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Method optimisation of dispersive liquid-liquid microextraction (DLLME) for the determination of polychlorinated biphenyls (PCBs) in groundwater samples: case of borehole waters in North West province, South Africa

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Method optimisation of dispersive liquid-liquid microextraction (DLLME) for the determination of polychlorinated biphenyls (PCBs) in groundwater samples: case of borehole waters in North West province, South Africa



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A dissertation submitted to the Faculty of Science,

University of Witwatersrand in fulfilment of the requirements for the degree of Master of Science in Chemistry

August 2025

DECLARATION

I, Iviwe Dofi (2529357) declare that this thesis is my own, unaided work. It is being submitted for the degree of master's degree in chemistry to the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted before for any degree or examination in any other university.



(Signature of candidate)

_____18_____day of ____August_____2025_____at__Pretoria_____

CONFERENCE PRESENTATION

ANALITIKA Conference 2024, Champagne Sports Resort, Drakensberg, South Africa. "Method optimisation for determining chlorinated dense non-aqueous phase liquids (DNAPLs) in groundwater by Dispersive Liquid-Liquid Micro Extraction (DLLME)". **I. Dofi**, L. Mdlalose, L. Chimuka. *Oral presentation*.

ABSTRACT

Dense non-aqueous phase liquids (DNAPLs) are a global environmental phenomenon. This is predominantly accurate regarding emerging countries, owing to various human activities that lead to their release in the environment. These chemicals frequently end up in various bodies of water, including groundwater. Polychlorinated biphenyls (PCBs) are a class under DNAPLs family, accountable for enumerable environmental ills owing to their toxicity and carcinogenicity. Consequently, their production and usage were internationally banned following the Persistent Organic Pollutants (POP) Convention of Stockholm, 2001. However, certain bodies of water still contain significant amount of PCBs due to their environmental persistence.

In the current work, a method for the detection of PCB's presence in groundwater in South Africa's North West province was developed. To this end, eight PCBs were selected, based on the availability of certified reference materials (CRMs). An extraction method had to be chosen which would give possible good results and also easy to perform and DLLME was considered to be an effective extraction method. To ensure optimum extraction of the desired compounds, "one-factor-at-a-time" approach was used during the optimisation process. Experimental parameters such as the extraction solvent, extraction solvent volume, dispersive solvent, dispersive solvent volume, sample volume, extraction time, drying salt addition and centrifuge speed and time were varied. To quantify the amounts of the extracted compounds, GC-MS (EI) and GC-μECD were utilized.

The use of dichloromethane (extraction solvent) and acetone (disperser solvent) with centrifugal speed of 1400 rev/min for 5 minutes gave the best extraction results. The extraction recovery was found to range between 80.6–106.3% with RSD values of 2.7–8.9%. Regression coefficient (r^2) of calibration curves for quantification ranged from 0.9936 to 0.9978. The detection limit ranged between 0.46 to 15 μg L^{-1} and limit of quantification ranged between 1.5 to 50 μg L^{-1}, whereas the enrichment factors (EF) were from 126 to 703 depending on each compound.

The method developed was then used on water samples from boreholes of the Northwest province, South Africa. The samples were collected from the local community borehole taps and in the nearby schools. The borehole water of Mokgojoa Secondary School was found to be

contaminated with 2-chlorobiphenyl (PCB 1) at 23.3 µg L^{-1} and 2,2,3,4,6-pentachlorobiphenyl (BZ #88) at 6.6 µg L^{-1}. BZ 88 was also found in the local community borehole tap 2 at an amount of 0.5 µg L^{-1}. These levels obtained were above the allowed established EPA's maximum residue limit for less than 0.5 µg L^{-1} for human use. This poses a significant health hazard because the residents and students in this community use these water sources for drinking purposes. This study is significant as it divulges a fast and easy sample extraction that is also sensitive and inexpensive for the PCBs analysis. In addition, the study could also assist in guiding policy decision that concern water usage and safety in the region.

DEDICATION

This thesis is dedicated to my supportive mother, my precious daughter, my sisters and niece.

ACKNOWLEDGEMENTS

I want to give thanks to God, the creator, for his guidance in bringing this dream to fruition. The path was challenging as I balanced work, studies, and single parenthood, yet through prayer, commitment, and effort, I managed to accomplish this task. I want to convey my appreciation to Prof. Luke Chimuka who supervised the work for his constant support, patience, and significant professional input. Dr. Lindani Mdlalose, thank you for your exceptional guidance, kindness and enthusiasm for my research as my co-supervisor. I also appreciate Dr. Songeziwe Ntsimango for his insightful contributions and expert advice. A heartfelt thank you to the entire Dofi family for their unwavering support and encouraging words when I was close to giving up.

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LIST OF ABBREVIATIONS

AMU	-	Atomic mass unit
ASE	-	Accelerated solvent extraction
BZ	-	Ballschmiter and Zell
CAS No	-	Chemical abstract service no.
CI	-	Chemical Ionization
CRMs	-	Certified reference materials
C-series	-	Carbon series
DB5	-	5% phenyl, 95% methyl siloxane column
DB-624	-	6% cyanopropyl phenyl, 94% polydiethyl siloxane column
DCM	-	Dichloromethane
DES	-	Deep eutectic solvents
DNAPL	-	Dense non-aqueous phase liquids
DWAF	-	Department of Water Affairs and Forestry
EF	-	Enrichment factor
EI	-	Electron impact ionization
EM	-	Electron Multiplier
EPA	-	Environmental Protecting Agency
FG	-	Fentograms
FDA	-	Food and Drug Administration
GC	-	Gas chromatography
GC-µECD	-	Gas chromatography-micro electron capture detector
GC-MS	-	Gas chromatography-mass spectrometry
GC-MS (EI)	-	Gas chromatography-mass spectrometry (electron ionisation)
GC-MS/MS	-	Gas chromatography-tandem mass spectrometry

He	-	Helium
HF-LPME	-	Hollow fiber-liquid-phase micro extraction
HPLC	-	High performance liquid chromatography
HRGC/HRMS	-	High-resolution gas chromatography coupled with high-resolution mass spectrometry
IDEM	-	Indiana Department of Environmental Management
LC-MS/MS	-	Liquid chromatography tandem mass spectrometry
LLE	-	Liquid-liquid extraction
LPME	-	Liquid phase micro extraction
LOD	-	Limit of detection
LOQ	-	Limit of quantification
MAE-OV	-	Microwave-Assisted Extraction in Open Vessel
MASE	-	Membrane-assisted solvent extraction
$MgSO_4$	-	Magnesium sulphate
MIPs	-	Molecular imprinting polymers
MOF	-	Metal organic framework
MSD	-	Mass selective detector
ND	-	Not detected
NPL	-	National priority list
NIST	-	National Institute of Standards and Technology
OHS	-	Occupational health and safety
PCB	-	Polychlorinated biphenyl
PDMS	-	Polydimethylsiloxane
POPs	-	Persistent organic pollutants
PDMS/DVB	-	Polydimethylsiloxane/divinylbenzene
PPB	-	Parts per billion

PPM	-	Parts per million
PTFE	-	Polytetrafluoroethylene
QC-Mix	-	Quality control mix
QC	-	Quality control
QUECHERS	-	Quick, easy, cheap, effective, rugged, and safe
RSD	-	Relative standard deviation
RT	-	Retention time
SANS	-	South African National Standards
SBSE	-	Stir-bar sorptive extraction
SDME	-	Single-drop micro extraction
SE	-	Size exclusion
SPE	-	Solid-phase extraction
SPME	-	Solid-phase micro extraction
STD	-	Standard
STD DEV	-	Standard deviation
S/N	-	Signal-to-noise
TDS	-	Total dissolved solids
UHPLC	-	Ultra-high performance liquid chromatography
U. S	-	United States
UV	-	Ultraviolet
USAEME	-	Ultrasound-assisted emulsification micro extraction
VALLME	-	Vortex-assisted liquid - liquid micro extraction
WHO	-	World Health Organization

CHAPTER ONE- INTRODUCTION

1.1 Introduction

One significant issue related to the extensive contamination of groundwater systems arises from the dense non-aqueous phase liquids (DNAPLs) formation (Bergman *et al*., 2012). This is a class of organic liquid contaminants having a density that exceeds that of water. Their presence in the environment raises significant concern in developing countries because of their ongoing and extensive use across numerous industries and human activities (Pretorius *et al*., 2008). Under the DNAPL umbrella, there are different families of compounds. Halogenated hydrocarbons and polychlorinated biphenyls (PCBs) are the most frequent contaminants ground water in and soils, respectively (Pretorius *et al*., 2008). These slow movements contaminants often remain undetected, as they are chemically stable and have a habit of being persistent in the surroundings (Kodavanti *et al*., 2022).

PCBs are a category of man-made organic compounds composed of two benzene rings connected via a biaryl axis, differing only in the degree of substitution with the chlorine atoms (IDEM, 2013). They can be generalized with the formula $C_{12}H_{10-n}Cl_n$, where n is a number between 1 and 10 chlorine atoms and a total 209 unique compounds are generated when ten atoms of hydrogen are substituted by chloride on biphenyl. In 2001, Stockholm Convention has identified PCBs amongst the first twelve list of compounds that are persistent organic pollutants (POPs) (Zhang *et al*., 2014). The classification was a result of their persistence in the environment, bioaccumulation and toxic properties (Claudine *et al*., 2011).

PCBs are widely used in significant industrial and technical processes such as electronic manufacturing, steel production, solvent production, dry cleaning chemical production, and pesticides/herbicide manufacturing (Bart *et al*., 2016). Stemming from their extensive use, they have also made their way into environmental drinking water systems. Environmental Protection Agency (EPA) has set limit of 500 ng g^{-1} in drinking water while the Food and Drug Administration (FDA) has recommended a range between 200 to 300 ng g^{-1} in food in the Unites States (US). The World Health Organization (WHO) has set the recommended daily amount of 6 ng g^{-1} for PCBs intake (Ahmad *et al*., 2019). Despite all these guidelines that were put in place to ensure that their disposal and production is controlled, detection of these compounds is rather expensive and time consuming.

Therefore, ongoing monitoring and assessment are not often conducted to adhere to the permissible limits, especially in developing countries. Their negative effects on human beings include immunotoxicity, endocrine disruption, carcinogenic, impaired reproduction and neurodevelopmental effects in infants (ATSDR, 2000). Due to these factors, PCB usage and manufacturing has been prohibited worldwide, however, these compounds are still existing in high amounts in transformers and outdated power capacitors (Iwabe, 2016). PCBs were never manufactured in South Africa, instead the PCB containing machinery and PCB oils were imported and used mostly in the country's electricity production industry. PCB containing equipment is still present and in use in the country even though PCBs are no longer produced internationally (DEA, 2011).

In the past, PCBs in South Africa were utilized as electrical insulating medium for transformers and capacitors by the country's largest electricity service provider, Eskom (Pretorius *et al*., 2008). However, problems occur whenever there is fire or an electrical equipment leakage, hence there are suggestions of their elimination (Pretorius *et al*., 2008). Even though PCBs were initially used by Eskom in transformers, their use is being phased off. Eskom intends to remove approximately 150.000 L of PCB containing oil by the year 2025. Certain industries, mines, railways and municipal equipment also use oils that may contain PCBs (DEAT, 2003). To help with the elimination of PCB usage, Eskom has convinced oil companies to reject any oil with PCB levels exceeding 20 µg L^{-1}. Even though Eskom provides training and raises awareness about the dangers of PCBs, there is a widespread ignorance about their danger (Pretorius *et al*., 2008). The absence of regulatory rules and policies that deal with aftermath of PCBs once they make their way to the environment exacerbates this (Pretorius *et al*., 2008).

Owing to PCBs persistent in the environment, concerns over their accumulation in various matrices remain (Begum Yurdakok-Dikmen *et al*., 2016). Numerous studies have been carried out both locally and internationally to determine PCBs in different matrices such as groundwater, transformer or oils from turbines, soil, and wastewater collected from a power station (Bice *et al*.,2019). These studies were conducted using techniques such as Gas Chromatography-Mass Spectrometry (GC-MS) and Gas Chromatography-micro-Electron capture detector (GC-µECD) for analysis. The combination of GC-MS and GC-µECD showed accurate, sensitive, fast and reliable findings in different matrices, hence it is recommended for screening of PCBs (Zhang *et al*., 2016). There is not much information available on PCB detection in South Africa. Therefore, it is imperative that such information be available to inform lawmakers and policy surrounding PCB environmental contamination in this country.

This is especially true in relation to water contamination. Water supply challenges in South Africa provided motivation to undertake this research. As pointed, there is a scarcity of literature reports providing information concerning PCBs in groundwater. Most research that has been carried out in the country regarding PCBs in the environment was undertaken long time ago which may be outdated with all these industrial developments. With limited information in the country, particularly in the Northwest province, this study entails the development of an extraction method for PCBs detection that is simple and fast even at trace levels.

This work looked at DLLME optimisation method for analysis of PCBs. This was performed by varying different parameters such as extraction solvent, extraction solvent volume, disperser solvent, disperser solvent volume, centrifuge time, centrifuge speed, drying salt and extraction time effect. GC-MS-EI and GC-μECD were used as measurement techniques. The developed method was tested in river water and boreholes from the Northwest Province of South Africa.

CHAPTER TWO- LITERATURE REVIEW

2.1 Literature Review

2.1.1 Characteristics of Polychlorinated Biphenyls (PCBs)

PCBs are a group of hydrocarbons consisting of carbon, chlorine, and hydrogen atoms. They are non-flammable, dense, highly stable and vicious liquids created when a double benzene ring molecule's hydrogen atom is replaced with a chlorine atom called biphenyl (Claudine *et al*., 2011). They enter into humans and animals through the mouth, nose, and skin (Lyon, 2016). There is no distinct treatment for PCB exposure; however, preventive steps and treatment of symptom are possible, and inhalation issues can be addressed by supplying oxygen (Lyon, 2016). The noted early symptoms include diarrhoea, dizziness, nausea, signs of depression, vomiting, headaches, anxiety, skin and eye lesions, stomach pain, nervousness, decreased immune response, irregular menstrual periods and fatigue (Carpenter, 2006).

PCBs are hazardous to the ozone layer and are found in the environment where they do not break down easily (Cassidy *et al*., 2002). They accumulate in the aquatic organisms resulting in harmful and lasting effects. Their health concerns include increasing cancer risks, decreasing fertility, affecting the immune system and causing harm in the development of foetus (USEPA, 1991). They can be light-coloured, thin liquids black or yellow solids that have no identifiable taste or odour (USAEPA, 2012). The physical and chemical characteristics of the eight major PCBs studied are provided in Table 1 and their structures are illustrated in Figure 1.

Table 1: Physical and chemical characteristics of PCBs (USAEPA, 2012)

PCB names	Chemical properties	Physical properties
2-Chlorobiphenyl or PCB 1	Molecular formula $C_{12}H_9Cl$ and mass of 188.653 g/mol.	A colourless to pale yellow appearance of greasy liquids or solids.
2,3-Dichlorobiphenyl or BZ6 or PCB 6	Molecular mass 222.653 g/mol and chemical formula $C_{12}H_8Cl_2$.	Oily colourless liquid.
2,4,5-Trichlorobiphenyl or PCB 29 or BZ 29	Molecular of 255.961 g/mol and chemical formula of $C_{12}H_7Cl_3$.	Oily liquid or solid that is colourless to light yellow.
2,2',4,4'-Tetrachlorobiphenyl or tetrachlorobiphenyl or PCB 47	$C_{12}H_6Cl_4$ with a molecular mass of 291.22 g/mol.	Colourless to light-coloured, viscous liquid with a mild, hydrocarbon odour.
2,2,3,4,6-Pentachlorobiphenyl or BZ 88	Molar mass of 326.4 g/mol.	White to off-white colour.
2,2,4,4,5,6-Hexachlorobiphenyl or BZ 154	$C_{12}H_4Cl_6$ with a molecular mass of 360.88 g/mol.	A colourless to light yellow solid.
2,2,3,3,4,4,6-Heptachlorobiphenyl or PCB 177 or BZ188	Molecular formula of $C_{12}H_3Cl_7$ and mass weight of 395.3 g/mol.	Oily liquid or solid that is colorless to light yellow.
2,2,3,3,4,5,6,6-Octachlorobiphenyl or PCB 198	Chemical and molecular formula of $C_{12}H_2Cl_8$ and 429.8 g/mol.	Oily liquid or solid that is colorless to light yellow

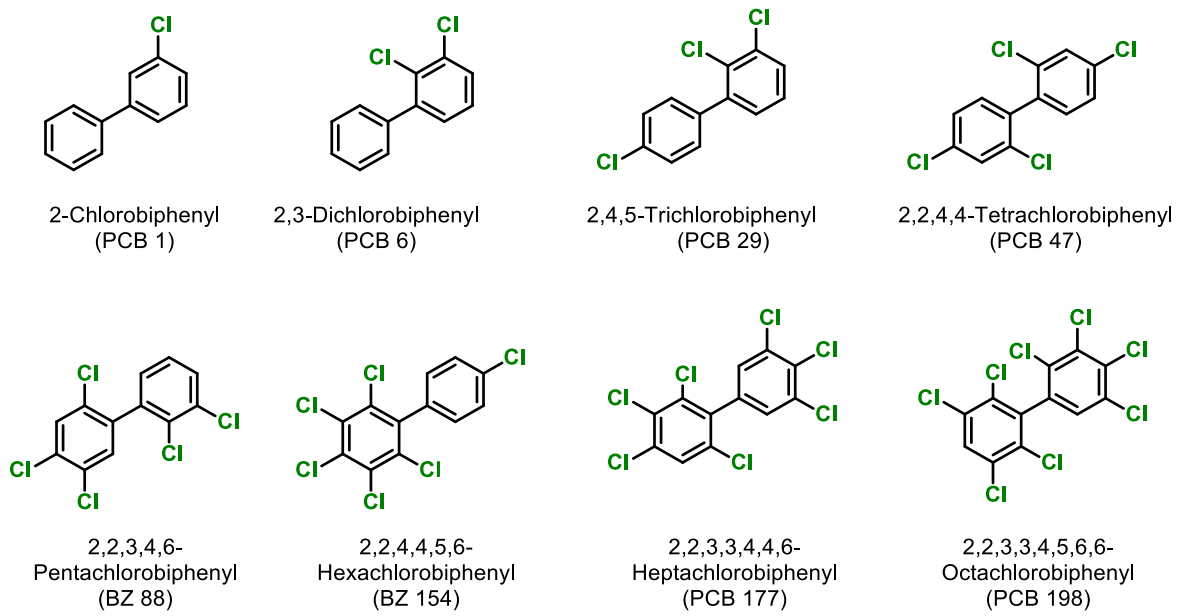


Figure 1: PCB structures used in the study

2.1.2 Occurrence of PCB contaminants and their uses

PCB compounds were manufactured between 1930 and 1977 in the United States (US) for numerous industrial applications (Rosalina *et al*., 2013). Their toxic nature to both humans and animals led to their prohibition in several countries during the 1970s. Many countries imposed stringent regulations governing the utilization and discharge of PCBs in the 1970s and 1980s. As a result, their environmental impact has decreased significantly; however, PCBs can still be released from contaminated reservoirs such as lakes and coastal sediments (Magdalena, 2007). Numerous studies have identified sources that are increasing in Africa because of leakages, improperly discarded transformers, biomass burning, shipwrecks and ongoing influx of electronic waste from the northern nations that contain PCBs. The volatile PCBs are vaporized as a result of waste recycling methods such as melting and open burning for metal contained within the waste (Rosalina *et al*., 2013). These vaporized PCBs can then accumulate in air, water, blood, breast milk and fish increasing their levels. Also, other industries like railways, mines and municipalities also use equipment consisting of PCBs (Pretorius *et al*., 2008). They have been extensively utilized in a range of industries and profitable fields because of their chemical resilience, non-combustible, good electrical insulating and higher boiling point. This includes paint plasticizers, plastics, rubber plastics, dyes, electric, heat transfer, carbon copy paper, to name a few (Al-Alam *et al*., 2020).

2.1.3 Transportation of PCBs into the environment

When a spillage of PCBs occurs from old equipment, urban runoffs, petrol stations, oil spillage tanks, industrial storages or pesticides fertilizer application they move down through the unsaturated zone during rainfall into the pore spaces (Kueper *et al*., 2003). The water table is then penetrated by these compounds and continues to move down the saturated zone where their soluble constituents' dissolves into the groundwater resulting into contamination of groundwater (Kueper *et al*., 2003). Another way of PCBs enters the environment is via the natural sources such as atmospheric, soil and aquatic systems because of inadequate disposal of waste removal. In the atmosphere, their routes are through wet deposition such as rain and dry deposition of fine particles (Langer, 2005). In addition, PCBs can enter the environment through gaseous phase, hence they can travel long distances from their point of origin to areas where they have neither been utilized nor produced (Langer, 2005). Schematic chart below in Figure 2 illustrates the whole transportation process.

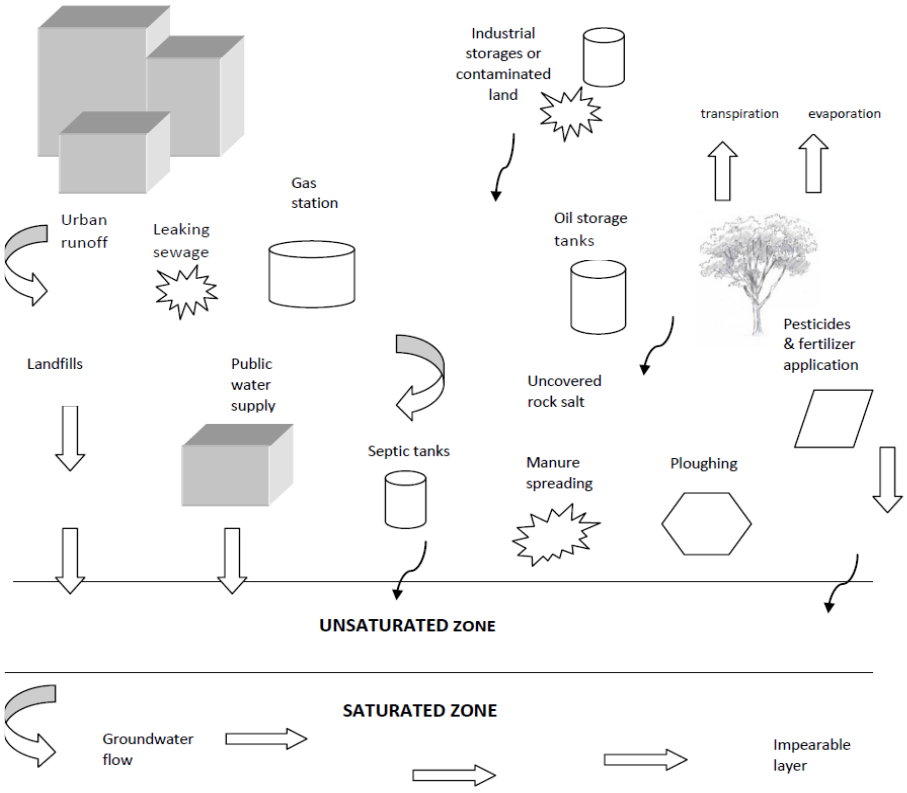


Figure 2: Schematic flowchart of PCBs into the groundwater (Reddy *et al*., 2019)

In humans, PCBs are ingested by consumption of water that is contaminated or via the intake of contaminated food items, including fish, meat, and dairy products. There is evidence that

some of these compounds may cause health threats and even at low concentrations, hence it is crucial for them to be monitored and detected (Mark *et al.*, 2001).

