winter) from two sampling sites on Vaal River

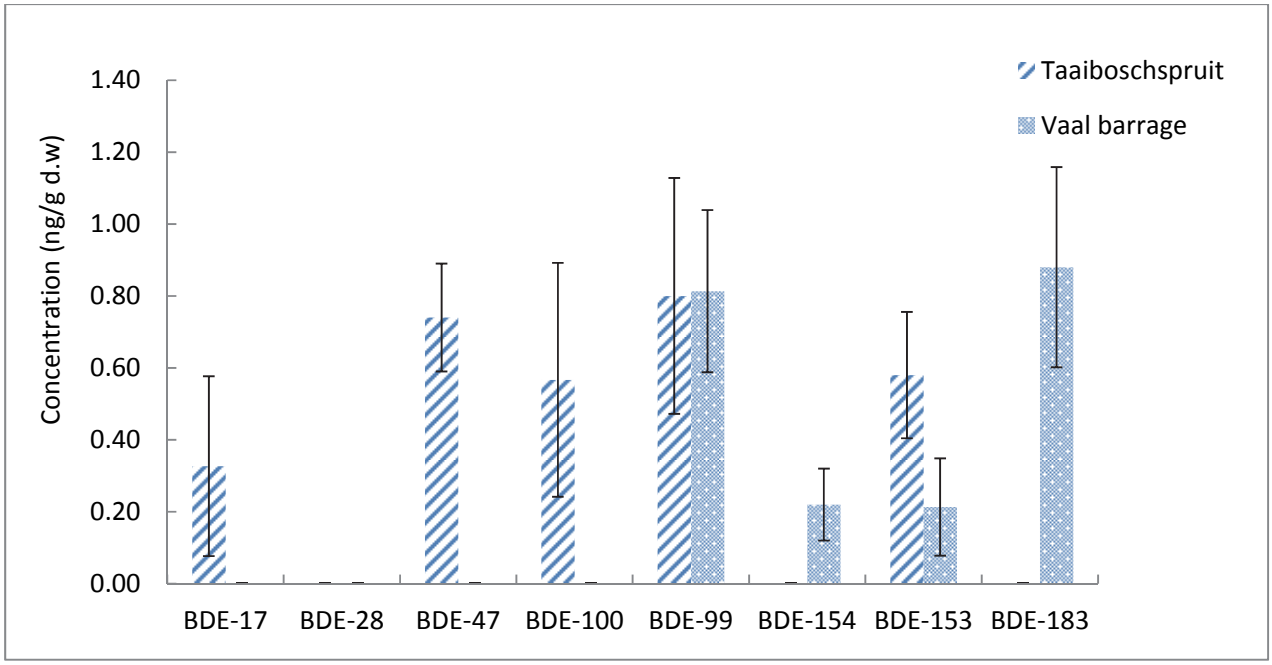


Figure 4.9: Mean concentration of PBDE congeners in sediment samples (summer) from two sampling sites on Vaal River

4.4.2.3 Alberton, Meyerton and Clarington river sediment

The concentrations of BDE detected in sediment samples obtained from Alberton, Meyerton and Clarington in winter are shown in Figure 7.15. As can be seen from Figure 7.15, Alberton and Meyerton exhibited the highest BDE value of 9.8.1 ng g^{-1} and 8.50 ng g^{-1} for BDE-100. The highest concentrations for BDE-209 and BDE-183 were also exhibited by the two aforementioned sites. BDE-47, 99, 100, 153, 154, 183 and 209 were all detected in the sediment samples, particularly in Meyerton site. The values recorded for sediment samples obtained from the aforementioned rivers are significantly higher than the values for Jukskei and Vaal Rivers. As was seen in the results of other rivers like Jukskei and Vaal, the observed BDE levels in Alberton, Meyerton and Clarington summer samples, as shown in Figure 7.16 were lower than in winter.

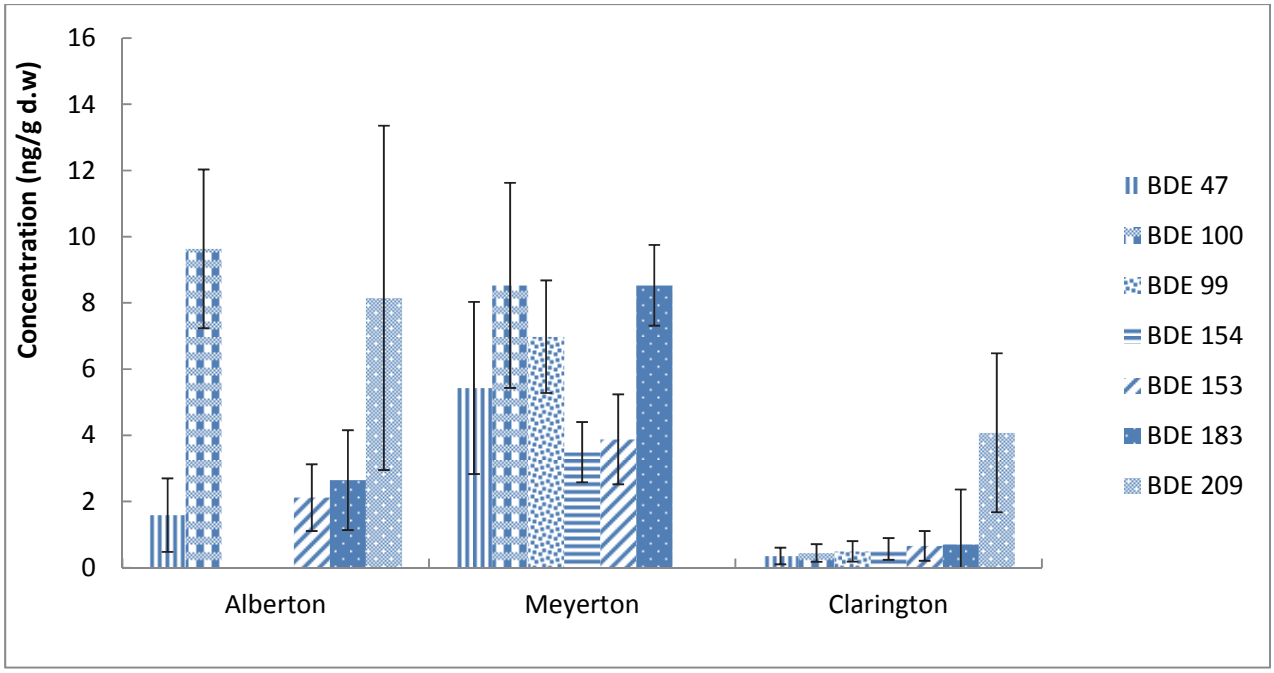


Figure 4.10: PBDE concentrations in river sediment samples (winter)

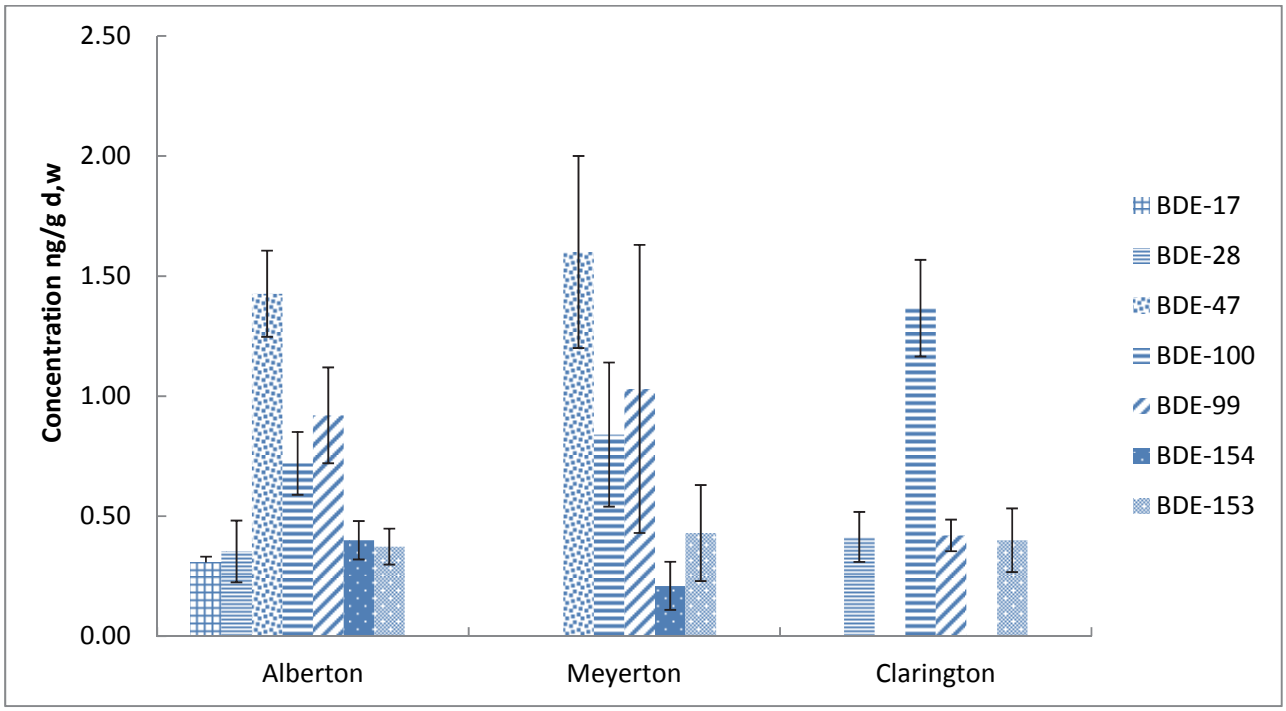


Figure 4.11: PBDE concentrations in river sediment samples (summer)

4.5 PBDE CONCENTRATIONS IN WETLAND SAMPLES

4.5.1 BDE concentrations in wetland water samples

Figure 4.12 shows typical chromatogram of BDEs detected in wetland samples. With respect to BDE in wetland water samples, the following BDE were detected: BDE-47, 99, 100, 153, 154, 183 and 209. Rietvlei wetland exhibited the highest levels followed by Bullfrog as can be seen in Figure 4.13. BDEs were found below LOD in Klip River and Karlspruit wetland samples. Summer samples also showed levels below limit of detection for all the four sites, except for Klip River that was not sampled for lack of access because the wetland was completely overgrown with vegetation.

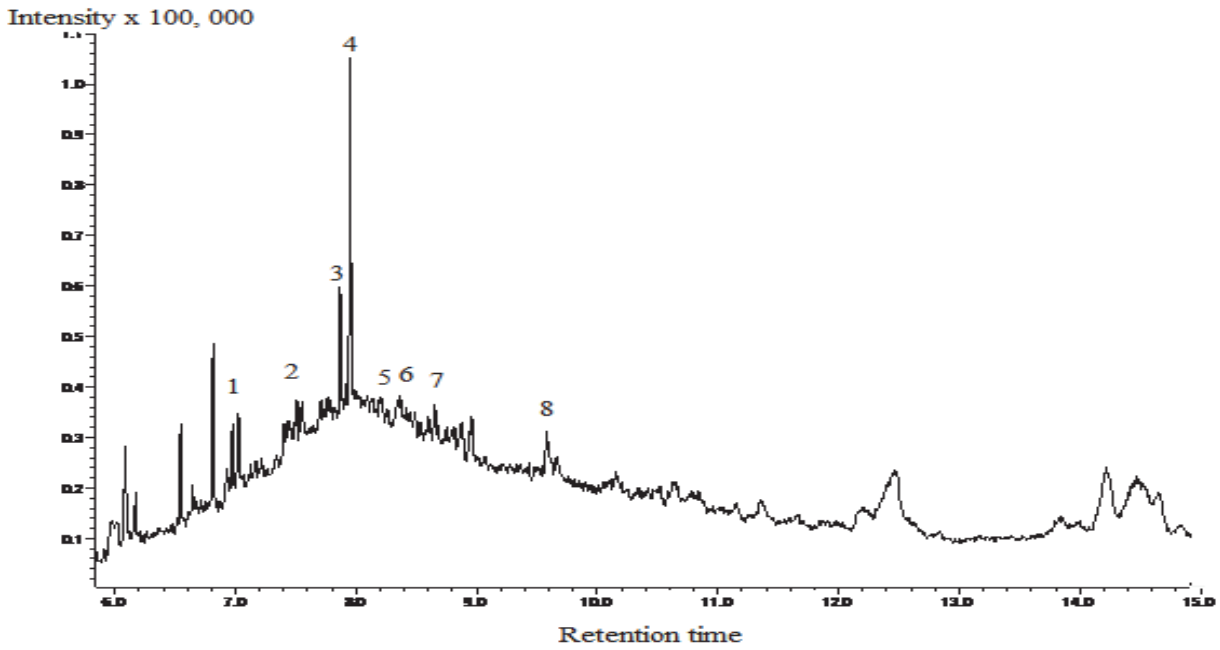


Figure 4.12: Typical chromatogram of PBDEs in wetland samples (1= BDE-47, 2= BDE100, 3= BDE-99, 4= BDE-118 (IS), 5= BDE-154, 6= BDE-153, 7= BDE-139L (IS), 8= BDE-183)

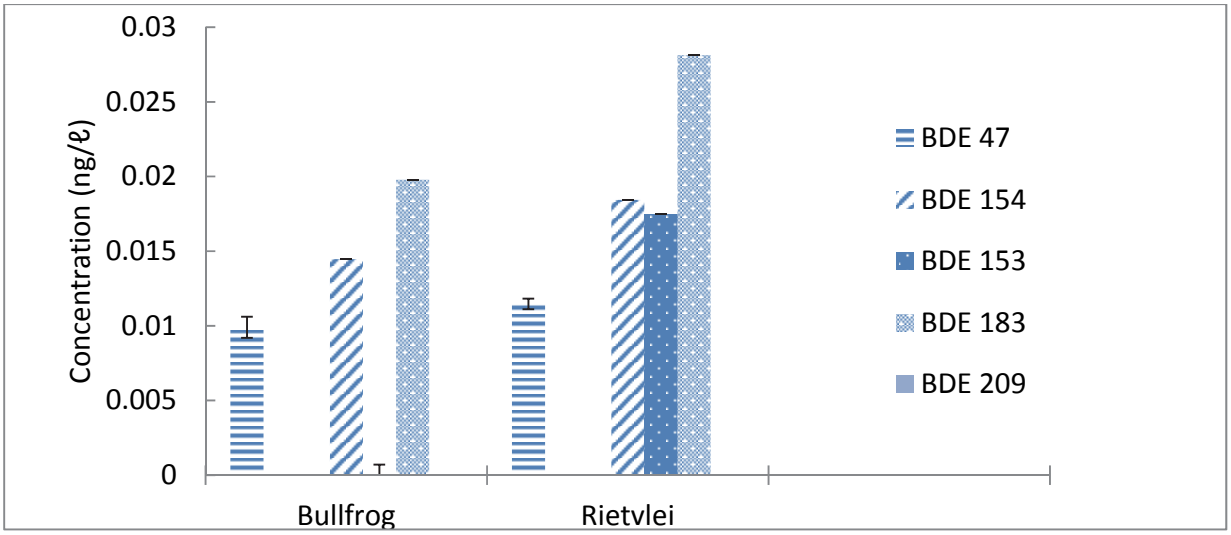


Figure 4.13: PBDE concentrations in wetland water (winter)

4.5.2 BDE concentrations in wetland sediment samples

With respect to BDE in wetland sediment samples, the following BDE were detected: BDE-47, -99, -100, -153, -154, -183 and -209 with Karlspruit wetland exhibiting the highest levels followed by Klip River wetland and finally Rietvlei as can be seen in Figure 4.14. Lower levels of BDEs were observed in summer compared to winter (Figure 4.15) with the exception of the Klip River that was not sampled because of lack of accessibility.

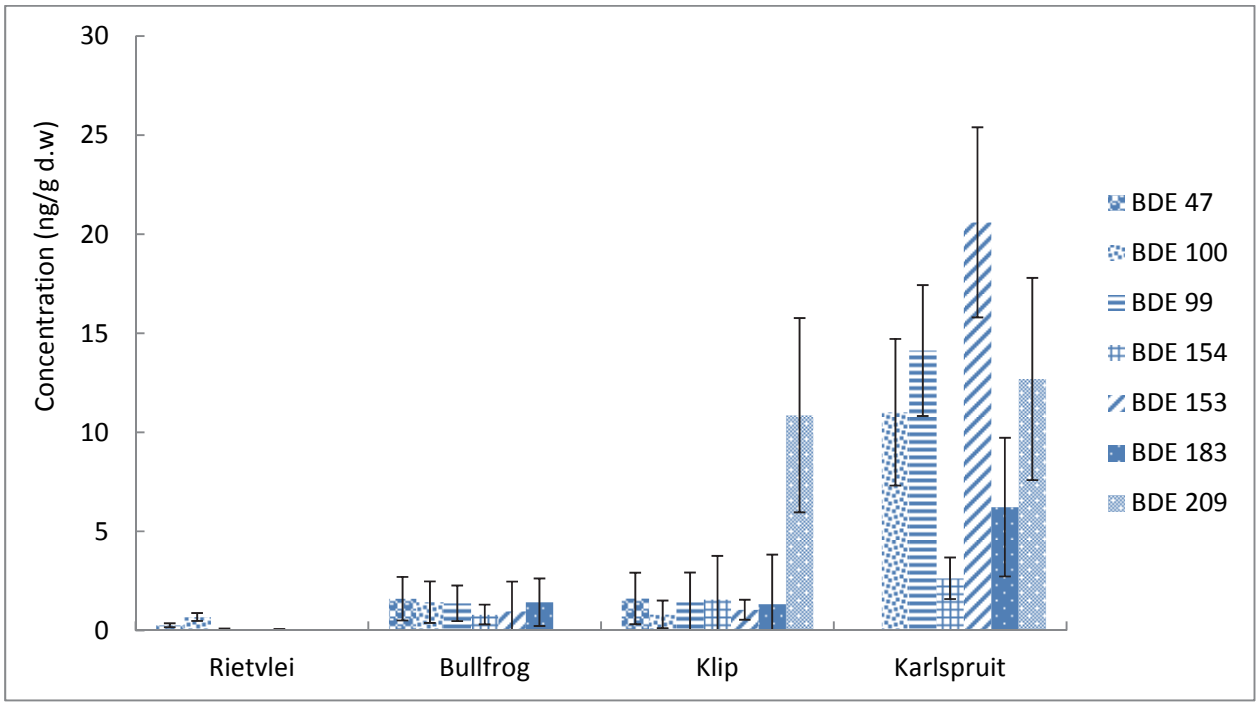


Figure 4.14: PBDE concentrations in wetland sediment (winter)

