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Determination of Poly Chlorinated biphenyls in sediment of Trondheim fjord (Norway) and Lake Tana (Ethiopia) using pressurized liquid extraction (PLE) and Gas Chromatography - Mass Spectrometry (GC-MS)

Meseret Zebeaman Birhanu

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Supervisor: Rudolf Schmid, IKJ
Co-supervisor: Oyvind Mikklesen, IKJ

Norwegian University of Science and Technology
Department of Chemistry

NTNU
Norwegian University of
Science and Technology

Faculty of Natural Science and Technology

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By: Meseret Zebeaman

A thesis submitted to Norwegian University of Sciences and Technology Department of Chemistry in partial fulfillment of the requirements for the degree of Master of Science in Environmental Chemistry.

ADVISOR:

Rudolf Schmid (Associate professor) /Supervisor/,

Øyvind Mikkelsen (Professor) /Co-supervisor/

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Trondheim, Norway

Declaration

I, the undersigned, declare that this thesis entitled with Determination of Poly Chloro Biphenyl in sediment of Trondheim fjord (Norway) and Lake Tana (Ethiopia) using GC-MS (Gas Chromatography – Mass Spectroscopy) is my own work and has not been presented for any degree in any other University and all the resource of material used for the thesis have been duly acknowledged.

Name: MESERET ZEBEAMAN BIRHANU

Signature: Date:

This thesis has been submitted for examination with our approval as University advisors.

Name: Rudolf Schmid /supervisor/

Signature: Date:

Name: Øyvind Mikkelsen /Co-supervisor/

Signature: Date:

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List of Abbreviation

ASE	Accelerated solvent extractor
DDT	Dichlorodiphenyltrichloroethane
EU	European Union
GC-ECD	Gas chromatography – electron capture detector
GCMS	Gas chromatography mass spectroscopy
PBT	Persistence Bioaccumulation Toxicity
HPLC-UV	High Performance Liquid chromatography- Ultra visible
IARC	International Agency for research on Cancer
ICPMS	Inductively Coupled Plasma
IUPAC	International Union of Pure and Applied Chemistry
LOD	Limit of Detection
LOQ	Limit of Quantification
NMR	Nuclear Magnetic Resonance
PCB	Polychlorinated biphenyls
PHAHs	Poly Halogenated Aromatic Hydrocarbons
PLE	Pressurized liquid extraction
POP	Persistent organic pollutants
SPMDs	Semi Permeable Membrane Devices
UNEP	United Nations Environment Programme

Abstract

The presence of polychlorinated biphenyls (PCBs) in the environment is of great concern, as they are compounds that are hazardous to the environment and human health. Environmental investigations of PCBs can be challenging as the analytes are found in trace amount, thus, it is important to have high sensitive and selective method for the determination of PCBs in environmental samples. In this work, an analytical method for the determination of PCBs in marine sediment samples was tested on sediment samples from Trondheim fjord, Norway, and Lake Tana Bahir Dar, Ethiopia. The samples were extracted by pressurized liquid extraction (PLE) and analyses were carried out by gas chromatography coupled with mass spectroscopy. The method made it possible to quantify the Dutch seven target PCBs (indicator PCBs) in marine sediments.

In the Trondheim fjord, from out of the Dutch seven indicator PCBs, six PCBs were found and quantified in samples from Trondheim Bay ranging from 13 - 141 ng/g in dry sediment weight, four PCBs are found and quantified in samples from Steinkjer ranging from 10.5-28.5 ng/g in dry sediment weight, and only PCB 118 is quantified in samples from Verdal fjord with amount of 14.1 ng/g in dry sediment weight. Samples from Ethiopia have only two quantified PCBs ranging from 10.8 – 14.4 ng/g in dry sediment weight. In all sediment samples the amount of PCB found is under the Effect Range Medium (ERM). ERM value for PCB is 180ng/g. Also according to the Norwegian climate and pollution agency (Klif), all sediment samples lies in moderately polluted (17-190ng/g) group. This indicates all study areas are environmentally safe relative to PCB contamination.

The analytical method was shown to have an acceptable instrumental precision, LOD (28 ng/g in dry weight), LOQ (82 ng/g in dry weight), and linearity (r^2= 0.97), while the method remains to be further developed in order to improve the accuracy and selectivity. Specially, PCB 28 and PCB 31 were overlapping and not separated in this study. Therefore, further work is recommended to be done in order to achieve a reliable method for the determination of PCBs in marine sediment samples using PLE and GC-ECD (gas chromatography – electron capture detector, which has high selectivity for PCBs) or tandem mass spectroscopy. May be a column of 60m is likely to improve the selectivity of the analyte, which is therefore recommended to test.

Chapter one:

1. Introduction

Nowadays, different analytical methods have been used for studying environmental pollutant. Some of the analytical method includes GCMS, HPLC-UV, and ICPMS. Some of the pollutant but not all includes POPs, trace-toxic metals (like, Hg, Pd, Cd), nanoparticles, radioactive compounds, micro-plastics and so on. The analytical method accompanies from small outdoor in suit instrument to large indoor instrumental method. In this paper work the amount of PCB, which is one of POPS from the dozen, was determined in the sediments collected from Norway's Trondheim fjord and Ethiopia's Lake Tana using indoor ASE, Turbovap, and GC-MS method.

1.1. Background of the study

Persistent organic pollutants (POPs) are environmental pollutants which are defined by United Nations Environmental Programme as "chemical substances that persist in the environment, bio- accumulate through the food web, and pose a risk of causing adverse effects to human health and the environment ". Many POPSs belong to the Organochloride pesticides (example; DDT, Dieldrin, Lindane), PCBs (polychlorinated biphenyls), PHAHs (Poly Halogenated Aromatic Hydrocarbons). According to the Stockholm convention (2001) and the Regulation 850/2004/EC of the European Parliament, polychlorinated biphenyls are listed as a one of prior POPs. International Agency for research on Cancer (IARC, 1987) classified PCBs as probable human carcinogens (2A group). Additionally, the Water Frame Work Directive (200/60/EC) sets strategies against pollution of water by these chemicals (Directive 2008/105/EC) and defines Environmental Quality Standards (EQSs). [1, 2]

The air, aquatic systems, soil, and sediments are main reservoirs of these compounds.[3] Specially, due to hydrophobic properties of this compounds, once they are introduced in to the water bodies, mostly, they attach themselves to particulates and settle down on the sea bead and become the part of sediment.[1] For environmental analysis of POPs, sediment samples are recommended rather than water or air samples.[4] Sediment cores are used to reconstruct the history of anthropogenic pollution and provide information on the historic loading of atmospheric pollutants through time.[5] Monitoring the distribution of PCB in the environment is important for evaluating their effects on human health and environment.[1, 3]

PCBs constitute a class of 209 persistent organic compounds (called congeners) having 1 to 10 chlorine atoms bound to a biphenyl molecule with different biological activity and toxicity, as a result of differences in the number and position of chlorine atoms in the molecular structure .[6, 7] The PCBs were widely used as a technical product in many countries since the 1930s and throughout the 20th century. At present, these substances are considered to be persistent organic pollutants. Despite prohibition of their industrial production and use since 1970s, they can still be found in the environment due to their high stability and tendency to accumulate. The potential ability of PCBs to be transported through trophic chains makes PCB control in environmental and biological materials an important concern. From the 209 PCB congeners, about 60 up to 150 can be found in the environment, and seven of them, including IUPAC No.'s 28, 52, 101, 118, 138, 153, and 180, have been selected as indicator congeners that are recommended for regular monitoring (UNEP 2003). [4, 7, 8] Selection of these PCB environmental monitoring indicators is not only due to they found mostly in the environment but also relatively high concentration in technical mixtures and their wide chlorination range (3-7 chlorine atoms per molecule).[9]

Due to their physico-chemical properties (high K_{0W}-values, low water solubility) a significant fraction of these contaminants can be adsorbed to particulate matter and subsequently accumulate in sediments, where many of them may persist for decades. In that sense, sediments can be considered as a sink for these chemicals. Analysis of this environmental compartment is therefore a key tool to evaluate the impact of human activities on aquatic systems. So far, there is no an EU directive that establishes criteria and levels for priority and/or emerging contaminants in sediments, other than some recommendations in Directive 2008/105/EC.[1]

The determination of these chemicals in sediments and other environmental solid matrices (soils, sludge) requires efficient extraction and sensitive analytical techniques due to the low concentrations expected (from a few ng/g to several µg/g). The use of advanced techniques such as pressurized liquid extraction (PLE) or it is also called accelerated solvent extraction (ASE) is encouraged as they reduce the amount of solvent and time required, can be easily automated, and increase the efficiency of the extraction process by operating at higher pressures and temperatures. Also it avoid extra time wastage for further clean up procedure, because it can do the cleanup at the same time by adding the cleanup agent inside the PLE cell before the extraction starts.[1]

After having the extracts, mostly gas chromatography is used to separate and identify the analytes as it can also be easily couple with different types of detector. The most commonly used detector nowadays is a mass spectrometer (GC–MS), which has encouraged the development of multiresidue methods as it is capable of analyzing many compounds within one single extraction and injection. [1]

Nowadays, to investigate POPs in environmental compartment the use of PLE coupled with GC-MS becomes a common method. To the best of our knowledge, however, there has been no study in Trondheim Fjord as well as sediment of Lake Tana Ethiopia with the methods used in this study. Our goal in this work was, therefore, to develop and validate a new method for the simultaneous extraction, cleanup, identification and quantification of PCBs in Trondheim Fjord (Norway) and Lake Tana (Ethiopia) sediments. Pollution of the water bodies in central Norway, and in particular the Trondheim fjord, so far, has been studied mostly for inorganic environmental contamination. In order to complete the picture, some knowledge of organic pollutants, and in particular POPs, would be needed. As part of the study and comparison, sediment samples from Ethiopia also included.

1.2. Objective of the study

1.2.1. General objective

The general objective of the project was to determine the concentration of Poly chlorinated biphenyl in sediment of Trondheim fjord, Norway, and Lake Tana, Ethiopia.

The project aims at performing the analyses in-house, and, therefore, part of the work was oriented towards method establishment, and procedure validation.

1.2.2. Specific Objectives

The specific objectives were described as the following;

- Sample collection and preservation
- Extraction and clean up using accelerated solvent extractor(ASE)
- Solvent evaporation using Biotage TurboVap evaporator.
- Sample and standard solution preparation
- Determining the concentration of selected PCB in sediments using GC-MS (Gas Chromatography-Mass Spectroscopy.

Chapter two:

2. Theory

2.1. **Poly Chlorinated Biphenyls**

Poly chlorinated biphenyls are not known to occur naturally. They are semi volatile chlorinated organic compounds synthesized by humans through chlorination of biphenyl with chlorine gas. Hence, productions of 209 discrete chemical compounds has been theoretically possible and they are called congeners, in which one to ten chlorine are attached to the biphenyl (Figure2.1).[10] These chemicals have a high chemical inertness and heat stability and hence they have been used for wide variety of applications before production and use were banned during 1970s. Some, but not all, applications included: dielectric fluids in capacitors and transformers, heat transfer fluids, lubricating oils and as additives in pesticides, paints and plastics (section 2.1.1).

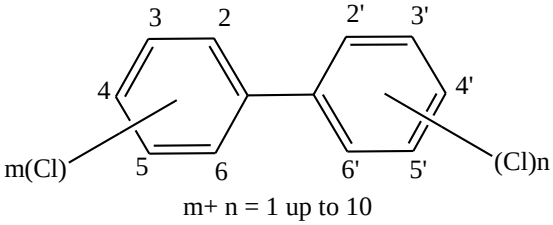

Figure 2.1. General structural formula of PCB.