2.2 Analytical methods for PCBS extraction in aqueous samples

Organic pollutants such as PCBs are found in various matrices within the environment, including water, milk, human serum, and blood. It is therefore imperative to select an effective extraction technique for PCBs so that they can be concentrated in preparation for analysis. There are numerous extraction techniques used for PCBs detection. These extraction techniques include liquid-liquid extraction (LLE), solid-phase extraction (SPE), solid-phase micro extraction (SPME) and liquid phase microextraction (LPME) techniques such as stir-bar sorptive extraction (SBSE), dispersive liquid-liquid microextraction (DLLME) and hollow fibre liquid phase microextraction (HF-LPME).

2.2.1 Liquid-Liquid Extraction (LLE)

LLE is a commonly utilized method of extraction for isolating compounds across two non-mixing liquid layers. After mixing the two phases by shaking vigorously or centrifuge, the mixture is left to settle for some time and organic layer that contains analytes is separated as indicated in Figure 3. According to Megahed *et al.*, (2015), this method has been employed for the extraction of PCBs from river water and surface water, achieving recovery rates of up to 98% and 91.4%, respectively. Despite the simplicity and easily accessible of its apparatus, it is work intensive and necessitates substantial volumes of solvent, which results to the creation of an emulsion. The larger solvent that needs to be disposed has a negative impact on the environment therefore LLE has been largely replaced with more modern micro extraction techniques such as liquid phase microextraction techniques (Rawa-Adkonis and Wolska, 2006).

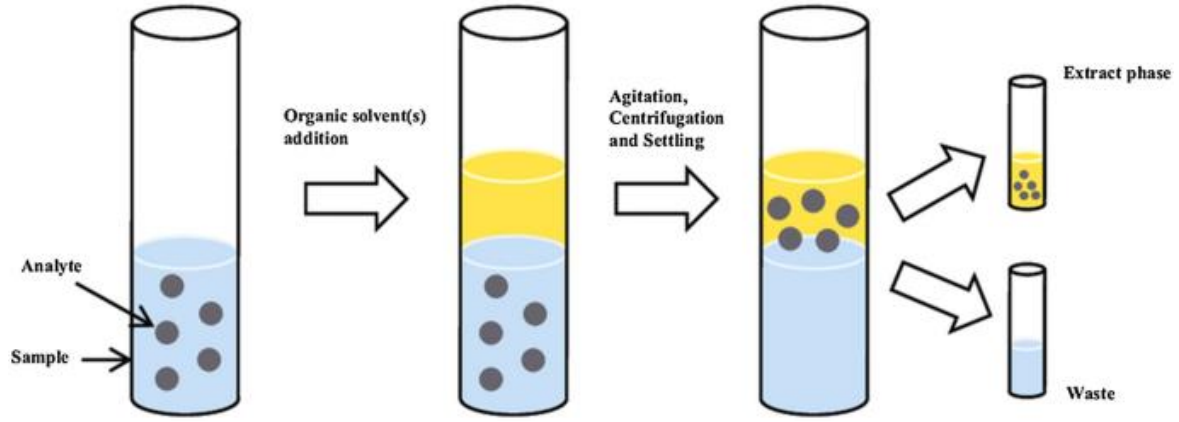


Figure 3: Schematic representation of LLE (Sofia *et al*., 2020)

2.2.2 Solid-Phase Extraction (SPE)

SPE is characterized by its speed and accuracy in extracting analytes from a solution, utilizing both a solid phase and a liquid phase to isolate a specific analyte or a particular type of analyte (Camel, 2003). It is employed to distinguish between compounds that are either suspended or dissolved in a liquid mixture and other compounds present in the mixture, relying on their physical and chemical properties. It is typically used to clean up a sample prior to chromatographic analysis. The concept of SPE is the same as of that of LLE, which involves the partitioning of solutes between two distinct phases. In contrast to the two immiscible liquid phases present in the LLE, SPE entails the separation between a liquid phase, which may contain either the sample or the solvent for the analyte, and a solid phase known as the sorbent (Camel, 2003). SPE method involves several steps such as the conditioning of a sorbent with a solvent, the loading of a solution into the SPE cartridge, removing undesirable elements, and lastly the elution of desired analytes is separated from the mixture by using a different solvent, which is then collected in a designated tube as shown in Figure 4. In contrast to LLE, SPE extraction method uses reduced solvent volume. Also, SPE method is not limited to organic compounds in environmental samples but also used for the removal of interferences in complex matrices to obtain a cleaner extract with the desired analytes (Swagata *et al*., 2023).

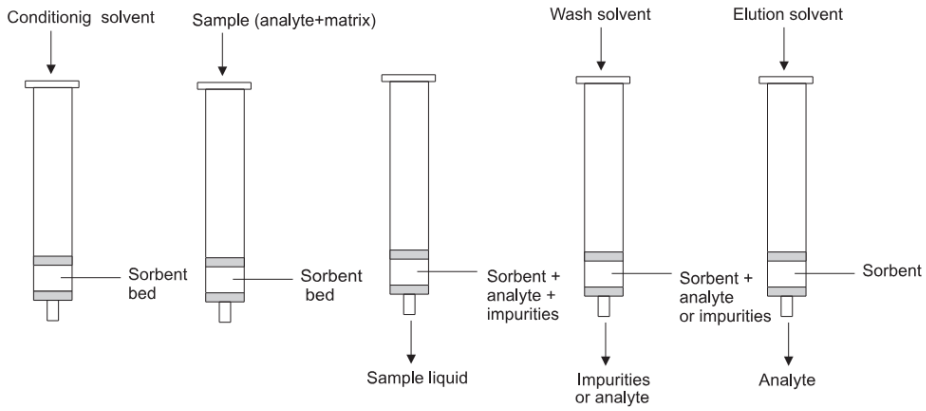


Figure 4: Solid phase extraction steps (Żwir-Ferenc *et al*., 2006)

On the study conducted by Dachs and Boyana, (1997), PCBs were extracted from milk and seawater using conventional SPE containing cartridge C18 octadecylsilane and the recovery of specific PCBs from milk resulted in recoveries exceeding 80% (Picó *et al*., 1995). C18 was employed in seawater for the pre-concentration of PCBs, achieving recovery rates between 60% and 70% (Dachs and Bayona, 1997). Non-polar solvents like hexane were employed for conditioning the SPE, while polar solvent such as methanol was used for the elution step.

However, SPE using C18 cartridge showed inadequate recoveries because of limited adsorption capacities and non-selective interactions between the sorbent and the target analytes. Hence there is transition towards more advanced and trustworthy sorbents that offers robustness, specificity, and improved recovery rates such as molecular imprinting polymers (MIPs). The MIPs emerged as improved sorbents that deliver the required selectivity to show size, shape, and functional group towards a specific target molecule. This makes them to be an excellent material for sample preparation, as they allow for customised sample treatment prior to the final analysis (Turiel and Martín-Esteban, 2010). SPE utilizing MIPs is increasingly recognized as a preferred method for sample preparation and is attracting significant interest across various disciplines (Ndunda *et al*., 2015).

It has thus far been employed for PCBs analysis in water samples from rivers and lakes, achieving recovery rates between 83.5% and 104.4% (Ndunda *et al*., 2015). The study reported by Gbadebo and others, (2018) revealed yet another challenge with SPE referencing on the work done by (Fatoki and Awofolu, 2003; Sibali *et al.*, 2008) for water extraction used LLE because it offered better results compared to the solid-phase extraction (SPE) method. They noted that during sample preparation for the SPE method, the SPE cartridges introduce interferences from phthalate esters, which originates from the material of the cartridge housing.

Consequently, LLE offered superior outcomes in comparison to the SPE (Sibali *et al*., 2008) compared the reliability in extraction recoveries between LLE and SPE, emphasising on the extraction of selected PCBs on river waters, soil and sediments of Msunduzi situated in the KwaZulu-Natal province during spring and winter season (Gbadebo *et al*.,2018).

2.2.3 Solid-Phase Micro Extraction (SPME)

SPME is defined as a no solvent sample preparation method that uses a fibre covered with a sorbent material during the extraction phase (Boyaci *et al*,.2015). It is capable of extracting a range of analytes from gaseous to liquid states, encompassing compounds that are volatile and non-volatile (Risticevic *et al*., 2009). Upon sample extraction, a fiber is placed inside the liquid or within the headspace of a liquid (headspace-SPME) to enable the partitioning of analytes between the sample matrix and extraction phase. Lastly, the concentrated extracts are injected onto an analytical instrument as shown in the step-by-step process in Figure 5. This method can be incorporated into an analytical technique to eliminate several steps seen in many techniques and eventually reduces time. Some examples that are reported on the utilization of the SPME technique involves extracting PCBs from fish feed used in aquaculture and from cultured marine species by the use of a silica fused fibre coated with polydimethylsiloxane (PDMS) (Li *et al*., 2015). Recoveries that were obtained were reported to range between 78–101% (Li *et al*., 2015). Niehus and others (2019) have also demonstrated the use of SPME method to extract of PCBs in sediment pore water. They obtained recoveries between 84% and 91%. In another study published by (Al-Alam *et al*.,2020) from snail extracts, lower recoveries of 55–80% were obtained.

Recent literature reports have focused on newer improved fibre coatings that are more stable, possessing a shorter equilibrium extraction time, a high adsorption surface area, which also show higher sensitivity. A case in point is the water extraction of PCBs with customized multimodal porous carbons sorbents (Cheng *et al*., 2019). PCB recoveries of 86–104% were reported (Cheng *et al*., 2019). In the same vein, Bashir and co-workers (2020) extracted PCBs in water using a chitosan-functionalized polypropylene membrane coated with multiwalled carbon nanotubes, achieving recoveries of 82.4–98.6%. Despite the progress that has made in SPME method, it is still faced with multiple challenges such as limited working temperature, swelling in solvents and breakages (Pawliszyn., 2002). Additionally, this method is costly, due to the need to constantly replace the expensive fibres (Pawliszyn., 2002).

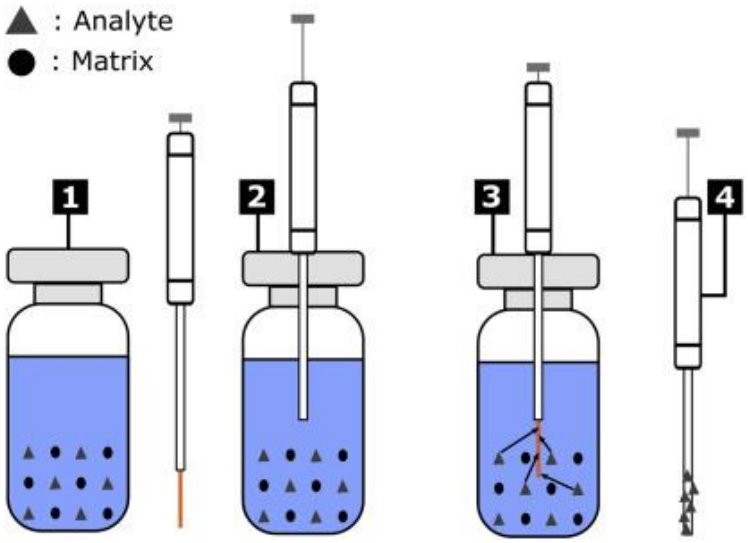


Figure 5: Diagrammatic representation of the general SPME direct immersion principle. 1) A sample is put inside the vial and enclosed. 2) The fiber is retracted before inserting the SPME needle. 3) The fiber is submerged in the sample with the relevant analytes. 4) SPME needle can be taken out and used for injection (Benedikt *et al*., 2020).

2.2.4 Liquid-Phase Micro Extraction (LPME)

LPME method can be considered as LLE in a much smaller scale. The LPME method operates in a much smaller solvent volume that can reach several microliters (Pacheco-Fernandez and Pino, 2020). LPME method can be subdivided into five categories namely a) Dispersive Liquid-Liquid Micro Extraction (DLLME) b) Vortex-Assisted Liquid-Liquid Micro Extraction (VALLME) c) Ultrasound-Assisted Emulsification Micro Extraction (USAEME) d) Hollow Fibre-Liquid-Phase Micro Extraction (HF-LPME) and e) Single-Drop Micro Extraction (SDME) (Yamini *et al*., 2019). The ensuing discussion will further discuss these categories.

2.2.4.1 Dispersive Liquid-Liquid Micro Extraction (DLLME)

DLLME entails dispersing fine droplets of extraction solvent introduced into an aqueous sample using a disperser solvent that is compatible with both phases. The greater interaction among the sample surface and the solvent used for extraction results in a more efficient extraction of the sample. The mixture results in a turbid solution, which is subsequently subjected to centrifugation to yield an extraction phase that encompasses the analytes of interest. The primary benefit of this technique is the significantly reduced volume of solvent, which results in the sample suitable for instrumental analysis (Quigley *et al*., 2016). The full

process is illustrated in Figure 6. This extraction method is regarded as valuable method due of its contribution to green analytical chemistry (Assadi *et al*., 2006). Additional advantages of this method comprise of low sample size usage, minimal energy usage, reduced solvent volume, ease of automatization, minimal generated waste, multi-analyte extractions, usage of greener reagents and reduced exposure to solvents (Rezaei *et al*., 2008).

DLLME has been utilized in the identification of PCBs with recovery rates in environmental water samples ranged from 81.9 to 109.7%, with a relative standard deviation of 1.7% to 11.8% (Hou *et al*., 2019). The method demonstrated an extensive linear range with an r^2 value exceeding 0.9989, along with low detection and quantification limits ranging from 0.16 to 2.17 μg L^{-1} and 0.53 to 7.16 μg L^{-1}, respectively under the optimum extraction conditions. Hexane was selected as the solvent, and GC-μECD was employed for the analysis. The calibration standards exhibited a concentration range of 0.005 to 1.0 μg mL^{-1}, with a limit of detection established at 0.005 μg mL^{-1}. The enrichment factors (EF) observed were notably high, varying between 63 and 116. The retrieval of 25 μg L^{-1} for every chosen PCB exhibited relative standard deviations (RSDs) of under 10.2%. The study revealed that the DLLME-GC-μECD is a potential method for routine analysis techniques for detection of trace concentrations of PCBs in environmental water bodies. Despite the DLLME being simple and fast, there are still challenges rising from the utilization of a disperser solvent presents due to the decreased analyte barrier coefficients in the solvent used for extraction. Issue associated with phase separation resulting from the creation of emulsions has also been cited as a potential barrier when using this method (Pacheco-Fernández and Pino, 2020).

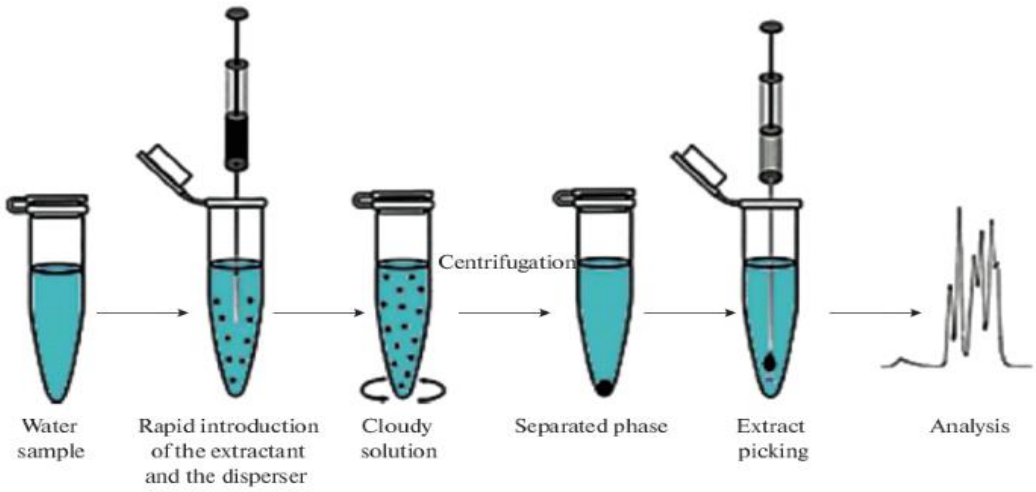


Figure 6: Schematic representation of DLLME (Galuszka *et al*.,2013)

2.2.4.2 Vortex-Assisted Liquid-Liquid Micro Extraction (VALLME)

VALLME entails adding microliters of a solvent that is insoluble in water to an aqueous sample using vortex agitation (Elefteria, 2010). Following the vortex agitation, the separation of two phases is done by the utilization of centrifuge, and the solvent used for extraction is collected for analysis. Step 1 in Figure 7 is showing the distribution of the extracting solvent in the aqueous sample via vortex agitation, leading to the formation of an emulsion as the drops break apart. Centrifugation speeds up the resulting emulsion's phase separation in step 2. After that, the extracting solvent is collected and put to use in chromatographic analysis. The extracting solvent is then collected and used for chromatographic analysis. This VALLME has been employed for the analysis of wastewater for PCBs (Ozcan, 2011). The optimal conditions involved the use of sample of 5 mL, mixed with extraction solvent 0.2 mL of chloroform.

The extraction process included centrifuging the mixture at a speed of 3000 rpm and increased to 4000 rpm for 5 minutes followed by vortexing for 2 minutes. On these optimise conditions, the obtained recovery was 96% (Ozcan *et al*., 2009). Furthermore, a recovery rate exceeding 80% was attained under optimised conditions, which included a 10 mL sample, 0.2 mL of chloroform, an extraction duration of 10 minutes at 25 °C, and a centrifugation period of 5 minutes at 4000 rpm was achieved (Ozcan *et al*., 2009).

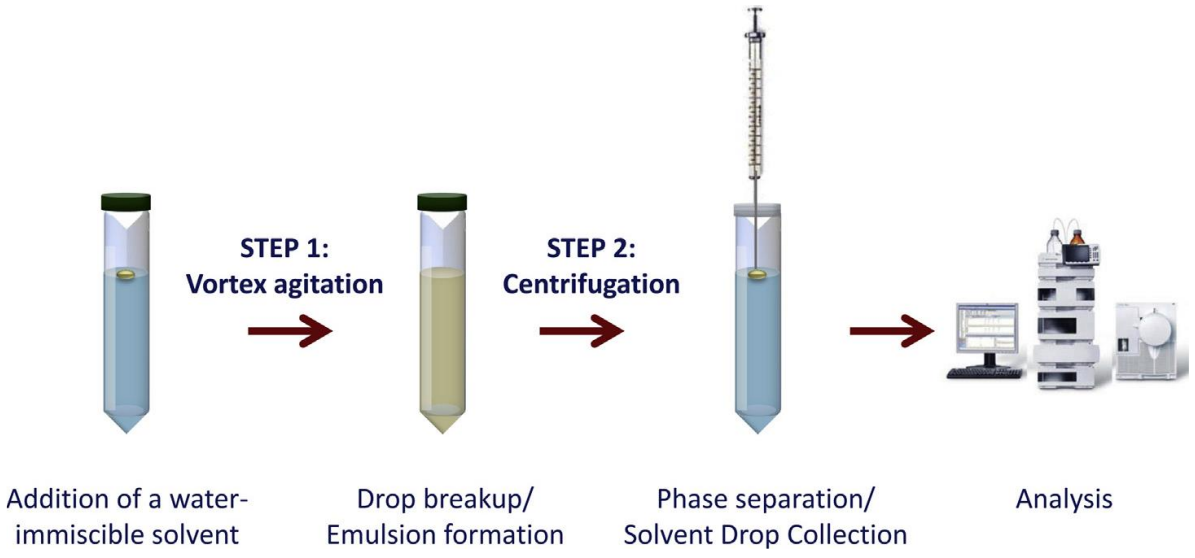


Figure 7: Diagrammatic illustration of the VALLME procedure (Rafael *et al*., 2021)

2.2.4.3 Ultrasound-Assisted Emulsification Micro Extraction (USAEME)

Another extraction technique is called the USAEME, which uses employs ultrasonic radiation as an alternative to a disperser solvent to facilitate the process of emulsification. It was developed as a straightforward and efficient method for the extraction and pre-concentration of trace compounds in aqueous matrices. The USAEME necessitates a minimal amount of extraction solvent, which is emulsified through the application of ultrasound waves. The waves enhance mass transfer from the aqueous phase to the organic phase by facilitating the formation of an extensive surface area as illustrated in Figure 8. USAEME has been applied effectively to analyse different compounds in various matrices including water (Ozcan *et al* 2009 a, 2009b).

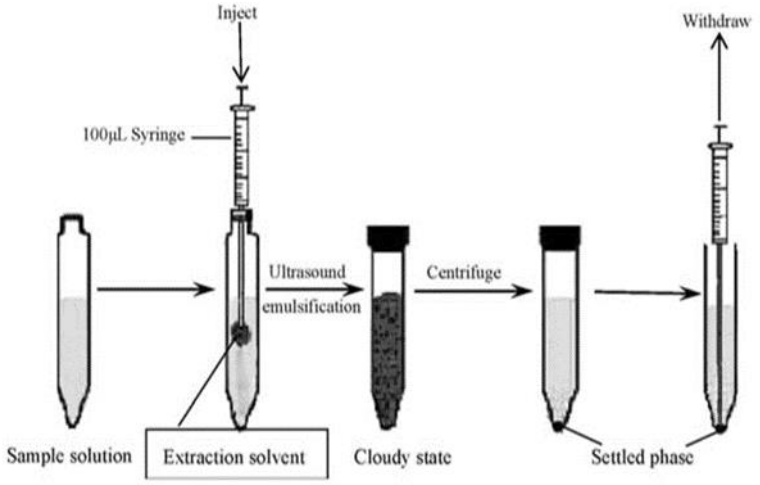


Figure 8: Schematic representation of the USAEME procedure (Gholamreza *et al*,.2016)

The USAEME method was primarily developed for PCBs in water but has also been applied to analyse other substances in samples such as beer, (Chung *et al*., 2012), wine, (Jofre *et al*., 2010), and biological (Seidi *et al*., 2012). It has demonstrated effectiveness, simplicity, speed, and cost-efficiency in the extraction process for gas chromatography (GC) analysis concerning PCBs. The ideal extraction parameters for a 10 mL sample were determined to be 200 µL of isooctane as the extraction solvent, followed by ultra-sonication for a duration of 10 minutes, centrifugation for 10 minutes, and conducted at a temperature of 25 °C without the addition of sodium chloride. The suggested method was also utilized for analysing samples of tap water, yielding mean recovery values ranging from 89.47% to 103.01%, by the use of USAEME. The study found that both the RSDs and recovery values were satisfactory leading to the conclusion that the study area was likely affected by PCBs contamination originating from outdated plumbing systems and water storage tanks (Begum *et al*,. 2016).

2.2.4.4 Hollow Fibre-Liquid-Phase Micro Extraction (HF-LPME)

To overcome the DLLME challenges mentioned in section 2.2.4.1 above the HF-LPME was employed. The HF-LPME employs a hollow fibre as an extracting device, usually made of polypropylene. The fibre is placed in contact with the liquid sample on the outside and contains the organic extraction solvent in the lumen or inside the fibre. The analytes diffuse through the organic liquid which is part of the pores into the lumen and the large particles found in the sample do not obstruct the pores of the hollow fibre as shown in Figure 9. The method has been employed for the analysis of PCBs in human's breast milk and water, achieving recoveries as high as 92% (Villegas-Álvarez *et al*., 2020). Additionally, for PCBs in water, recoveries ranged from 85.9% to 92.0%, with enrichment factors between 718 and 840-fold (Li *et al*., 2008). Membrane-assisted solvent extraction (MASE), closely related to HF-LPME, transfers analytes from the aqueous phase into a small amount of organic solvent via the membrane made of propylene material. This technique removes interferences, is inexpensive, and requires a small amount of extractant (Carro *et al*., 2009).