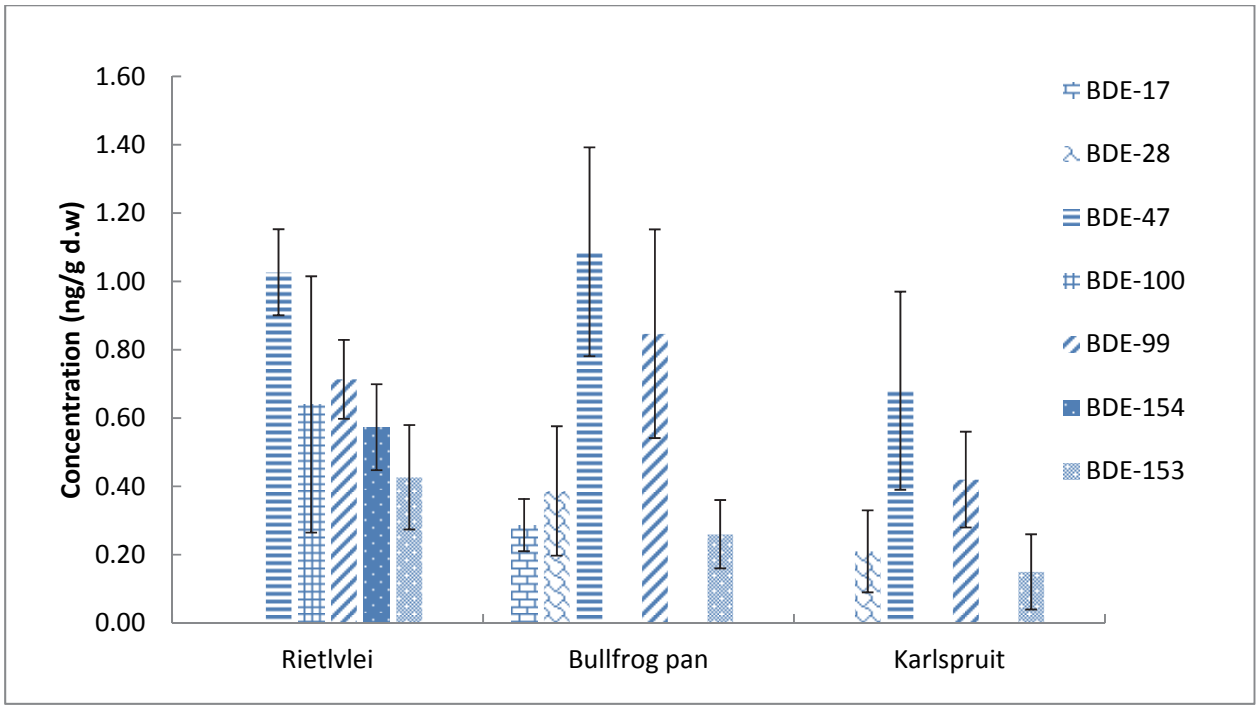


Figure 4.15: PBDE concentrations in wetland sediment (summer)

The BDE concentrations in wetland sediment sampled from Soshanguve are shown in Figure 4.16. As can be seen in Figure 4.16, all the congeners were detected except BDE-154 and 183. Winter sediment sample could not be collected since the wetland was dry.

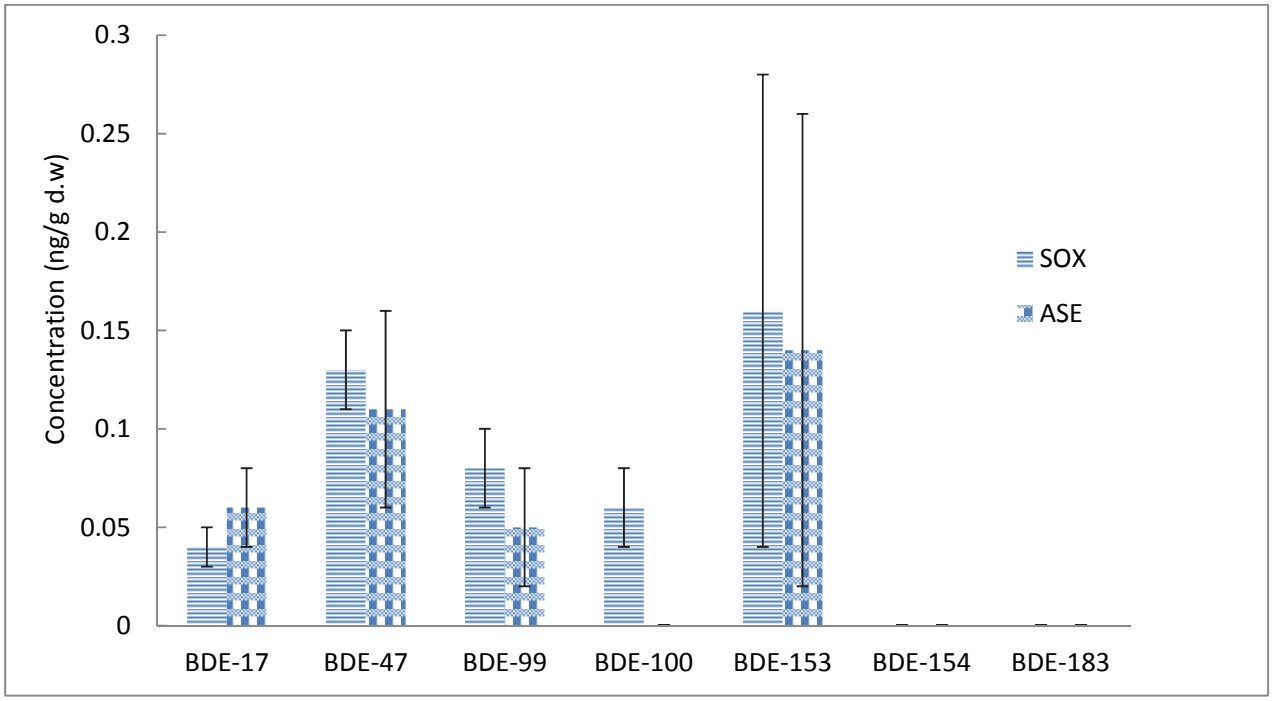


Figure 4.16: PBDE concentrations in Soshanguve wetland sediment (summer)

4.6 BDE CONCENTRATIONS IN LANDFILL SAMPLES

4.6.1 BDE concentrations in landfill leachate (water) samples

Figure 4.17 shows typical chromatograms of BDEs in landfill leachates. Figure 4.18 shows BDE concentrations in leachate samples collected in summer from Hatherly and Soshanguve. As can be seen from Figure 4.17, BDE-47 exhibited the highest concentrations from both landfill sites. The order for the other congeners was as follows: BDE-154> -99>153>100>17>183. BDE-183 was not detected in Soshanguve leachates and 209 from either of the landfill sites. Generally, the BDE levels in Hatherly were slightly higher than the levels in Soshanguve. In contrast, PBDE levels in summer samples for the remaining sites were observed to be around the limits of detection and, therefore, not quantified. Dilution effect as a result of infiltration of rain into the landfills may have contributed to the observed outcome.

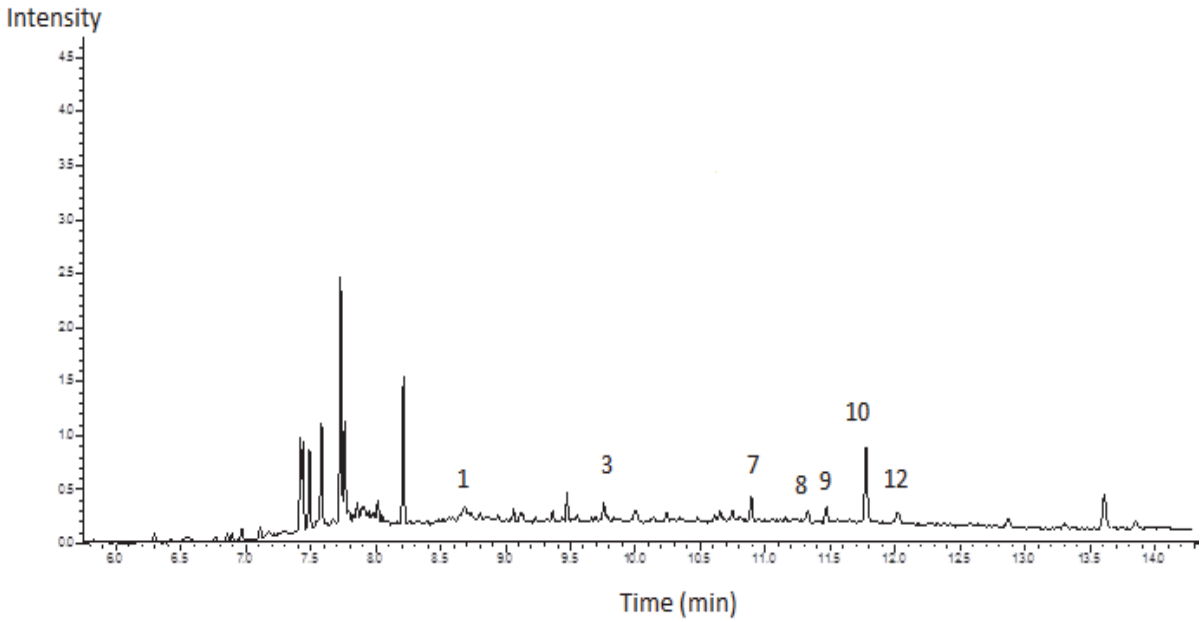


Figure 4.17: Representative chromatograms (TIC) of BDEs in leachate for summer samples (5= BDE-47, 8= BDE-100, 9= BDE-99, 12, BDE-154, 13= BDE-183).

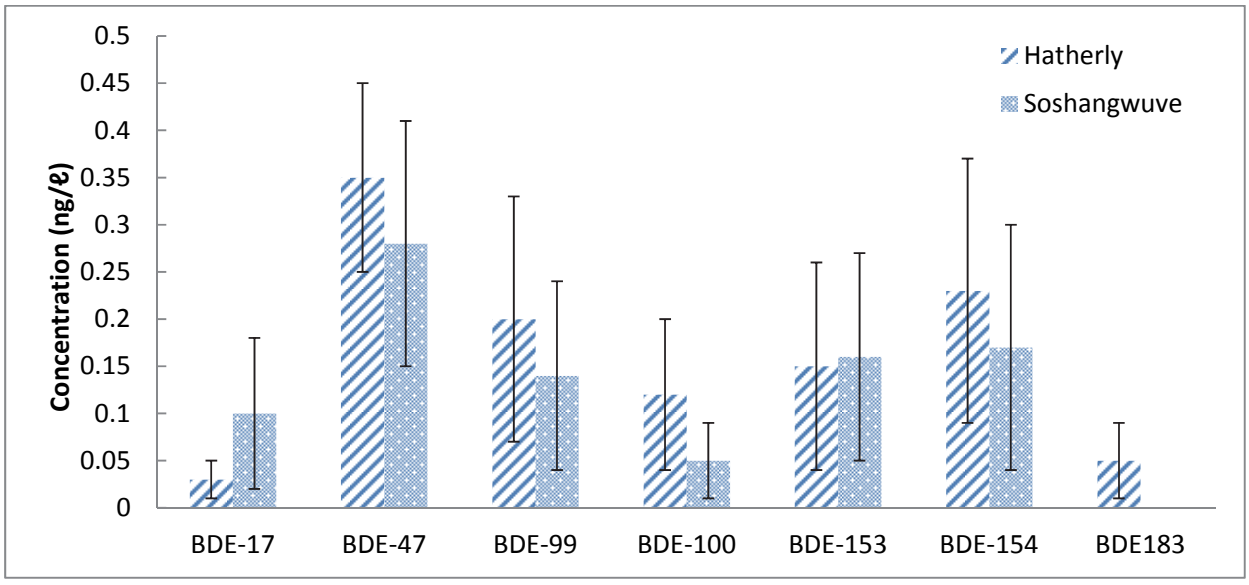


Figure 4.18: PBDE concentrations in leachate samples (summer)

Figure 4.19 shows typical chromatograms of BDEs in landfill leachates while Figure 4.20 shows levels of BDE in landfill leachates for winter. BDE-47, 99, 100, 153, 154, 183 and 209 were all detected in the leachate samples. BDE-209 was the highest overall concentration of 1.9 ng g^{-1} for Chloorkop. This was followed by BDE-100 with a concentration of 1.32 ng g^{-1}, BDE-47 at 0.98 ng g^{-1} and BDE-209 for Robinson deep at 0.51 ng g^{-1}. The profiles of PBDE congeners detected at each of the six landfill sites are as shown in Figure 4.20.

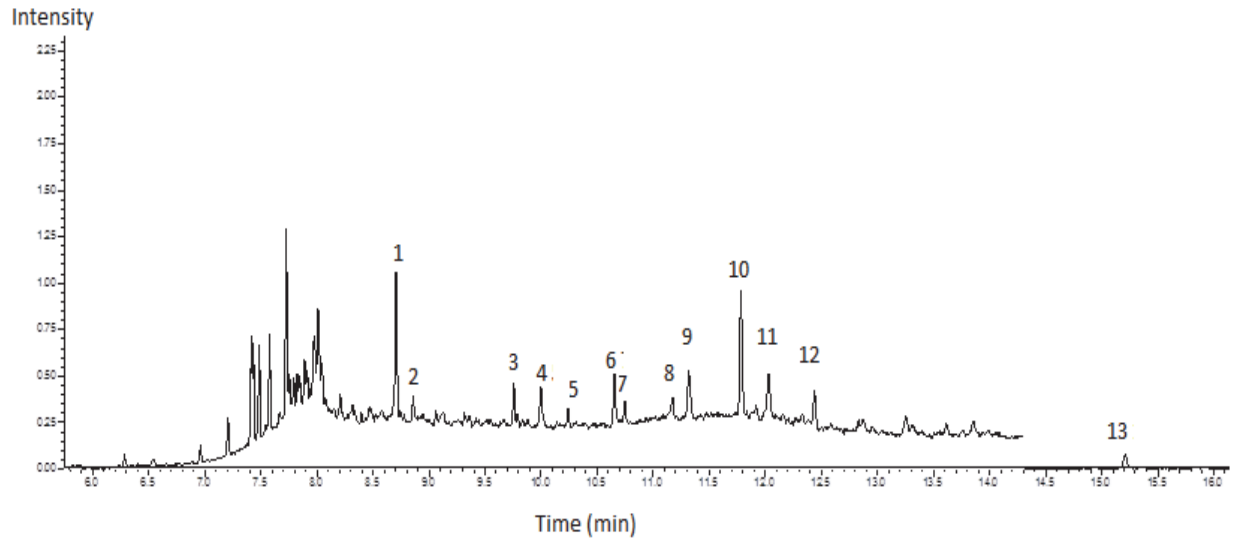


Figure 4.19: Representative chromatograms (TIC) of BDEs in leachate for summer (A) and winter (B) samples (5= BDE-47, 8= BDE-100, 9= BDE-99, 12, BDE-154, 13= BDE-183)

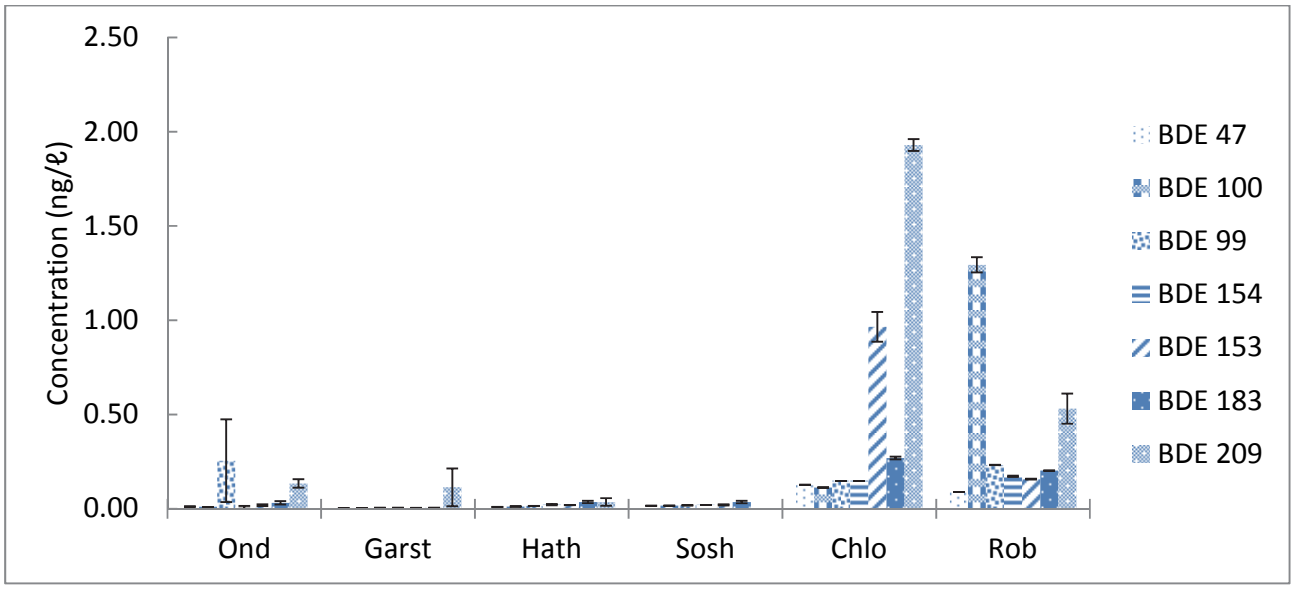


Figure 4.20: Mean concentration of BDE in landfill leachates samples (winter)

4.6.2 PBDE concentrations in landfill sediment samples

Figure 4.21 shows the BDE levels in winter (dry season) sediments. As can be seen from Figure 4.21, BDE-47, -99, -100, -153, -154, -183 and -209 were all detected from the landfill sites sampled. The highest value (4.2 ng g^{-1}) was recorded for BDE 209 from Garstkloof. This was followed by BDE-153 from Robinson deep (3.8 ng g^{-1}). Garstkloof landfill site is one of the oldest landfill sites within the City of Tshwane Metropolitan Municipality and handles about 45,000 ton/month of building, garden and household wastes. Large volume and different types of waste dumped into Garstkloof may have contributed to the observed high values. From Figure 4.21, it can be seen that BDE-209 was the highest in Garstkloof landfill site. Surprisingly, Chloorkop landfill site which receives hazardous waste exhibited low levels of BDE compared to Garstkloof which is a municipal landfill site. Hatherly showed the lowest BDE values compared to other sites. This site is believed to be lined with geomembrane material. It is possible that some adsorption of the contents of the leachate may have occurred and thus the observed low levels in the winter sample. Soshanguve landfill site which is not lined and is 10 years old showed significantly higher levels of BDE compared to Hatherly which is about 8 years old and receives building, garden and household wastes.