Once these chemicals are released to the environment, after giving their use, they persist and bioacumlate in food web and cause toxic effect on human and the environment. Organic substances which fulfill the criteria of persistence, bioaccumulation and toxicity (the PBT criteria) are called POPs. They cause concern and undergo risk assessment to determine whether they are harmful or not (Council of the European Union, 2006; Stockholm Convention, 2013). The definitions (in brief) of these terms, according to the Stockholm Convention, are as follows: [11]

Persistence: the substance should have a half-life in water >2 months or >6 months in marine sediment. They are not easy to degrade.

Potential for long-range transport: Data from air, water or migratory species in remote areas showing long-range transport, or physical-chemical properties of the substance/results from models that indicate a potential for long-range transport.

Bioaccumulation: The bioconcentration or bioaccumulation factor should be >5000, i.e. the log of the partitioning coefficient octanol-water (K_{0w}) >5, or if monitoring of species should indicate bioaccumulation.

High Toxicity (adverse effects): Toxicity data or evidence for (potential) impact on human and/or environmental health.

Since PCBs fulfill the above criteria, the United Nations Environment Programme (UNEP) in the Stockholm Convention in 2001 classified PCB as POP and which in turn a convention was made that had the aim of restricting and eliminating production, use, release and storage of PCBs since the 1970s. United States Congress banned domestic PCB production in 1977. However, due to their persistence criteria nowadays these compounds exist in the micro environment (air, soil, dust, sediment, food, tissue) and it attracts the attention of researchers for study.

2.1.1. Analytical chemistry, Synthesis and use of PCBs

Analytical chemistry procedures for PCB determine how much PCB is in the sample. Hence quantification of PCB in the environmental samples usually consists of three distinct steps: (1) extraction of PCB from the sample matrix by solvent or a combination of solvent, (2) cleanup of PCB (removal of impurities) on single or multiple column, and (3) quantification by gas chromatography with suitable detector, mostly electron capture detector (ECD) and mass spectroscopy (MS). Researchers may report PCB concentration as Aroclors, as sum of homologs, or as individual congeners. Qualitatively PCBs are either oily liquids or solids and are colorless to light yellow. PCBs are volatile and may exist as a vapor in air. They have no known smell or taste to the human nose or tongue. They are also chemically inert and heat resistant. [12]

Regarding PCB synthesis, PCBs were synthesized for commercial and laboratory purpose following different reaction procedures. Commercially PCBs were synthesized by batch chlorination of biphenyls with chlorine gas (figure 2.2). For the sake of information, all 209 congener have been synthesized individually. For laboratory use, to study toxic and biological activity of PCB, there are different procedure to follow accordingly. For example the Suzuki or the Ullman coupling reaction is used to synthesize only non–dioxin or ortho substituted PCB for laboratory use only. The Suzuki coupling reaction yields a 78-99% of 2, 2'-dichlorobihenyl (figure 2.3) at 110^0C. The limitation of Suzuki coupling reaction is it

doesn't give multiple ortho substituent (3 or 4 chlorine in ortho position). However, the Ullman coupling reaction gives multiple ortho substituted PCB. After synthesis their identity and structure is confirmed by NMR spectroscopy and X ray crystallography while the purity is determined by GC, and HPLC. [10, 13]

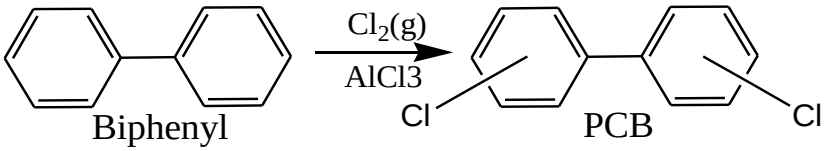

Figure 2.2. Typical technical Synthesis of PCB from chlorination of biphenyl.

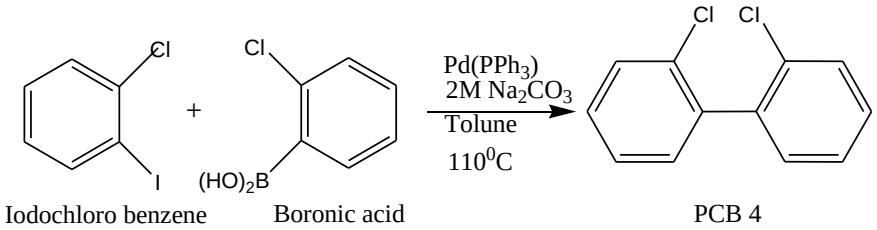

Figure 2.3. Suzuki coupling reaction.

Use of PCB is uncountable. This is because of their chemical inert and heat resistance. Some of the application includes; dielectric fluids in capacitor and transformer, hydraulic fluids, lubricating and cutting oils, and as additives in pesticides, paints, copying paper, carbonless copy paper, adhesives, sealants and plastics.

2.1.2. Structure and Nomenclature of PCBs

The general chemical formula of PCB is $C_{12}H_{10-X}Cl_X$. where x is number of chlorine. The general structural formula of PCB is given above in figure 2.1. When the 209 PCB congeners are sub divided by degree of chlorination, the term homolog is used. There are ten homologs counting from mono- to deca-chloro biphenyl. PCBs of a given homolog with different chlorine substitution position is called isomers. For homologs 2 to 46 possible isomer could be found. Distribution of chlorine atoms in the two rings of biphenyl is described in table 2.1 along with figure 2.4. The Table also shows the diagonal line pointing to the homologs with its total isomer found by summing up the numbers that lies above the diagonal line.

Table 2.1. Distribution of chlorine atoms in the two rings of biphenyl. (adopted from[10])

Chlorine on Ring B	Chlorine Atoms on Ring A: 0	1	2	3	4	5
0		3	6	6	3	1
1	Mo	6	18	18	9	3
2	Di	Tri	21	36	18	6
3		Tetra-	Penta-	21	18	6
4			Hexa	Hepta	6	3
5				Octa	Nona	1

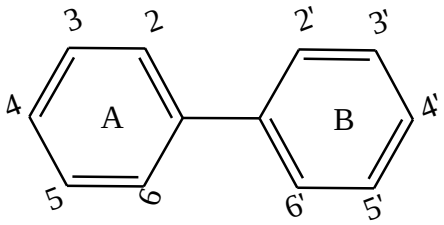

Figure 2.4 biphenyl

As a result of the differences in number and position of chlorine atom in the molecular structure, all 209 congeners have different biological activity and toxicity. Most of these PCBs are detected in the environment and may be quantified as Aroclors or individual congeners. Thus, mixtures of PCBs are often assessed based on a chemical analysis of the "Dutch seven PCBs", also called "indicator-PCBs". These compounds are assumed to be a suitably representative for all PCBs as they are predominant congeners in biotic and abiotic matrices. Also these seven kinds of PCB mixtures include 35% of all the PCBs commercially produced and 98% of PCBs sold in the United States since 1970.The molecular structures of the Dutch seven PCBs are shown in Figure2.5 and their full name and IUPAC number are presented in Table2.2. [4, 7, 9, 12, 14]

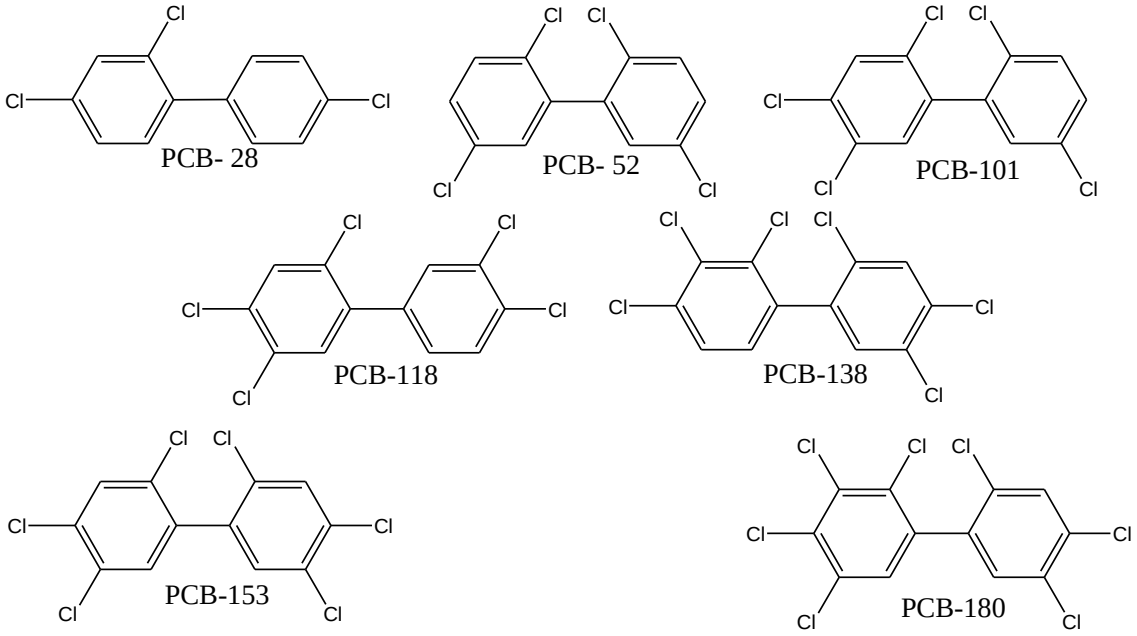

Figure 2.5. Structural formula of the Dutch Seven PCBs.

Table2.2. IUPAC Number and Name of the Dutch seven PCBs.

IUPAC Number	IUPAC Name	Number of Chlorines
PCB- 28	2,4,4'-Trichlorobiphenyl	3
PCB- 52	2,2',5,5'-Tetrachlorobiphenyl	4
PCB-101	2,2',4,5,5'-Pentachlorobiphenyl	5
PCB-118	2,3',4,4',5-Pentachlorobiphenyl	5
PCB- 138	2,2',3,4,4',5'-Hexachlorobiphenyl	6
PCB- 153	2,2',4,4',5,5'-Hexachlorobiphenyl	6
PCB- 180	2,2',3,4,4',5,5'-Heptachlorobiphenyl	7

The term PCB is used to refer to the entire class (209 congeners) or any subset of one or more compounds. Admitting that mono is not poly; however the three monochlorobiphenyls (table 2.1) have been included as member of the class. Biphenyl is not counted as PCB, although for matter of completeness it is included in table 2.1, zero chlorine on the two biphenyl rings. PCBs are listed in chemical abstract under "1, 1'-biphenyl, chloro derives'' with a generic CAS registry number of 12767-79-2. The abbreviation PCB can be used for other materials and chemicals, which can lead to confusion in the literature and, especially, in computerized

keywords searches. Notable examples include printed circuit board and pentachlorobenzene. In some foreign literature, the abbreviation from the native language yields “PBC” or other variants. [10]

Naming of the 209 PCB congeners was first started by Ballschmiter and Zell (1980) followed by IUPAC numbering. The idea behind the two naming system is the same except they differ on naming 11 PCBs (I.e.PCB-33, 34, 76, 98, 122, 123, 124, 125, 177, 196, and 201). Generally, first the 209 congeners were separated based on the homolog (from mono to decachlorobiphenyl) followed by arranging the congeners ascending numeric order, from PCB-1 to PCB-209. The first PCB has one chlorine and the last PCB has ten chlorines. For example, 2,3,3’,4,5,5’,6-heptachlorobiphenyl and 2,3,3’,4’,5,5’,6- heptachlorobiphenyl may be easily confused but in the short hand are referred to as PCB 192 and PCB 193, respectively. [10, 15]