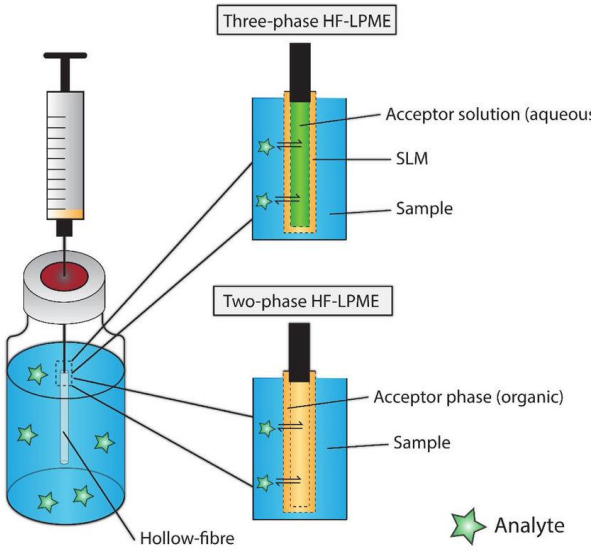


Figure 9: A schematic diagram of HF-LPME, two and three phases (Frederik *et al.*, 2020)

2.2.4.5 Single-Drop Micro Extraction (SDME)

SDME is another extraction method that entails the usage of an organic solvent at the micro syringe's tip to draw out analytes of interests from an aqueous phase. It uses either direct immersion (DI-SDME) or headspace (HS-SDME) modes on a magnetic stirrer as shown in

Figure 10. The DI-SDME involves the direct injection of micro syringe into the solvent whereas the HS-SDME involves the injection onto the headspace solvent vail. After extraction, a micro-drop of organic solvent is drawn into the syringe once more and is ready for injection (Sarafraz-Yazdi *et al*., 2010). In a study by (He *et al*., 2013) for trace amounts of PCBs in water, green ionic liquids utilized in HS-SDME mode attained recoveries ranging from 77.6% to 103.3%, LODs varied between 0.005 and 0.07 µg L^{-1}.

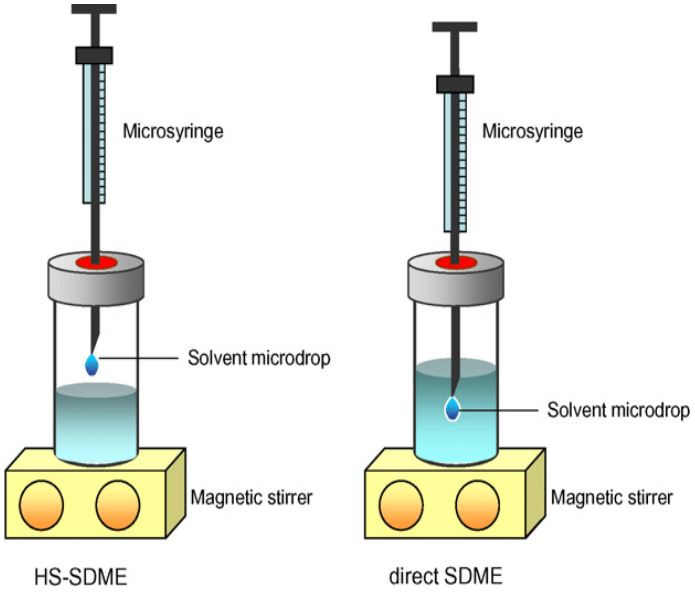


Figure 10: Schematic diagram of the single-drop microextraction (SDME) technique. Direct and headspace SDME (José *et al*., 2008).

2.3 Separation and identification of PCBs techniques

Following sample preparation, extracts are analysed using various analytical methods for the identification and determination of the exact concentrations of organic contaminants in diverse matrices. The use of High-Performance Liquid Chromatography (HPLC) or Gas Chromatography (GC) attached to different detectors namely; the Mass Spectrometry (MS) and Electron Capture Detector (ECD) have been employed for PCBs analysis. Table 2 summarises the findings on various methods for quantifying trace levels of PCBs in different environmental applications, numerous matrixes and recoveries.

Table 2: An overview of PCB's quantification in different environmental matrices and analytical techniques

Extraction technique	Instrument	Sample Matrix	% Recovery	LOD	Reference
Microwave-Assisted Extraction in Open Vessel (MAE-OV)	GC-ECD	Soil	84.7-117.3	0.03-0.27 ng g^{-1}	(Ahmed *et al*., 2018)
Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS)	Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)	Conifer needles	60 -121	<20 ng g^{-1}	(Baroudi *et al*., 2020)
Stir bar sorptive extraction (SBSE)	High-performance liquid chromatography (HPLC) with ultraviolet-visible (HPLC-UV)	River water	72.6 -120	0.048 – 0.22 µg L^{-1}	(Lei *et al*., 2015)
Size Exclusion (SE)	High-Resolution Gas Chromatography coupled with High-Resolution Mass Spectrometry (HRGC/HRMS)	Fish	84 - 115	<1.52 pg g^{-1}	(Wang *et al*., 2010)
Accelerated Solvent Extraction (ASE)	GC-ECD	Caulk and glazing material	45 - 170	-	(Popp *et al*., 2005)
DLLME	GC-µECD	Water	81.9-109	0.05 µg m^{-1}	(Osemwengie *et al*., 2019)
Dispersive solid-phase extraction (DSPE)	Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS)	Tea	70.2-114.7	0.2-1.5 µg kg^{-1}	(Zhixin *et al*., 2023)
Magnetic solid-phase extraction (mSPE)	GC-MS/MS	Beverages	87.0-115.1	0.1-0.2 ng L^{-1}	(Shuangying *et al*., 2022)

2.3.1 Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS is an analytical technique that combines the gas chromatography features with a mass spectrometry detector for the analysis of compounds in a sample mixture. These compounds include the volatile organic compounds, esters, fatty acids, alcohols, aldehydes, terpenes, pesticides, chemical warfare agents, to name a few (Sparkman *et al*., 2011). This analytical technique is employed to determine contaminants at trace levels and consist of wide libraries for further confirmation of analyses. Being employed for the trace analysis, it is a sensitive technique that offers high resolution, precision, accuracy and reproducibility. The GC–MS ion source can operate in either Chemical Ionization (CI) or with electron impact ionization (EI) mode. The CI being a softer ionization whereas EI is the hard ionization technique. The MS detector is a universal detector capable of scanning a wide range of different compounds in various applications.

This technique has been employed to evaluate trace concentrations of PCBs in air, soil, and surface water matrices, achieving detection limits of 2.0×10^{-5} to 1.0×10^{-5} ng m^{-3} for air, 2.0×10^{-5} to 1.5×10^{-4} ng L^{-1} for water, and 3.0×10^{-5} to 1.0×10^{-4} ng g^{-1} for soil (Ullah *et al*., 2020). On another study, PCB in fresh root and leafy vegetables varied from 90.9 to 234 ng g^{-1} with detection limits between 10 and 22 ng g^{-1} (Olatunji, 2019). The presence of PCBs in surface water varied from concentrations of 32.17 to 199.4 ng L^{-1}, with detection limits between 10 and 22 ng L^{-1} (Habibullah-Al-Mamun *et al*., 2019). PCB concentrations in commercial seafood were assessed using Gas Chromatography-Tandem Mass Spectrometry (GC–MS/MS), with limits of detection achieved between 0.011 and 0.014 ng g^{-1} (Li *et al*., 2017).

2.3.2 Gas chromatography Electron Capture Detector (GC-ECD)

GC-ECD is a useful technology for analysing halogenated chemicals in various environmental matrices. It is a selective detector that is sensitive for halogens, organohalides, and nitrogen-containing compounds (Qadeer *et al*., 2019). Studies have been done by the use of GC-ECD to measure the amounts of PCBs in urban river surface sediments. Winter and summer concentrations obtained were from 7.90 to 30.21 ng g^{-1} and 5.53 to 32.67 ng g^{-1}, respectively, whereas detection limits ranged from 0.1 to 0.9 ng g^{-1} (Qadeer *et al,*. 2019). In addition, with detection limits of 0 to 0.8 ng g^{-1}, PCB levels in Bering flounder from the Okhotsk Sea varied from 24.7 to 279 ng g^{-1} (Lukyanova *et al*., 2018). A more improved, latest developed and sensitive GC with micro ECD (GC-µECD) coupled with a headspace was used in this study to prevent the loss of some volatile's presence in the samples. The GC-µECD is an extremely

sensitive technique ideal for detecting halogenated organic chemicals and aromatic contaminants down to lowest levels such as femtogram.

2.3.3 High Performance Liquid Chromatography (HPLC)

A technique utilized to isolate mixtures of substances that are structurally similar and identify the components within a mixture is called an HPLC. This technique is sensitive, adaptable and suitable for extremely complex mixtures. Frequently, GC or GC-MS are preferred instruments for analysing volatile and semi-volatile organic compounds. However, HPLC is capable of managing a much broader array of combinations, including those that contain non-volatile or thermally unstable molecules. Derek in (2007) used an HPLC equipped with a C18 column for the separation of different PCB in soil and water samples. In the study, the PCBs classes were eluted in less than 20 minutes at standard HPLC backpressures. C18 columns are therefore an excellent alternative choice for the analysis of PCBs. Another investigation conducted by Al-Janabi (2016) utilized HPLC with the ultraviolet (UV) detector at wavelength of 225 nm and was applied in river water. On the standard mixture of eight PCBs with the duration of 12 minutes and by addition of PCBs standard combination of distilled water, the precise HPLC retention durations for the targeted analytes were ascertained with the LODs achieved varying between 0.10 and 0.38 µg L^{-1} (Al-Janabi, 2016). Both studies came to the conclusion that, in the absence of GC, the HPLC-with the UV method is a useful alternative for PCB analysis. Additionally, it can be used to analyse other hazardous organic compounds present in water in small amounts.

2.3.4 Ultra-High Performance Liquid Chromatography (UHPLC)

UHPLC is a modern liquid chromatographic system, which is simply the modification of HPLC for the investigation of environmental non-volatile compounds in food industries, pharmaceuticals, agricultural and drug industries for sample analysis. In UHPLC, particle sizes, column dimensions, flow rates, back pressure and detection parameters are smaller than HPLC which provides a better resolution, speed and sensitivity compared to HPLC (Durai *et al*., 2020). UHPLC is a type of column chromatography where a solvent, which is a mobile phase is allowed to flow through a column filled with an adsorbent under pressure. The compounds are separated within the column, and their separation relies on the differing levels of interaction with the stationary phase, leading to varying elution rates for each unique component (Kupiec, 2004). After separation, a detector is used to quantify analytes (Swartz, 2010). Studies

conducted by Lei *et al*. (2016) involved the determination of PCBs in water samples using UHPLC coupled by the Ultra Violet (UV) detection. The obtained PCB concentration varied between 880 to 55 ng L^{-1} and LODs between 48 to 220 ng L^{-1}. Similarly, PCB concentrations in river water were determined using UHPLC with UV detection and found showing a range between 111 and 6700 ng L^{-1}, with the LODs from 110 to 620 ng L^{-1} (Al-Janabi, 2016).

CHAPTER THREE- GENERAL AND SPECIFIC OBJECTIVES

3.1 General Objective

The general objective of the study was to develop and optimise a dispersive liquid–liquid microextraction method coupled to GC-µECD for the determination of PCBs in borehole water samples in the Northwest Province.

3.2 Specific Objectives

- To optimise various parameters that affect the DLLME method for the extraction of selected PCBs on real water.
- To develop a separation and quantification method to measure PCBs using GC-µECD then further validation using GC-MS (EI).
- To apply the developed method to quantify PCBs presence in boreholes from Northwest Province, South Africa.

3.3 Research Rationale and Motivation

PCBs can endure various conditions typically encountered in industrial processes. These include being resistant to high temperature, high pressure and stability when exposed to harsh acids and bases. As a result, they were appealing to different applications in dielectric fluids, plasticizers, heat transfer fluids and flame retardants to mention a few. However, being stable also relates to them not degrading over a period of time and consequently accumulation in the environment. The environmental risk assessment of chlorinated compounds such as PCBs requires reliable validated analytical methods for detection and there is limited data published on their detection in South Africa. Hence the study was seeking for new improved methods and techniques used for their analysis. Traditional methods of preparing of liquid samples such as liquid-liquid extraction (LLE) can be time consuming and use significant quantity of solvents. Other techniques like SPE exhibit lower recoveries because of the interactions between the sorbent and the target analytes that is non-selective, especially when compared to newer microextraction methods. As a result, DLLME has been developed to address the shortcomings of traditional extraction methods which include usage of more solvent volumes, time consuming and expensive. The GC technique was applied offered a high resolution,

reproducibility, high precision and accuracy. In this study, the DLLME method was modified for water analysis in boreholes.

3.5 Hypothesis and Research Question

3.5.1 Hypothesis

- The boreholes that collect water from underground in Northwest province are contaminated with PCBs due to possible use of PCBs in the local mines in surrounding area.
- The dams in this area are polluted with PCBs due to excessive burning of wood, combustion of petroleum products, and coal mining nearby.

3.5.2 Research Question

- Can the optimised DLLME coupled to the GC-µECD be sensitive enough to detect PCBs in borehole water from the Northwest Province?

CHAPTER FOUR- RESEARCH METHODOLOGY

The methods and experimental procedure are presented in this chapter. The chapter covers study area, sample collection, chemicals and reagents, quality assurance, method validation, using actual samples and the optimised method as well as sample extraction by DLLME methods followed by analysis using GC-MS and GC-μECD.

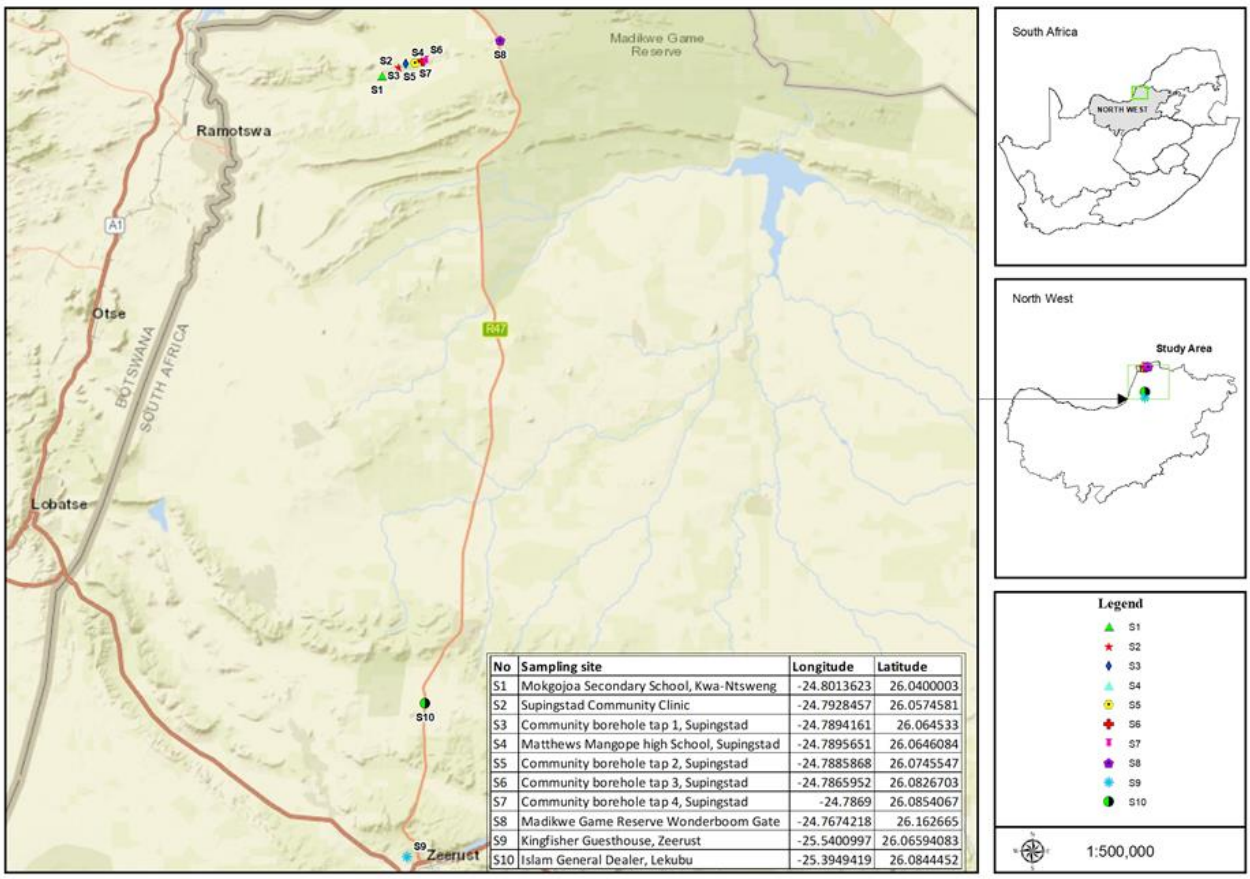
4.1 Study area

Supingstad is a town the Ngaka Modiri Molema District Municipality located in the North West province of South Africa. It is the South African city in closest proximity to Botswana, more precisely near Gaborone. The two countries share a high-potential Karst aquifer known as the "Ramotswa Dolomitic Aquifer" which is located in South Africa's North West province and in Botswana's South East district. Within the municipality, there are approximately 3238 boreholes in which various towns and villages depend on groundwater (Altchenko *et al*., 2017). Samples were collected from the taps installed on boreholes. This water comes from this aquifer, which covers the towns and other settlements within this region. The aquifer is an important source of water in this region and it serves to meet the community's drinking water needs. The areas were selected due to bad water quality and poor maintenance of the infrastructure (Altchenko *et al*., 2017). Ten sampling areas were chosen for the study and their exact coordinates are presented in the below Table 3 and their map is illustrated in Figure 11.

Table 3: Details of sampling locations

No	Sampling site	Longitude	Latitude
S1	Mokgojoa Secondary School, Kwa-Ntsweng	-24.8013623	26.0400003
S2	Supingstad Community Clinic	-24.7928457	26.0574581
S3	Community borehole tap 1, Supingstad	-24.7894161	26.064533

S4	Matthews Mangope high School, Supingstad	-24.7895651	26.0646084
S5	Community borehole tap 2, Supingstad	-24.7885868	26.0745547
S6	Community borehole tap 3, Supingstad	-24.7865952	26.0826703
S7	Community borehole tap 4, Supingstad	-24.7869	26.0854067
S8	Madikwe Game Reserve Wonderboom Gate	-24.7674218	26.162665
S9	Kingfisher Guesthouse, Zeerust	-25.5400997	26.06594083
S10	Islam General Dealer, Lekubu	-25.3949419	26.0844452



No	Sampling site	Longitude	Latitude
S1	Mokgojoa Secondary School, Kwa-Ntsweng	-24.8013623	26.0400003
S2	Supingstad Community Clinic	-24.7928457	26.0574581
S3	Community borehole tap 1, Supingstad	-24.7894161	26.064533
S4	Matthews Mangope high School, Supingstad	-24.7895651	26.0646084
S5	Community borehole tap 2, Supingstad	-24.7885868	26.0745547
S6	Community borehole tap 3, Supingstad	-24.7865952	26.0826703
S7	Community borehole tap 4, Supingstad	-24.7869	26.0854067
S8	Madikwe Game Reserve Wonderboom Gate	-24.7674218	26.162665
S9	Kingfisher Guesthouse, Zeerust	-25.5400997	26.06594083
S10	Islam General Dealer, Lekubu	-25.3949419	26.0844452

Figure 11: Map showing the sampling sites for this study along the North West province, South Africa (created using ArcGIS, ArcMap version 10.6)

4.2 Sample Collection

Samples were collected in ten different areas from borehole taps. Newly purchased 50 ml plastic bottles were used for sample collection and rinsed with acetone and were left to dry before rinsing with deionized water. Samples were put in an ice-filled cooler and taken for analysis to the laboratory. After collection, samples were kept at 4°C until analysed and filtered using a size 0.45-µm filter paper.

4.3 Chemicals and Reagents

A PCB mixture of 500 µg mL^{-1} in acetone containing reference 8 compounds (2-chlorobiphenyl (99.5%); 2,3-dichlorobiphenyl (99.5%); 2,4,5-trichlorobiphenyl (99.0%); 2,2,4,4-tetrachlorobiphenyl (99.0%); 2,2,3,4,6-pentachlorobiphenyl (99.6%); 2,2,4,4,5,6-hexachlorobiphenyl (99.8%); 2,2,3,3,4,4,6-heptachlorobiphenyl (99.6%) and 2,2,3,3,4,5,6,6-octachlorobiphenyl (98.8%)) was purchased from Dr Ehrenstorfer GmbH, (Augsburg,

Germany). HPLC grade acetone, acetonitrile and n-Hexane with 99% purity were purchased from Merck (Darmstadt, Germany). Dodecanol (98.0%), methanol (99.8%) and undecanol (98.7%) were sourced from Sigma-Aldrich (Burghausen, Germany). Dichloromethane (99.5%) from Macron fine chemicals (Gliwice, Poland). Hexadecane (99.0%) and Magnesium sulfate (99.5%) were obtained from Sigma-Aldrich (St Louis, USA). Ultrapure deionized water system (Siemens Labostar, USA) was used throughout the experiment.

4.4 Optimisation of DLLME Method

To optimise the extraction efficiency of DLLME method, parameters such as selection of extraction solvents, the effect of extraction solvent volumes, selection of disperser solvent, the centrifuge time, extraction time and drying salt amount were optimised.

4.4.1 Optimisation of extraction solvent type and volume

The selection of extraction solvent was conducted by using five solvents for PCBs extraction (Rezaei *et al.*, 2008). This included hexane, dichloromethane, hexadecane, undecanol and dodecanol. The spiking with CRMs was done with a 0.1 µg L^{-1} concentration and analysed on GC. The highest peak area responses obtained were chosen as the best solvent of choice to be used further for extraction. Based on the fact that DCM has the highest density compared to hexane, it was selected as the optimal extraction solvent. The DCM was further varied with the following volumes 0.01, 0.02, 0.03, 0.05, 0.1, 0.2 and 0.3 mL respectively. The extraction volume that gave the highest sediment layer was selected as an ideal volume for the experiment.

4.4.2 Optimisation of disperser solvent type and volume

During the optimisation of disperser solvents, three solvents were chosen for the study. The solvents used were acetone, methanol and acetonitrile. The extraction solvent and disperser solvent were combined and added to an aqueous matrix. The extraction solvent volume was kept constant and the volume of dispenser solvent 0.5, 1.0, 2.0 and 2.5 mL. In varying the disperser solvent, the best solvent was chosen based on the amount of sediment layer volume recovered.

4.4.3 The effect of extraction time

Selected time intervals of 1, 2, 5, 10, and 15 minutes were used to examine the impact of extraction time. This was conducted by observing the time it took to form a cloudy solution

after mixing the disperser and extraction solvents. The cloudy solution was centrifuged to separate and retained the sediment phase for analysis. Other parameters such as sample volume, spiking concentration, centrifuge speed, extraction solvent and its volume were kept constant.

4.4.4 Centrifuge time

The centrifuge speed was not optimised but kept at 1400 rev/min because the 3-in-1 Eppendorf concentrator plus/vacufuge plus equipment that was used came with only that speed setting from the manufacturer. The centrifuge time was varied between 2 and 15 minutes while other parameters such sample volume, spiking concentration, extraction time, extraction solvent and its volume were kept constant. The time taken to get a clear separation from the centrifuge time was taken as the optimum centrifuge time.