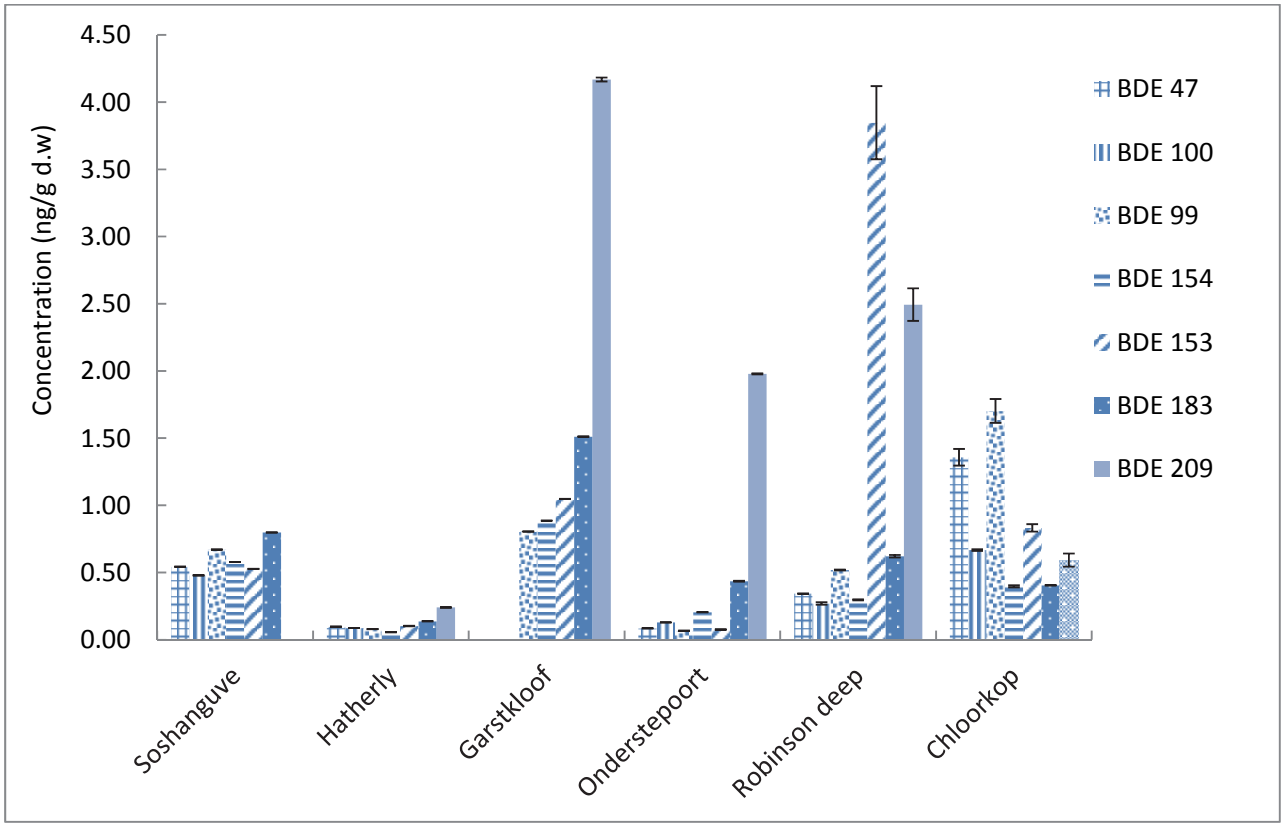


Figure 4.21: PBDE profile of landfill sediment (winter)

Figure 4.22 shows PBDE profile in summer (wet season) sediment samples and Figure 4.23 shows the ∑PBDEs observed per site in the sediment samples for the two seasons i.e., summer and winter. As can be seen in Figure 7.22, ∑PBDEs were higher in winter samples for the landfill sites compared to summer except for Hatherly and Soshanguve. It is unclear what brings about this difference.

A comparison of the observed PBDE concentrations (Figure 4.24) between landfill leachates and sediment was carried out and as can be seen Figure 4.24, landfill sediment samples exhibited higher levels of BDE. This is expected since sediments are considered as sinks for contaminants compared to the liquid component of any water system. Further comparison was performed to explore whether there was a relationship between high/low PBDE concentrations in leachates with the corresponding high/low PBDE levels in sediment. The results (Appendix A) however, showed a negative correlation between leachates and sediment concentrations of PBDE except for Garstkloof landfill site (r = 0.27).

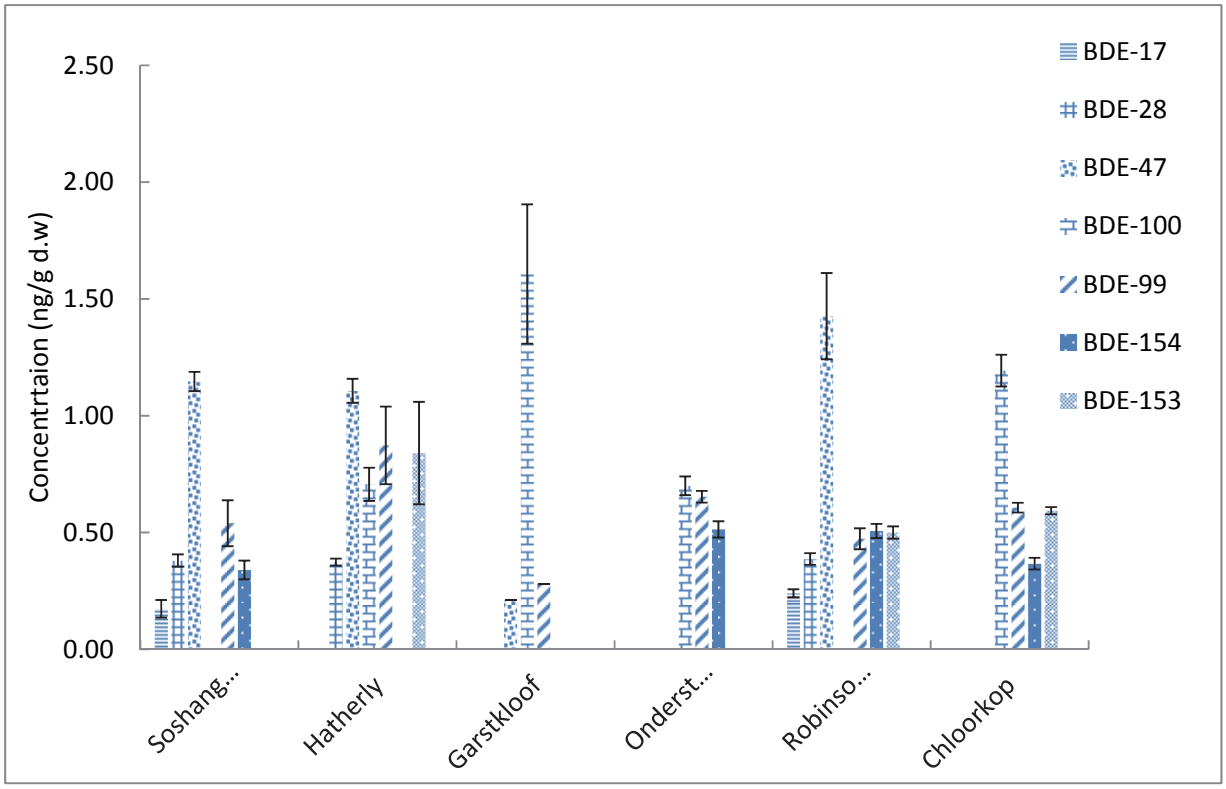


Figure 4.22: PBDE profile of landfill sediment (summer)

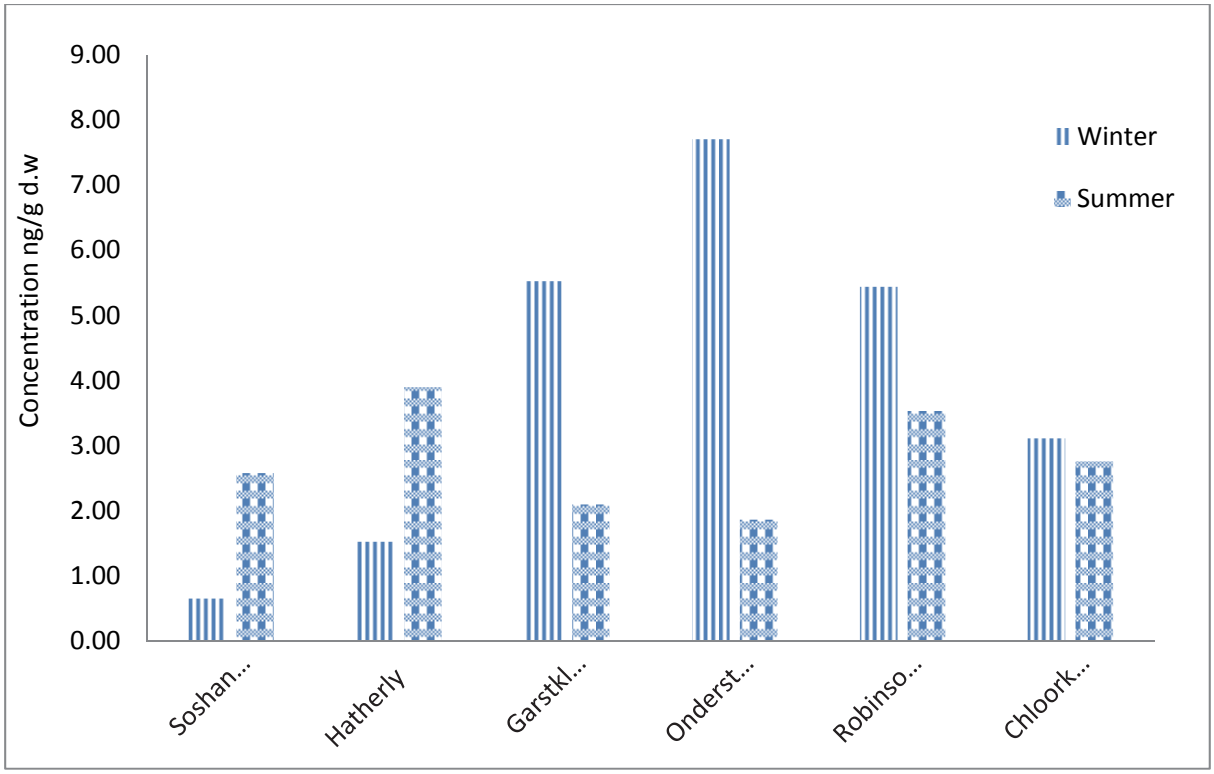


Figure 4.23: Observed ∑PBDE per site in landfill sediment samples for winter (dry) and summer (wet) seasons.

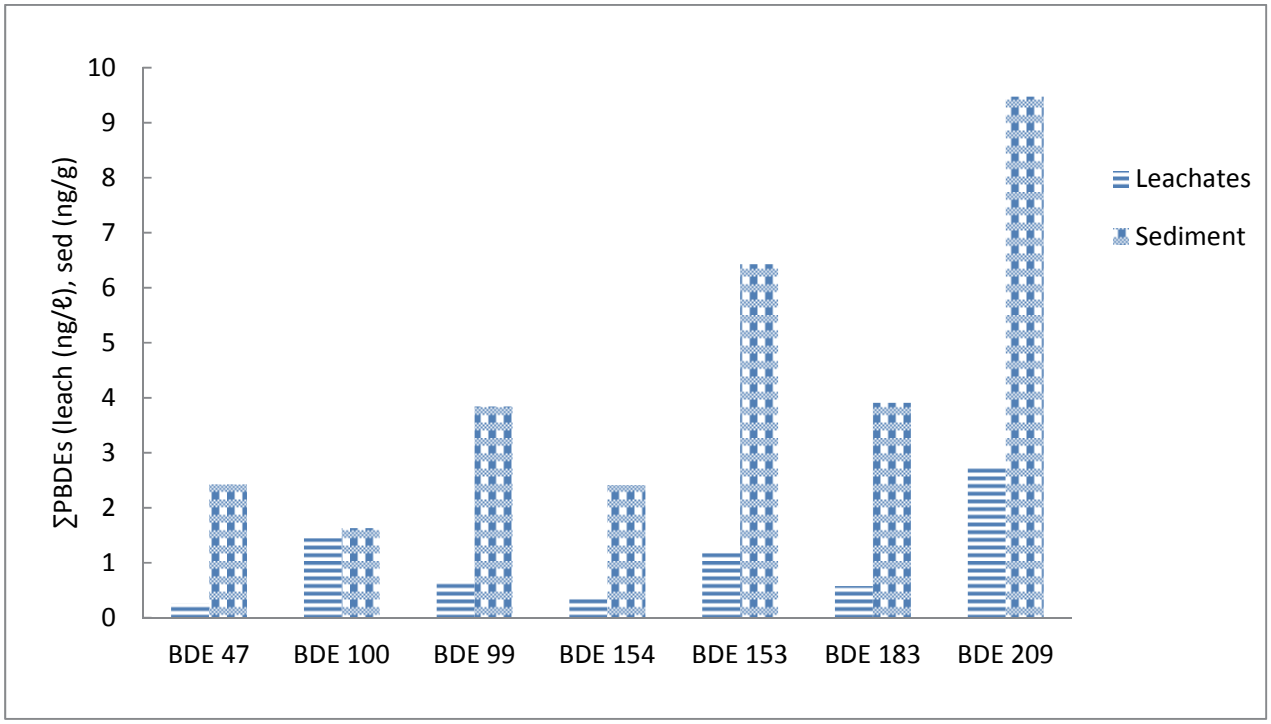


Figure 4.24: Sum PBDE concentrations in landfill leachates and sediment

The detection of BDE-17, -47, -99 and BDE-153 in all the samples indicated that these were the most common BFRs in the samples analysed. One could, therefore, suggest that these congeners can be used as preliminary screening indicators of BFRs contamination in environmental matrices. The levels of BFRs obtained in this study, however, were found to be lower than the values (23.7-2253 ng g^{-1} d.w) obtained from other parts of the world (Binelli et al., 2007; Chen et al., 2006; Li et al., 2010; Samara, 2006). The heavy rain experienced in late 2012 and early 2013 may have affected the levels of PBDEs observed in this report.

4.7 FISH SAMPLE ANALYSIS

4.7.1 Fat removal

The fat from the muscle tissue was determined gravimetrically to determine the level of fat from the sample. The fat contents in 12.5 g of tissue muscle are tabulated in the Table 4.3.

Table 4.3: Percentage fat contents in tissue muscle samples

g/g		%Fat
0.62	0.05	5
0.52	0.04	4.1
0.54	0.04	4.3

For fat removal, several removal methods were tested and the best results for was exhibited by the combination of aminopropyl cartridges with silica gel treatment. The procedure removed ~99% lipids from the extracts with %RSD of 0.198 as shown in Table 4.4.

Table 4.4: Optimum results after silica gel with aminopropyl cartridge for fat removal

Mass fat	% fat	% fat removed	Avg %fat removed	Std dev	%RSD
0.0045	0.036	99.19			
0.0038	0.030	99.32			
0.0025	0.02	99.55			
0.0055	0.044	99.02			
0.0036	0.028	99.35	99.27	0.197	0.198

Std dev = standard deviation; RSD = Relative standard deviation

4.7.2 Recovery studies

Heptafluorobutyric anhydride (HFBA) was chosen for the present study because of its rapid and quantitative reaction, the formation of stable products, excellent chromatographic properties for the targeted analytes as well as the availability of the reagent**.** The chromatograms are shown in Figure 4.25 and the selected ions monitored are shown in Table 4.5. The recoveries of targeted compounds as shown in Figure 7.25 ranged from 5.5%(TBBPA)-78.7% (PBB49).