Commercially for domestic and industrial use PCBs are named not by their individual congeners IUPAC name rather by their mixture name mostly Aroclor. For example, the name Aroclor 1254 means that the mixture contains approximately 54% chlorine by weight, as indicated by the second two digits in the name. The first two digits show the number of carbon, which are always 12. [12]

2.1.3. Exposure and Toxicity

Although PCBs are no longer manufactured since 1970s, humans and animals can still be exposed to PCB through inhaling contaminated air, skin contact (at work place and non-intentional exposure from spills), and consumption of polluted marine and terrestrial food. This is because of the persistente nature of PCB, which makes them still available in our environment and called as one of POPs. Biomedical data from human and laboratory animal studies provide strong evidence of the toxic potential of exposure to PCB. Health effect associated with exposure to PCB in humans and animal includes hepatic effect (liver), endocrine effect (thyroid), dermal and ocular effect, immunological alteration, neurodevelopmental effect, developmental and reproductive toxicity effect, and cancer. Among many real world incidents that indicates health effect of PCB are by consumption of contaminated rice oil in Japan (the Yusho incident) and Taiwan (the Yu-Cheng incident). [12]

The biological activity of PCBs is congener specific, and, therefore, different mixtures of PCBs will have different biological and toxicological activity. Many of the effects of PCBs

are mediated through interaction with the arylhydrocarbon receptor (AhR). The effects mediated through the AhR are described as “dioxin-like”. Like TCDD(2,3,7,8 Tetra chloro dibenzodioxin), non-ortho substituted and, in some cases, mono-ortho-substituted congeners that are substituted in the 3, 4, or 5 lateral positions (3, 4, 5 or 3', 4', and 5' positions) can exist in a planar conformation (i.e., the coplanar PCBs) and bind to the AhR.[16]

The potency with which individual PCB congeners elicit dioxin-like effects (compared with the potency of TCDD itself) gives rise to the concept of TCDD-toxic equivalents, or toxic equivalence factors (TEF). These factors provide a means of pooling and comparing different mixtures of PCB congeners (Table 2.3). The congeners that exhibit the highest TEF values tend to be the planar, most highly substituted forms, with lateral chlorine substitution. Non coplanar congeners and congeners with low levels of chlorination are rated at very low TEF values. [16, 17]

In addition to the effects of planar PCBs, studies have shown that non coplanar PCBs elicit neurotoxic effects in exposed animals and in cell cultures. But, the toxicity of the non-coplanar PCBs is not mediated by the AhR rather by the signal transduction pathways.[16]

Table 2.3. The 12 PCBs which are considered dioxins or dioxin-like compounds by the World Health Organization (2005) with their TEF value.

Non-ortho substituted PCBs	TEF value,
PCB 77	0.0001
PCB 81	0.0003
PCB 126	0.1
PCB 169	0.03
Mono-ortho substituted PCBs	
PCB105, 114, 118, 123, 156, 157, 167, 189	0.00003
TCDD	1

PCBs have been shown to affect tyrosine kinase, protein kinase C, and phospholipase A2. Intracellular calcium homeostasis is also affected by non-coplanar PCBs. Some PCB congeners also appear to have estrogenic and antiestrogenic effects, possibly mediated by interactions with one or more steroid receptors. PCBs affect the metabolism of thyroid

hormones through the induction of enzymes involved in thyroid hormone metabolism. PCBs also affect the immune system. Effects on the immune system seem to occur through both AhR- and non AhR-mediated mechanisms. PCBs might also increase oxidative stress, which might contribute directly to carcinogenesis. [16]

2.1.4. Behavior of PCB in the environment

The first behavior of PCB in the environment is, as even the group implies persistent organic compounds, they persist in the environment after they are being released. This is because of their non-degradable nature. This also allows them to bioaccumulate and become biomagnified in food chains. The other behavior is they are potential for long range transport. This means they cycle between air, water and soil. Consequently, these compounds have been found in areas far away from their emission sources such as the Arctic. These makes PCBs are found all over the world. [18]

In general, the lighter the type of PCBs, the further and the faster they may be transported from the source of contamination. PCBs are present adsorbed on solid particles or as a vapor in the atmosphere. They will eventually return to land and water by settling as dust or in rain and snow. In water, PCBs may be transported by currents and evaporate into air, or due to gravity they are attached to bottom sediment or particles in the water. Heavy kinds of PCBs are more likely to settle into sediments while lighter PCBs are more likely to evaporate to air. Sediments that contain PCBs can also release the PCBs into the surrounding water. PCBs stick strongly to soil and will not usually be carried deep into the soil with rainwater. They do not readily break down in soil and may stay in the soil for months or years; generally, the more chlorine atoms that the PCBs contain, the more slowly they break down. Evaporation appears to be an important way by which the lighter PCBs leave soil. As a gas, PCBs can accumulate in the leaves and above-ground parts of plants and food crops. PCBs are taken up into the bodies of small organisms and fish in water, called bioaccumulation. They are also taken up by other animals that eat these aquatic animals as food, called biomagnification. PCBs especially accumulate in fish and marine mammals (such as seals and whales) reaching levels that may be many thousands of times higher than in water. PCB levels are highest in animals high up in the food chain. [12]

2.2. Determination of PCBs in marine sediment

2.2.1. On-line cleanup and extraction with accelerated solvent extractor

In sample preparation much time and solvent is required. However, Accelerated solvent extraction (ASE) improves these problems. Accelerated solvent extraction, also known as pressurized fluid extraction (PFE) or pressurized liquid extraction (PLE), is a method used for extracting either organic or inorganic compounds from a variety of solid or semisolid samples. In brief, the sample is placed inside the cell and the extraction is proceeded by filling the cell with extraction solvent under elevated temperature and pressure. The elevated pressure maintains the heated solvent in liquid state even though it was heated over its boiling point, not changed into gas. This is one of the unique characters of the ASE. Also, desorption of analytes from the matrix and dissolution of analytes in solvent is accelerated at high temperatures compared to room temperature. After some minutes, a compressed gas usually nitrogen is used to purge the extract into a collection vial (Figure 2.6). [19, 20]

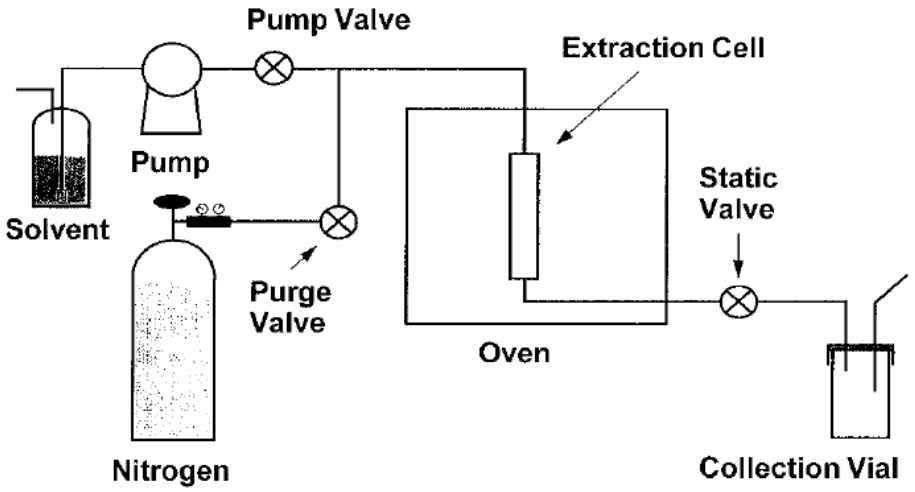

Figure2.6: Schematic representation of the ASE system.[19]

The technique has become popular in environmental analyses as it uses less solvent and is more rapid than traditional solvent based procedures such as Soxhlet extraction. In addition, the ASE system can be fully automated and is easy to understand and operate. As shown in figure 2.6 the system has one extraction cell however there are also some systems that include carousel for holding multiple extraction cells and collection vials in such a way that the system can extract a sequence of samples without the presence of an operator. [19]

The other advantage of using ASE is that it can perform online clean up to remove impurities. It is also called in-cell clean up. It is simply adding the sorbent (cleaning agent), such as

silica, florisil, alumina and copper into the cell beneath the sample and filter before extraction starts to remove impurities like sulfur and lipids. This reduces the time it takes for separate clean up procedure. [1]

Once extraction is done concentration of extract is necessary before analysis, especially when the analytes are found at trace levels. It is achieved by, example using Rota evaporator or Turbovap. The Biotage TurboVap LV Evaporator is a system for automated concentration of multiple extracts in parallel. The samples are placed in a water bath, and gas nozzles provide a stream of nitrogen to each sample vial, which makes the solvent evaporate.

2.2.2. Gas chromatography-Mass spectroscopy

To separate and quantify the PCBs mostly gas chromatography coupled with mass spectroscopy or electron capture detector is used. The general principle in brief of how GC-MS work is described as the following (see also figure 2.7). First the liquid extract get vaporized in GC inlet under high heat while mixed with a gas mobile phase and let allowed to pass across a long column made with liquid or solid material stationary phase where the analyte get adsorbed, and hence the analyte get start to separated based on their partitioning difference to the stationary phase. After leaving the GC column, the gaseous molecule get ionized by letting the molecule to collide with an electron beam that comes from a heated filament in the ion source and then the ionized molecule will then transferred to mass analyzer where the ionized molecule get separated based on their mass and transferred to a detector, that gives a characteristic peak for the interest of the analyte.

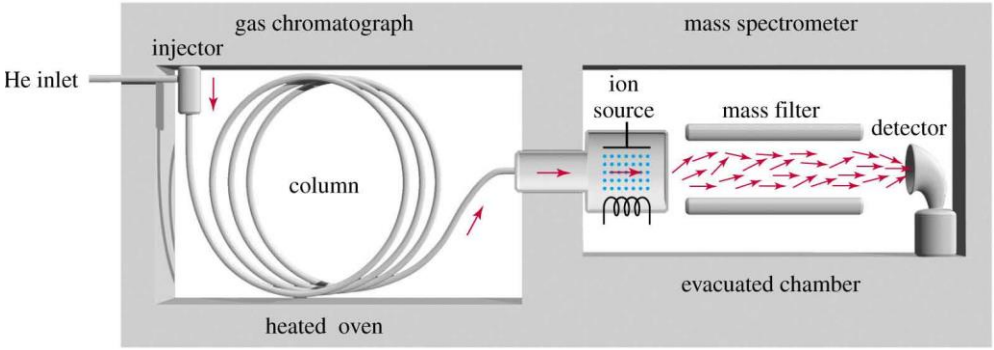

Figure 2.7. The GC/MS schematic over view.[21]

See for example The Essence of Chromatography by Poole (2003)[22] for more information on the principles of GC-MS.

2.3. Current Application Used for Remediation of PCB contaminated sediment

Remediation of PCB contaminated sediment is needed when PCB concentration in the sediment exceeds certain amount of concentration level called Effect Range Low (ERL) or Effect Range Medium (ERM). ERM value for PCB is 180ng/g.[23] The Norwgian Climate and Pollution Agency (Klif) also set guidelines for classification of metal and organic pollutants in sea water and marine sediments. It classify the pollution level in five groups, Background (<5ng/g), Good (5-17ng/g), Moderate (17-190ng/g), Polluted (190-1900ng/g), and Highly polluted (1900ng/g).[24] Some Current Application Used for Remediation of PCB contaminated sediment is reviewed as described in the following.