4.4.5 Drying Salt

Optimisation of drying salt was done by varying the amount of magnesium salt used as drying agent of the extract. The salts amount used were 5, 10, 20 and 50 mg while keeping other parameters constant. After filtering with different salt amount, the sediment layer with the highest obtained amount of the extracts was taken as an optimum amount of salt. The drying process was done by pushing the extract through a Pasteur pipette packed with cotton wool and $MgSO_4$ salt with the aid of a rubber nipple. The volume of the obtained sediment layer was measured by the removed sediment layer obtained, and the extract was transferred into a low insert vial using the hypodermic sterile needle.

4.5 Analysis of PCBs

4.5.1 Analysis of PCBs on GC-MS (EI)

The analysis of PCBs was carried out in triplicates (5 mL) and injected in triplicates utilizing Helium (He) as a carrier gas with a flow rate of 0.9 mL/min. The starting injection temperature was maintained at 250 ºC. The initial oven temperature was set at 40 ºC, maintained for 2 minutes, and subsequently raised to 280 ºC at a ramp rate of 10 °C/min making an overall time to be 36 minutes. The mass detection was scanned from 40 to 550 atomic mass unit (amu). The mass spectra electron energy was 70eV; the ion source was electron impact (EI) of mass scan ranges between 40 to 550 amu with 3.50 minutes of solvent delay. The source temperature of

the MS was set to 230 ºC, while the temperature of the quad was at 150 ºC and peak identification and data processing were done using the National Institute of Standards and Technology (NIST) library software and databases. Retention times of compounds were used to identify peaks for specific ions.

4.5.2 Analysis of PCBs on GC-µECD

The analysis of PCBs was carried out in triplicates (5 mL) and injected onto GC-µECD using nitrogen as a carrier gas with the starting injection temperature was maintained at 250 ºC. The initial oven temperature was set at 40 ºC, maintained for 2 minutes, and subsequently raised to 280 ºC at a ramp rate of 10 °C/min making an overall time to be 36 minutes. The GC-µECD was linked to a headspace alongside an oven; loop/valve and transfer line temperatures of 90 ºC; 100 ºC and 110 ºC. The GC cycle; valve equilibration; pressure equilibration and injection time of 45 min; 20 min; 0.10 min and 0.50 min with a vial size of 10 or 20 mL.

4.6 Quality Assurance

The optimisation of the chromatographic instruments as part of quality assurance was achieved by using GC-MS (EI) and GC-µECD. Prior to their usage, instruments were well maintained. This included the installation of new non-polar HP-5ms columns, the use of a new 10 µL syringes, replacement of the liners, putting new septa and cleaning of the ion source. To check the selectivity and sensitivity of MS ion source, instrument was auto tuned, and the obtained tune report was recorded.

To check the performance of the HP-5ms column in both techniques, a QC-Mix plus C-series (carbon chain) were ran in both techniques. The QC test solution consisted of a 1000 µg L^{-1} of six compounds; namely 2,6-dimethylphenol (DMP); 5-chloro-2-methylaniline (CMA); tributylphosphate (TBP); dibenzothiophene (DBT); malathion (MAL) and methyl stearate (MST) and a C-series standard is composed of carbons chains from C_8-C_{30} carbon chains prepared in-house. The QC mixture was prepared in hexane. To prepare the 10 µg L^{-1}, the QC

mixture plus C-series were prepared in hexane by taking a 10 µL of each into a 980 µL of hexane to make a 1 mL total volume.

4.7 Analytical Instruments

4.7.1 Gas Chromatography-Mass Spectrometer (electron ionisation)

An Agilent 5975C inert MSD mass spectrometer equipped with a triple axis detector functioning in the positive electron ionisation (EI) mode connected to an Agilent 7890A GC system utilizing ChemStation software (Agilent MSD ChemStation G1701EA, E.02.01.177) was employed. The gas chromatograph was equipped with a non-polar HP-5 ms column with dimensions of 30 m, length; 0.25 mm I.D; 0.25 µm film thickness; stationary phase, 5% phenyl, 95% methyl siloxane. The gas chromatograph had a non-polar HP-5 ms column with dimensions of 30 m in length, 0.25 mm inner diameter, and 0.25 µm film thickness; the stationary phase consisted of 5% phenyl and 95% methyl siloxane. A 99.999% pure helium gas was utilized as a carrier gas with a steady flow of 0.9 mℓ/min.

4.7.2 Gas Chromatography-Micro Electron Capture Detector-Headspace

An Agilent GC system with Openlab CDS Chemstation edition software rev.c.01.10 [201] utilizing headspace (G4557A) was employed. The gas chromatograph had a non-polar HP-5 ms column with dimensions of 30 m in length, 0.25 mm inner diameter, and 0.25 µm film thickness; the stationary phase consisted of 5% phenyl and 95% methyl siloxane. A 99.999% pure helium gas was utilized as a carrier gas with a steady flow of 0.9 mℓ/min.

4.8 Preparation of stock standard solution and calibration curves

Hexane, dichloromethane, hexadecane, undecanol and dodecanol were used to prepare stock standard solution (1 µg L^{-1}) consisting of a mixture of 8 PCBs compounds. The solution was vortex for 5 minutes and decanted onto 2 mL GC vials for analysis. To prepare calibration standards, a 50 µg L^{-1} working calibration standard stock solution was prepared from a newly purchased 500 µg L^{-1} stock. This working calibration standard stock was prepared by pipetting a 100 µL into 900 µL of dichloromethane to a final volume of 1mL vial. Calibration curves were plotted using the prepared working ranges of some PCB standards from 50 µ g L^{-1} to 3.9 $x10^{-1}$ µg L^{-1} as tabulated in Appendix 1. Concentration fitting points of standards were prepared in a 1 mL vial from the 50 µg L^{-1} working calibration solution. To ensure the linearity and reliability of the calibrations curve, a standard control was used and recoveries were calculated. The control had the same compounds as the standard and was prepared with the same

concentration as the middle standard to calculate the % recovery. The accepted recovery criteria range between 80-120%.

4.9 Method validation

To ascertain the performance of the developed DLLME parameters in real sample matrix, water samples were collected from Hennops River in Centurion, Pretoria, South Africa. Samples were initially filtered through a 0.45 µm PTFE (polytetrafluoroethylene) membrane to eliminate any suspended solid particles and were then thoroughly homogenized using a vortex for 2 minutes before sub-sampling. A 0.01 µg L^{-1} spiked solution was prepared from a 1 µg L^{-1} working stock solution to spike the river water sample in triplicates. The enrichment factors (EF) of each PCBs after spiking were calculated by observing the change in concentration through the obtained area responses in the spiked concentration sediment phase and initial concentration in aqueous phase. The enrichment factor for each PCB was calculated using the formula:

Enrichment factor (EF) = C_{sed}/C_0 where, C_{sed} is Concentration in sediment phase and C_0 is Initial Concentration in the aqueous phase.

The determination of LOD and LOQ was done by preparing standards in different concentrations from the highest (50 µg L^{-1}) and diluted down to the lowest (3.9×10^{-5} µg L^{-1}), where it was no longer detected by the instrument or close to the noise. All standard preparations in different concentrations are shown in Appendix 1. The working standards were prepared from a 500 µg L^{-1} stock and analyzed by the GC-µECD. The instrumental limit of detection (LOD) was calculated based on international guidelines and determined as 3 times signal to noise ratio whereas the LOQ was estimated as 10 times signal to noise ratio (US EPA ,2014).

4.10 Application of the optimised method to real samples

Prior to application of DLLME optimised method to real samples, the samples were measured for their physical and chemical properties by determining pH, turbidity, total dissolved solutes (TDS) and conductivity. Thereafter, sample aliquot (5 mL) was filtered (in triplicates) and shaken vigorously for homogeneity. A 500 µL of acetone was added (disperser solvent) and 200 µL of dichloromethane (extraction solvent) were added and vortexed for 5 minutes. The mixture was then centrifuged at 1400 rev/min for 5 minutes. A sterile needle was used to

remove the extract and 5 mg of $MgS0_4$ was added onto the recovered sediment layer to eliminate excess water and lastly the extract was subsequently placed into a low insert GC vial for analysis.

CHAPTER FIVE- RESULTS AND DISCUSSION

5.1 Results on the Optimisation of DLLME

5.1.1 Extraction solvent selection by evaluating their compatibility with separation techniques

Upon completion of the quality assurance results of the chromatographic system, which gave satisfactory results, we commenced with the optimisation studies of the DLLME method that was employed in the study. At this point, that aim was to identify the best extraction solvent. The results from these solvent optimisation studies are disclosed in Figures 12 and 13. These figures shows that hexane gave better area responses followed by DCM. The least performing solvents were the DES (deep eutectic solvents) namely hexadecane, undecanol and dodecanol. These solvents were selected on the basis of six basic requirements. These properties include solvents that possess a density higher than that of water (greater than 1 g/cm^3), solvents that are insoluble in water and ability to form a cloudy solution when combined with a disperser solvent. Also, solvents that are able to dissolve the target compounds, solvents that have an ability to form a stable two-phase solution and offers good resolution such as of compatible with the column or detector.

Even though the DES are less toxic by adhering to green chemistry principles, they were not be used due to the lower area responses which is equivalent to lower concentration detected as shown in Figures 12 and 13. The poor performance of undecanol and dodecanol in dissolving some PCBs could have arisen from the tendency of these fatty alcohols to solidify at ambient temperatures. Also, fatty alcohol shares the same structural similarities with PCBs which could results into co-extraction and co-elution with the compounds of interest (Mudge.,2005). Dodecanol solidified in a few minutes after it was left in the fume cardboard and therefore, hexane and DCM were selected based on their density factors, no co-extract and no interferences with the compounds of interest. DCM has a density of 1.33 g/cm^3 while hexane has a 0.766 g/cm^3, which is lower than that of water. As a result, DCM had an added advantage as an extraction solvent, because it easily formed the bilayer with the aqueous layer. Additionally, it was easy to remove it from the aqueous layer unlike hexane.

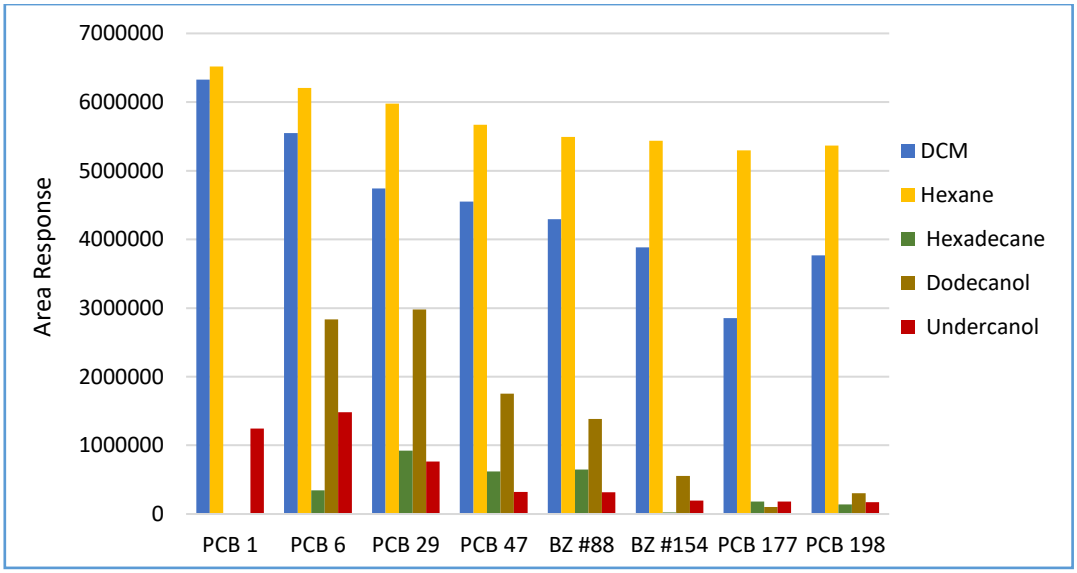


Figure 12: Summary results of 1000 µg/L of PCBs prepared in different solvents analysed on GC-MS (EI)

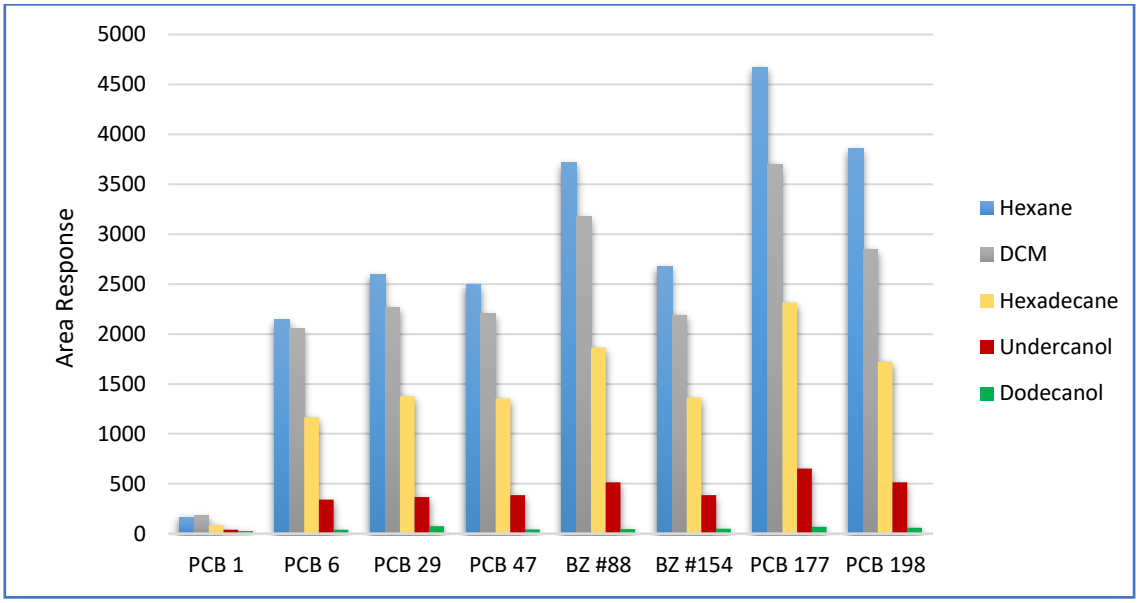


Figure 13: Summary results of 1000 µg/L of PCBs prepared in different solvents analysed on GC-µECD

5.1.2 Volume of the extraction solvent

After DCM was selected as an extraction solvent, the solvent volume was then optimised. This was experimented by varying the DCM and the sediment volume as depicted in Figure 14 and at volume of 200 µL of DCM, the results were satisfactory. It was observed that the sediment layer volume increased from a smaller volume of 0.04 mL at 100 µL to 0.123 mL at 200 µL.

It was further observed that the higher the extraction solvent volume, the higher the sediment layer volume. According to the study by Rezaei and others in 2008, the solvent volume used for extraction should not be set very low that the sediment phase volume would become less than 5 µL, which might cause difficulties in the removal with a micro syringe resulting into systematic errors.

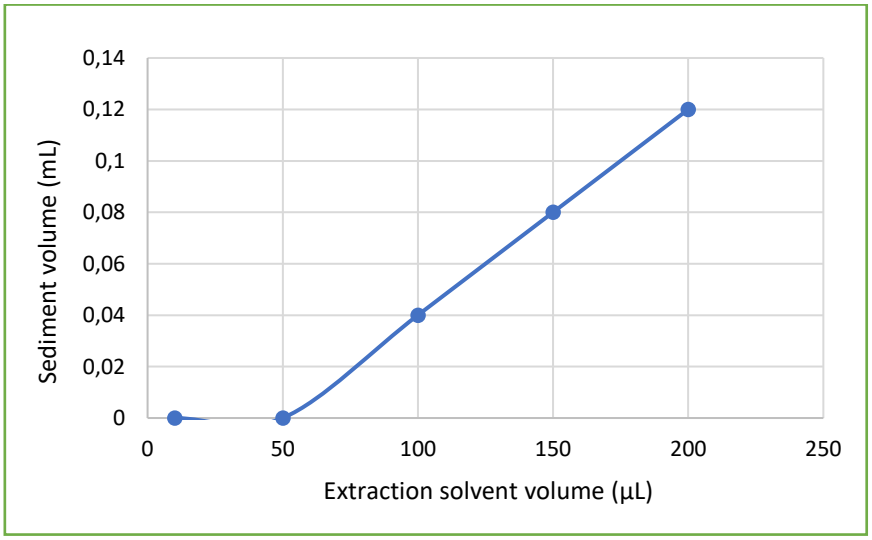


Figure 14: Extraction solvent volume versus the recovered volume

5.1.3 Selection of disperser solvent and volume

After determining the optimum volume for the extraction solvent, the focus was shifted to the selection of the disperser solvent and its volume. One of the characteristics of the disperser solvent is its miscibility in water and the ability to dissolve the extraction solvent. Acetone, acetonitrile and methanol exhibit these properties; thus, the study focused on them and results obtained are displayed in Figure 15. As it can be seen, acetonitrile consistently outperformed both acetone and methanol when considering area responses and sediment layer. It was followed by acetone, which also gave good area responses in recovering PCBs. Moreover, acetone also gave a higher sediment layer volume to work with for the DLLME as shown in Table 5. Due to the toxicity and expensiveness of acetonitrile, acetone was selected as the solvent of choice among these solvents since it is less toxicity and cheaper (Rezaee *et al.*, 2006).

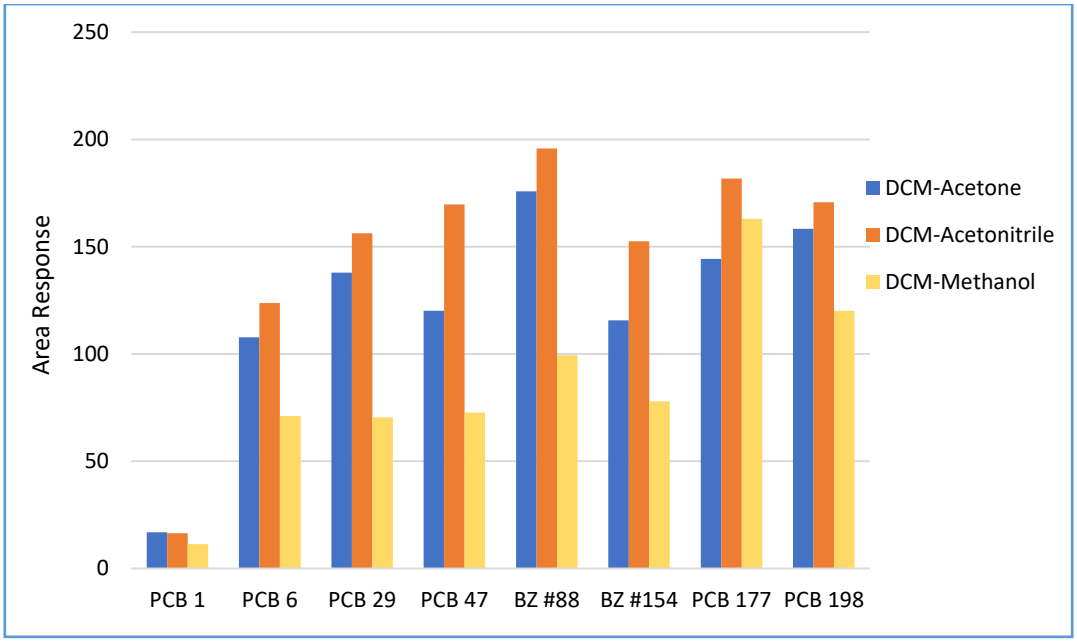


Figure 15: Addition of disperser solvents on extraction solvent DCM

After determining disperser solvent, the optimal volume of that solvent was then investigated. Table 4 summarizes the results obtained. To evaluate the impact of the disperser solvent amount, the extractions were performed over a range of volumes, from the lowest to the highest volume of 10-500 µL following the work done (Rezaei et *al.*, 2008). The addition from 10 µL up to 50 µL of the disperser solvent showed no separation as can been seen in Table 4. Separation was noticed when 5 mL of water added into 100 µL of acetone and upwards. It was noted that the higher the disperser volume added, the cloudier the solution became and the larger the sediment layer.

Table 4: Effect of acetone volume on layer separation

Volumes (µL)	Observations
10	No separation
20	No separation
30	No separation
50	No separation
100	Separation

200	Separation
300	Separation
500	Clear separation

The extraction solvent volume DCM of 200 µL combined with 500 µL of acetone gave the best results as illustrated in the Figure 16. According to the results obtained, it was concluded that at the highest disperser volume of 500µL as the separation was clear which increased the solubility of PCBs. Thus, in this study the acetone volume of 500 µL was selected as the optimum disperser solvent volume. A 1 mL disposable sterile non-bacteria, non-toxic, non-pyrogenic syringe was used with a hypodermic sterile needle for the extract removal.

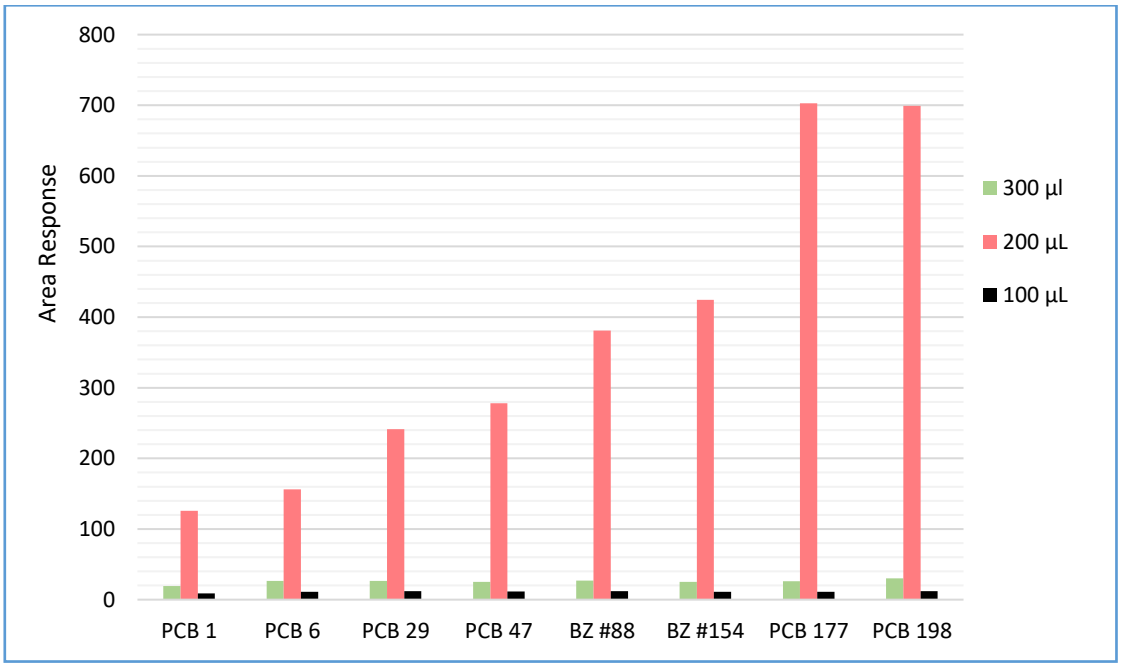


Figure 16: Enrichment Factor (EF) of 100 µL, 200 µL & 300 µL of DCM with acetone

5.1.3.1 Settling effect of different combinations of extraction solvents-disperser solvents

Table 5 in the next page shows the settling effect on different combination of extraction solvents and disperser solvents in different disperser solvent volumes. At lower volumes between 10 µL and 50 µL there was very little sediment layer formed. However, as the extraction solvent volume increases a clear sediment layer was observed and with methanol it started at volume 200 µL and 300 µL and a white turbid sediment was formed. The white turbid

sediment formed was not suitable for any direct instrumental injection as it would clog the instrument and interference with the results. Therefore, acetone was chosen as the optimum disperser solvent used due to its settling effect and clear sediment with no white turbid generated. For the best DLLE, the extraction solvent needs to be immiscible in water, have affinity with the analytes and preferably have a good chromatographic behaviour whereas the disperser solvent needs to be water soluble and DCM and acetone resembles these properties (Mark *et al*.,2001).