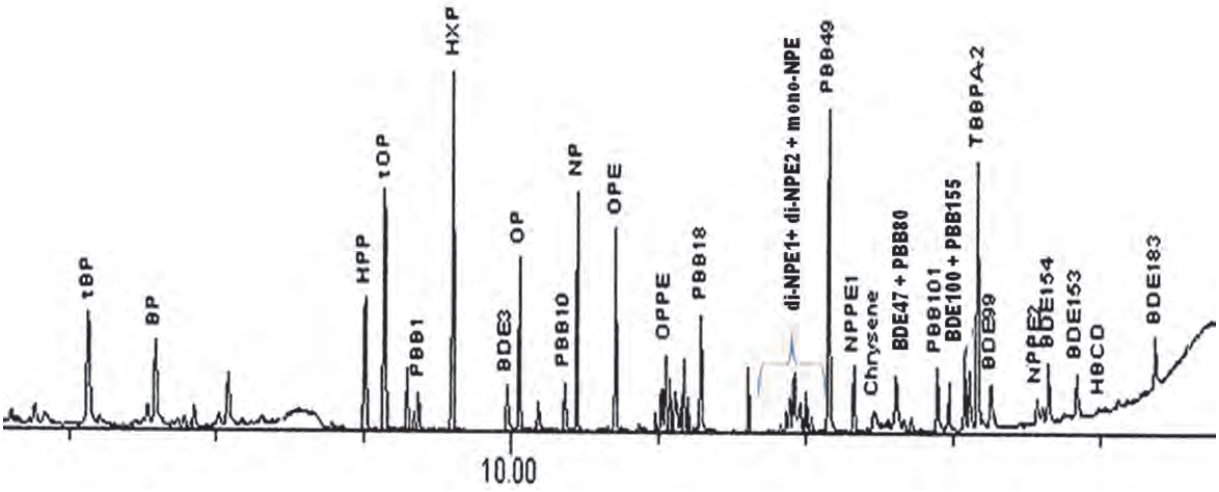


Figure 4.25: Typical chromatogram of derivatized APEs, TBBPA in the presence of PBBs, PBDEs and HBCD

Table 4.5: Ions for selected ion monitoring of heptafluorobutyric derivatives

Compound	Fragment ions *m/z*		**Compound**	Fragment ions *m/z*
t-BP	331.1; 303		PBB1	232
n-BP	303.1; 345		PBB10	311.9
HXP	303.1; 374.2		PBB18	310.9; 232; 389.8
t-OP	331		PBB-49	309.9; 469.7; 388.8
HPP	302.90; 387.9		PBB101	228; 389; 468.7; 549
OP	303.1; 402.1		PBB155	642; 307; 467
NP	302.9; 416.1		BDE3	248
OPE	375.1		BDE28	405.8; 245
OPPE	389.1; 375.1; 361.1; 431.1		BDE-47	485.7
di-NPE2	433.1; 419.1; 405.1; 475.1		BDE99/100	404; 564
di-NPE1	419.1; 433.2; 405.1; 475.4		BDE153/154	483.7; 643
mono-NPE	433.1; 461.1		BDE183	561.7; 723.6
NPPE1	463.2		BDE209	642; 721
NPPE2	551.1		TBBPA	489; 724; 739
			HBCD	239.1; 560.8

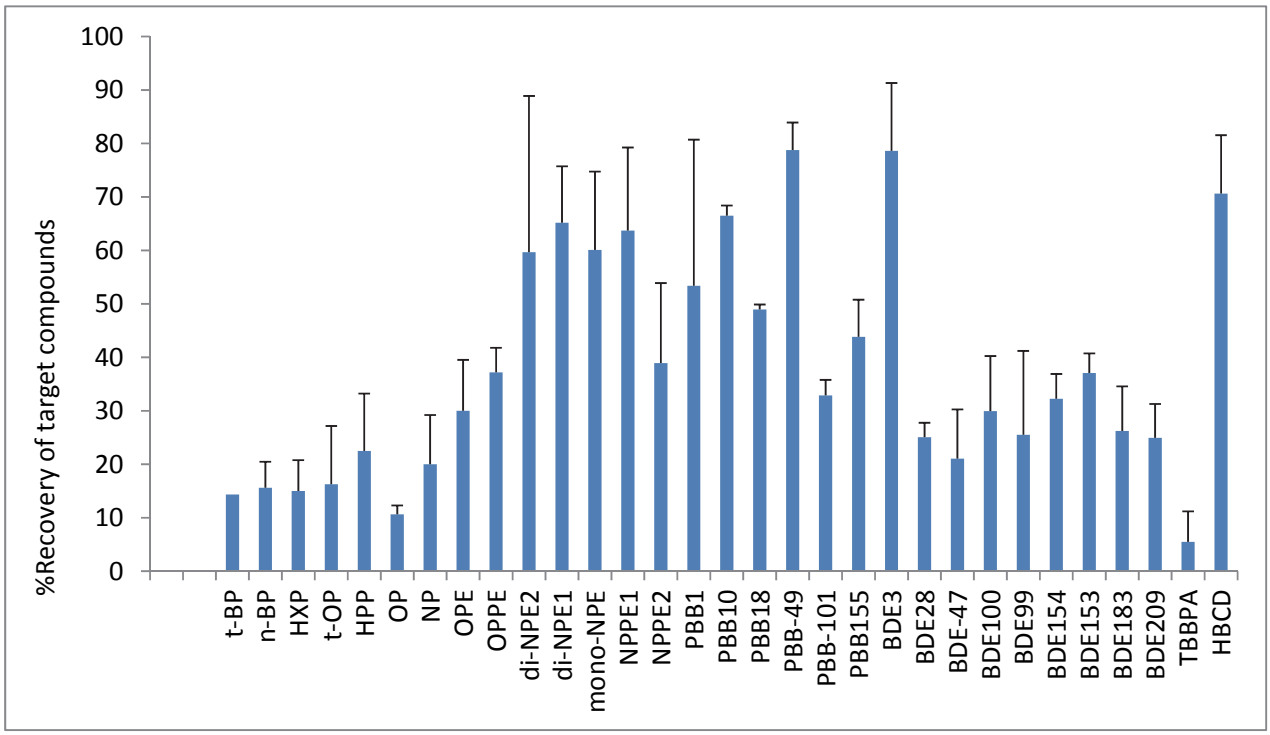


Figure 4.26: Recoveries of targeted compounds from fish sample

4.7.3 Analysis of targeted compounds in fish samples

Four bottom feeders, *Labeo umbratus* (% fat~ 2.4; n=3), collected at the Vaal Barrage, were extracted, purified and analyzed by GC-MS. These samples (as presented in Figure 4.27) indicated that nonylphenol ethoxylates isomers are major pollutants of the APEs while PBB101, BDE (-3, -28, -99, -100 and -183) and HBCD were major pollutants for the BFRs. The concentrations of these analytes ranged from 0.061 (BDE-3) to 4.6 ng g^{-1} (di-NPE1). The method as developed can be extended to determine the mentioned analytes from a range of fish samples around the Vaal River catchment.

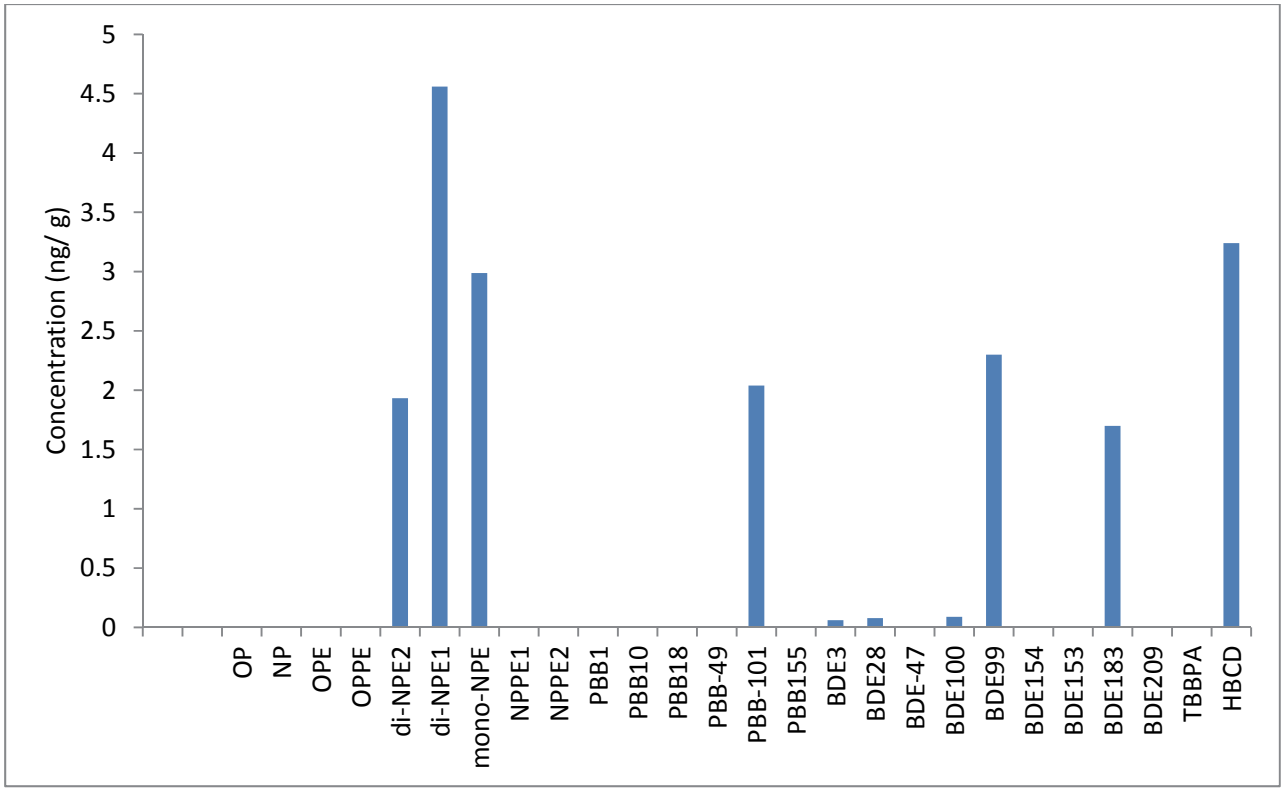


Figure 4.27: Concentrations of APEs and BFRs in Labeo umbratus, a sediment feeder, from the Vaal Barrage

4.7.4 General discussion of results

The results obtained from the fish sample were compared to other results reported in the literature in different types of fish (Table 4.6). Compared to other studies, the levels of NP1E and NP2E detected in the present study are comparably lower than the levels reported in carp fish (Datta et al, 2002; Rice et al, 2003), mussel (Cathum & Sabik, 2001) Wayelle (Schmitz-Afonso et al, 2003) and in *Orechromis sp* (Peng et al, 2006). However, the comparison is not a true reflection as most studies used fish like *carp*; that has a different feeding pattern like the *Labeo umbratus*, to analyze the level of the compounds in fish matrix. It can be seen in Table 7.6 that HBCD ranks second in concentration to NP2E in the current study.

Table 4.6: Comparison of detected concentrations (ng g-1) of APEs and BFRs with other studies

NP1E	∑NP2E	PBB - 101	BDE -100	BDE -99	BDE -183	HBCD	Fish type	Ref
9.4	11	n.a	n.a	n.a	n.a	n.a		
82	23	n.a	n.a	n.a	n.a	n.a		
98	17	n.a	n.a	n.a	n.a	n.a		
100	20	n.a	n.a	n.a	n.a	n.a	Carp	Rice et al, 2003
550	240	n.a	n.a	n.a	n.a	n.a		
350	120	n.a	n.a	n.a	n.a	n.a		
250	100	n.a	n.a	n.a	n.a	n.a		
<MDL	177	n.a	n.a	n.a	n.a	n.a		
<MDL	823	n.a	n.a	n.a	n.a	n.a	Mussel	Cathum & Sabik, 2001
2075	567	n.a	n.a	n.a	n.a	n.a		
700	295	n.a	n.a	n.a	n.a	n.a	Carp	Datta, Loyo-Rosales & Rice, 2002
248	473	n.a	n.a	n.a	n.a	n.a		
	2400	n.a	n.a	n.a	n.a	n.a	Wayelle	Schmitz-Afonso et al, 2003
n.a	n.a	n.a	2.51	0.82	0.107	n.a		
n.a	n.a	n.a	4.21	1.39	0.421	n.a		
n.a	n.a	n.a	4.25	5.4	1.03	n.a	*Oreochromis sp*	Peng et al, 2007
n.a	n.a	n.a	10.7	6.5	1.03	n.a		
n.a	n.a	n.a	24.2	21.6	0.728	n.a		
n.a	n.a	n.a	4.67	3.37	1.15	n.a		
2.98	6.49	2.04	0.09	2.3	1.7	3.24	*Labeo umbratus*	***This study***

n.a = not applicable

CHAPTER 5: DEVELOPMENT AND TESTING OF SAMPLE PRE-CONCENTRATION EXTRACTION KIT

5.1 INTRODUCTION

The unique differences in the chemical properties of emerging organic pollutants tend to necessitate the need for different analytical techniques such as liquid and gas chromatographic techniques for their analysis. Due to these challenges, the cost of analysis associated with the determination of these pollutants is constantly increasing on an annual basis. Therefore, the need for the development of improved analytical protocols that can allow for the simultaneous determination of a wide range of emerging organic pollutants in a single extraction step cannot be overemphasized. The developed sample pre-concentration extraction, which entirely favours gas chromatographic technique, offers this unique advantage through the *in-situ* chemical transformation of certain polar organic compounds in the presence of some non-polar organic compounds

This section describes the development of a pre-concentration extraction kit for monitoring persistent organic pollutants and emerging organic pollutants. For this experiment, tetrabromobisphenol A (TBBPA) was targeted because of its non-volatility. Generally, the use of hyphenated techniques based on liquid chromatography mass spectrometry has remained the method of choice for the determination of TBBPA (Gorga et al., 2013). However, there are certain drawbacks such as signal suppression often caused by sample matrix that is associated with this technique. Furthermore, the structural elucidation of this compound and its degradation products is more problematic because the libraries of LC-MS are less complete than those available for GC-MS (Noche et al., 2011). Consequently, the use of gas chromatography continues to gain increased relevance for its determination due to its enhanced separation capabilities and availability in major laboratories around the world. As shown in Figure 5.1, the two hydroxyl groups attached to the phenyl groups need to be completely replaced with relatively non-polar substituents in order to obtain a good chromatography.

Generally, there are two approaches to achieve the derivatization of the phenolic compound in aqueous media. The first approach involves the extraction of the TBBPA into an organic solvent that is immiscible with water. This procedure often involves several additional steps of extraction and extracts clean-up which could make the entire analytical procedure cumbersome and labour-intensive. The approach employed in this exercise involves the use of an in situ derivatization technique where a water-soluble derivatizating reagent is employed to produce non-polar TBBPA derivatives. The newly modified compound may then be preferentially extracted into an appropriate organic solvent. In this study, we aim to explore the advantages of

this unique extractive in situ derivatization technique to develop a single-step sample preparation technique for TBBPA in aqueous matrices prior to GC-MS analysis.

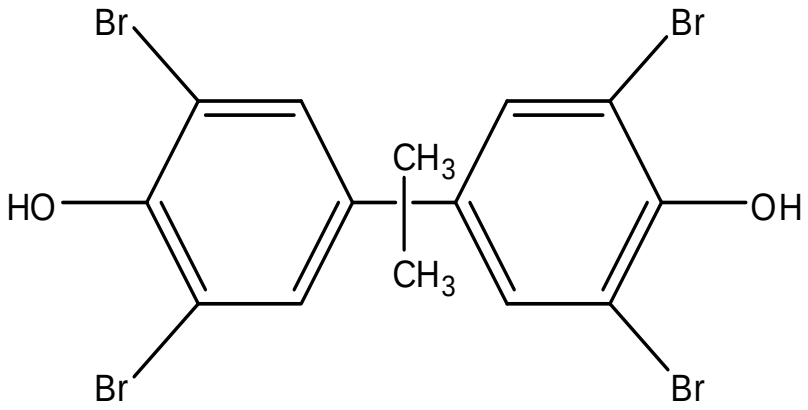


Figure 5.1: The chemical structure of Tetrabromobisphenol A (TBBPA)

5.2 METHOD DEVELOPMENT AND OPTIMIZATION

5.2.1 Reagents and other materials

HPLC grade organic solvents (n-hexane, isooctane, dichloromethane, toluene and methanol) were purchased from Sigma-Aldrich (South Africa). Unlabelled TBBPA, BDE 47, 99, 153, 183 and 209 were purchased from Wellington Laboratories (Ontario, Canada). ^{13}C-BDE 139 employed as internal standard was purchased from Cambridge Isotope Laboratories (MA, USA). Acetic anhydride (>98% purity) and acetic anhydride (for GC derivatization, 99.9%) were purchased from Sigma-Aldrich (South Africa). Florisil® (60-100 mesh), ascorbic acid (99% purity), anhydrous sodium sulphate and potassium carbonate (99.9% purity) were also purchased from Sigma-Aldrich (South Africa). SPE cartridges, including Supelco ENVI-18™ and Phenonemon Strata FL-PR® were supplied by Sigma-Aldrich (South Africa) and Separation (Pty) Ltd. (South Africa), respectively.

5.2.2 Working solutions

A solution of 0.2 ng $\mu\ell^{-1}$ TBBPA prepared in methanol was employed for the optimization of the *in situ* derivation procedures. To investigate the effect of the derivatizing reagent on other BFRs which were simultaneously extracted with the TBBPA derivative, a spiking of 0.2 ng $\mu\ell^{-1}$ of BDE -47, -99, -153 and -183 and 2 ng $\mu\ell^{-1}$ of BDE 209 and HBCDD was prepared. Finally, the concentration of ^{13}C-BDE 139 employed as internal standard for quantitative analysis was 0.1 ng $\mu\ell^{-1}$.

5.2.3 Samples

The preliminary laboratory studies were based on the use of deionized water (EQOUAVA Water Technologies, Barsbüttel, Germany) as the aqueous medium. However, the developed *in situ* derivatization technique was applied to landfill leachate samples which were collected from the Robinson landfill site situated in central Johannesburg during the 2014 winter season.

5.2.4 Chromatographic conditions

The analysis of TBBPA derivative and other BFRs was performed on Ultra-trace 2010 Shimadzu Gas Chromatograph equipped with QP 2010 Ultra mass spectrometer operated in electron ionization mode. All the target compounds were separated on a DB-5 MS (15 m, 0.25 mm i.d., 0.1 µm film thickness) capillary column. High purity helium (99.999%) was used as a carrier gas at a flow rate of 2.33 mℓ min^{-1}. The oven temperature was programmed initially from 90^{o}C (held for 1 min) to 200^{o}C at 40^{o}C min^{-1}, it was further ramped at 25^{o}C min^{-1} to 250^{o}C. Finally, the temperature was increased to 310^{o}C at 7.5^{o}C min^{-1} and was held for 5 min. The GC injector was maintained at 270^{o}C and was operated in splitless mode. The mass spectrometer was operated in electron ionization mode (EI: 70 eV). The interface temperature was maintained at 280^{o}C, while the ion source temperature was set at 250^{o}C. The injection of target compounds was recorded in both full-scan (200-1000 amu) and SIM modes under similar chromatographic conditions, where the characteristic ions for each target compound were selected. To assess the efficiency of the derivatization, it was necessary to monitor the characteristic ions of both the TBBPA derivative and the underivatized TBBPA using the scan mode. The details of the target as well as the confirmation ions for each target compound, which were employed for identification and quantitative purposes, are presented in Table 5.1.

Table 5.1: Identification parameters employed for target compounds

Mode of analysis	Target compounds	Retention time (min)	Target ions (m/z)	Confirmation ions (m/z)
Scan	TBBPA derivative	7.710	543.00	528.00, 546.00
SIM	BDE 47	5.439	326.00	486.00, 324.00
	BDE 99	6.220	406.00	404.00, 566.00
	BDE 153	7.218	484.00	482.00, 643.00
	^{13}C-BDE 139 (IS)	7.332	496.00	498.00, 658.00
	Total HBCDD*	7.464	239.00	237.00, 399.00
	TBBPA derivative	7.710	544.00	529.00, 546.00
	BDE 183	8.439	562.00	564.00, 723.00
	BDE 209	14.919	799.00	401.00, 797.00

IS – internal standard employed for quantitative analysis; * - sum of α, β and ϒ-HBCDD isomers.

In all cases, data acquisition was performed with Shimadzu Labsolution (GCMS solution) and the target compounds were identified by comparison with the mass spectrum generated by a standard solution of the derivatized compound and with that of the NIST 2008 database in the MS library for other compounds.