2.3.1. Activated Carbon

Work of Beckingham, et al., shows remediation of contaminated sediments by using activated carbon reduces bio uptake of PCBs in benthic organisms. After treatment with activated carbon applied at a dose similar to the native organic carbon of sediment, bioaccumulation in freshwater oligochaete worms was reduced compared to pre amendment conditions by 69 to 99%, and concentrations of PCBs in water at equilibrium with the sediment were reduced by greater than 93% at all treatment sites for up to three years of monitoring. They also said the traditional ways of remediation like excess heat field burning is destructive for natural resources and it is not applied to sediment under water.[25]

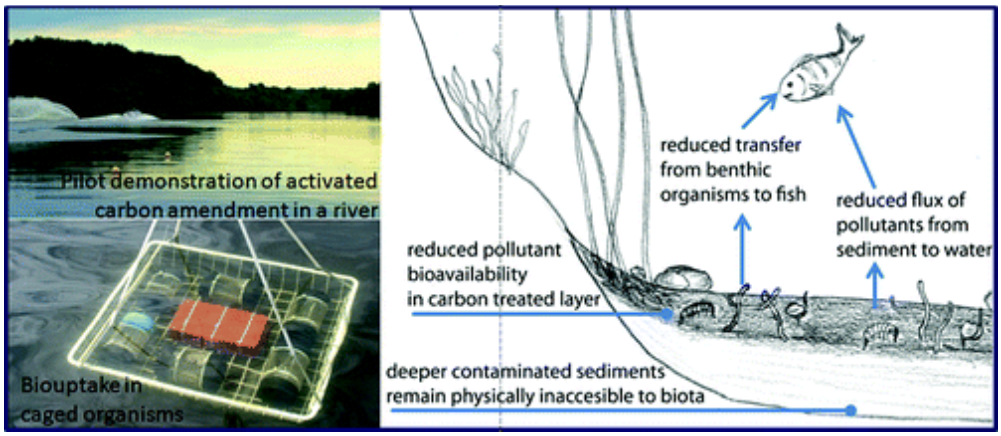

Figure 2.8 Pilot demonstration of activated carbon.[25]

2.3.2. PCB remediation technology

As Guangping, et al., reviewed shows they divide PCB remediation technology in to two categories as shown in the following figure. [26]

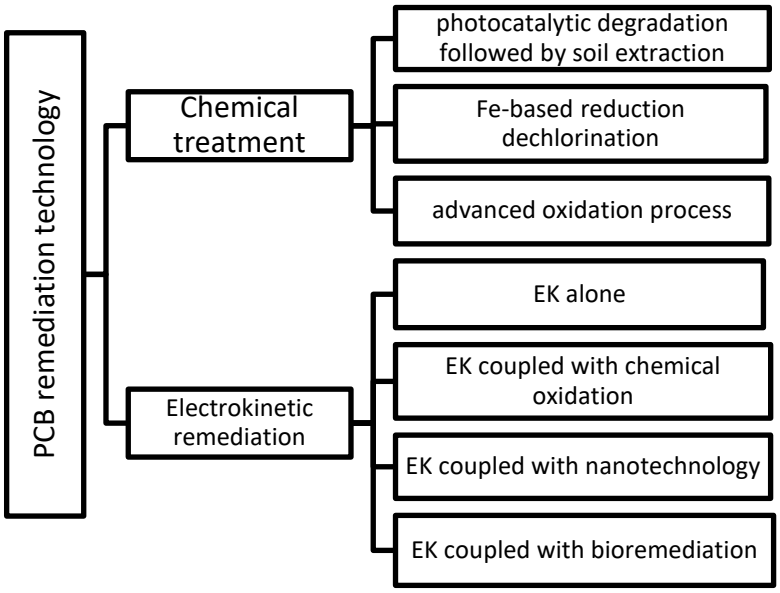

Figure 2.9 PCB remediation technologies.

2.3.3. Bioremediation dechlorination

As Rayford, et al., work reveled bioremediation of sediments contaminated with commercial PCBs is achieved by concurrent dechlorination using bioaugmentation with anaerobic halorespiring "Dehalobium chlorocoercia" DF1 and aerobic Burkholderia xenovorans LB400. These two bacterias were added to 2-liter laboratory mesocosms containing weathered Aroclor-contaminated sediment from Baltimore Harbor, and they gave a dechlorination in an 80% decrease by mass of PCBs, from 8 mg/kg to less than 2 mg/kg after 120 days. [27]

2.3.4. Phytoremediation

It is a remediation by planting plants that can uptake PCBs from sediment. For example according to Liu et al., work, the ability and mechanisms of Kandelia candel (mangrove), seedlings planted in sediments contaminated with a series of concentrations of PCB 47 and PCB 155, to remediate PCB47 and PCB155 were studied. At the end of a 180-day experiment, the residual concentrations of PCB47 and PCB155 were lower in the sediments planted with Kandelia candel than in non-planted sediments. The residual concentrations of PCB47 and PCB155 in planted sediments were 53.99-528.37μg·kg-1 and 68.25-682.90μg·kg-1, respectively, which were 10.40%-15.46% and 6.10%-11.94% lower than control (with addition of $HgCl_2$). [28]

2.3.5. Pressurized Ozonization

Hydrophobic nature of PCB makes the treatment difficult by hindering the polar treatment agent to reach the PCBs. Pressurized ozonization breaks this barrier and the treatment does work. The pressure-assisted technique removed 96% of PAHs from river sediments within 1 h; it completely removed both PAHs (16 mg kg^{-1} initially) and PCBs (5.1 mg kg^{-1} initially) from the Waukegan Harbor sediment in 0.5 h. Conventional ozonation reached maximum 60% and 40% removal of PAHs from the Passaic River (40 mg kg^{-1} initially) and St. Louis River sediment (520 mg kg^{-1} initially), respectively, in 1 h; however, removals ceased despite prolonged treatment for 2 h.[29]

2.4. Review of similar previous studies

Using water-exposed semipermeable membrane devices (SPMDs) and in bed sediment samples the occurrence of thirty two polychlorinated biphenyls, hexachlorobenzene (HCB) and pentachlorobenzene (PeCB) were analysed in the River Alna (Oslo, Norway). Performance reference compound-corrected data from the passive samplers deployed at three sites along the river were used to track PCB contamination in the overlying water. SPMDs were able to detect an increase in dissolved PCB concentrations at the site furthest downstream that was corroborated by bed sediment concentrations. The study also concludes the Alna River is a continuous source of PCBs to the Oslofjord. [30]

In the following paragraphs the review covers a study done in Europe but not in Norway and the idea of those paper is the base for this study. Pintado-Herrera et al., study shows simultaneous extraction and cleanup is possible using ASE. Also they found out simultaneous determination of 97 contaminants using GC coupled with tandem mass spectroscopy is possible. In their five solvent comparison, based on the recovery percent, they found out Dichloromethane(DCM) is good solvent and that is why in this study DCM was used as extracting solvent. Under optimal conditions, their quality assurance showed good recovery percentages (70–100%), linearity (>0.99) and limits of detection below 1 ng g^{-1} for all compounds. In general, after the above quality assurance value is obtained analysis of the sediment samples, taken from coastal areas from Andalusia (Spain), is started and twenty five compounds out of 98 were detected in all samples, with the endocrine disruptor nonylphenol and the fragrance galaxolide showing the highest concentrations, up to 377.6 ng g^{-1} and 237.4 ng g^{-1}, respectively.[1]

A study conducted in Italy by Salvatore Barreca, on the determination of PCB using automated Soxhlet and GC-MS, shows a detection limit of 36ng/gm and a recovery of not less than 60%. [6]

2.5. Quantitation/Quality assurance

In GC-MS, quantitation is based upon establishing the relationship between the amount or concentration of analyte passing the detector and the detector response, using peak height or peak area. The peak height can vary with mobile phase flow rate in the detector mass spectrometry. However, peak area is not and hence no error occurs with the variation in flow rate if peak area is used in quantitation. In general, quantitation or analyte amount determination can be calculated using two ways as described in the following;

2.5.1. Calibration curve or linearity test

In order to determine the concentration of an analyte it is necessary to prepare a calibration curve with standard solutions of known concentrations. When using the internal standard method a constant amount of internal standard is added to each standard solution, and the calibration curve is constructed by plotting the various concentrations of the analyte against the ratio of the peak area of the analyte and the peak area of the internal standard. An internal standard is a compound of similar chemical properties of the analyte, which is added to the sample prior to injection or extraction. The linearity test is based upon constructing a calibration curve with six or five calibration solution, spiked with constant amount internal standard (z) solution. The five or six calibration solution must be made with factorial increase in concentration. Then checking the regression (r^2) of the calibration curve by constructing signal area ratio versus concentration ratio of analyte and internal standard. It would be nice if r^2 is as possible as close to one. Finally the amount of analyte in the unknown (a) is determined by using the calibration equation after having signal area of unknown in combination with signal area of internal standard.

In general, let say

Y=mx + b, be the calibration line equation. Then

$$Y = \frac{Signal\ area\ of\ analyte(Sa)}{\text{Signal area internal standard(Sisd)}}$$

And X= Concentration of Analyte

Then X (amount of analyte) becomes;

$$X = \frac{(Y - b)}{\text{m}}$$

Where m is the slop and b is the y-intercept.

2.5.2. Using response factor

In quantitative analysis with GC-MS is it necessary to establish a relationship between the amount of analyte passing the detector and the detector response. The internal standard method is a commonly used method for converting peak areas into analyte concentrations.

The response factor f_i is defined as[31]

$$f_{i=\frac{Ci}{As(i)}}$$

Where

f_i : Response factor of analyte i in the standard

C_i : concentration of the analyte i in the injection standard volume V and

As(i): area of the signal in the standard chromatogram of the analyte i

The relative response factor f_r relative to ISTD is defined as

$$f_{r=\frac{C(i).As(ISTD)}{C(ISTD).As(i)}}$$

Here by

$C_{(ISTD)}$: Concentration of the ISTD in the injected standard volume V and

$As_{(ISTD)}$: Signal area of the ISTD

Via conversion of the formula, the amount of analyte Mi can be calculated by;

$$M_{i=\frac{M(ISTD).As(i).\text{fr}}{C(ISTD).As(ISTD)}}$$

Here by, M_{ISTD} is the amount of the ISTD.

In an analogous manner, the response factor $f_{r(RSTD)}$ of the ISTD relative to the recovery standard RSTD can be defined as:

$$f_{r(RSTD)}=\frac{C(ISTD).As(RSTD)}{C(RSTD).As(ISTD)}$$

Here by is

C(RSTD) is concentration of the RSTD in the injected Standard volume V.

As(RSTD) is signal area of the RSTD.

The recovery R is then given as

$$R(\%) = \frac{M(RSTD).As(ISTD).fr(RSTD)}{M(ISTD).As(RSTD)}x100$$

Hereby $M_{(RSTD)}$ is the amount of the RSTD.

Chapter three:

3. Materials and Method

3.1. Chemicals and Equipment

3.1.1. Chemicals

Acetone, Analytical grade, VWR Chemicals

Acetone, Technical grade, VWR Chemicals

Dichloromethane, Analytical grade, Sigma Aldrich Corporation, German

Ethyl acetate, Analytical grade, VWR Chemicals

Hydrochloric acid, 37%, analytical grade, Fisher scientific UK

Aluminum oxide(Alumina), activated, Sigma Aldrich Corporation, German

Diatomaceous Earth, Sigma Aldrich Corporation, German

Copper powder, Sigma Aldrich Corporation, German

Dutch Seven PCB mixture, S-4236-100-IO, 100µg/mL in Isooctane, CHIRON AS

Marker-7 PCB mixture (EC-5375) (^{13}C, 99%) 1000 ng/ml in nonane, Cambrige isotope laboratory

EN-1948-4 PCB Recovery standard (^{13}C, 99%) 100 ng/ml in nonane, Cambrige isotope laboratory

Standard reference material, 1941b, organics in Marine Sediment, NIST

3.1.2. Equipment

Accelerated Solvent Extractor: Dionex ASE 150, Thermo Fisher Scientific, With 22 ml stainless cells, 60mL collection vials and 22mm cellulose filter.