Table 5: Settling effects of different combinations of extraction solvents-disperser solvents

	Disperser Solvents		
Solvent Volumes (µL)	**Acetone**	**Acetonitrile**	**Methanol**
10	No sediment	No sediment	No sediment
20	No sediment	No sediment	No sediment
30	No sediment	No sediment	No sediment
50	No sediment	No sediment	No sediment
100	Clear sediment	Clear sediment	Clear sediment
200	Clear sediment	Clear sediment	White turbid sediment
300	Clear sediment	Clear sediment	White turbid sediment

5.1.3.2 Sediment layer volumes after extraction

According to the results shown in Figure 17, an addition of a higher volume such as 300 µL acetone as a disperser solvent was found to be optimum. In Figure 17, it was observed that methanol exhibited the least recovery, whereas acetonitrile and acetone had the highest recovery at 300 µL. The volume was deliberately not adjusted further to less than 300 µL. This was to avoid the sediment phase being less than 5 µL, which may cause challenges in its removal by the use of micro syringe and causing systematic errors (Rezaei *et al.*, 2008).

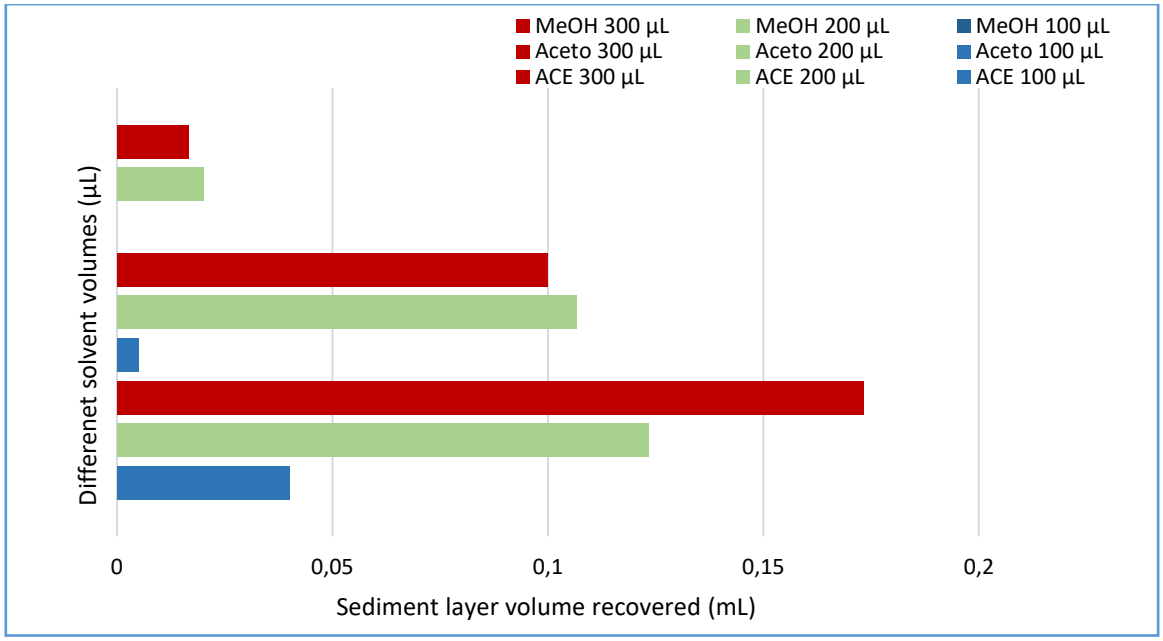


Figure 17: Sediment layer volume after extraction

5.1.4 Centrifuge speed and time

The centrifuge speed was kept at 1400 rev/min because the 3-in-1 Eppendorf concentrator plus/vacufuge plus equipment used had a default speed setting. The speed of 1400 rev/min was sufficient for DLLME separation. According to (Hou *et al*., 2019), centrifuge speed has a little effect on the extraction efficiency. The effect of centrifuge time was measured as it is regarded an essential parameter in eliminating emulsification. During the centrifuge process, there is incomplete collection of the organic phase from the sediment layer with inadequate centrifuge time (Hou *et al*., 2019). In this study, centrifugal time was varied between 2 and 15 minutes. The best results were visually noticed at 5 minutes as demonstrated in the image in Figure 18 below because the separation was clearly visible at this time and testing up to 15 minutes is a little excessive as it adds unnecessary time without improving results.



Figure 18: Image of a centrifuge used and separation results after centrifuge

5.1.5 Drying Salt

To remove any residual water that could interfere with the analysis remaining in the organic layer, magnesium sulphate ($MgSO_4$) was used. It was varied at amounts from 5 mg-50 mg. The results were assessed through monitoring the sediment layer volume as discussed in section 5.1.3.2 above. This was to ensure a phase separation between organic solvent and water. It was observed visually that the lesser the sediment layer volume, the less the amount of $MgSO_4$ was needed for the removal of water. DCM is denser than water, therefore the separation was visible and 5 mg of salt was used as an optimum drying salt amount. The sediment layer was removed and placed into a 2 mL GC low insert vial using a hypodermic sterile needle and loaded into the instrument for analysis. It was concluded that the higher the volume of extraction solvent, the clearer the separation and the lesser the drying agent needed as a result, in several studies (Hou et al., 2019) DLLME procedure was carried out with no addition of salt.

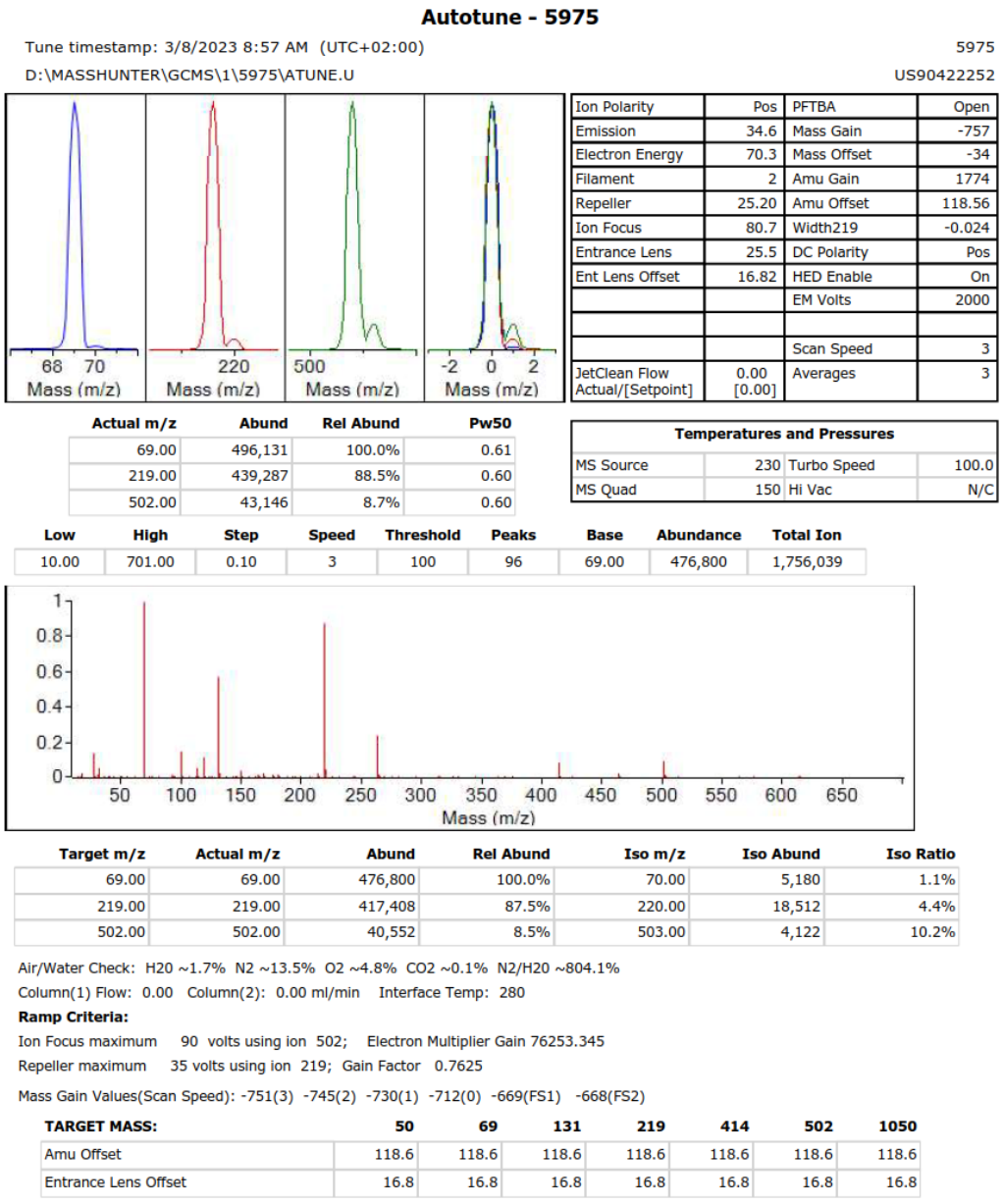
5.1.6 The effect of extraction time

In this study, it was observed that the transfer of the analytes from the aqueous phase to the extraction phase was fast and equilibrium was achieved quickly. Consequently at 2 minutes the cloudy solution was formed when mixing the extraction and disperser solvents and was selected as an optimal time on a range between 1-5 minutes. The cloudiness confirms successful phase separation or analyte transfer. The time period of 2 minutes is in full agreement with the studies on the DLLME for the determination of PCBs in water (Rezaei *et al*., 2008). Therefore, the DLLME does not consume time and the extraction time has no significant effect on the efficiency of extraction for all target compounds and this is the most outstanding benefit of this method.

5.2 Quality assurance results of the chromatographic system

5.2.1 Auto tune report

The tune report obtained from GC-MS system in Figure 19 shows that parameters such as the target masses of 69, 219 and 502 m/z, the air/water leaks of the system. The Electron Multiplier (EM) volts indicated that the ion source was free of contaminates, no faulty filament, vacuum was enough and EM was passing for it to be used below 3000 volts.



Autotune - 5975

Tune timestamp: 3/8/2023 8:57 AM (UTC+02:00) 5975

D:\MASSHUNTER\GCMS\1\5975\ATUNE.U US90422252

Ion Polarity	Pos	PFTBA	Open
Emission	34.6	Mass Gain	-757
Electron Energy	70.3	Mass Offset	-34
Filament	2	Amu Gain	1774
Repeller	25.20	Amu Offset	118.56
Ion Focus	80.7	Width219	-0.024
Entrance Lens	25.5	DC Polarity	Pos
Ent Lens Offset	16.82	HED Enable	On
		EM Volts	2000
		Scan Speed	3
JetClean Flow Actual/[Setpoint]	0.00 [0.00]	Averages	3

Actual m/z	Abund	Rel Abund	Pw50
69.00	496,131	100.0%	0.61
219.00	439,287	88.5%	0.60
502.00	43,146	8.7%	0.60

Temperatures and Pressures			
MS Source	230	Turbo Speed	100.0
MS Quad	150	Hi Vac	N/C

Low	High	Step	Speed	Threshold	Peaks	Base	Abundance	Total Ion
10.00	701.00	0.10	3	100	96	69.00	476,800	1,756,039

Target m/z	Actual m/z	Abund	Rel Abund	Iso m/z	Iso Abund	Iso Ratio
69.00	69.00	476,800	100.0%	70.00	5,180	1.1%
219.00	219.00	417,408	87.5%	220.00	18,512	4.4%
502.00	502.00	40,552	8.5%	503.00	4,122	10.2%

Air/Water Check: H20 ~1.7% N2 ~13.5% O2 ~4.8% CO2 ~0.1% N2/H20 ~804.1%

Column(1) Flow: 0.00 Column(2): 0.00 ml/min Interface Temp: 280

Ramp Criteria:

Ion Focus maximum 90 volts using ion 502; Electron Multiplier Gain 76253.345

Repeller maximum 35 volts using ion 219; Gain Factor 0.7625

Mass Gain Values(Scan Speed): -751(3) -745(2) -730(1) -712(0) -669(FS1) -668(FS2)

TARGET MASS:	50	69	131	219	414	502	1050
Amu Offset	118.6	118.6	118.6	118.6	118.6	118.6	118.6
Entrance Lens Offset	16.8	16.8	16.8	16.8	16.8	16.8	16.8

Figure 19: GC-MS autoune report as part of the quality assurance procedure

5.2.2 Results of column performance evaluation on GC-MS

To ascertain the performance of the column, a GC HP-5 column that was used and 10 µg L^{-1} QC-Mix plus C-series mixtures was run. The QC test mixture consisted of six compounds 2,6-dimethylphenol (DMP); 5-chloro-2-methylaniline (CMA); tributylphosphate (TBP); dibenzothiophene (DBT); malathion (MAL) and methyl stearate (MST); whereas the C-series consisted of carbon chains from C_8 to C_{30}. The six QC compounds were selected based on their different physical, chemical and retention properties and their retention times are in Table 6.

This was to check the performance of the column prior to injection of target compounds on the instruments by making sure that there are no interferences or any contaminations present in the system. Results of the column check ran on GC-MS as part of quality assurance are shown in Figures 20. GC-MS is a universal detector therefore, all the QC compounds were detected, and the method was fit for its intended use.

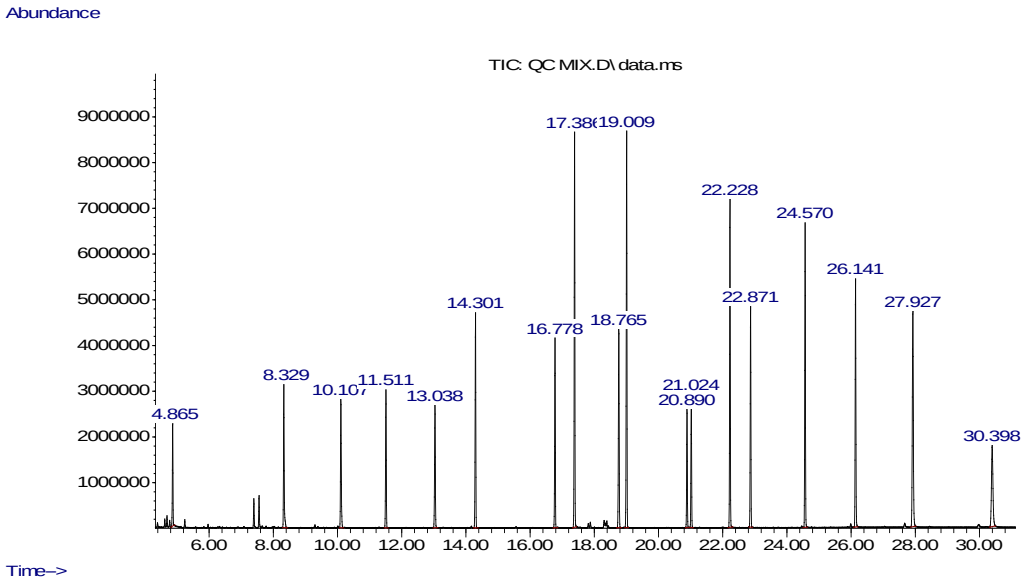


Table 6: Retention times and compound names of the QC-mix along with the C series analyzed on GC-MS

Retention time (min)	Compound name	Retention time (min)	Compound name
4.865	C8	19.009	C18
8.329	C10	20.890	MAL
10.107	DMP	21.024	C20
11.511	C12	22.228	C22
13.038	CMA	22.871	MST
14.301	C14	24.570	C24
16.778	C16	26.141	C26
17.386	TBP	27.927	C28
18.765	DBT	30.398	C30

5.2.3 Column performance check results on GC- µECD

GC-µECD is a sensitive and a selective detector for compounds containing only electronegative groups such as halogens, therefore when running the QC-mix. The GC-µECD detected only one chlorine containing compounds which is the 5-chloro-2-methylaniline (CMA) at retention time of 23.073 minutes and is shown in Figure 21. Consequently, based on the chromatogram presented, it was determined that the method's appropriateness for its intended use prior to analysis, as PCBs are compounds that contain chlorine similar to CMA.

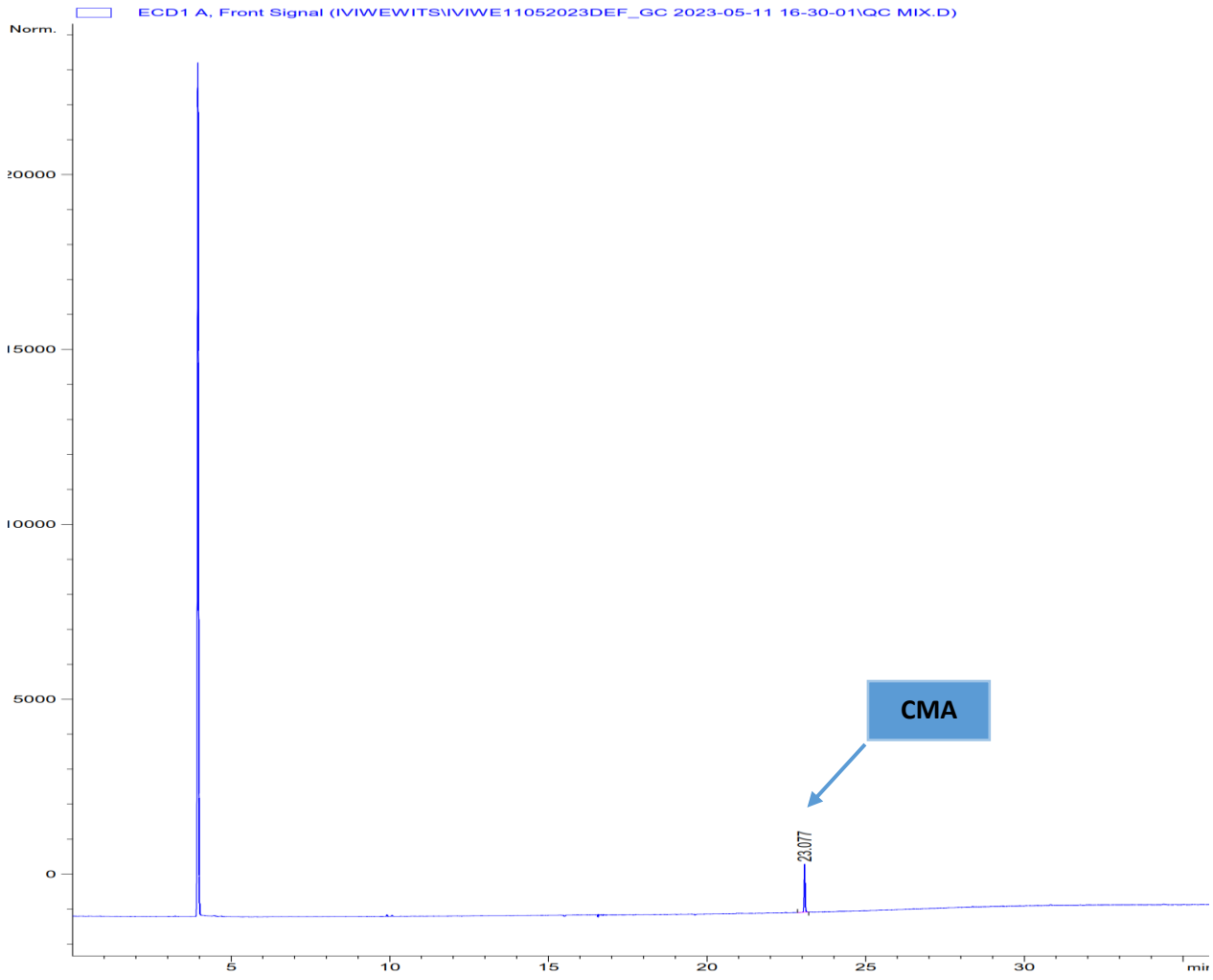


Figure 21: A QC-mix chromatogram on GC-µECD

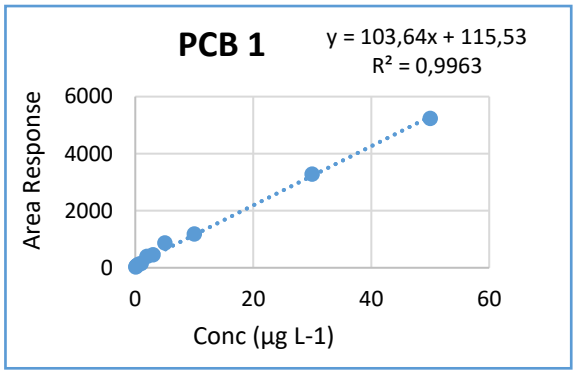
5.3 Calibration curve results

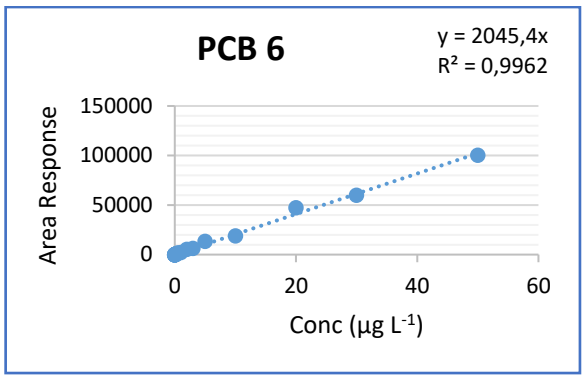
The obtained calibration curves are shown in Figure 22. The results show linearity in the concentration range between 50 µg L^{-1} and 3.9 x 10^{-1} µg L^{-1} as tabulated in Appendix 1. The

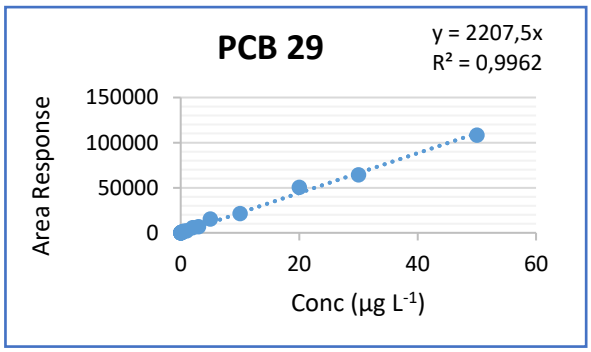
obtained recoveries were within the accepted range between 80-120% as tabulated in the Table 7.