5.2.5 Derivatization and extraction

One hundred millilitres of MilliQ water was measured into a conical flask (or a separatory funnel for liquid-liquid extraction (LLE)) and was fortified with 500 µℓ of methanol. On addition of 500 µℓ of 0.2 ng $µℓ^{-1}$ TBBPA, the set-up was allowed to equilibrate for approximately 30 min. After the equilibration, 2.0 g K_2CO_3 and 2.5 g of ascorbic acid in this order were added and the resulting solution was vigorously shaken until all the additives were completely dissolved. The addition of the carbonate provides the alkaline condition that is required for the derivatization, while the ascorbic acid often acts as a strong oxidising agent to deprotonate the TBBPA. To investigate the efficiencies of the derivatizing reagent, different volumes (2.5, 5.0, 10.0, 20.0 and 30.0 mℓ) of the reagent were tested. Once the reagent is added, the solution is shaken and the derivatizing reagent is allowed to react with the TBBPA in the solution for a specific time.

Both LLE using separatory funnel and solid-phase extraction (SPE) techniques were employed in this study. The preliminary optimization of the *in situ* derivatization was performed using the LLE where the efficiencies of different extraction solvents, namely n-hexane, dichloromethane and toluene were investigated. However, the efficiencies of the different SPE-based approaches which were also evaluated were mostly based on the optimized conditions from the LLE technique. For clarity, pre-concentration columns were packed with 5 g of deactivated silica gel and 5 g of Florisil, respectively. These were compared with two different commercial SPE cartridges, namely ENVI-18 and FL-PR. Prior to their use these SPE gadgets were preconditioned with suitable organic solvents to remove trapped air and possible interfering contaminants. Specifically, the commercial SPE cartridges were conditioned with 5 mℓ n-hexane, 5 mℓ dichloromethane, 5 mℓ methanol and 5 mℓ MilliQ water in that particular order. Similarly, the self-made SPE glass columns were conditioned with 20 mℓ of methanol followed by 20 mℓ of MilliQ water.

Following the completion of the reaction time, the TBBPA derivative was extracted with 20 mℓ of dichloromethane using a separatory funnel. The extraction was repeated using the same extractant volume. The combined extract which was collected over anhydrous Na_2SO_4 was then concentrated under vacuum to a suitable volume using a rotary evaporator. The concentrated extract was quantitatively transferred into a pre-weighed amber vial and the final weight of the vial was also determined prior to the GC-MS analysis. It is, however, worth mentioning that the LLE technique was only employed for the extraction of the TBBPA derivative but was not employed for the simultaneous extraction of the TBBPA derivative and other BFRs as it was the case for the SPE techniques employed.

For both the commercial SPE cartridges and the self-made SPE kits, the spiked sample or sample was loaded at a uniform flow rate of approximately one drop per second. The sample container was rinsed with 10 mℓ of MilliQ water which was also loaded onto the SPE cartridge and kit. After the extraction, the SPE cartridges and kits were vacuum dried for about 45 min. The adsorbed target compounds were then eluted with 20 mℓ and 50 mℓ of n-hexane for the commercial SPE cartridges and the self-made SPE kits, respectively. The eluate was concentrated under vacuum to a suitable volume using a rotary evaporator. The concentrated extract was then quantitatively transferred into an amber vial and 200 µℓ of isooctane was added as a keeper to prevent analyte loss during further concentration steps. The extract was further concentrated to incipient dryness under a gentle stream of pure nitrogen gas. The dried extract was reconstituted with 500 µℓ of 100 ng $mℓ^{-1}$ of ^{13}C-BDE-139 employed as an internal standard and the resulting solution was vortexed prior to instrumental analysis. One microlitre of the final solution was injected into the GC-MS for quantitative analysis.

5.2.6 Method performance and validation

To evaluate the effectiveness of the *in situ* derivatization procedure, the conventional method of derivatization based on direct heating of the derivatizing reagent with the compound of interest was employed. In this study, this method was employed for the preparation of eight (8) calibration solutions against which the efficiency of the *in situ* derivatization procedure was evaluated. Precisely, a total volume of one millilitre containing different proportions of the target compounds and a uniform volume of the derivatizing reagent was prepared for each calibration level. In order to ascertain the completeness of the derivatization, the peaks of both the TBBPA and its derivatized product were simultaneously monitored in scan mode, and in all cases, no traces of the TBBPA was observed.

5.3 RESULTS AND DISCUSSION

5.3.1 Sensitivity and recovery tests

The analysis of the derivatized TBBPA resulted in enhanced sensitivity and improved chromatographic performance than the underivatized TBBPA which often produced broad peaks during the preliminary studies in Figure 5.2 -5.5. The evaluation of the analytical performance of the *in situ* derivatization in this study is limited to the estimation of the linearity and percent yield of the TBBPA derivative.

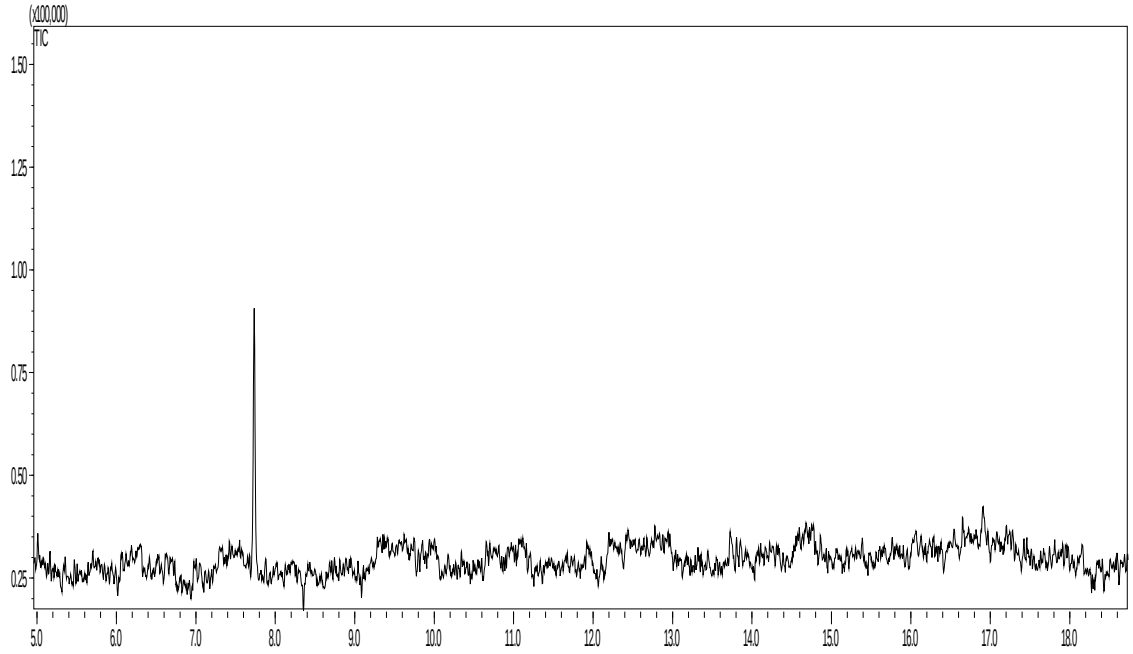


Figure 5.2: Total Ion chromatogram of TBBPA derivative

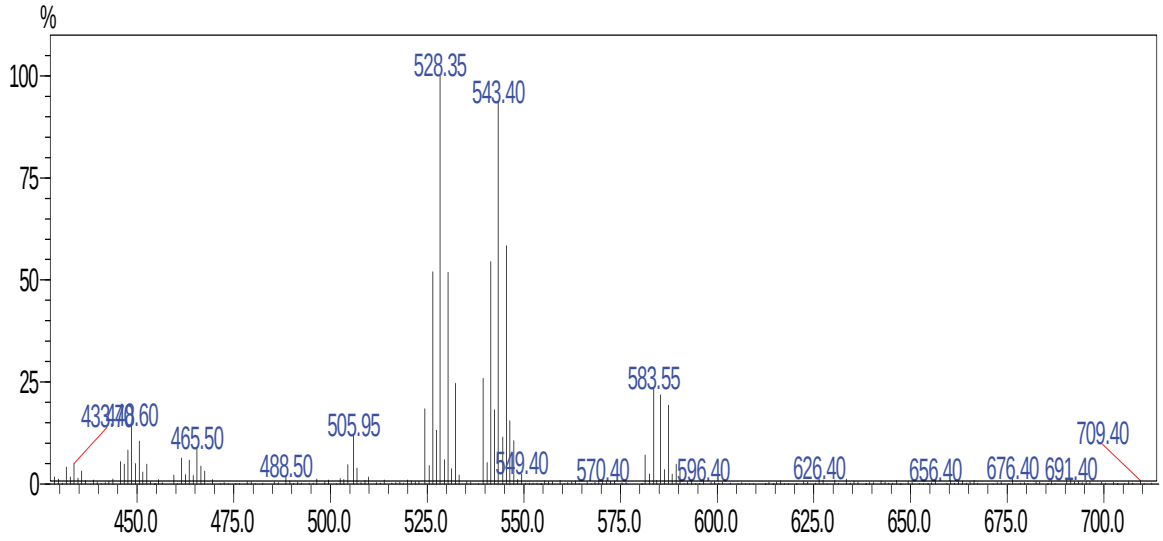


Figure 5.3: Mass spectrum of the TBBPA derivative

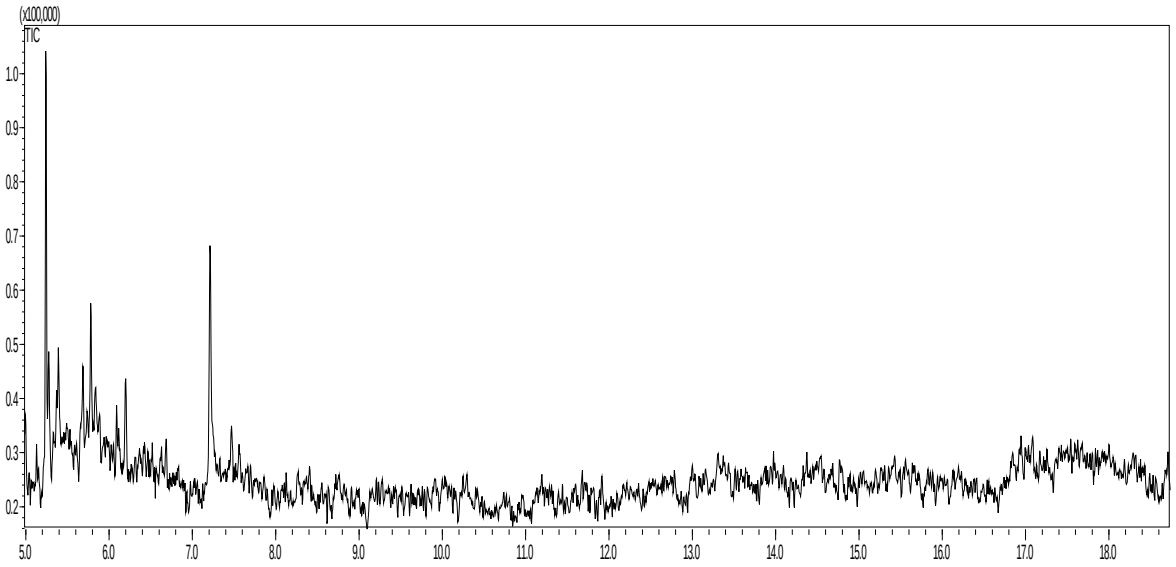


Figure 5.4: Total Ion chromatogram of underivatized TBBPA

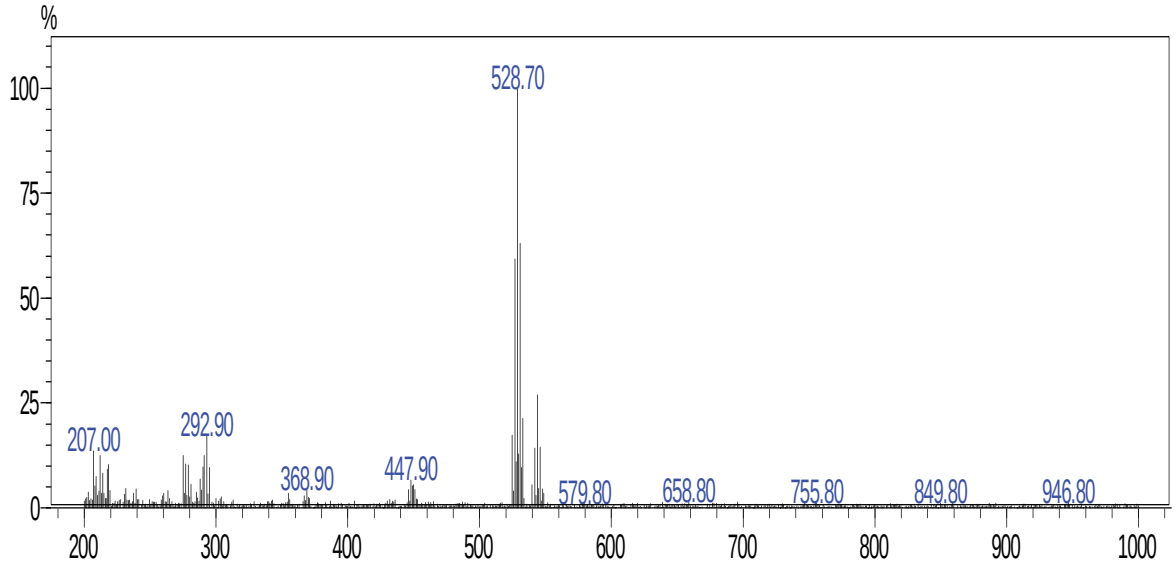


Figure 5.5: Mass spectrum of the underivatized TBBPA

As indicated in Table 5.2, the linearity of all the target compounds, including the TBBPA derivative was greater than 0.9978.

Table 5.2: Calibration parameters of the target compounds employed for the in situ derivatization procedure

Target compounds	Linear equation	Correlation co-efficient (R^2)	RSD	Calibration range
BDE 47	f(x) = 2.70x – 0.10	0.9985	8.18	10-200
BDE 99	f(x) = 2.00x – 0.03	0.9979	8.04	10-200
BDE 153	f(x) = 0.99x – 0.02	0.9985	8.76	10-200
BDE 183	f(x) = 0.25x + 0.01	0.9988	21.99	10-200
BDE 209	f(x) = 0.06x – 0.02	0.9985	19.71	100-2000
HBCDD*	f(x) = 2.70x – 0.10	0.9993	26.63	100-2000
TBBPA derivative	f(x) = 0.91x – 0.14	0.9981	22.29	10-200

RSD – relative standard deviation of the response factors; * - sum of α, β and ϒ-HBCDD isomers

Besides the determination of the linearity of the TBBPA derivative, the estimation of its percent yield will also give a good indication of the suitability of the *in situ* derivatization as an alternative to the traditional methods of analyte derivatization. In this study, the percent yield of the TBBPA derivative regardless of the extraction methods employed was estimated using equation 1.

$$\text{Percent yield (\%)} = \frac{\text{Actual concentration}}{\text{Theoretical concentration}} \text{ x } 100 \qquad \text{(Equation 1)}$$

5.3.2 Method optimization *in situ* derivatization

During the preliminary studies, several important parameters, including the amount of derivatizing reagent, reaction time, the type of extraction solvent, amongst others were optimized. The influence of these parameters on the yield of the TBBPA derivative was investigated. However, it is important to emphasize that other parameters such as the volume of the extraction solvent employed, the quantities of additives (ascorbic acid and K_2CO_3) and the degree of mixing remained the same throughout the preliminary studies.

5.3.3 Influence of amount of the derivatizing reagent

The amount of the derivatizing reagent is an important factor that requires adequate consideration during method optimization for the *in situ* derivatization procedure. To avoid unnecessary wastage of the reagent, it is important to determine the optimum volume of the derivatizing reagent that would be required for the complete derivatization of TBBPA. As previously mentioned, different volumes of the reagent were evaluated.

The results obtained from the preliminary studies presented in Figure 5.6 showed that the use of 20 mℓ of the derivatizing reagent gave the best yield of the TBBPA derivative. Consequently, this volume was employed for subsequent LLE as well as the SPE-based studies. In all cases, triplicate analyses were performed except for the experimental procedure involving the use of 30 mℓ of the derivatizing reagent, in which case, a single analysis was performed.

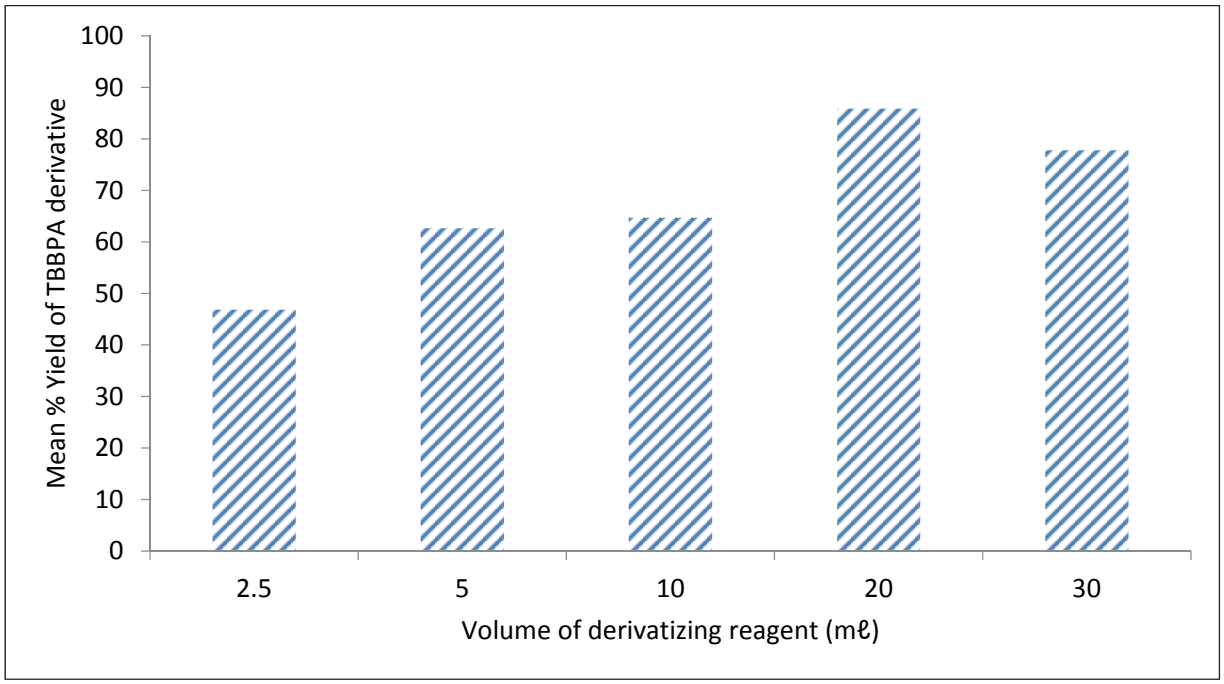


Figure 5.6: Percentage yield of TBBPA derivative at different volumes of the derivatizing reagent

5.3.4 Effect of reaction time

Acetic anhydride is a soluble reagent that reacts vigorously with water. These properties make it a unique derivatizing reagent that has been widely explored for *in situ* derivatization. However, the reaction time between the derivatizing reagent and the target compound needs to be properly evaluated in order to achieve the desired outcome. In this study, different reaction times ranging from 10 to 50 min were evaluated. As indicated in Figure 5.7, the optimum reaction time was 10 min beyond which there was possible disintegration of the TBBPA derivative.