Biotag Turbovap LV Evaporator with vial racks for ASE vials

Gas chromatography coupled with Mass spectrometry, TRACE Ultra GC with TriPlus Autosampler and ITQ 1100 Ion Trap Mass Spectrometer, Thermo Fisher Scientific

Column: DB-5MS fused Silica (5%phenyl/95%methylsiloxane) open tubular capillary columns, 30m x 0.25mm x 0.25µm film, Agilent J&W

GC-MS Injection syringe: Hamilton Microliter 701, 10µL

Helium gas 6.0 quality

GC-MS Data processing software: Xcalibur 3.0

Balance: Sartorius BL210S Analytical Balance

Measuring Syringe: Hamilton Microliter 701, 100µL

Sample Vials: 2mL, Screw top Vials with bonded caps, Agilent technology

Glassware: Various Beakers, glass pipettes and test tubes. All glasswares was rinsed with water followed by technical acetone before use. Micro syringes and beakers were rinsed with the respective solvent prior to use.

3.2. Sampling places

The sediments were collected in Norway, on the Trondheim fjord which covers around 130km. Specifically the sediment samples were taken from three places around three cities that includes (1) Trondheim, (2) Steinkjer, and (3) Verdal. The cities are located along side of the Trondheim fjord (Figure 3.1).

Trondheim is city and municipality in Sør-Trøndelag county located in central Norway at 63° 25′ 47″ N, 10° 23′ 36″ E and has a total population of 187,353 (January 1, 2016). Steinkjer city is municipality in the Nord-Trøndelag county, Norway, located in the inner part of the Trondheim fjord at 64° 3′ 29″ N, 11° 43′ 8″ E and has a population of 21,151(2011). Verdal is a municipality in Nord-Trøndelag county, Norway, located at 63° 47′ 53″ N, 11° 56′ 57″ E and has a population of 14,334(2011).

The other study place is found in Ethiopia specifically Lake Tana and Blue Nile River. Lake Tana is the first largest lake in Ethiopia while Blue Nile is the world longest river. Bothe the River and Lake is part of the city called Bahir Dar, with a population of 243,300 (2015). The city is located in north western Ethiopia (Figure 3.2).

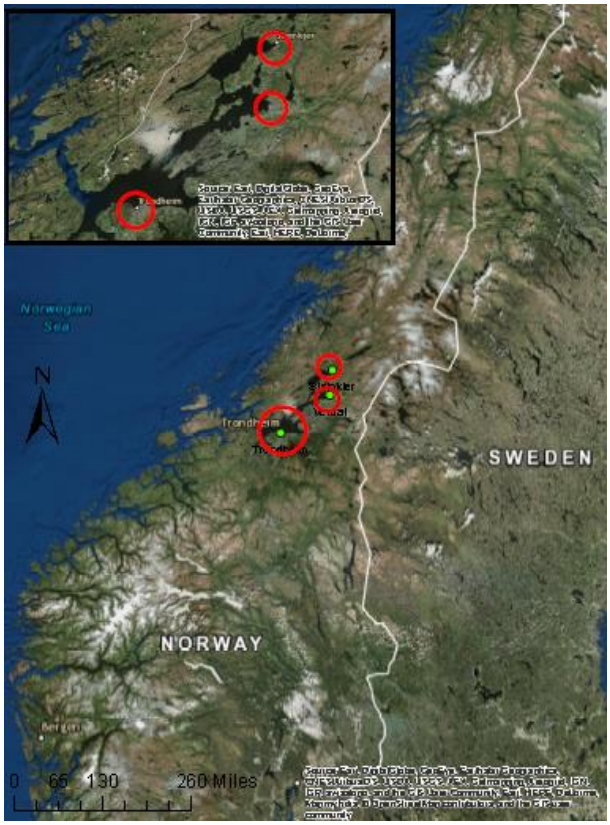

Figure 3.1. Norway Sampling place location. Trondheim (The first bottom green dot), Verdal (the middle green dot), Steinkjer (the upper green dot).

Figure 3.2. Map of Ethiopia. In the small rectangular layout map the red circle indicates the location of BahirDar city, northwestern Ethiopia.

3.3. Sampling and Preservation method

In this study pilot survey sampling, ISO 5667-19:2004(E) Water quality: part 19; Guidance on sampling of marine sediments, was applied as a guide line for sampling method with some deviations. The sediment samples were collected in May 9-11, 2016 using Box corer during Gunnerus research vessel sample collection cruises (NTNUs own ship). Prior to sample collection in the field, aluminum boxes (15cm x6cmx6cm) were washed first with technical acetone followed with analytical grade acetone, to avoid wastes and contaminants, and let dry in the hood. After the sediment is withdrawn from the box corer, a Teflon knife was used to cut out the sediment layer from top to bottom having 2cm height and then transferred to and stored in aluminum box. In Trondheim sampling place mostly the first three layers were taken (0-2cm, 2-4cm, 4-6cm) whereas in Steinkjer and Verdal the first top layer was taken (0-2cm). Finally, the sediment sample was stored in freezer at -18 to -24^{0}C. It is clear that after every single sample is collected the sampling material is also washed with the sea water to avoid cross contamination between different sampling positions. In addition to avoid internal cross contamination with in a single sampling position the Teflon knife was repeatedly washed with sea water in between taking out each sediment layer for a single sampling place. Similarly, samples from Ethiopia are collected from Lake Tana and Blue Nile river by using Van veen grab. Sampling place, code, GPS coordinate and depth of each study places are summarized in tables (appendix A; Table A.1-A.4). Approximate sampling locations, with the help of ArcMap10.4, is also showed on maps for each study place (appendix A; FigureA.1-A.5).

3.4. Activation of dispersing agent and sorbents

3.4.1. Diatomaceous earth

Following method EPA-3545A, this dispersing agent was activated by heating 200g DE in crucible at 400^{0}C for four hour in oven and stored at room temperature in desiccator until use. [32]

3.4.2. Alumina

According to Method EPA-3610B alumina powder was purified by heating it in oven at 400^{0}C for sixteen hour, then, poured to reagent bottle tightly sealed and cooled at room temperature until use. [33]

3.4.3. Copper

Method EPA- 3660B was used to activate copper powder. It was done by treating the powder with concentrated hydrochloric acid (37%) to remove the oxides. Approximately one gram per five mill liter acid was used. Then the mixture was rinsed with organic free distilled water until no acid left. It was confirmed by using litmus paper. Finally, it was rinsed once with acetone and collected in a reagent bottle.[34]

3.5. Determination of Organic Carbon Content

It was determined by following NS-EN 159358: 2012 method. In brief, freeze dried 2g sample with 20ml 10% HCl heated to 60^0C in 40mL beaker until no more carbon dioxide effervescence showed. Transferred to a pre washed, dried and accurately weighted porcelain Gooch filter. Excess acid was washed with organic free distilled water using a suction flask. To remove excess water the filter with the sediment was dried in heating cabinet at 105^0C for 12hr. Cooled down to room temperature in desiccator, the filter with sediment sample was then weighted. Afterwards the filter was placed inside a furnace at 550^0C for 3hr and then cooled in a desiccator. Finally, the filter with the sample was weighted. Total organic carbon content is then calculated using the following equation.

$$\text{TOC } (\%) = 100 - 100(\frac{NWAA}{NWBA})$$

Where NWAA; Net Weight After Ashing

NWBA; Net Weight Before Ashing. Net weight is weight obtained by deducting the Gouch filter weight from sum of sample and Gouch filter weight.

3.6. Online in cell cleanup and extraction using accelerated solvent extractor (ASE)

The samples were extracted and cleaned up by using ASE, where details on equipment can be found in section 2.2.1. The extraction conditions were taken from Pintado-Herrera et al., with dichloromethane as extracting solvent. In brief, first the cell is loaded with filter then copper (2g) followed by filter then alumina (2g). Finally, previously freeze dried 5g sediment sample homogenized with activated 2g DE and 10ng internal standard was loaded and sealed (Figure 3.3). The sealed cell is the finally loaded in to the ASE instrument and processed under the ASE conditions tabulated in table 3.1. A method blank which contains the dispersing agent and sorbent only also extracted without internal standard and used for checking the purity of all the dispersing agent, alumina, and copper from PCB contamination.

Table 3.1 ASE conditions used for extraction of PCBs in sediments.

Parameter	Value
Temperature	100^0C
System pressure	1500psi
Static time	5min
Static cycle	2
Purge Volume	60%
Cell size	22 Ml
Filter size	27 mm
Solvent	Dichloromethane
Total time per sample	20min
Total solvent per sample	40Ml

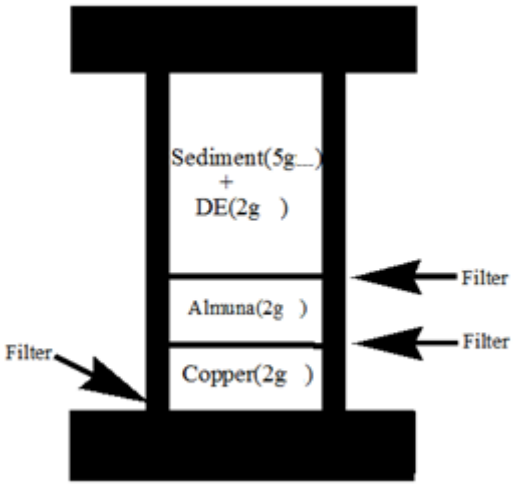

Figure 3.3 Schematic over view of cell loading with sediment and sorbents in ASE cell.

3.7. Concentration of Extracts

It was done by using Turbovap evaporator, where the detail about it is found in section 2.2.1. After having the extract from ASE, the extracting solvent is evaporated by placing the extract sample vials on the Turbovap and hence the solvent will evaporate under the nitrogen purge exposure. The temperature in the water bath was set to 35 ^{0}C and the nitrogen gas flow was set to 12Psi. After evaporating down to Ca.1mL extract left in the vials, the inner wall of the

vials was rinsed with 2 to 3 mL ethyl acetate in order to minimize loss of sample, and concentrated back to Ca. 1.5mL and transferred to small vials. Finally, a 10ng recovery standard was added as injection standard, to determine the percent of recovery, and run on GCMS.

3.8. GC-MS Analysis

First a calibration solution of 5, 25, 50, 100, 150, 200ng/mL was prepared gravimetrically by using standard calibration solution, which contains the Dutch Seven PCBs. Each calibration solution also contains 0.2ml of 100ng/ml isotope (^{13}C) labeled internal standard. Then, to determine the retention time (Table 3.2) of each Dutch seven PCB first a full scan GCMS was run using the highest concentration of calibration solution. After knowing the retention time of the Dutch seven PCB in the highest concentration (200ng/mL), SIM (selected ion monitoring) was run for the rest analysis of the samples.

Table 3.2. Retention time and two SIM m/z values for the Dutch seven PCBs and isotopically labeled internal Dutch Seven PCB standard.