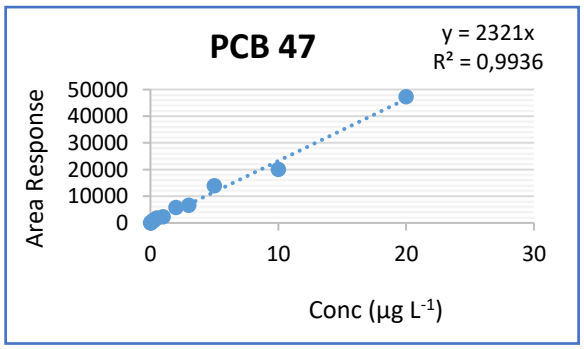
Table 7: PCBs recoveries obtained

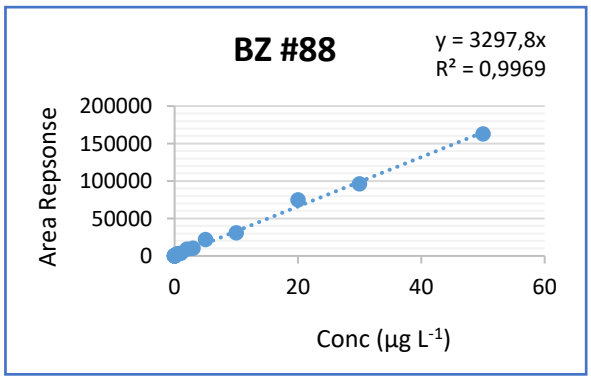
PCB name	Recoveries (%)	PCB name	Recoveries (%)
PCB 1	80.6	BZ# 88	105.7
PCB 6	103.7	BZ# 154	88.0
PCB 29	104.0	PCB 177	97.6
PCB 47	106.3	PCB 198	104.3

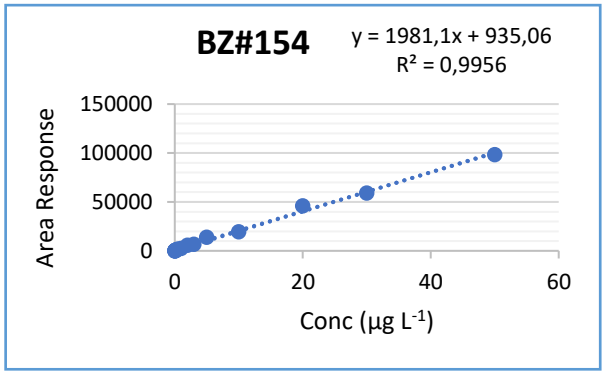












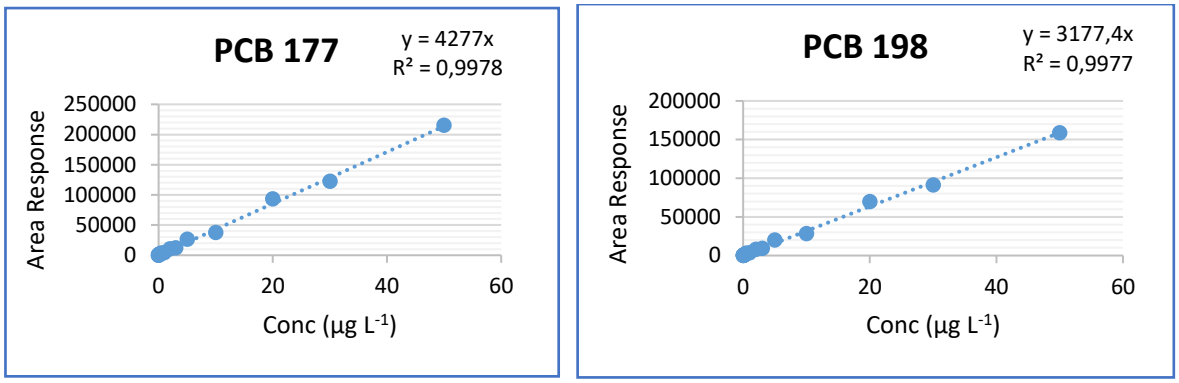


Figure 22: PCBs on GC-µECD Calibration curves

5.4 Method Validation

The river water sample was collected at Hennops River in Centurion in a clean 1L schott duran laboratory bottle. It was placed in a cooler bag and then transported to the laboratory. It was analyzed in the same day and stored in a refrigerator below 10 °C. 5mL of the sample was taken in triplicates and spiked with 0.01 µg L^{-1} PCBs CRMs to validate the applicability and accuracy of the method. The obtained RSD values were between 2.7- 8.9% and were within acceptable range of less than 10% at ppb levels, as shown in Table 8. The calibration data could fit a linear model for all PCBs with a linearity regression coefficient (r^2) between 0.9936 and 0.9978 which is accepted because values for linear regression typically falls within the range of 0.95 to 1.00 (Manisha *et al.*,2023). These values indicate a perfect fit, meaning all data points falls precisely on the line.

The investigation was conducted in 5 mL water samples and this method provided a good performances of high enrichment factors (126-703) for all eight PCBs. The obtained limits of detection were between 0.46 to 15 µg L^{-1} and limit of quantifications were between 1.5 to 50 µg L^{-1}. The LOD was calculated based on 3 times the ratio of the signal to noise of measured blank and the LOQ was estimated as 10 times the signal to noise ratio of the LOD as tabulated in Table 8.

Several studies on the extraction of PCBs in water used gas chromatography for analysis. For example, Zaater *et al.* (2005) used GC-ECD and GC-MS for analysis, while their extraction method was LLE. The authors reported values below 200 ng L^{-1} in surface water for four PCBs, PCB; 1232, 1242, 1254, 1262 for LODs (Zaater *et al.*, 2005). In a separate report, Popp *et al.* (2005) disclosed the use of LLME extraction method, were GC-MS was used for analysis, the LOD was found to be 40 ng L^{-1} for the determination of PCB 77, PCB 126 and PCB 169. Ten

PCBs, namely, PCB 28, 52, 101, 105, 118, 126, 138, 153, 170 and 180 were detected at concentrations in the range 1-2 ng L^{-1} using DLLME on GC-ECD (Rezaei *et al*., 2008).

Therefore, the obtained results in relation to brevity of time of operation, use of less solvents and less sample volume were comparing with other methods in – advantages afforded by the DLLME extraction method. These results pointed to the DLLME in combination with GC-µECD as a sensitive, reliable, fast and reproducible approach for the detection of PCBs in traces and ultra-traces. An added advantage of this approach is its evasion of labour intensive, time-consuming and unnecessary steps.

Table 8: Results acquired for LOD, LOQ, RSD and EF of PCBs

	PCB 1	**PCB 6**	**PCB 29**	**PCB 47**	**BZ #88**	**BZ #154**	**PCB 177**	**PCB 198**
LOD (µg L^{-1}) GC- µECD	15	3.7	1.9	1.9	0.94	0.94	0.46	0.94
LOQ (µg L^{-1}) GC- µECD	50	12.5	6.3	6.3	3.1	3.1	1.5	3.1
RSD (%)	7.7	3.0	2.7	3.1	3.0	3.8	3.3	8.9
Enrichment Factor (EF)	126	156	242	278	381	424	703	699

5.5 Application of the optimised DLLME method to real samples results

The Table 9 below summarizes the results obtained on the physical and chemical properties of the water samples. According to the South African National Standards (SANS) 241, the obtained results for all sample parameters were within the accepted range for drinking water quality.

Table 9: Physical and chemical properties of the water samples

Samples	**Sample Area names**	**pH**	**Temp (°C)**	**Turbidity (NTU)**	**Conductivity (µS/m)**	**TDS (µg L^{-1})**
Sample 1	Mokgojoa Secondary School, Kwa-Ntsweng	7.41	19.2	0.64	396	203
Sample 2	Supingstad Community Clinic	7.75	18.8	0.50	905	452

Sample 3	Community borehole tap 1, Supingstad	7.55	19.4	0.79	770	385
Sample 4	Matthews Mangope high School, Supingstad	7.68	19.8	0.26	793	399
Sample 5	Community borehole tap 2, Supingstad	7.98	19.8	0.66	778	391
Sample 6	Community borehole tap 3, Supingstad	7.63	20.1	0.23	799	400
Sample 7	Community borehole tap 4, Supingstad	8.31	21.2	0.32	740	380
Sample 8	Madikwe Game Reserve Wonderboom Gate	7.96	20.7	0.35	780	363
Sample 9	Kingfisher Guesthouse, Zeerust	7.88	20.6	0.82	487	243
Sample 10	Islam General Dealer, Lekubu	8.63	21.0	0.69	631	316

The application of the developed method on the above samples detected some targeted compounds and some were not detected. Out of the ten places in the study area, Mokgojoa Secondary School, Kwa-Ntsweng contained 2-chlorobiphenyl or (PCB 1) with the concentration of 23.2 µg L^{-1} ± 1.4 and 2,2,3,4,6-pentachlorobiphenyl (BZ #88) with 0.5 µg L^{-1} ± 0.1. The Community tap 2 at Supingstad had PCB 1 at 6.6 µg L^{-1} ± 2.1. Both these areas have exceeded the allowed level of less than 0.5 µg L^{-1} in public drinking water supplies according to the Environmental Protecting Agency (EPA).

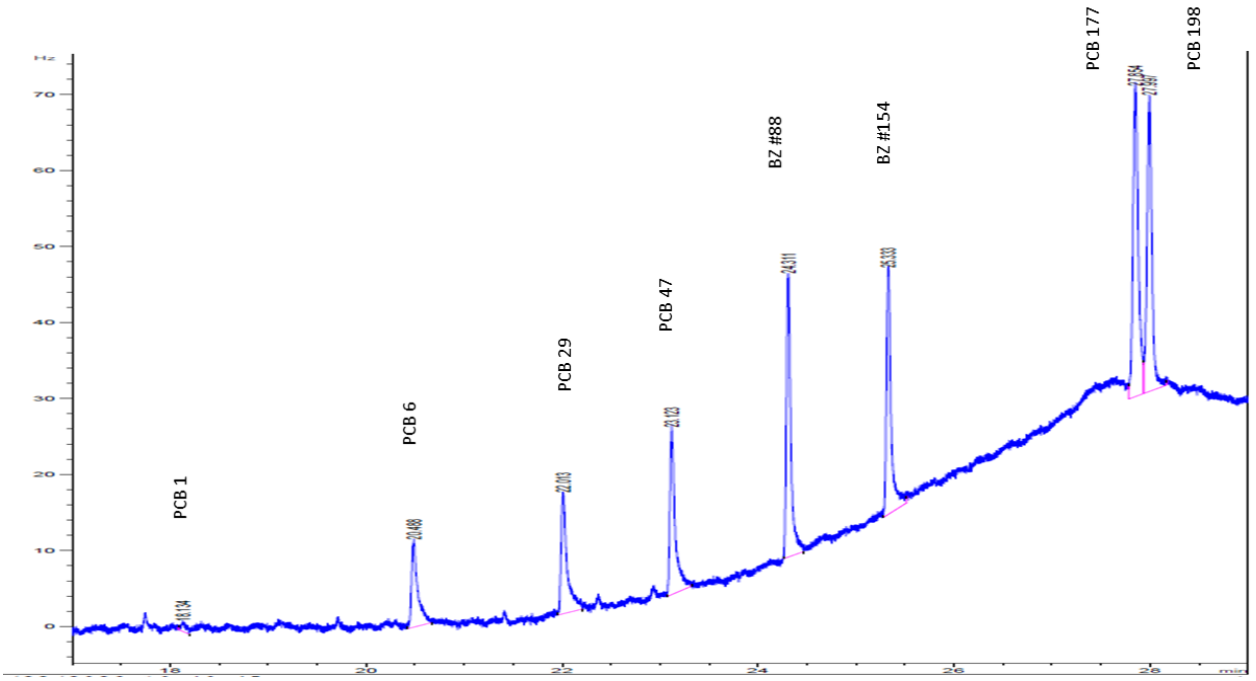
Figure 23 presents the chromatograms for (a) the river water spiked at 0.01 µg L^{-1} and (b) Mokgojoa Secondary School, Kwa-Ntsweng. Any detected PCB by the use of retention time on target compounds were reported and traces of PCBs on the blank were subtracted to determine the precise area of the compound and result compounds with retention times are tabulates in Table 10.

Table 10: The resulting chromatograms obtained for all PCBs in terms of retention times

PCBs	Retention times (minutes)
PCB 1	18.1
PCB 6	20.4
PCB 29	22.0
PCB 44	23.1
BZ #88	24.3
BZ # 154	25.3
PCB 177	27.8
PCB 198	28.0

The elution order of PCBs was from the lowest chlorine containing 1 chlorine PCB 1 to the highest 8 chlorine containing PCB 198. The data obtained in the study indicated that the recommended method can be used in the analysis of PCBs in the environmental water samples with no matrix effect observed. More chromatograms are attached in Appendices (2-10) and some no PCBs were not detected on other study areas. PCBs are recorded to be detrimental to the immune and nervous system; they also damage the reproduction of humans and animals. Therefore, if PCBs exceed the allowed limit for human consumption could cause harm to the environment. This is very concerning considering that these are public areas and children consumes water directly from them and further investigation is needed for the identification of their possible source in these areas. In various studies, the origins of PCB contamination were examined, and it was presumed that old plumbing systems and storage tanks were the source. (Begum Yurdakok-Dikmen *et al*., 2016).

(a)



(b)

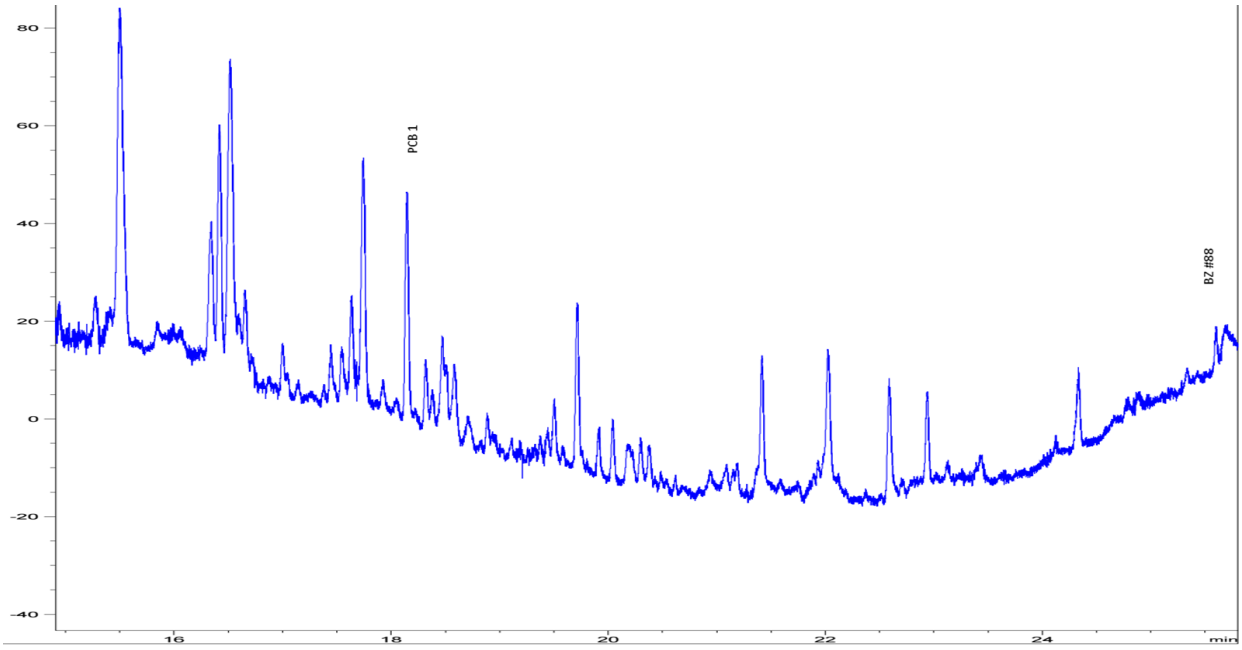


Figure 23: Chromatograms obtained (a) the river water spiked at 0.01 µg L^{-1} and (b) Mokgojoa Secondary School, Kwa-Ntsweng with PCB 1 retention time of 18.142 minutes and BZ #88 at 24.3 minutes.

The effectiveness of the method's performance in microextraction and determination of PCBs from water was contrasted with other methods. The sample volume taken, RSDs, LODs, and the estimated time needed for sample preparation were all taken into consideration. The obtained RSDs were lower than the other methods by the use of GC-ECD (Rezaei *et al*., 2008). Additionally, they were comparable with regard to the fact that sample preparation time and volume consumption have been significantly reduced. As anticipated given the highly sensitivity of the GC-μECD, the LODs were lower when compared to other methods like GC-ECD in other studies (Zaater *et al*., 2005). The calculated p value was 0.16 when comparing the data using the t-test, which is considered not statistically significant because it is less than 0.5. The application of the latest technique GC-μECD in South African borehole waters has not received much attention. Therefore, more work is required to employ this highly sensitive, accurate and reliable technique.

CHAPTER SIX- CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study demonstrated DLLME as a reliable, accurate and reproducible extraction method for PCBs in spiked deionized and river water with good recoveries. The versatility of this method was demonstrated through its application in real samples that were taken from the North-West Province of South Africa. Coupling the DLLME extraction method and the latest GC-µECD for analysis proved a great combination that allowed for the detection of PCBs even at lower concentrations. The glaring advantages of this approach are its use of small sample sizes, short extraction and analysis times, reduced volume solvents, use of green reagents and ability to analyse multi-analytes.

The study also established the GC-µECD technique as superior to GC-MS (EI) in detecting PCBs. However, GC-MS (EI) played an important role in the study to compensate for the absence of the NIST library in the GC- µECD used in the study. It was therefore necessary to employ GC-MS (EI) to confirm the presence of these compounds based on their retention times, chemical names and relative matches. There were minimal shifts on retention times and same elution order between the two techniques as the instrumental conditions applied were the same. Two PCBs were detected in the borehole taps that is used for drinking water by the community members. However, the possible sources remain unsure whether it is the burning of wood, combustion of petroleum products or the coal mining nearby therefore further investigation is needed.

6.2 Recommendations

- More utilization and investigation on application of DLLME and GC-µECD on real water samples using the optimised methods to better understand the behaviour of PCBs so that a valid conclusion can be drawn;
- Continued monitoring has to be conducted in order to ensure the prolonged effects of PCBs and further determine the accumulation as they are known to be persistent in the environment;
- More studies to be done on Supingstand water and its surrounding areas, in order to investigate possible sources of these two PCBs as to obtain a comprehensive perspective on PCB-related water pollution in the North West province overall.

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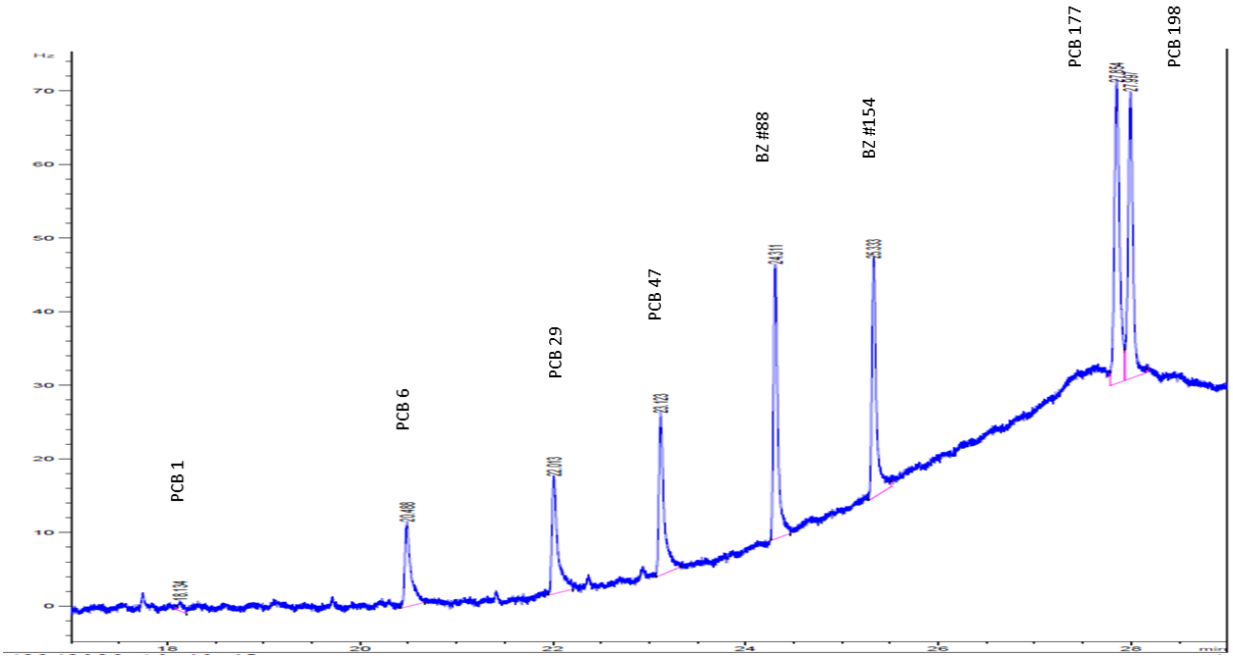
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APPENDICES

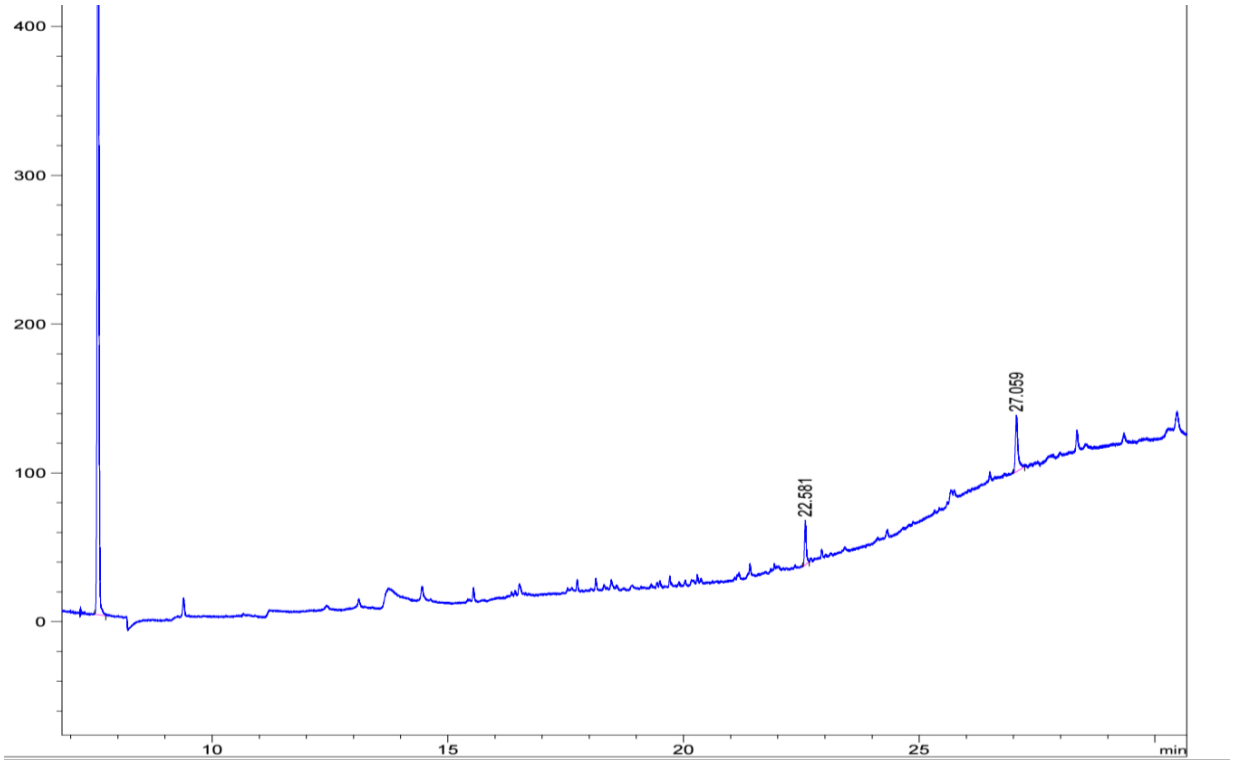
Appendix 1: Different concentrations were prepared in DCM from a 500 µg L^{-1} stock standard solution