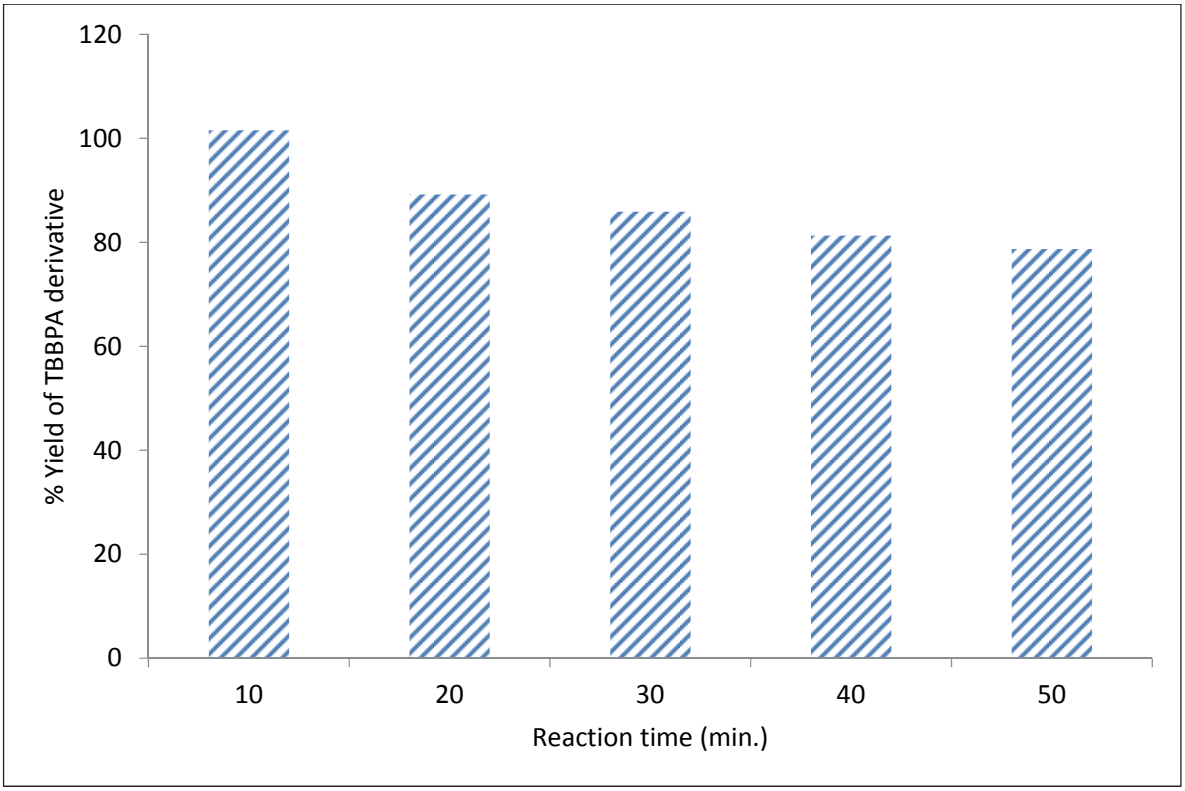


Figure 5.7: Influence of reaction time on the yield of TBBPA derivative

A unique trend on the yield of the TBBPA derivative over time was observed. In particular, a gradual decrease in the percent yield of the TBBPA derivative with increasing reaction time indicates the limited stability of the derivatized product. Furthermore, the derivatized product may reverse to its original reactants during and after the extraction process. This possibility was investigated during the preliminary studies when a sample vial containing derivatized TBBPA was reanalyzed after three (3) days. In the resulting chromatogram, traces of TBBPA which were initially absent in the original chromatogram reappeared when monitored in the scan mode.

The influence of the reaction time on the total yield of the TBBPA derivative must be given a top priority, especially while choosing appropriate extraction methods for the isolation of TBBPA derivative in aqueous media. This is because the choice of extraction technique that will unnecessarily prolong the reaction time may result in decreased yield of the TBBPA derivative as observed in the present study.

5.3.5 Choice of extraction solvent

The choice of appropriate organic solvent for the isolation of the TBBPA derivative is another important factor that requires a careful consideration. In this study, the efficiencies of three (3) individual organic solvents, namely: n-hexane, dichloromethane and toluene were evaluated. Generally, the polarities of the organic solvent as well as that of the target compound are primary factors for consideration while choosing a suitable extraction solvent. Besides, the possible health risks associated with exposure to these organic solvents must also be taken into consideration. The results of the preliminary studies focused on the evaluation of the extraction efficiencies of the different organic solvents employed are presented in Figure 5.8. In terms of the polarity of the extraction solvent, n-hexane is the least polar solvent and it gave the best yield of the TBBPA derivative with a mean (± standard deviation) of 98.51 (± 19.40). In the same vein, the percent yields of the TBBPA obtained for both dichloromethane and toluene were 63.21±4.53 and 78.22±21.36, respectively. Consequently, n-hexane was adopted as the extraction solvent of choice for the isolation of the TBBPA derivative in the subsequent LLE experiments and SPE-based studies.

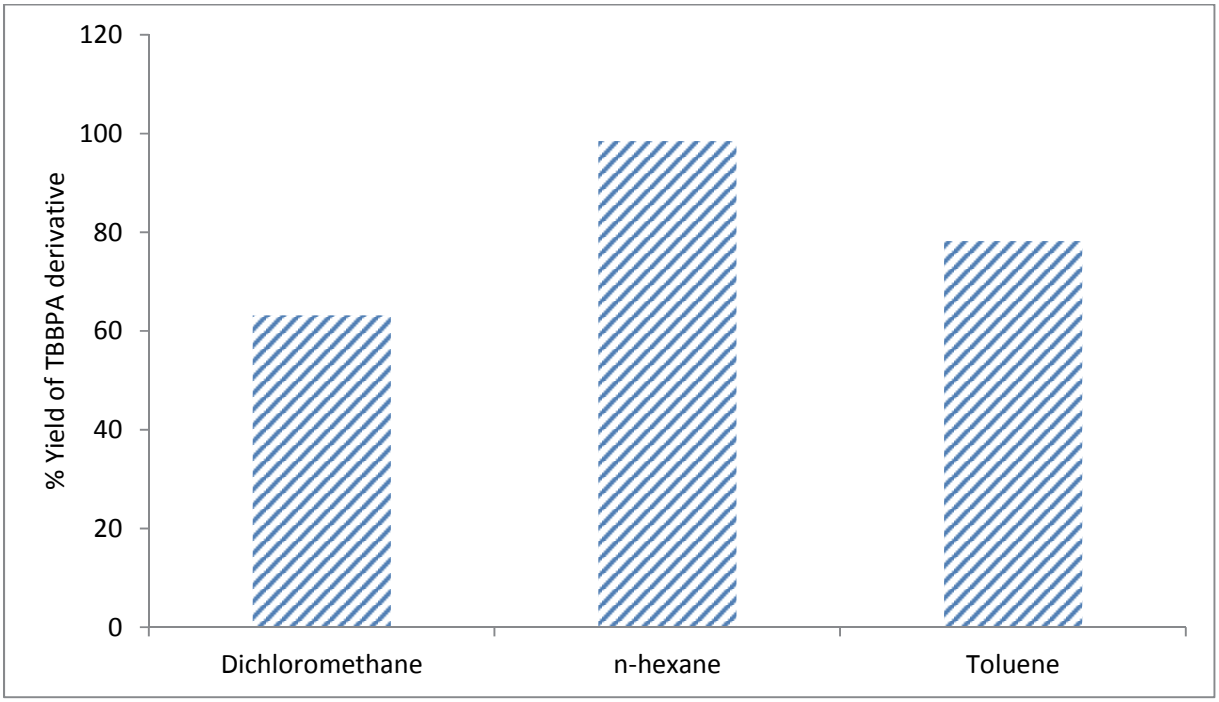


Figure 5.8: Extraction efficiencies of different organic solvents employed

5.3.7 Development and evaluation of the sample pre-concentration extraction kit

The extraction of aqueous matrices with SPE techniques offers some unique advantages over the conventional LLE technique. The use of relatively small volumes of the extraction solvent and its enhanced analytical recoveries of the target compounds are some of the major benefits of the technique. Based on these comparative advantages, different types of the SPE techniques were evaluated in this study. As previously mentioned, the efficiencies of four (4) different variants of the SPE techniques comprising two commercial SPE cartridges and two self-made SPE glass columns were evaluated. The performances of these SPE techniques were assessed by employing MilliQ water spiked with known concentrations of both TBBPA and other BFRs. In this case, all the target compounds were subjected to *in situ* derivatization prior to their extraction. The percent yield of the TBBPA derivative and the recoveries of other BFRs which were simultaneously extracted with the different SPE techniques are presented in Table 5.3.

Table 5.3: Comparison of the mean (± standard deviation) recoveries (%) of the target compounds using the different SPE techniques

	Commercial SPE cartridges		Pre-concentration glass column	
	ENVI-18	Strata FL-PR	Deactivated silica gel	Florisil
BDE 47	65.00±40.45	73.10±27.95	60.00±11.12	22.60±6.54
BDE 99	64.65±19.79	59.36±11.18	67.50±3.66	15.20±1.59
BDE 153	55.50±7.93	51.13±2.94	67.00±6.35	16.65±0.78
BDE 183	87.99±32.38	89.25±31.88	104.50±9.31	52.90±22.97
BDE 209	53.23±15.14	45.68±7.80	92.00±8.21	41.41±2.15
HBCDD*	25.58±8.66	29.68±11.96	18.30±1.90	19.19±2.12
TBBPA derivative	19.45±4.38	28.17±5.23	13.10±2.18	14.05±1.22

* - sum of α, β and Ɣ-HBCDD isomers

The extraction efficiencies of the different SPE techniques investigated in this study showed some quite interesting results. While the recoveries of both HBCDD and the TBBPA derivative were generally poor, relatively better recoveries were obtained for all the PBDE congeners except for the florisil-based self-made SPE technique. The poor recoveries obtained for both HBCDD and the TBBPA derivative may be due to their relatively high solubility compared with the PBDE congeners investigated. Although, this possibility may not hold for the TBBPA derivative whose original characteristics have been chemically modified during the *in situ* derivatization. More so, the choice of the extraction solvent was evaluated during the preliminary studies, the volume of the preferred solvent for elution was not optimized. Therefore, the possibility of obtaining better recoveries for these target compounds cannot be completely ruled out with the use of more elution solvent.

The analytical results obtained from the SPE-based studies clearly suggest that the technique is not suitable for the isolation of the TBBPA derivative from aqueous matrices. However, excellent recoveries of the TBBPA derivative (>98% yield) were obtained with the LLE performed with a separatory funnel when an optimum reaction time of 10 min was employed. Although this method requires the use of relatively large volumes of the extraction solvent, its rapid transfer of the TBBPA derivative into the organic phase during sample extraction favours this technique. Unlike the SPE techniques, shorter extraction times are usually possible with the LLE which is an advantage for the extraction of less stable derivatives from the aqueous matrices. A comparison between the LLE and the SPE techniques in terms of their extraction efficiencies for the TBBPA derivative is presented in Figure 5.9. As indicated in Figure 5.9, the LLE technique outperformed all the SPE-based techniques. Consequently, the use of LLE is recommended for the extraction of TBBPA derivative from *in situ* derivatization.

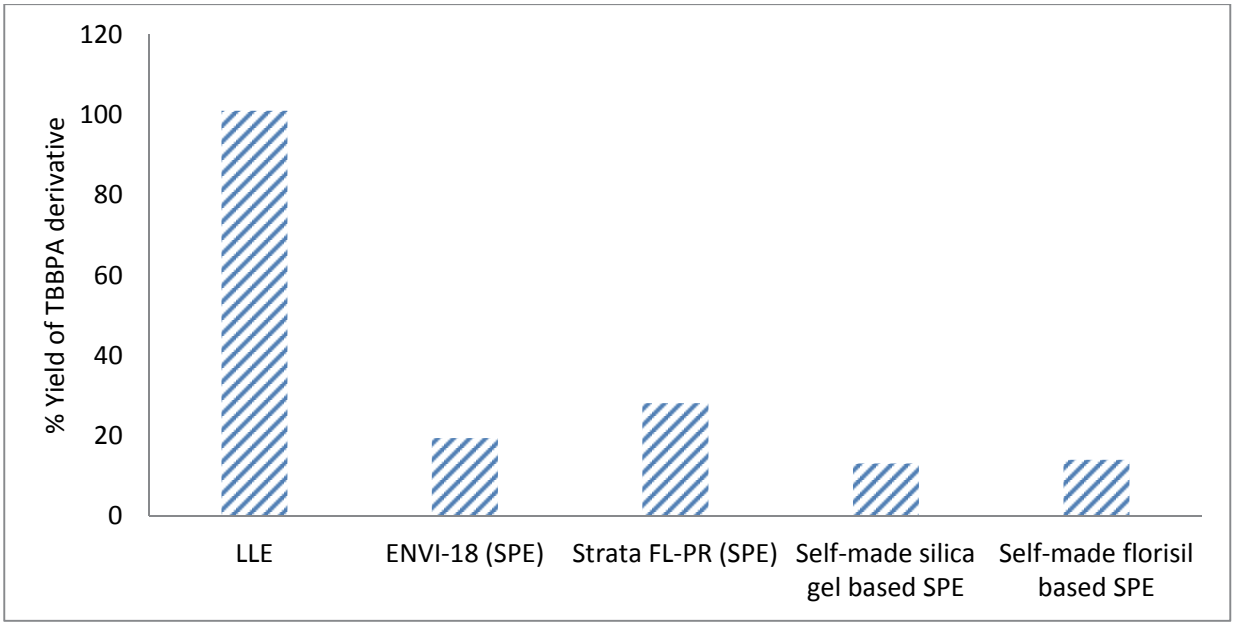


Figure 5.9: A comparison of the percentage yield of the TBBPA derivative with different extraction techniques

5.4 ANALYSIS OF REAL ENVIRONMENTAL SAMPLES

Despite the poor recoveries of the TBBPA derivative with the SPE techniques, this method of extraction, particularly the self-made silica gel based SPE was applied to landfill leachate samples. Triplicate spiked and unspiked leachate samples were employed to evaluate the performance of the extraction method. The performance of this SPE technique was markedly influenced by matrix effect as relatively low recoveries of all the target compounds, including the PBDE congeners were observed. To quantitatively determine the degree of these losses, the percentage losses due to the matrix effect were estimated by comparing the recoveries of the target compounds in spiked MilliQ water and leachate samples. As indicated in Table 7.9,

relatively high percentage losses were recorded for all the target compounds except the HBCDD which had less than 4% loss.

Table 5.4: Mean (±standard deviation) recoveries of target compounds in leachate samples and matrix effect estimates

Target compounds	Mean recoveries	% losses due to matrix effect
BDE-47	8.1±3.36	86.5
BDE-99	21.09±8.11	68.74
BDE-153	18.42±0.85	72.74
BDE-183	24.79±7.74	76.27
BDE-209	24.52±16.98	73.37
HBCDD*	17.65±1.00	3.28

*Sum of α, β and ϒ-HBCDD isomers

Landfill leachate is a complex environmental matrix containing high concentrations of dissolved organic substances, including humic and fulvic substances. The presence of these dissolved organic matters can considerably influence the analytical recoveries of these target compounds.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

The following conclusions were reached at the end of the project:

- The most common analytical methods for PBDE analysis in water and sediment, landfill leachate and sediment were identified;
- This was followed by the successful calibration of the analytical equipment using accredited BDEs standards;
- The validation of the analytical methods was successfully conducted using certified reference material and gas chromatographic technique used was optimized by varying the chromatographic parameters such as columns, retention times, carrier gas flow rates and other for optimal response;
- Once the method development was successfully conducted, the determination of common BDEs in sediment samples using optimized chromatographic parameters was carried out;
- BDEs were not detected in water samples, however, they were detected in sediment samples collected in winter and summer from Jukskei and Vaal Rivers and winter samples and these were significantly higher than the summer samples;
- Also the levels of BDE in landfill sediment samples were significantly higher than that of leachates and that unlined landfill sites showed higher BDE than lined landfill site, probably because of slow adsorption by soil in the former compared to a faster adsorption by geomembrane in the latter;
- A method for the analysis of APEs and BFRs in fish matrix was developed and applied to detect the levels of these compounds in bottom feeder type of fish;
- The feasibility of in situ derivatization of TBBPA with acetic anhydride as an extractive sample preparation procedure was demonstrated; and the simultaneous extraction of the resulting TBBPA derivative together with other commonly investigated BFRs was also performed;
- The application of different extraction methods for the isolation of the target compounds indicated that the use of SPE techniques as pre-concentration tools was characterized with poor recoveries and pronounced matrix effect;
- The use of LLE employing separatory funnel showed improved percent yield of the TBBPA derivative, although the extraction method was not employed for the simultaneous extraction of other BFRs.

6.2 RECOMMENDATIONS

- Chemical profile of water and sediment samples with respect to trace metals should be carried out in order to establish whether there is any relationship between the analytes of interest and other contaminants;
- The developed sample pre-concentration extraction kit should be subjected to a mixture of other emerging contaminants to test its ruggedness;
- Work should be done on the so called “novel flame retardants” that are currently used to replace the legacy flame retardants have been reported in water systems in developed countries, but not in any developing country;
- Phosphorous flame retardants which have also replaced the BFRs should be monitored in water systems since information on these is still scarce in South Africa
- The use of separating funnel extraction for the isolation of TBBPA derivative resulting from in situ derivatization is recommended in order to obtain acceptable analytical results.