Dutch Seven PCB	Retention time	SIM value(m/z)	SIM value for internal standard
PCB28	13.13min	256, 258	268, 270
PCB52	14.00min	290, 292	302, 304
PCB101	16.51min	324, 326	336, 338
PCB118	18.50min	324, 326	336, 338
PCB138	20.06min	360, 362	370,372
PCB 153	19.19min	360, 362	370,372
PCB180	22.23min	394, 396	406,408

A total of 53 samples were analysed by GC-MS in order to detect the presence of PCBs in the sample and determine the elution order, retention time and quantity of the individual congeners. Details on GC-MS equipment is found in section 3.1.2 and 2.2.2. Full over view of the analytical GC-MS conditions are shown in table 3.3.

Table 3.3. GC-MS conditions used for the analysis of the Dutch seven PCBs in the sediment.

Parameter	Value
Injection system	
Injection mode	Splitless, 4.5 min splitless time
Temperature	280^0C
Split flow	15mL/min
Injection volume	1.0µL
Injection technique	Autosampler
Washing solvent	Ethyl acetate
Column	
Temperature program	70^0C for 3.5 minute
	25^0C/min to 180^0C
	5^0C/min to 300^0C, hold for 4min
Carrier gas	Helium, 1.0mL/min
Total program time	36min per sample
Detector	
Type	Ion Trap Mass spectrometer
Ionization technique	Electron ionization (EI)
Auxiliary temperature	300^0C
Start time	8min
Ion Temperature	200^0C

3.9. Calculation

3.9.1. Quantification by the internal standard method

Quantification was carried out using the results from the GC-MS analyses. Analytes (PCBs) were quantified by applying the internal standard method. The internal standard method was used in order to establish a relationship between the magnitude of the detector signal and the sample amount. The advantage of this method is that quantification can be carried out despite variations in injected sample volumes. In addition, the extraction volumes do not have to be constant.[22] Before quantifying the analyte in the sediment, the method (extraction and GC-MS condition) was validated following the following Criteria.

Criteria for Method validation

The criteria used for evaluation of the analytical method were as follows:

1. Limit of detection: lowest measureable concentration

2. Limit of quantification: lowest concentration that allows quantification

3. Linearity: linearity of the calibration curve

4. Recovery: extraction efficiency

5. Accuracy: It is deviation from the true value. It is measured using the equation given in section 3.9.1.3. The lower the percent the more it's accurate. Accuracy or Percent error of below 15% is accepted as a good accuracy.

6. Precision: Repeatability which is measured by standard deviation and it should be cloth to zero.

3.9.1.1. Limit of Detection and Limit of Quantification

The limit of detection (LOD) is often defined as the concentrations were the signal-to-noise ratio(S/N) reaches an acceptable value, which is typically set to 3. Moreover, the limit of quantification is often set to 10 times the signal-to-noise ratio. The LOD and LOQ were determined from the GC-MS chromatogram of the lowest calibration standard for each of the Dutch seven PCBs. The height of the noise was estimated by measuring the height of the noise band close to the peak of interest. This is illustrated in Figure 3.4.

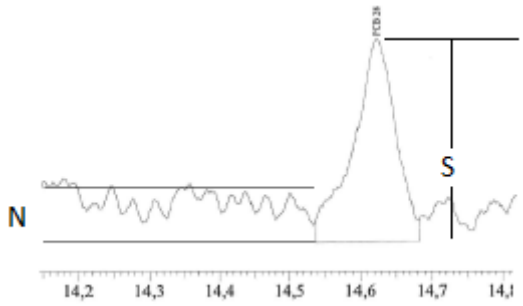

Figure 3.4. Illustration of how the height of the noise band was estimated in order to calculate the limit of detection (LOD) and limit of quantification (LOQ).

Mathematically concentration of LOD and LOQ was determined using lowest concentration of standard solution the following formula below:

$$C_{LOD} = 3.5 * \sigma$$

$$C_{LOQ} = 10 * \sigma$$

Where: σ is standard deviation of signal area of lowest concentration of calibration solution.

3.9.2. Recovery

The loss of analytes during extraction and clean-up was determined from the response factor (Fr) of the internal standard (ISTD) relative to the recovery standard (RSTD), given by the following equation:

$$f_r = C_{ISTD} \cdot A_{RSTD} / C_{RSTD} \cdot A_{ISTD}$$

Where C_{ISTD} and C_{RSTD} are the concentrations of the internal standard and the recovery standard in the standard solution and A_{ISTD} and A_{RSTD} are the signal areas of the internal standard and the recovery standard in the chromatogram of the standard solution. The recovery, R, for every sample is further given by

$$R(\%) = \frac{M(RSTD).As(ISTD).fr(RSTD)}{M(ISTD).As(RSTD)} x100$$

Where M_{ISTD} and M_{RSTD} are the amounts of internal standard and recovery standard added to the sample and A_{ISTD} and A_{RSTD} are the signal areas of the internal standard and recovery standard.

3.9.3. Accuracy

Accuracy is defined as the deviation from the true value. The deviation between the determined PCB concentration and the certified PCB concentration in the standard reference material or prepared standard solution was calculated as:

$$E(\%) = \frac{|\text{Determined value} - \text{Exact value}|}{\text{Exact value}} x100$$

Chapter four

4. Result and Discussion

4.1. Total Organic carbon content

During organic carbon content analysis the effervescence observed is due to the evolution of carbon dioxide formed by reaction of hydrochloric acid and the calcium carbonate found in the sediment. This in turn removes the inorganic carbon from the sediment as to use to determine the organic carbon content only. The organic carbon content study showed Trondheim bay has more carbon (2.7%) than Steinkjer(2.4%) and Verdal(2%) sampling sites(see figure 4.1-4.3). Due to hydrophobic nature PCBs adhere themselves to organic materials. And hence the probability of PCB contamination in sediment of Trondheim bye will be higher if equal PCB concentration distributed all over the fjords.

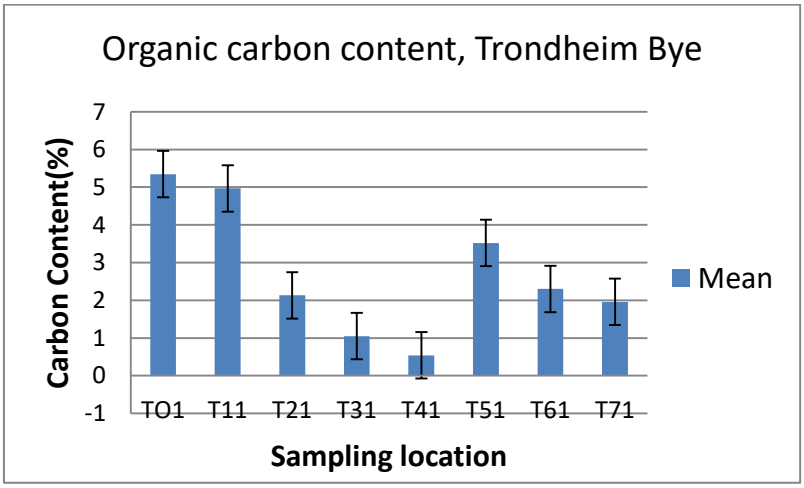

Figure 4.1. Total organic carbon content (%) of sediment samples taken in Trondheim Bay

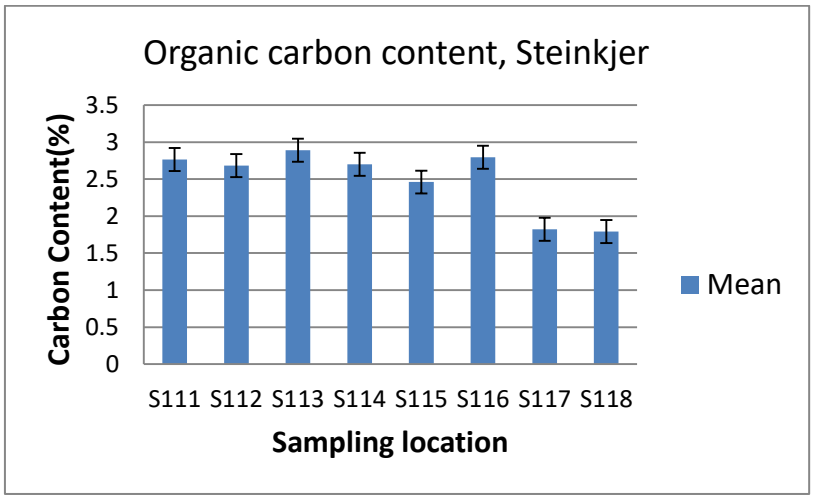

Figure 4.2. Total organic carbon content (%) of sediment samples taken in Steinkjer.

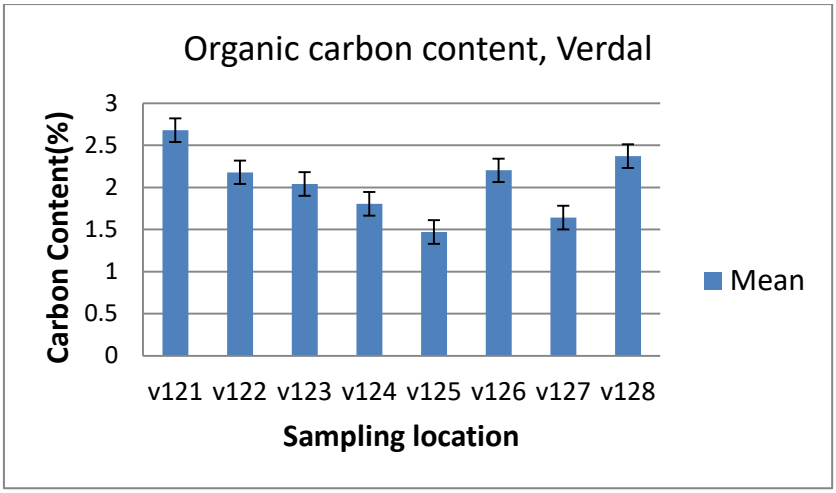

Figure 4.3. Total organic carbon content (%) of sediment samples taken in Verdal.

4.2. Evaluation of extraction condition

The sample size was chosen to be as large as possible since the PCB concentration in the sediment was expected to be low. 5gm was chosen as the sample size which is 2.5 times larger than the sample size used by Pintado-Herrera *et al.*(2016). Similarly the amount of diatomaceous earth (dispersing agent) was increased from 1gm to 2gm, the amount of alumina and copper which are cleanup agent (sorbents) increased from 1g to 2g. The nitrogen purge time was increased from 60s to 90s. In addition, the extraction cycles was reduced from three to two. This is because, first, 5g sediment sample is used which is 2.5 times higher and second reason is to save time. The solvent dichloromethane was used in this work. This is because Pintado-Herrera et al. (2016) work choses it from other solvent such as acetone, methanol, propan-2-ol, hexane based on its good recovery, which is more than 90%. The use of diatomaceous earth is a dispersing agent that allows the solvent to reach to every part of the sediment. The use of copper is to remove the sulfur from the extract. Similarly, alumina is used to remove lipids and other polar compounds from the extract.

The recovery calculation showed each Dutch Seven PCB has a recovery of above 98% which tells the extraction condition is efficient (see appendix B). Additionally, the blank extraction showed no PCB contamination of either the dispersing agent or the sorbents. (See Appendix D, Figure D.3)

4.3. Evaluation of GC-MS analysis

Calibration of the method was done using internal standard method. Thus the linearity of the calibration curve was acceptable (see appendix C) which indicates the GCMS conditions are good and hence the sample analysis was carried out using the above GCMS conditions (see table 3.3). The elution order of the PCBs is the lowest PCB number elutes first while the larger come later. This means PCB 28 elutes first then PCB 52, PCB101, PCB118, PCB153, PCB 138, and finally PCB180 (see Table 3.2). Here the exception arises for hexa chloro biphenyls that is PCB 153 elute before PCB 138 (See Appendix D, Figure D.1 & D.2.). The DB-5 column can't separate PCB 28 and PCB 31. They overlap and give one peak. The calibration curve of PCB 28 had unexpected response for 100ng/mL standard, considered as outliner. Excluding the "outliner'' would improve r^2 and result in somewhat lower LOD, LOQ, and measured concentrations.