Conc (µg L^{-1})	Dilution preparation	Volume of standard taken (µL)	Solvent used	Volume of solvent (µL)	Final volume (µL)
50	prepared from stock	100	DCM	900	1000
30	prepared from stock	60	DCM	940	1
20	prepared from stock	40	DCM	960	1
10	prepared from stock	20	DCM	980	1
5	prepared from stock	10	DCM	990	1
3	prepared from 30 µg L^{-1}	100	DCM	900	1
2	prepared from 20 µg L^{-1}	100	DCM	900	1
1	prepared from 10 µg L^{-1}	100	DCM	900	1
0.5	prepared from 5 µg L^{-1}	100	DCM	900	1
0.3	prepared from 3 µg L^{-1}	100	DCM	900	1
0.2	prepared from 2 µg L^{-1}	100	DCM	900	1
0.1	prepared from 1 µg/L)	100	DCM	900	1
0.05	prepared from 0.5 µg L^{-1}	100	DCM	900	1
0.03	prepared from 0.3 µg L^{-1}	100	DCM	900	1
0.02	prepared from 0.2 µg L^{-1}	100	DCM	900	1
0.01	prepared from 0.1 µg L^{-1}	100	DCM	900	1
0.005	prepared from 0.01 µg L^{-1}	500	DCM	500	1
0.0025	prepared from 0.005 µg L^{-1}	500	DCM	500	1
0.00125	prepared from 0.0025 µg L^{-1}	500	DCM	500	1
0.000625	prepared from 0.00125 µg L^{-1}	500	DCM	500	1
0.0003125	prepared from 0.00625 µg L^{-1}	500	DCM	500	1
0.00015625	prepared from 0.0003 µg L^{-1}	500	DCM	500	1
0.000078125	prepared from 0.00015 µg L^{-1}	500	DCM	500	1
0.000039063	prepared from 0.000078 µg L^{-1}	500	DCM	500	1
Check	prepared from stock	2	DCM	998	1

(a)

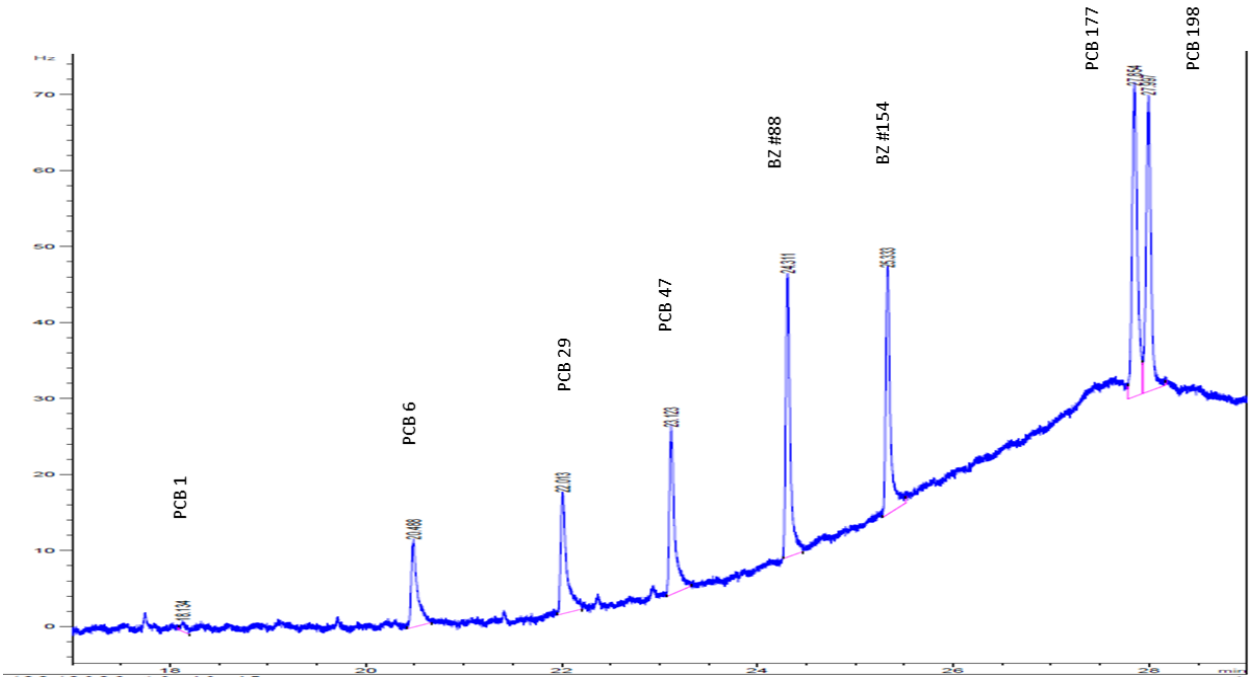


(b)

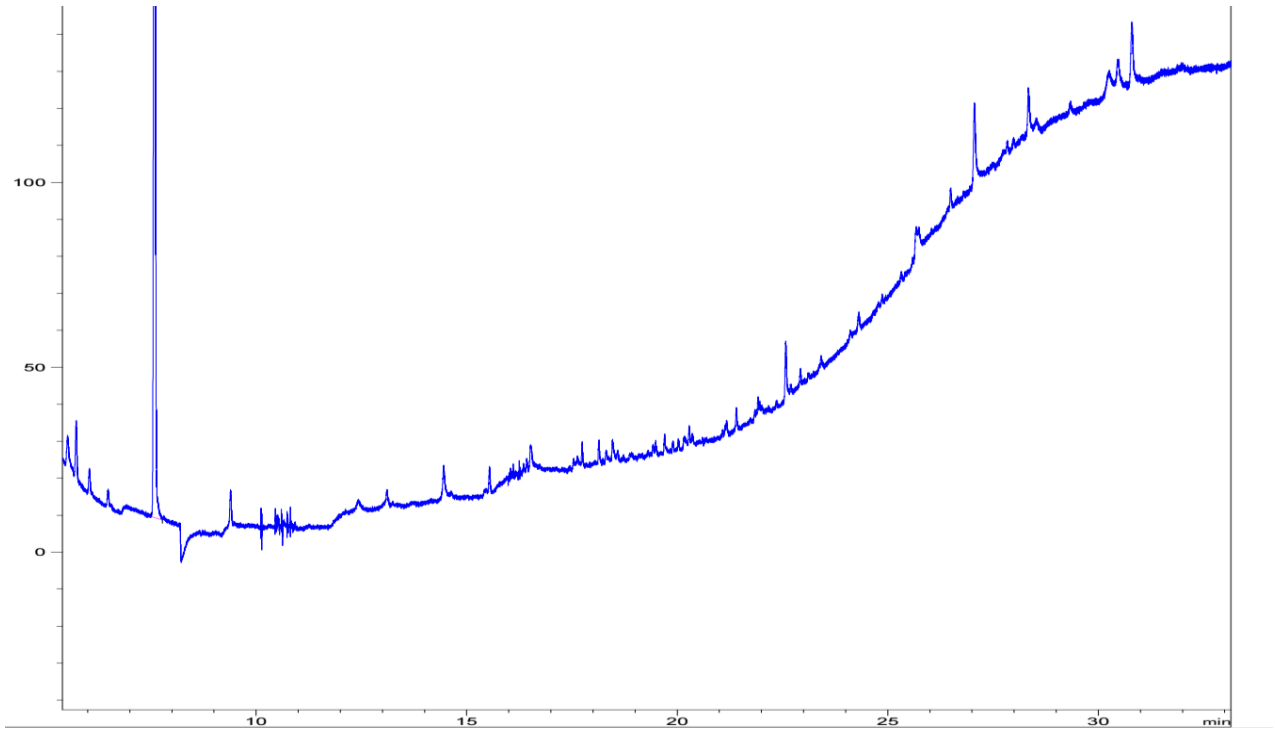


Appendix 2: Chromatograms obtained (a) the river water spiked at 0.01 µg L^{-1} and (b) Supingstad Community Clinic

(a)

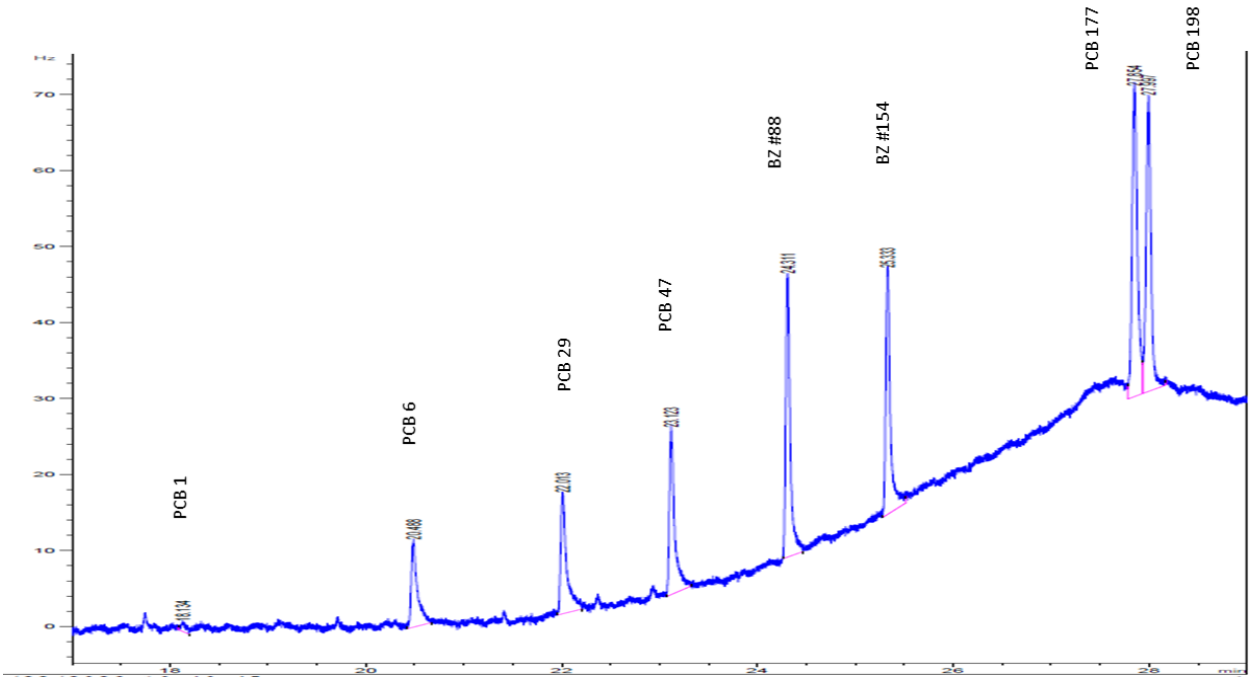


(b)

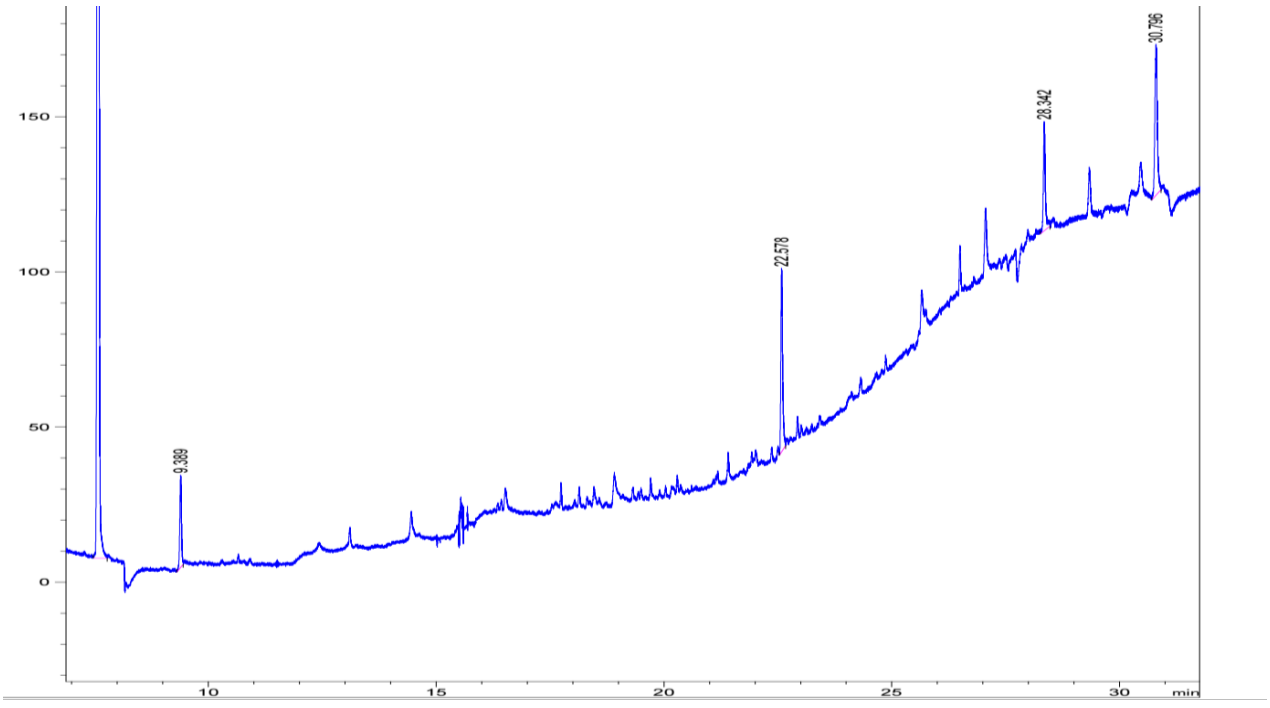


Appendix 3: Chromatograms obtained (a) the river water spiked at 0.01 µg L^{-1} and (b) Community borehole tap 1, Supingstad

(a)

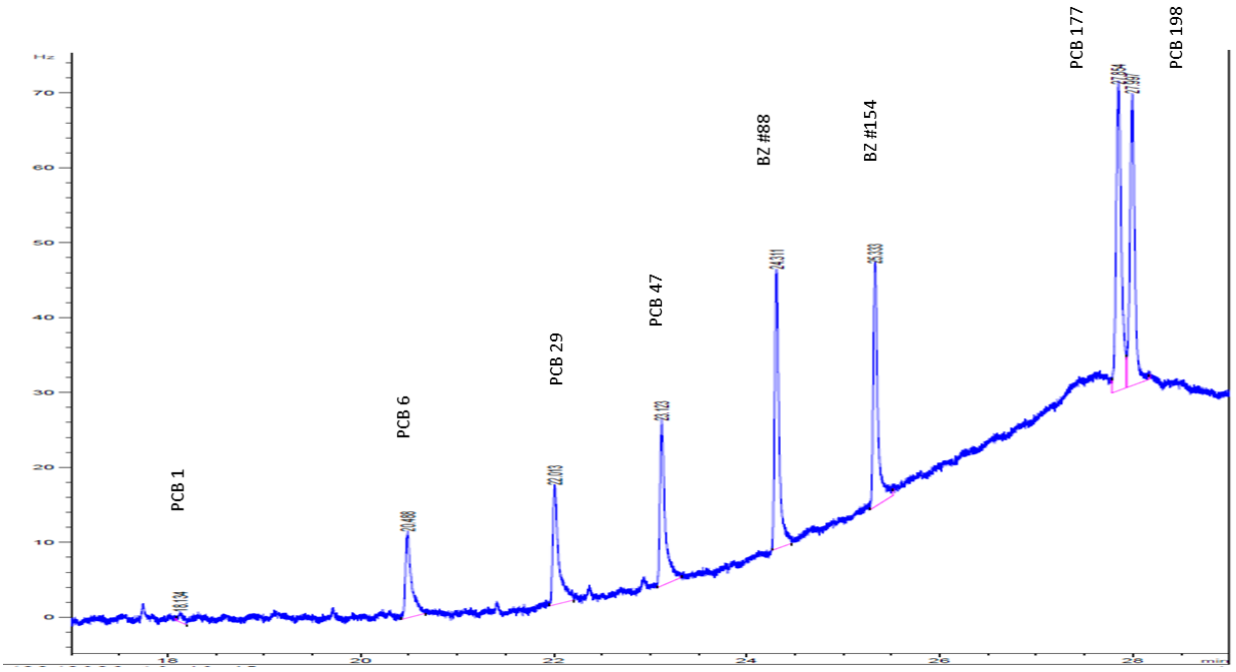


(b)

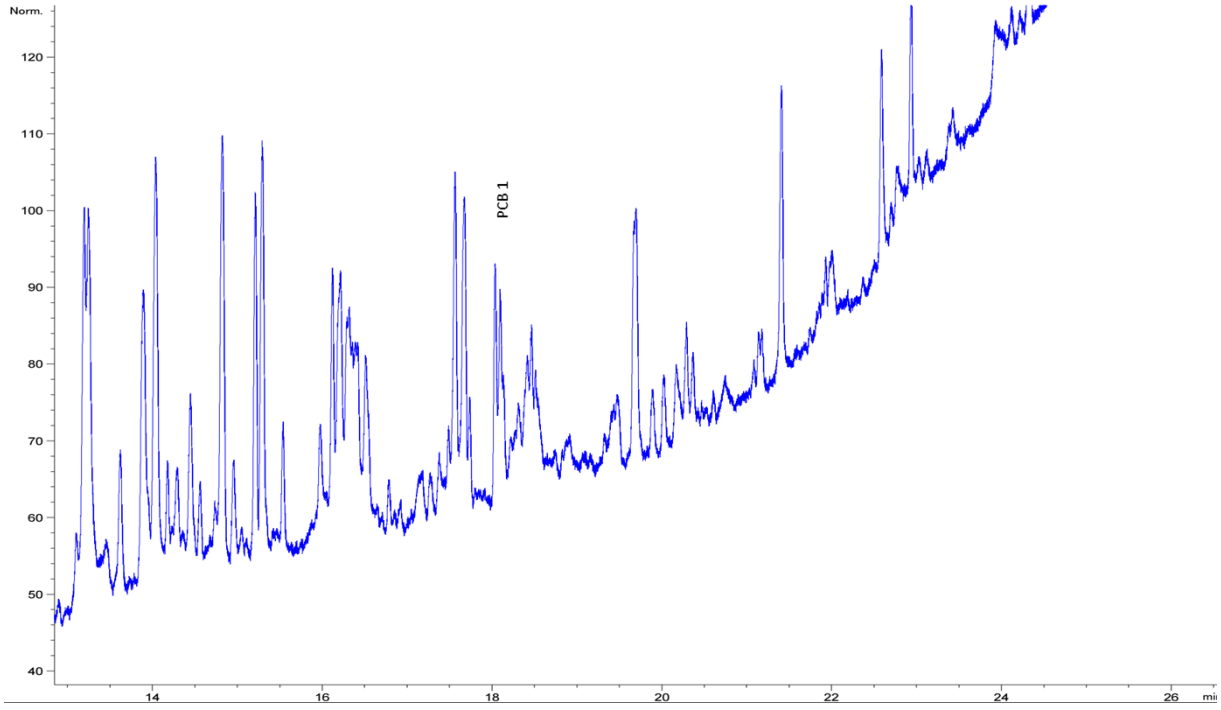


Appendix 4: Chromatograms obtained (a) the river water spiked at 0.01 µg L^{-1} and (b) Matthews Mangope high School, Supingstad

(a)

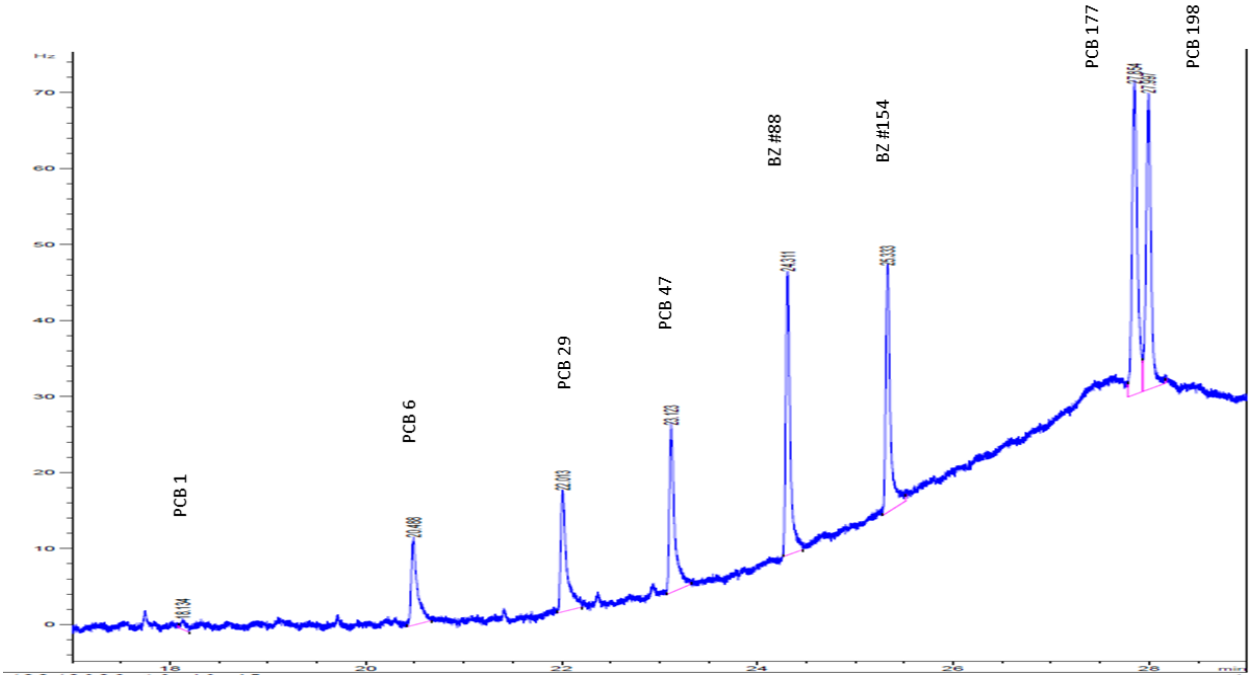


(b)

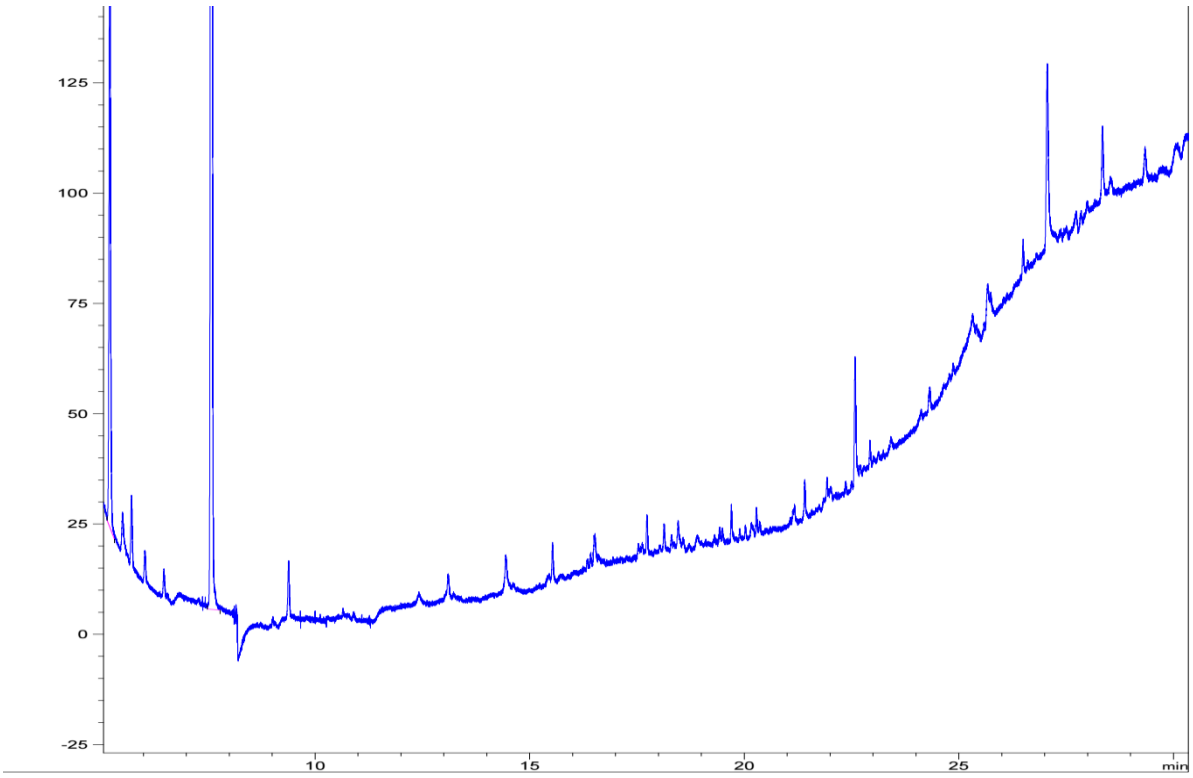


Appendix 5: Chromatograms obtained (a) the river water spiked at 0.01 µg L^{-1} and (b) Supingstad Community borehole tap 2, Supingstad with PCB1 obtained at retention time of 18.139 minutes