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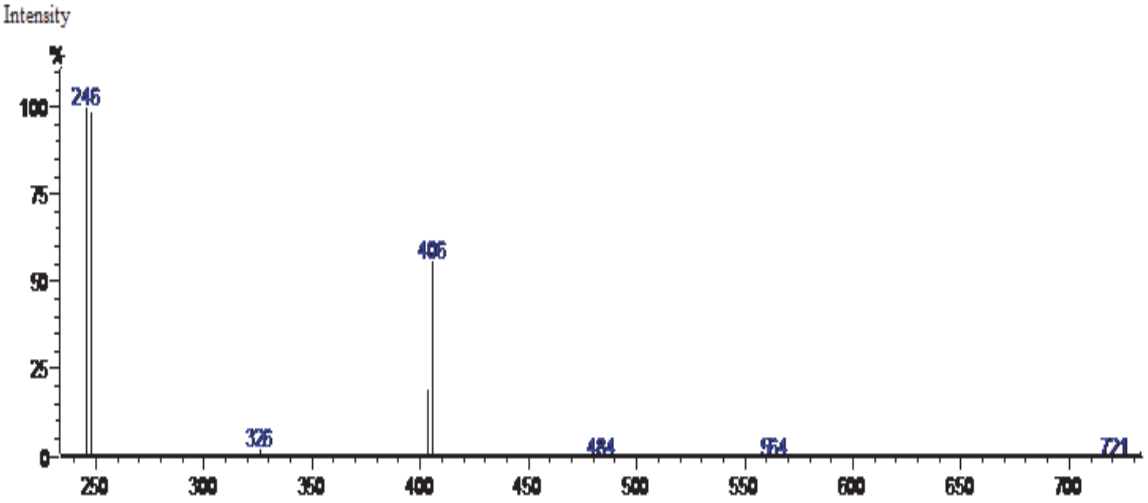
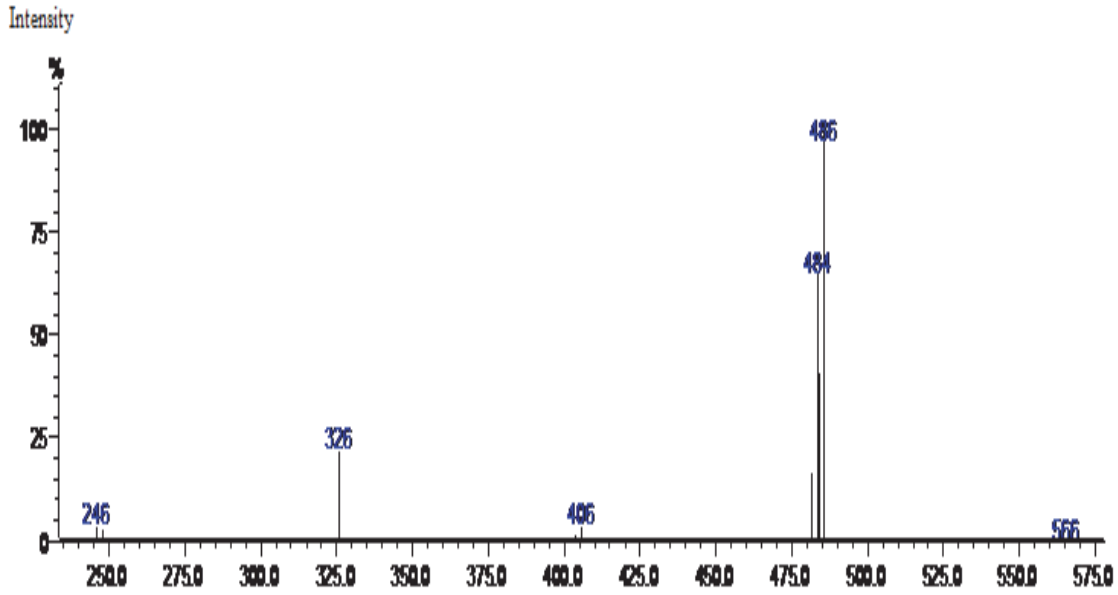
APPENDICES

APPENDIX A: Mass spectra of some target PBDEs



Molecular ion and fragment pattern of BDE-17



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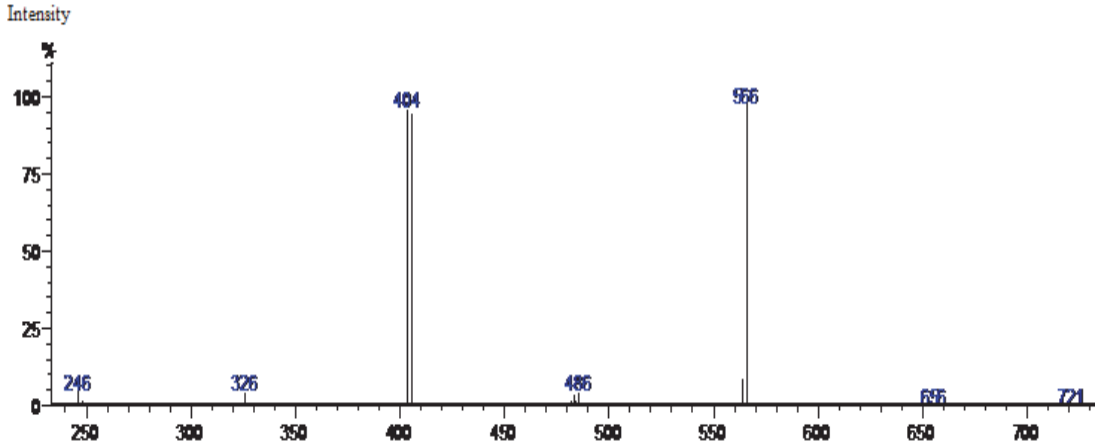

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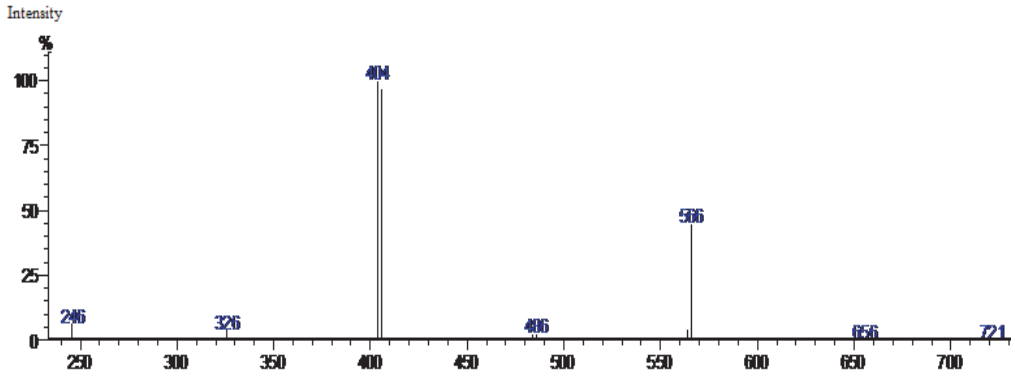

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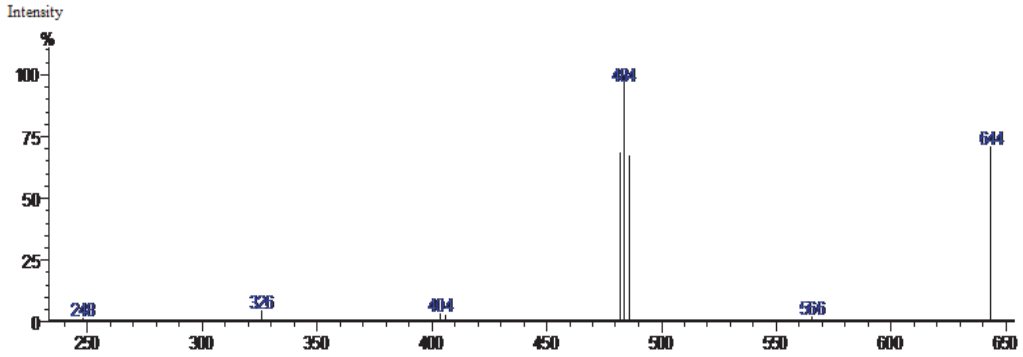

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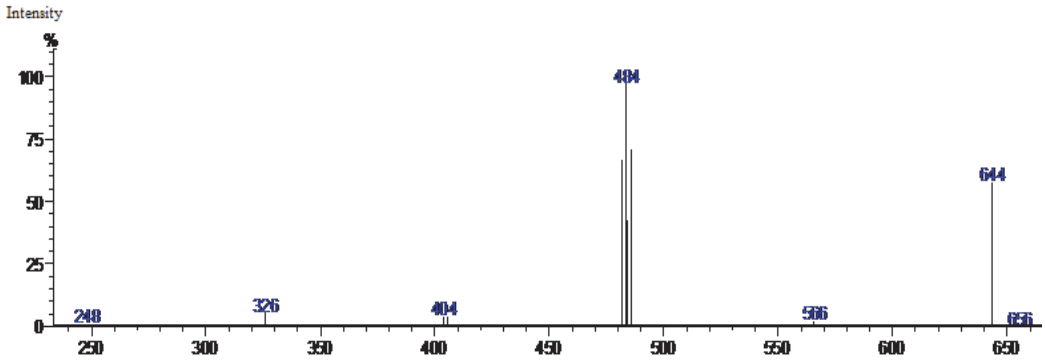

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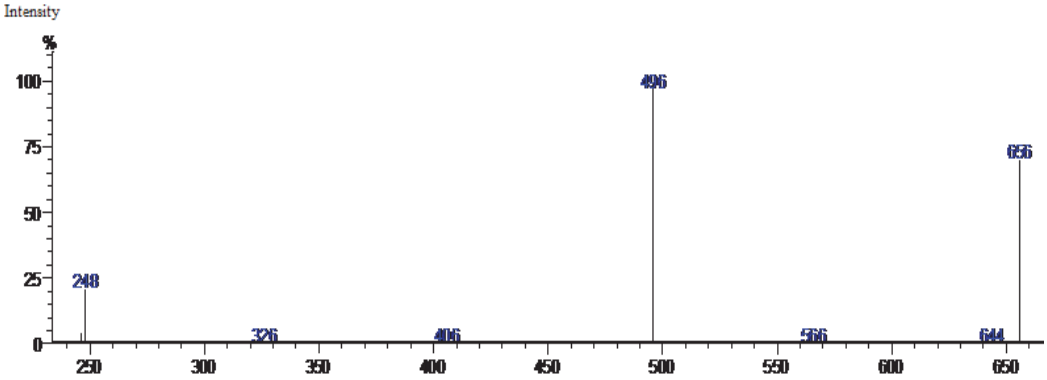
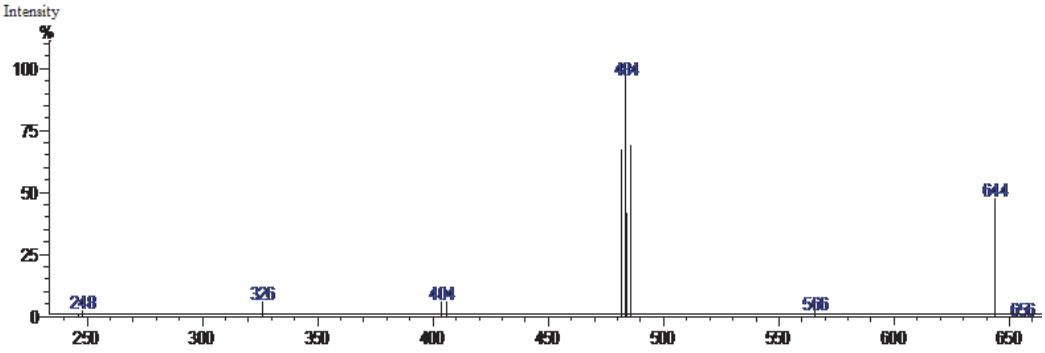


Molecular ion and fragment pattern of C^{13} BDE-139



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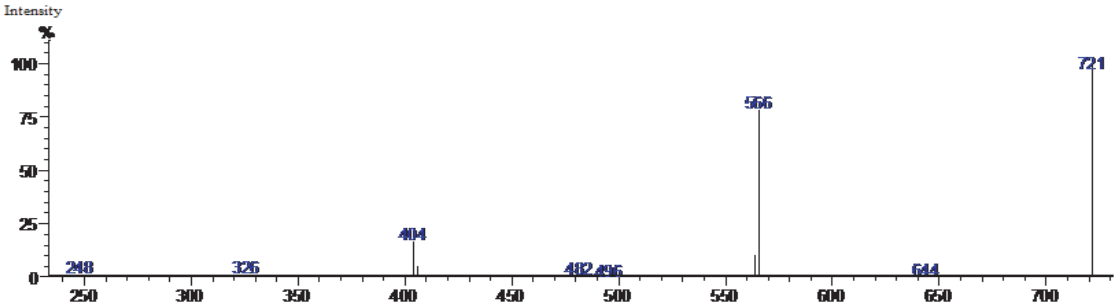

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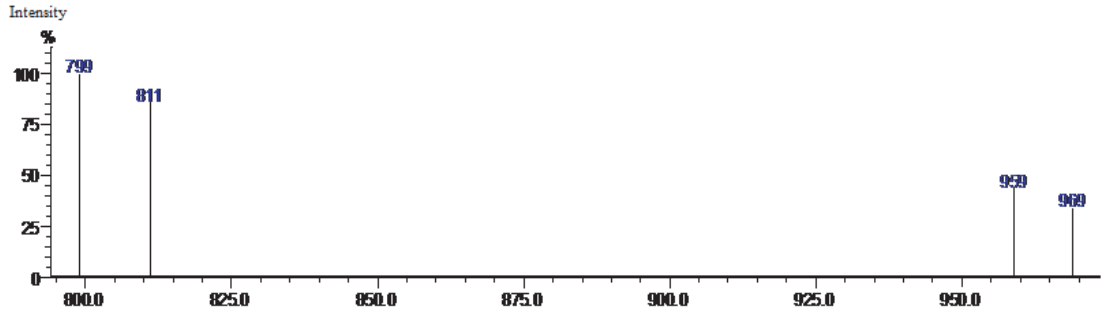
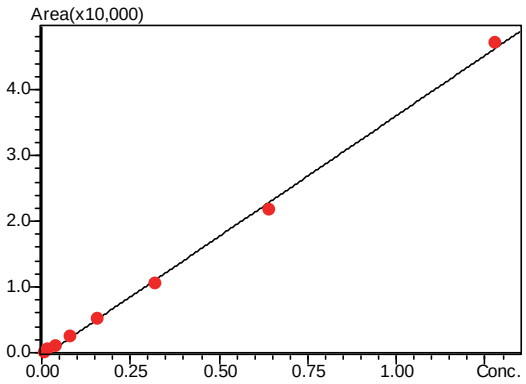


Molecular ion and fragment pattern of BDE-209

Method calibration curves of investigated PBDEs



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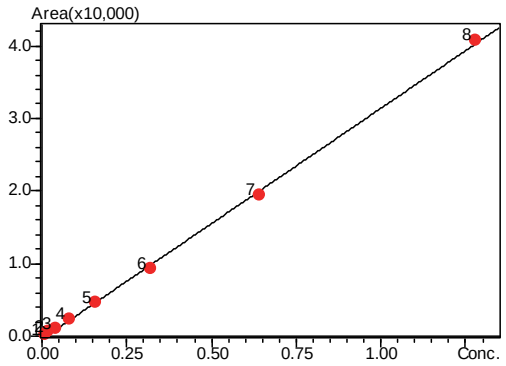

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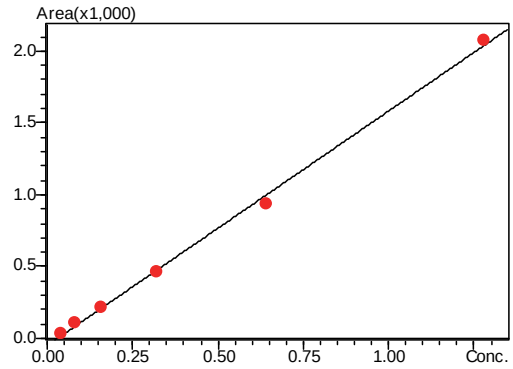

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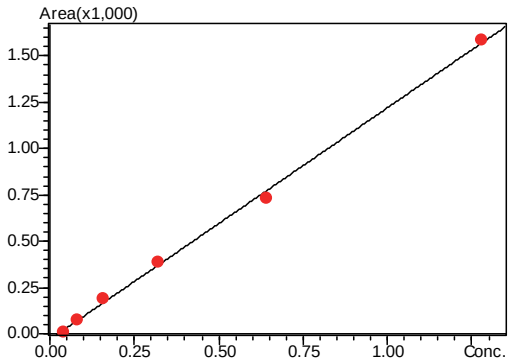

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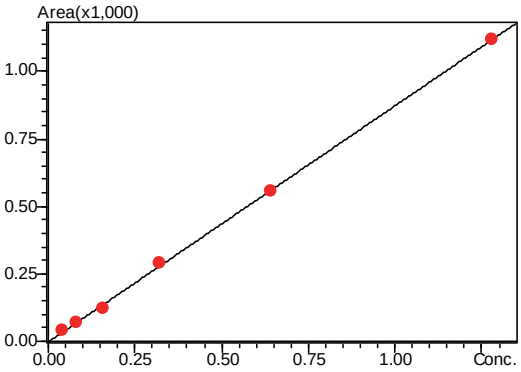

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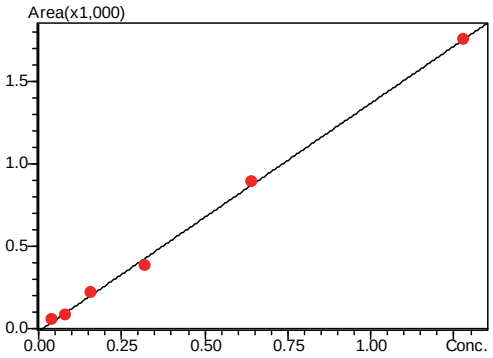

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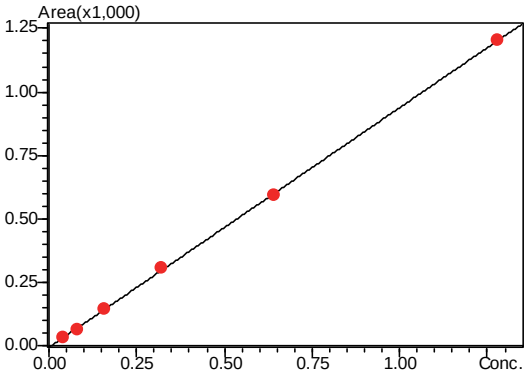