The LOD and LOQ of the method are tabulated below in Table 4.1. The equation used to determine LOD and LOQ is found in section 3.9.1.1. The method developed in this study has acceptable LOQ (28 ng/g) and LOD (82ng/g). This is because the LOQ and LOD values fall in the average range, moderate, which the Norwegian Climate and Pollution Agency (Klif) sets for pollution level. That is the Background (<5ng/g), Good (5-17ng/g), Moderate (17-190ng/g), Polluted (190-1900ng/g), and Highly polluted (1900ng/g).

Table 4.1. LOD and LOQ of the method used in this study.

Dutch seven PCB	LOD(ng/g)	LOQ(ng/g)
PCB28	4	11
PCB52	3	10
PCB101	3	10
PCB118	4	12
PCB138	5	14
PCB153	5	14
PCB180	4	11
Sum	28	82

To determine accuracy of the method the reference sediment sample form NIST was used. The reference material has known amount of PCBs. Accuracy of the method is tabulated below in table 4.2. The equation used to determine accuracy is found in section 3.9.1.3.

Table 4.2. Accuracy (Percent error) of the method used in this study based on analysis of the reference material.

Dutch seven PCBs	Exact value (ng/g sediment)	Determined value (ng/g sediment)	Accuracy (%)
PCB28	4.52 ± 0.57	Not detected	-
PCB52	5.24 ± 0.28	4.64	11.4
PCB101	25.55 ± 0.34	4.26	16.5
PCB118	5.11 ± 0.19	3.56	15.8
PCB138	3.6 ± 0.28	4.69	30.2
PCB153	5.47 ± 0.32	3.8	30.5
PCB180	3.24 ± 0.51	3.36	3.7

Precision of the method (Table 4.3) is determined by analysing extracts of the reference material three times in the given GCMS conditions and calculating the standard deviation.

Table 4.3. Precision of the method used in this study based on analysis of the reference material.

Dutch seven PCBs	Average determined value (ng/g sediment)	STDV (n=3)
PCB28	Not detected	-
PCB52	4.64	0.08
PCB101	4.26	0.79
PCB118	3.56	0.09
PCB138	4.69	0.67
PCB153	3.8	0.85
PCB180	3.36	0.05

4.4. PCBs in sediment samples

The method described in this work made it possible to detect the Dutch seven PCBs in sediment. In the tables found below NQ stands for not quantifiable but it is detected. The '-' sign indicates not detected at all.

Table 4.4. Trondheim Bay sediment sample PCB concentration in ng/g of dry weight.

Sample Code	PCB28	PCB52	PCB101	PCB118	PCB138	PCB153	PCB180	Sum
01	-	-	14	NQ	-	-	-	14
11	-	-	-	21	-	74	26	121
21	NQ	-	NQ	NQ	64	-	-	64
31	22	-	-		-	-	-	22
41	-	-	13	18	-	-	-	31
51	NQ	-	-	34	14	-	-	48
61	18	-	-		-	-	-	18
71	-	-	-	NQ	-	-	-	
81	-	-	-	21	-	-	-	21
91	-	-	-	-	-	-	-	
101	-	-	-	-	-	-	-	
02	91	-	-	-	86	-	-	177
12	-	-	-	-	-	141		141
22	-	-	-	-	-	-	-	
32	-	-	-	-	-	-	-	
42	-	-	-	-	-	-	-	
52	-	-	-	-	-	-	-	
62	-	-	-	-	-	-	-	
72	-	-	-	-	-	-	-	
82	-	-	-	-	35	14		49
92	-	-	14	-	-	-	-	14
102	-	-	12	-	-	-	-	12

Table 4.5 Steinkjer sediment sample Dutch Seven PCB concentration in ng/g of dry weight.

Sample code	PCB28	PCB52	PCB101	PCB118	PCB138	PCB153	PCB180	Sum
111	-	-		-	-	-	-	
112	-	-	20	-	-	-	-	20
113	-	-	-	-	-	-	-	
114	-	-	-	14	-	-	-	14
115	NQ	10	-	-	-	-	NQ	10
116	-	12	-	-	-	-	-	12
117	-	NQ	-	-	-	-	-	
118	-		-	13		-	-	13
119	-	NQ	-	28	14	-	-	42
1110	-		-	NQ	-	-	-	

Table 4.6 Verdal sediment sample Dutch Seven PCB concentration in ng/g of dry weight.

Sample code	PCB28	PCB52	PCB101	PCB118	PCB138	PCB153	PCB180	Sum
121	-	-	NQ	NQ	-	-	NQ	
122	-	-	-	14	-	-	-	14
123	-	-	NQ	-	NQ	-	NQ	
124	-	-	NQ	-	-	-	-	
125	-	-	NQ	-	NQ	-	NQ	
126	-	-	NQ	-	-	-	NQ	
127	-	-	-	NQ	-	-	NQ	
128	-	NQ	NQ	-	-	-	-	
129	-	NQ	NQ	NQ	-	-	-	
1210	-	NQ	NQ	NQ	-	-	-	

Table 4.7 Lake Tana and Blue Nile river (Ethiopia) sediment sample Dutch Seven PCB concentration in ng/g of dry weight.

Sample code		PCB28	PCB52	PCB101	PCB118	PCB138	PCB153	PCB180	Sum
Lake Tana	131	-	-	-	-	-	-	-	
	132	-	-	NQ	-	-	-	NQ	
	133	-	NQ	NQ	NQ	-	-	-	
	134	-	NQ	10	12	-	-	-	22
	135	-	NQ	NQ	-	-	-	NQ	
	136	-	-	NQ	NQ	-	-	NQ	
Blue Nile River	141	-	NQ	11	NQ	-	NQ	NQ	11
	142	-	NQ	NQ	14	-	-	NQ	14
	143	-	NQ	NQ	NQ	-	-	NQ	
	144	-	NQ	11	-	-	-	NQ	11

According to this study all the Dutch Seven PCBs, except PCB52, is detected and quantified in the first layer, sample code ending with one, of Trondheim Bay sediment sample. The second bottom layer, sample code ending with three, of Trondheim bay has four quantified PCBs.

In Steinkjer sediment sample, out of the Dutch Seven PCBs only four PCB (52,101,118,138) are detected and quantified. In Verdal sediment samples out of the Dutch seven PCBs only PCB 118 is quantified and PCB 52, 101, 138, 180 is detected. Sediment samples from Ethiopia have two quantified PCBs (PCB 101 and 118), three detected only PCBs (PCB 52,153, 180), and two not detected PCBs (PCB 28 and 138).

In comparison between the sampling places Trondheim bay sediment has a bit higher PCBs (but it is not mean polluted) than Steinkjer followed by Verdal. The possible reason is it may be due to the city Trondheim is more populated and hence more construction, trade, and shipping that contain PCB may take place before PCB production and use is banned. Also there is a nearby train station and harbor that could be a source of leakage for PCB. Ethiopia sediment sample has a bit higher PCBs (but it is not mean polluted) than Verdal and less PCBs than Trondheim and Steinkjer.

Chapter 5:

5. Conclusion and Recommendation

The study and monitoring of environmental pollutant is important to keep the world healthy and confortable. The common pollutants include POPs, trace-toxic metals (like, Hg, Pd, Cd), nanoparticles, radioactive compounds, microplastics, and so on. Among dozens of POPs, PCB is one. Environmental investigations of PCBs can be challenging as they are often present at trace level. The method that was tested in this work made it possible to identify the Dutch seven PCBs in marine sediment samples. In this study to quantify PCB in the sediment of Trondheim fjord (Norway) and Lake Tana (Ethiopia) in house method development and method validation was done. The three main step sample collection and preservation, sample extraction and preparation, and quantification using GC-MS were followed.

By the GCMS result, from out of the Dutch Seven PCBs six PCBs were quantified in eight samples from Trondheim bay ranging from 13 – 141 ng/g in dry weight. Four PCBs are quantified in samples from Steinkjer ranging from 10 - 28 ng/g in dry weight. Only PCB 118 is quantified in one sample from Verdal fjord with amount of 14 ng/g in dry weight. Samples from Ethiopia have only two quantified PCBs ranging from 10 – 14 ng/g in dry weight. In all sediment samples the amount of PCB found is under the Effect Range Medium (ERM). ERM value for PCB is 180ng/g. Also according to the Norwegian climate and pollution agency (Klif), all sediment samples lies in moderately polluted (17-190ng/g) group. This indicates all study areas are environmentally safe relative to PCB contamination.

The analytical method was shown to have an acceptable instrumental precision, LOD (28 ng/g in dry weight), LOQ (82 ng/g in dry weight), and linearity (r^2= 0.97) for individual Dutch Seven PCBs, while the method remains to be further developed in order to improve the accuracy and selectivity. Specially, PCB 28 and PCB 31 were overlapping and not separated in this study. Therefore, further work is recommended to be done in order to achieve a reliable method for the determination of PCBs in marine sediment samples using ASE and GC-ECD (gas chromatography – electron capture detector, which has high selectivity for PCBs) or tandem mass spectrometry. Maybe a column of 60m or DB-1 (100% dimethylpolysiloxane) is likely to improve the selectivity of the analyte, which is therefore recommended to test.

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Appendix

A. Sampling location.

Table A.1. Trondheim sampling place code, GPS coordinate and depth.

Sampling Place	Code	Sediment layer	GPS coordination	Depth
Trondheim: Munkholmen Date: May 9, 2016 Weather: 14-19^{0}C First gradient: From Turistskipskaia to Munkholmen island and second gradient: crossing the first gradient with Ca. 90^0)	01	01(0-2cm)	N63^{0}26,482' E10^{0}23,895'	23m
		02(2-4cm)		
	11	11(0-2cm)	N63^{0}26,523' E10^{0}23,817'	30m
		12(2-4cm)		
		13(9-11cm)		
	21	21(0-2cm)	N63^{0}26,584' E10^{0}23,696'	37m
		22(2-4cm)		
		23(7-9cm)		
	31	31(0-2cm)	N63^{0}26,672' E10^{0}23,523'	41m
		32(2-4cm)		
		33(4-7cm)		
	41	41(0-2cm)	N63^{0}26,753' E10^{0}23,362'	22m
		41(2-4cm)		
	51	51(0-2cm)	N63^{0}26,524' E10^{0}23,242'	62m
		52(2-4cm)		
		53(8-10cm)		
	61	61(0-2cm)	N63^{0}26,578' E10^{0}23,430'	50m
		62(2-4cm)		
		63(9-11cm)		
	71	71(0-2cm)	N63^{0}26,684' E10^{0}23,772'	38m
		72(2-4cm)		
		73(8-10cm)		
	81	81(0-2cm)	N63^{0}26,753' E10^{0}23,980'	34m
		82(2-4cm)		
Trondheim: Ostmarknes Date: May 9, 2016 Weather: 16^{0}C	91	91(0-2cm)	N63^{0}27,454' E10^{0}25,720'	106m
		92(2-4cm)		
		93(28-30cm)		
Trondheim: Korsvika Date: May 9, 2016 Weather: 18^{0}C	101	101(0-2cm)	N63^{0}27,094' E10^{0}25,378'	53m
		102(2-4cm)		
		103(8-10cm)		
	Total = 30 samples			

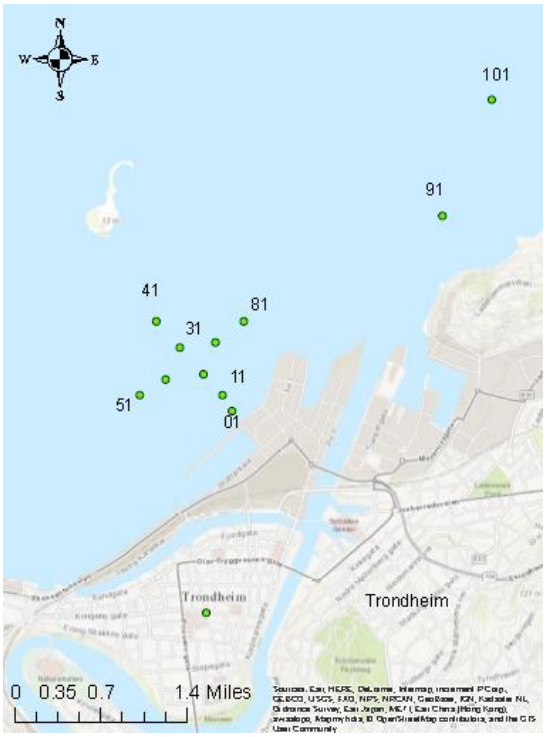


Figure A.1. Trondheim sampling locations on Trondheim fjord (green dots, total 11 sampling place).