(a)

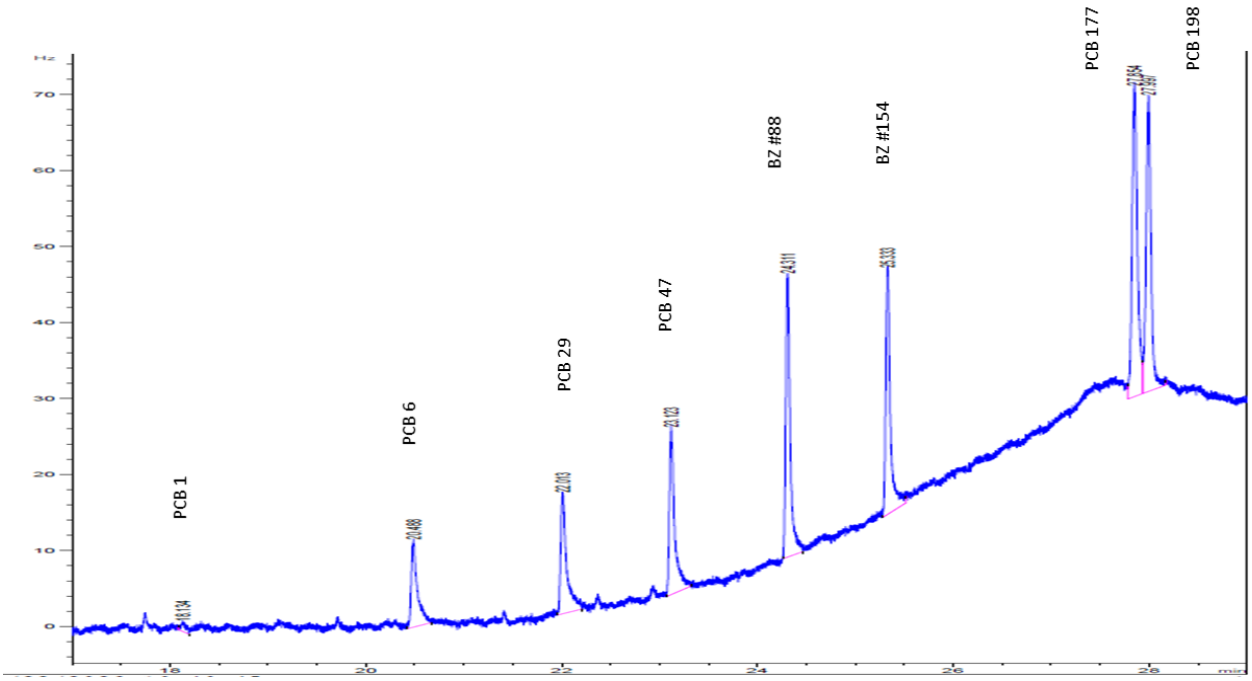


(b)

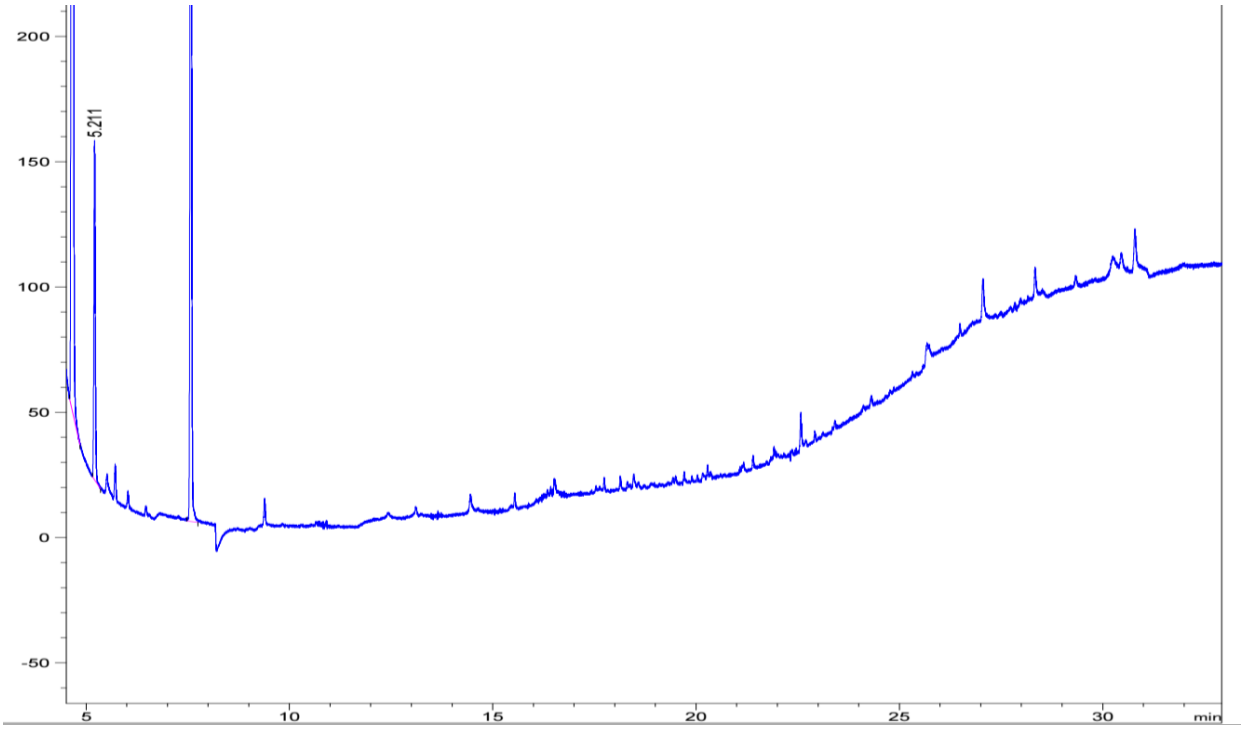


Appendix 6: Chromatograms obtained (a) the river water spiked at 0.01 µg L^{-1} and (b) Supingstad Community borehole tap 3, Supingstad

(a)

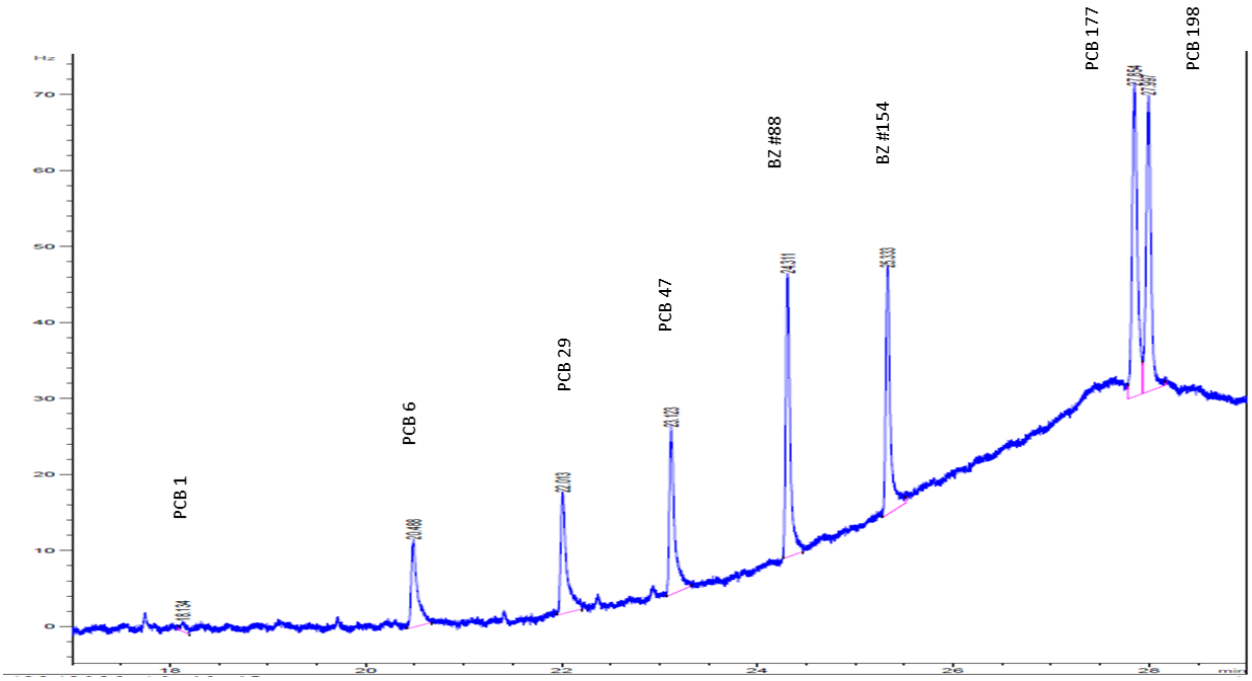


(b)

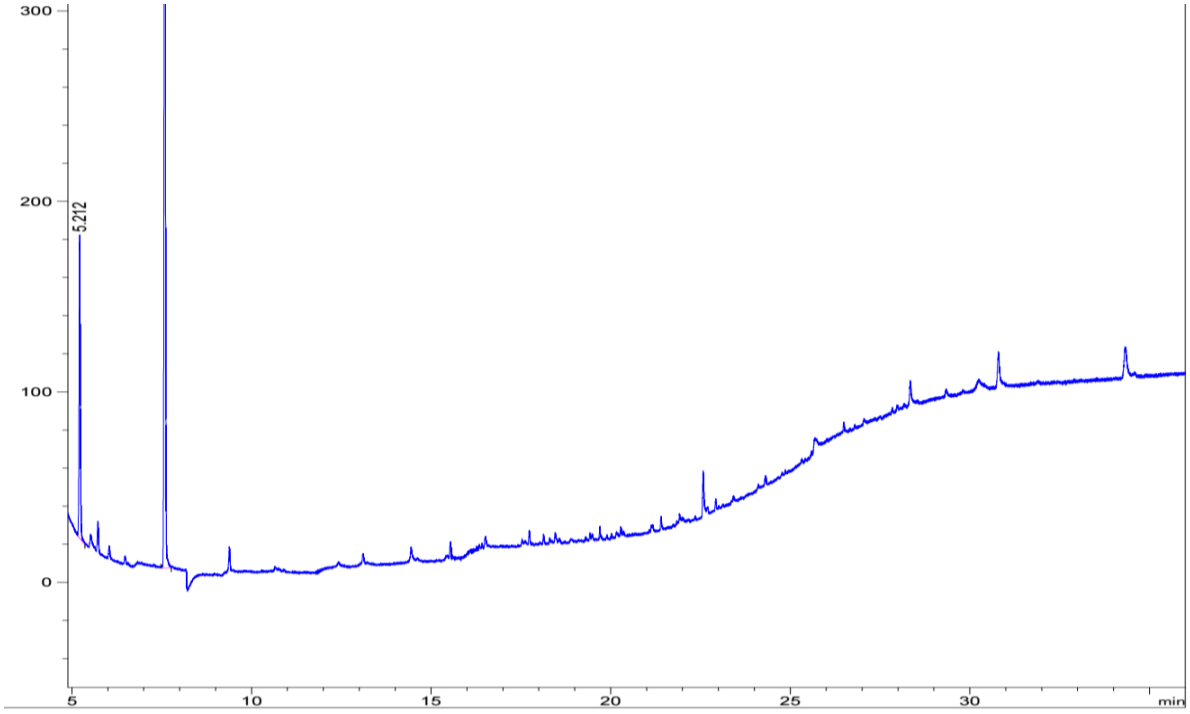


Appendix 7: Chromatograms obtained (a) the river water spiked at 0.01 µg L^{-1} and (b) Community borehole tap 4, Supingstad

(a)

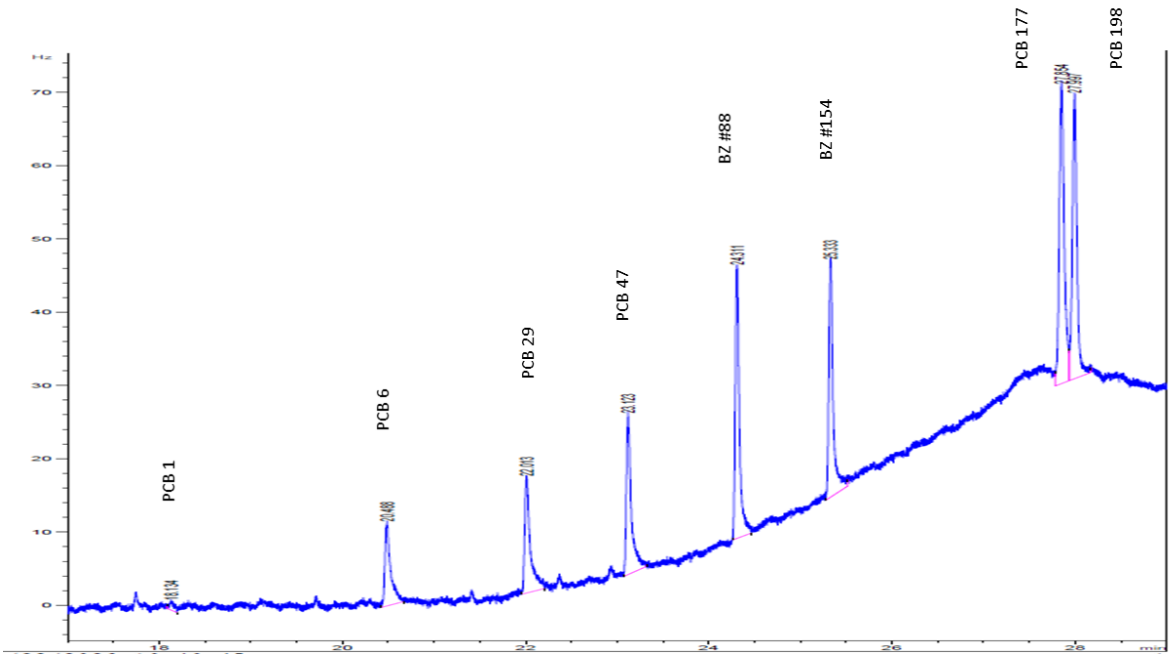


(b)

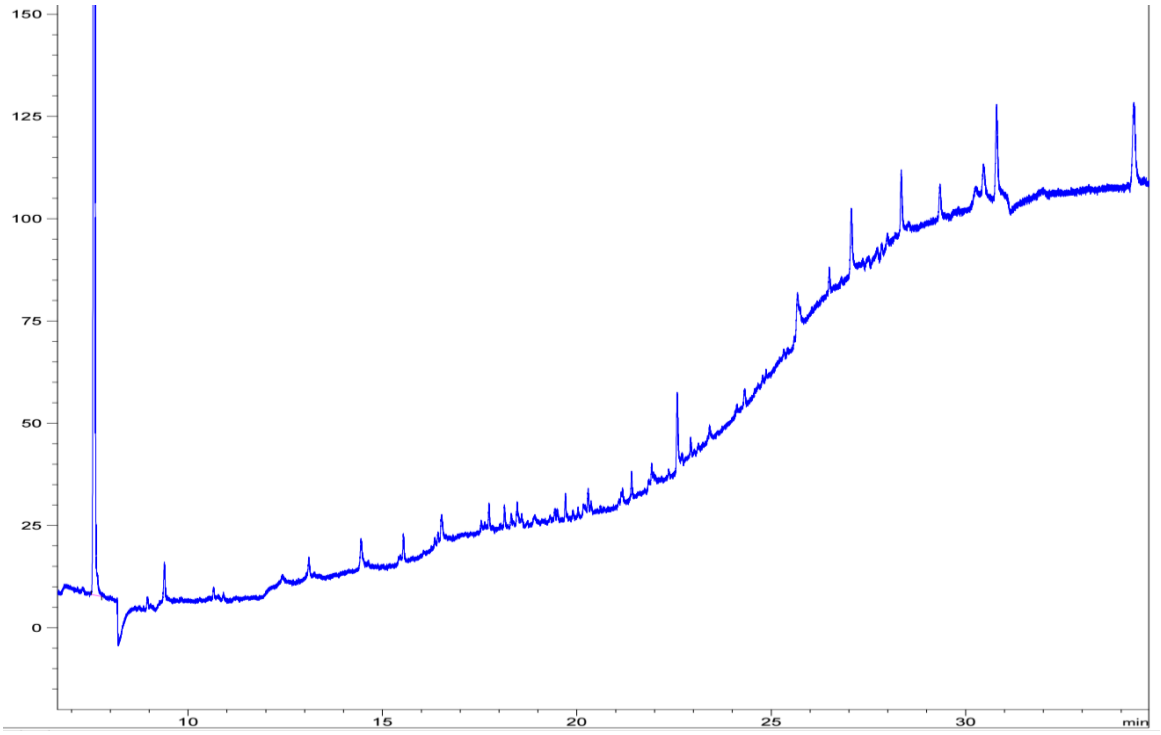


Appendix 8: Chromatograms obtained (a) the river water spiked at 0.01 µg L^{-1} and (b) Madikwe Game Reserve Wonderboom Gate

(a)

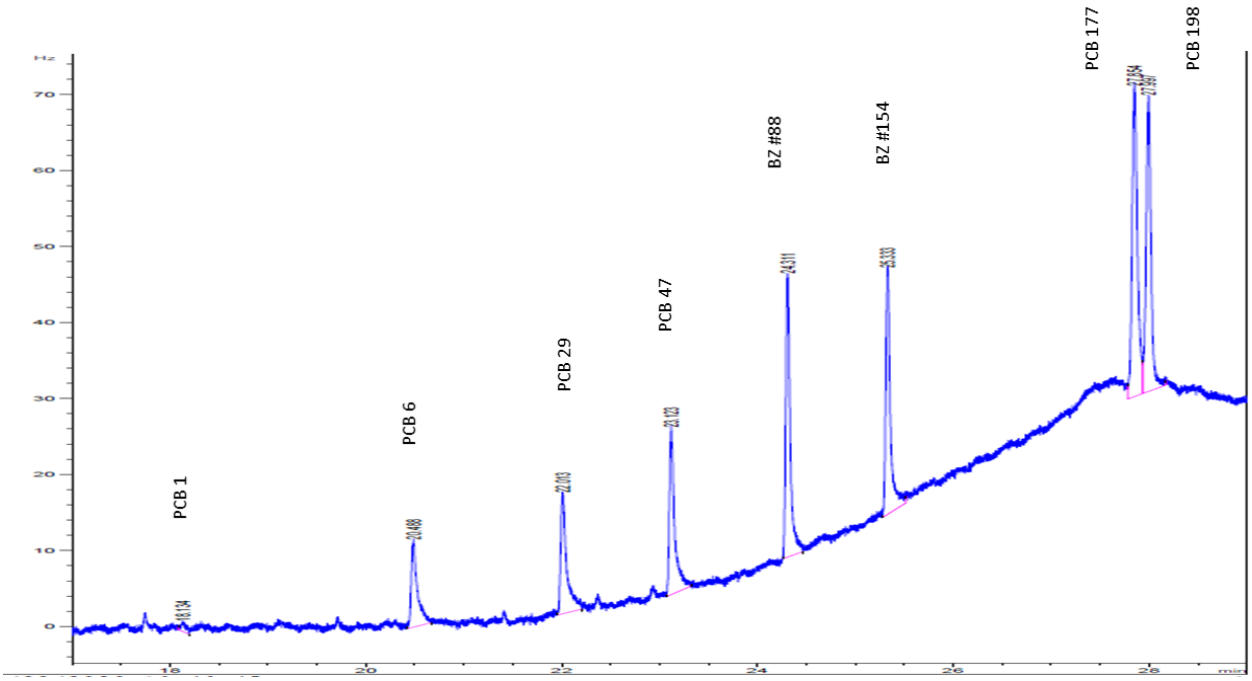


(b)

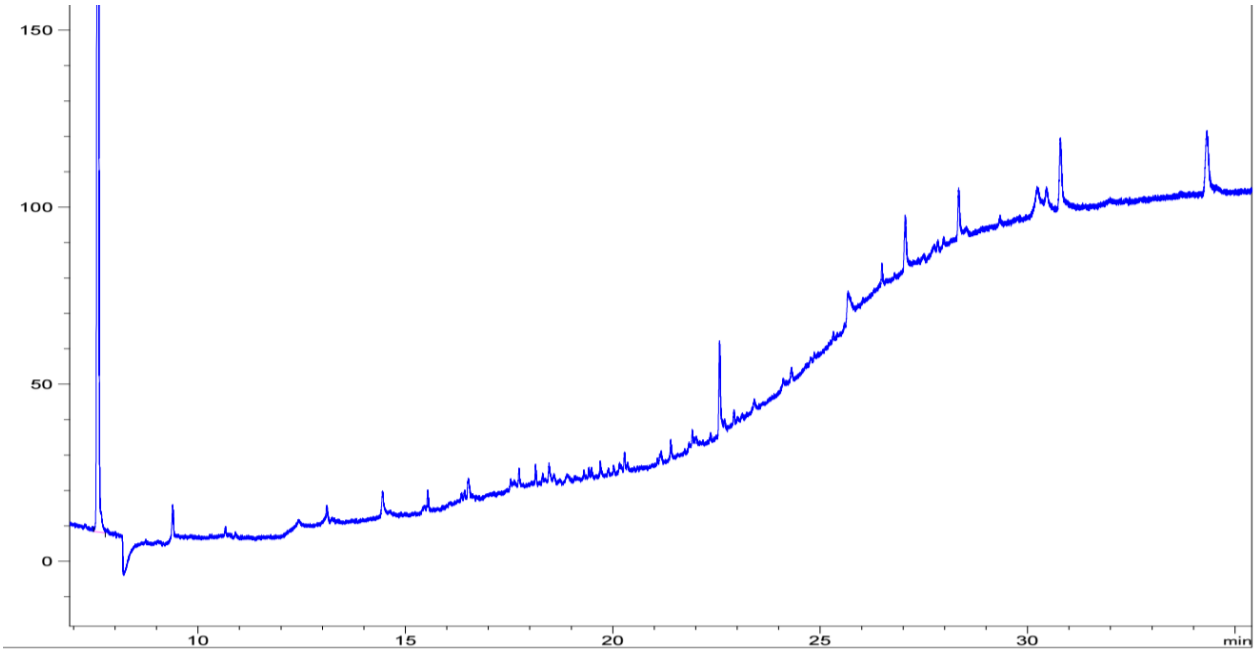


Appendix 9: Chromatograms obtained (a) the river water spiked at 0.01 µg L^{-1} and (b) Kingfisher Guesthouse, Zeerust

(a)



(b)



Appendix 10: Chromatograms obtained (a) the river water spiked at 0.01 µg L^{-1} and (b) Islam General Dealer, Lekubu