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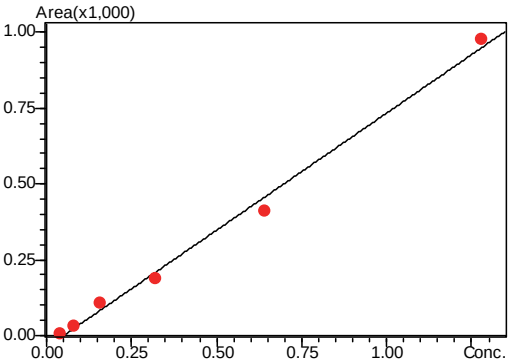
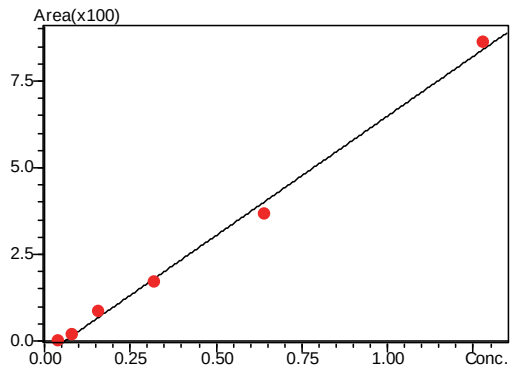


C^{13} BDE-139



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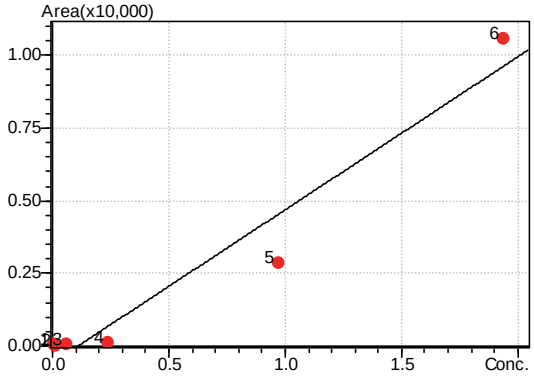
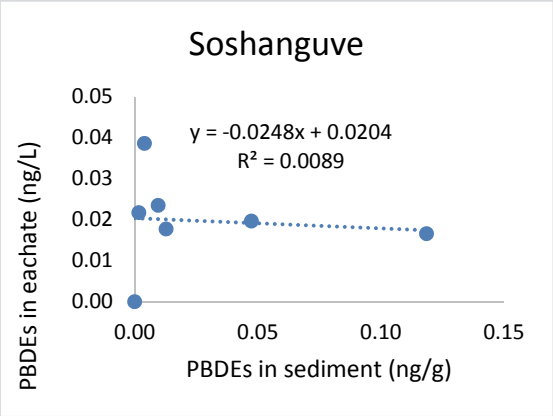


BDE-209

APPENDIX B: Evaluation of relationships between PBDE concentration in landfill leachates and sediment from the six MSWLS (A-F)



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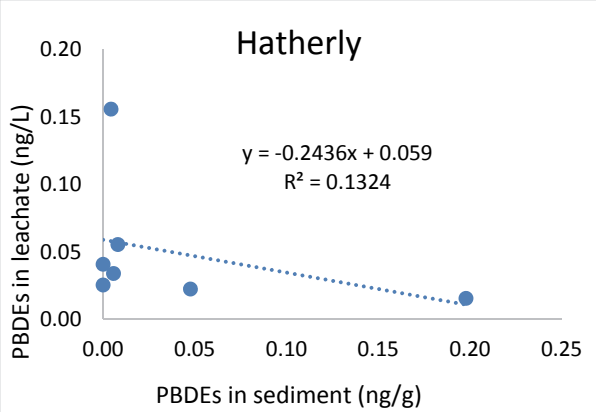

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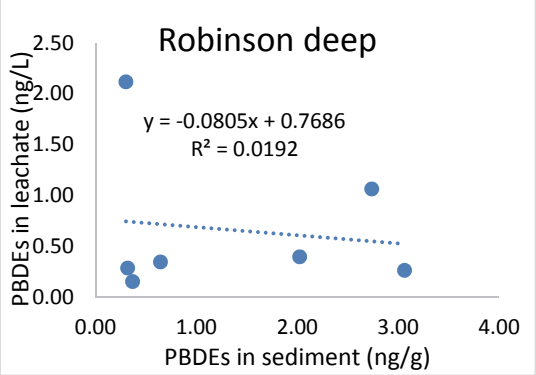

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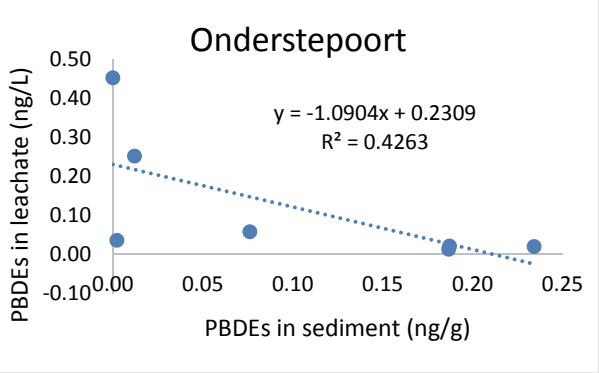

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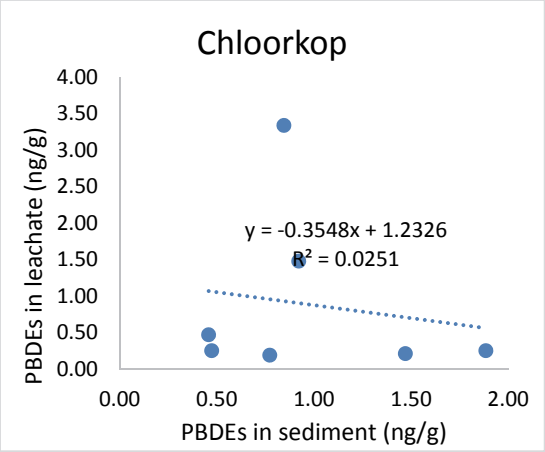

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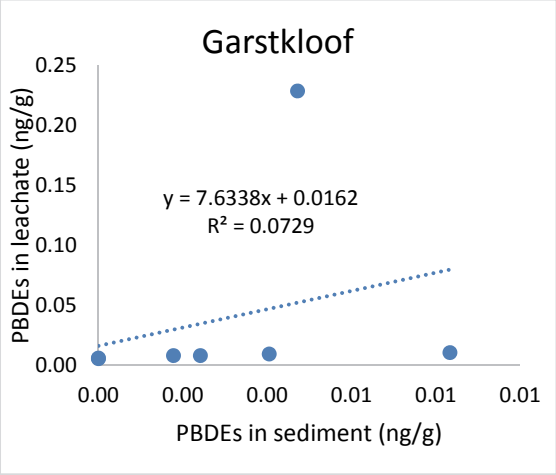
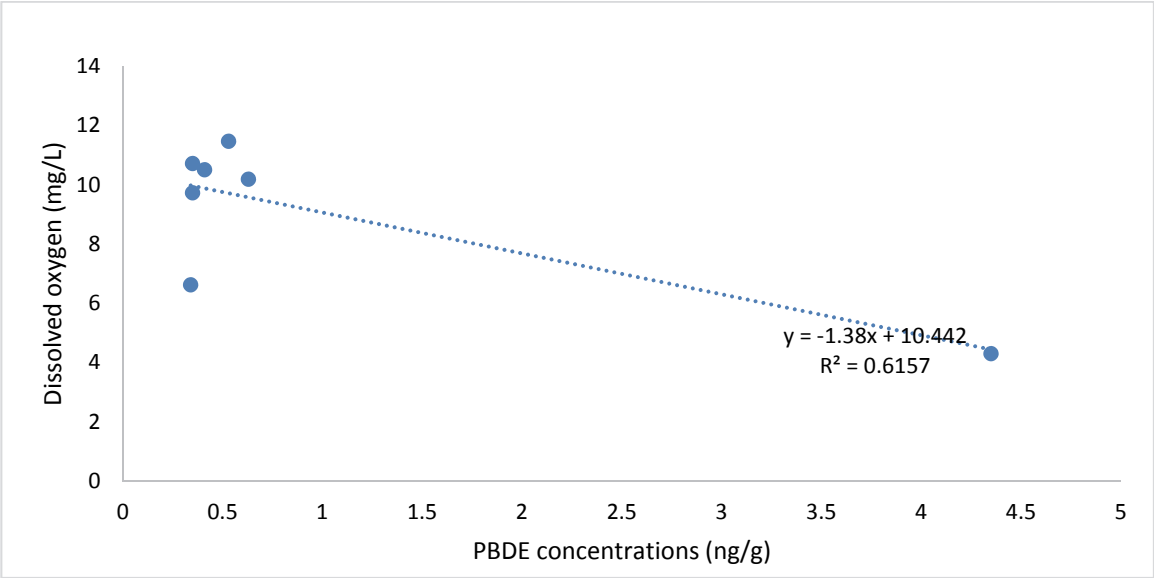
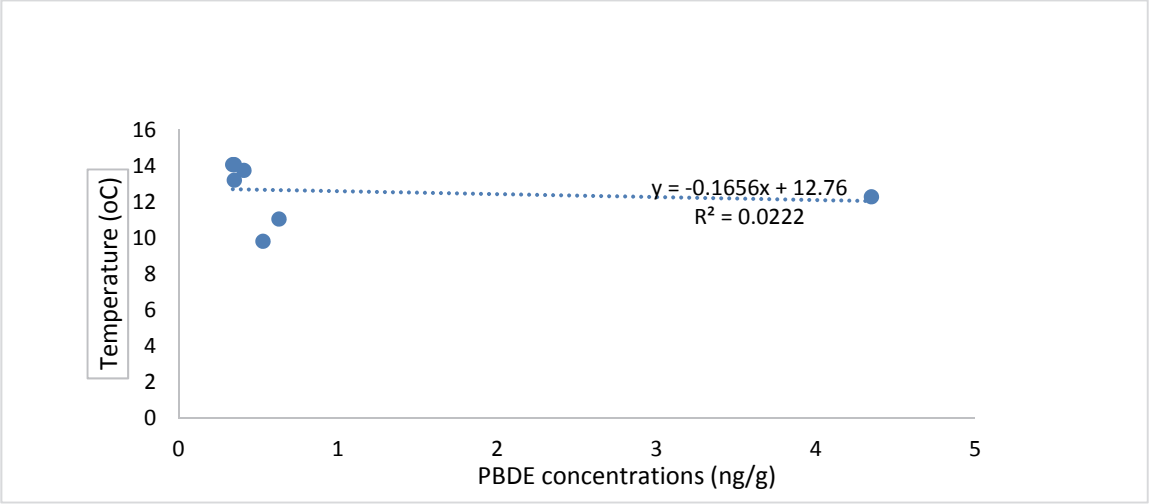


F

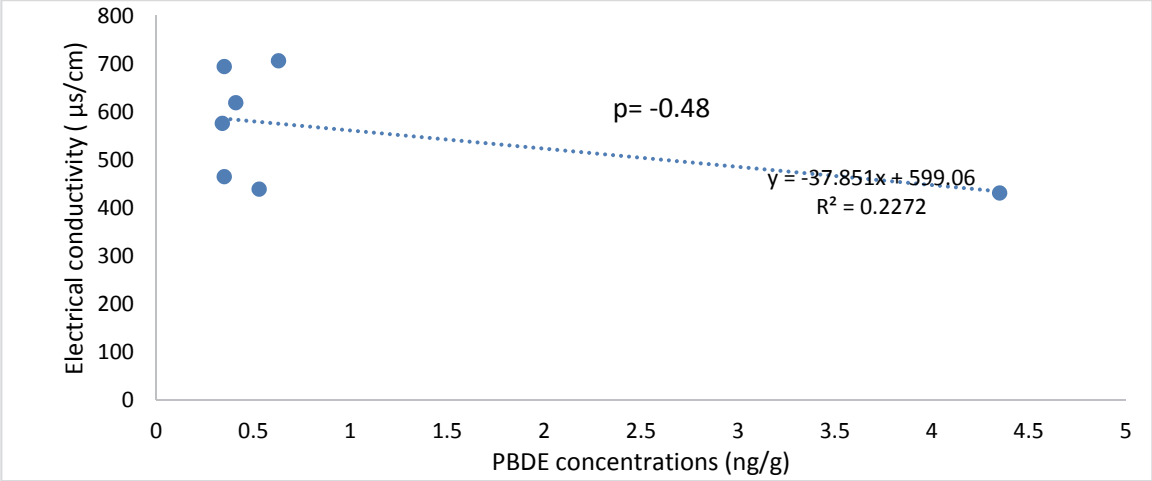
APPENDIX C: Relationship between dissolved oxygen, temperature and electrical conductivity and sum PBDEs in Jukskei River sediment



Relationship between dissolved oxygen and PBDE concentrations in Jukskei River sediment



Relationship between temperature and PBDE concentrations in Jukskei River sediment



Relationship between electrical conductivity and PBDE concentrations in Jukskei River sediment

APPENDIX D: Physico-chemical parameters of samples taken in winter and summer

Physico-chemical water quality parameters of sampled water and leachates in mg ℓ^{-1} (winter)

Site	pH	EC (µs)	F^-	Cl^-	NO^{2-}	Br^-	NO^{3-}	PO_4^{3-}	SO_4^{2-}
WETLANDS									
Klip River wetland	7.19	426	0.179	71.075	19.468	0.192	13.123	1.727	17.687
Karlspruit	6.97	560	65.828	n/d	n/d	n/d	n/d	4.901	13.764
Bulfrog Pan	9.17	5.66* ms	3.531	4597.37	n/d	n/d	n/d	n/d	0.82
Rietvlei	7.23	211	0.168	19.468	0.355	0.134	0.357	n/d	6.521
RIVERS									
Kyalami	7.35	434	0.449	48.009		0.157	33.957	0.443	20.077
Bruma Lake	7.16	288	0.129	30.696	n/d	0.02	37.83	0.999	7.857
Buccleuch	6.98	319	0.145	25.591	0.351	0.021	18.138	0.486	11.385
Eastgate(Marlboro)	8.04	353	0.17	39.75	n/d	0.074	8.524	n/d	16.018
Eastbank	7.48	294	0.169	26.026	0.345	0.06	8.268	0.77	12.544
Klip River	6.46	426	0.104	30.266	0.35	0.089	2.038	0.131	38.354
Alberton	6.62	649	0.209	65.789	0.494	0.129	2.417	n/d	121.555
Meyerton	7.25	691	0.282	87.836	1.325	2.666	n/d	10.194	10.59
Rietklip	6.71	651	0.231	47.537	1.219	0.181	2.583	n/d	113.306
Turffontein	8.39	667	n/d	n/d	n/d	n/d	n/d	n/d	n/d
GROUNDWATER									
Wonderboom park	7.43	n/d	0.241	39.939	0.342	0.17	35.668	n/d	15.444
Eastling	7.23	n/d	0.153	178.708	n/d	0.529	82.818	n/d	115.57
Doornrandjie	6.87	n/d	0.167	190.033	n/d	0.431	87.765	n/d	123.395
LANDFILL SITES									
****Onderstepoort**	7.58	539	1.057	123.919	n/d	0.418	2.06	n/d	15.02
****Garstkloof**	7.34	510	n/d	28.058	n/d	0.124	4.865	n/d	31.051
****Hatherly**	8.46	630	0.354	49.063	n/d	n/d	2.051	n/d	3.289
****Soshanguve**	7.58	1423	1.36	755.585	n/d	1.736	1.362	n/d	35.142
****Robinson deep**	8.20	23.4*	15.9	5306.2	n/d	30.4	15.4	25.8	114.9
****Chloorkop (Spring)**	12.8	7.97*	16	2144.3	n/d	166	11.3	n/d	29.4

n/d = not detected, *ms/cm

Physico-chemical water quality parameters of sampled water and leachates in mg ℓ^{-1} (summer)

Site	pH	EC	F^-	Cl^-	NO^{2-}	Br^-	NO^{3-}	PO_4^{3-}	SO_4^{2-}
Wetlands									
Klip wetland	na	na	na	na	na	na	na	na	na
Karlspruit	6.5	610	71.6	15	n/d	n/d	4	6.7	20.1
Bullfrog Pan	8.84	2.95 mS/cm	2.5	330	n/d	4.32	9.45	n/d	5
Rietvlei	6.84	261 µS/cm	0.34	29.86	0.2	n/d	11.91	0.42	40.72
Rivers									
Kyalami	4.82	5.89 mS/cm	12	29.4	n/d	n/d	5	n/d	1677
Bruma Lake	4.64	10.16 mS/cm	18.7	27.4	n/d	n/d	3.4	n/d	1909
Buccleuch	4.99	4.24 mS/cm	12	29.4	n/d	n/d	5	n/d	1677
Eastgate	4.88	5.30 mS/cm	15.1	26.4	n/d	n/d	17.35	n/d	1424
Midrand			33.25	70.2	n/d	n/d	45.2	n/d	3661
Eastbank	4.86	5.63 mS/cm	0.52	50.73	n/d	0.05	9.55	n/d	49.35
Alberton	7	443 µS/cm	0.32	47	n/d	0.04	17	n/d	192
Meyerton	7.53	261 µS/cm	0.55	57.72	4.9	n/d	65.34	12.9	18.26
Claring-ton	7.57	429 µS/cm	0.49	51	0	0.08	3.2	0.37	29.43
Groundwater									
Wonder-boom	7.97	951 µS/cm	0.75	45.7	n/d	n/d	35.81	n/d	403
Landfills									
Onderste-poort	7.5	685 µS/cm	0.4	39.5	0.18	0.008	40.5	0.5	6.9
Garst-kloof	7.63	325 µS/cm	0.41	17.8	4.985	n/d	n/d	n/d	35.2
Hatherly	7.63	836 µS/cm	0.596	68.362	n/d	n/d	n/d	n/d	8.41
Soshangu-ve	8.18	11.77 mS/cm	0	1425.52	0	8.85	0	8.83	4.48
Robinson deep	8.66	16.91 mS/cm	17.5	3045.3	0	6.4	0	7.3	101
Chloor-kop	8.25	10.61 mS/cm	0	1745.5	0	3.8	0	15	1.7

na= not tested