Table A.2. Steinkjer sampling place code, GPS coordination and depth.

Sampling Place	Code	Sediment layer	GPS coordination	Depth
Steinkjer Date: May 10, 2016 Weather: ^{0}C	111	0-2cm	N64^{0}00,409’ E11^{0}28,912’	11m
	112	0-2cm	N64^{0}00,350’ E11^{0}28,657’	16m
	113	0-2cm	N64^{0}00,288’ E11^{0}28,379’	19m
	114	0-2cm	N64^{0}00,226’ E11^{0}28,113’	20m
	115	0-2cm	N64^{0}00,168’ E11^{0}27,843’	16m
	116	0-2cm	N64^{0}00,116’ E11^{0}28,566’	16m
	117	0-2cm	N64^{0}00,200’ E11^{0}28,475’	19m
	118	0-2cm	N64^{0}00,407’ E11^{0}28,212’	23m
	119	0-2cm	N64^{0}00,505’ E11^{0}28,076’	17m
	1110	0-2cm	N64^{0}00,577’	13m

			E11^{0}27,976’	
	Total = 10 samples			

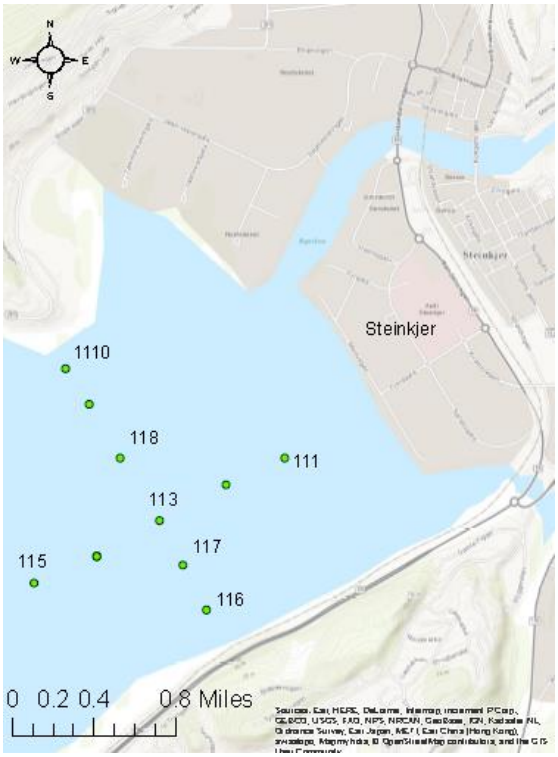


Figure A.2. Steinkjer sampling location on the fjord (Green dots).

Table A.3 Verdal sampling place code, GPS coordination and depth.

Sampling Place	Code	Sediment layer	GPS coordination	Depth
Verdal Date: May 11, 2016 Weather: ^{0}C	121	0-2cm	N63^{0}46,903’ E11^{0}25,481’	34m
	122	0-2cm	N63^{0}47,140’ E11^{0}25,517’	44m
	123	0-2cm	N63^{0}47,330’ E11^{0}25,554’	34m
	124	0-2cm	N63^{0}47,466’ E11^{0}25,476’	31m
	125	0-2cm	N63^{0}47,593’ E11^{0}25,340’	33m

	126	0-2cm	N63^{0}47,751’ E11^{0}25,262’	30m
	127	0-2cm	N63^{0}47,892’ E11^{0}25,244’	19m
	128	0-2cm	N63^{0}48,022’ E11^{0}25,365’	24m
	129	0-2cm	N63^{0}48,170’ E11^{0}25,357’	26m
	1210	0-2cm	N63^{0}48,321’ E11^{0}25,324’	21m
	Total = 10 samples			

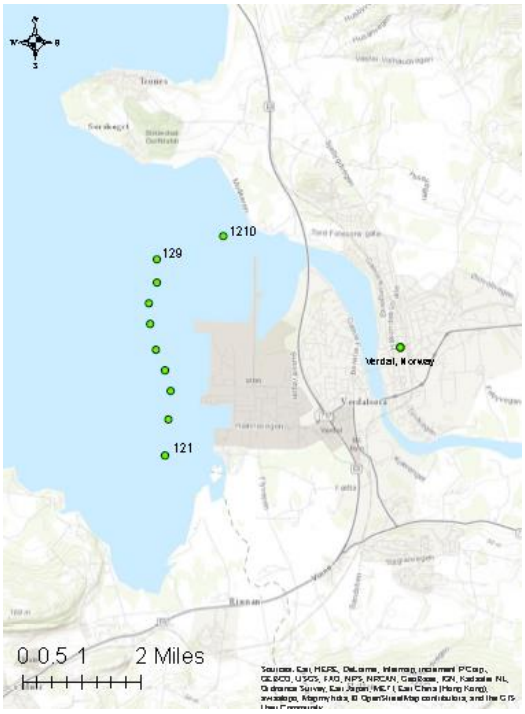


Figure A.3. Verdal sampling place on the fjord (green dots).

Table A.4. Lake Tana and Blue Nile river sampling place code, GPS coordination and depth.

Sampling Place	Code	Sediment layer	GPS coordination	Depth
Lake Tana, Bahir Dar Date: Weather: ^{0}C	131	0-2cm	N11^{0}35’56.6’’ E37^{0}23’26.3’’	
	132	0-2cm	N11^{0}36’01.8’’ E37^{0}23’20.3’’	
	133	0-2cm	N11^{0}36’06.6’’ E37^{0}23’15.3’’	
	134	0-2cm	N11^{0}36’12.7’’ E37^{0}23’08.4’’	
	135	0-2cm	N11^{0}36’17.6’’ E37^{0}23’03’’	
	136	0-2cm	N11^{0}36’22.8’’ E37^{0}22’58.3’’	
Blue Nile river, Bahir Dar	141	0-2cm	N11^{0}36’24.4’’ E37^{0}24’28.2’’	
	142	0-2cm	N11^{0}36’20.5’’ E37^{0}24’28.6’’	
	143	0-2cm	N11^{0}36’18.4’’ E37^{0}24’28.7’’	
	144	0-2cm	N11^{0}36’15.2’’ E37^{0}24’29’’	
	Total = 10 samples			

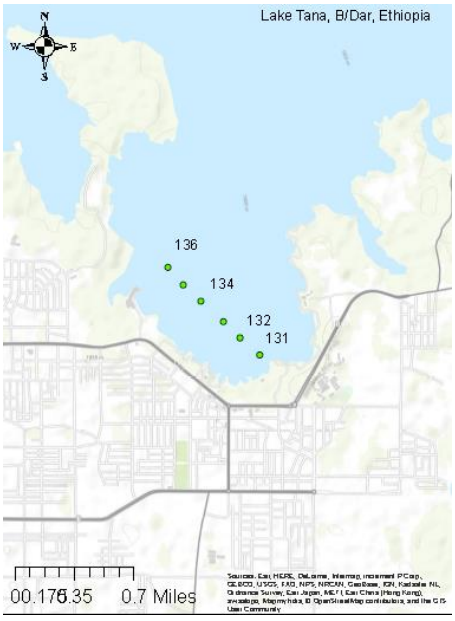


Figure A.4. Lake Tana Sampling place.

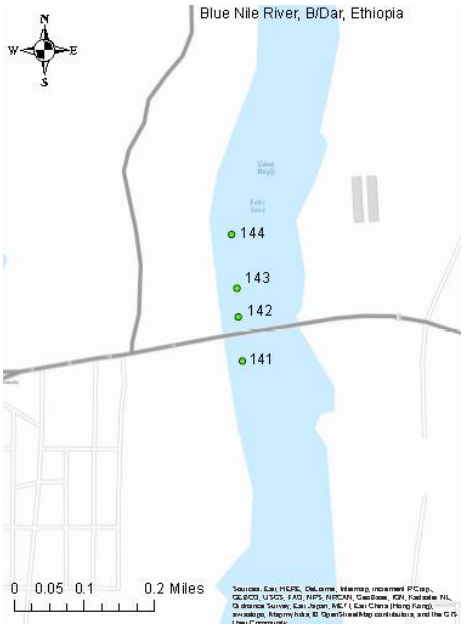


Figure A.5. Blue Nile river sampling.

B. Recovery(%) of the Dutch seven PCBs.

(A_{ISD}=area of internal standard, A_{RSTD}=Area of recovery standard, STDV=standard deviation, Con.isd= concentration of internal standard, Con.rstd= concentration of recovery standard).

CON. ng/ml	PCB 28						
	A_{ISD}	A_{RSTD}	fr(reseponse factor)	%R(recovery	average	STDV	
0	98248	20226	0.3	96.17	97.66	1.402	
5	53990	Nf					Con. Isd
10	21342	11193	0.78	98.1			15ng
50	22602	5346	0.35	98.97			
100	7625	Nf					Con. Rstd
150	22072	13847	0.94	98.89			
200	45624	13322	0.43	96.17			10ng

CON. ng/ml	PCB 52						
	A_{ISD}	A_{RSTD}	fr(reseponse factor	%R(recovery	average	STDV	
0	39852	20226	0.7612	98.98	98.69	0.4101	
5	24143	Nf	-				
10	0	11193	-				Con. isd
50	0	5346	-				15ng
100	0	Nf	-				Con. rstd
150	0	13847	-				10ng
200	16281	13322	1.2273	98.4			

CON. ng/ml	PCB 101						
	Aisd	Arstd	fr(reseponse factor	%R(recovery	average	STDV	
0	69687	17859	0.384	98.89	98.5475	0.4042	
5	35196	19541	0.832	98.9			
10	Nf	27367	-				
50	Nf	11361	-				Con. Isd
100	21284	15028	1.05	98.14			15ng
150	Nf	27496	-				Con. rstd
200	23375	19154	1.22	98.26			10ng

CON. ng/ml	PCB 118						
	Aisd	Arstd	fr(reseponse factor	%R(recovery	Average	STDV	
0	90116	17859	0.297	96.57	98.28	0.900067	
5	49748	19541	0.58	98.96			
10	14568	27367	2.81	98.72			Con. Isd
50	19399	11361	0.87	98.04			15ng
100	31490	15028	0.715	98.88			Con. Rstd
150	Nf	27496					10ng
200	28880	19154	0.99	98.51			

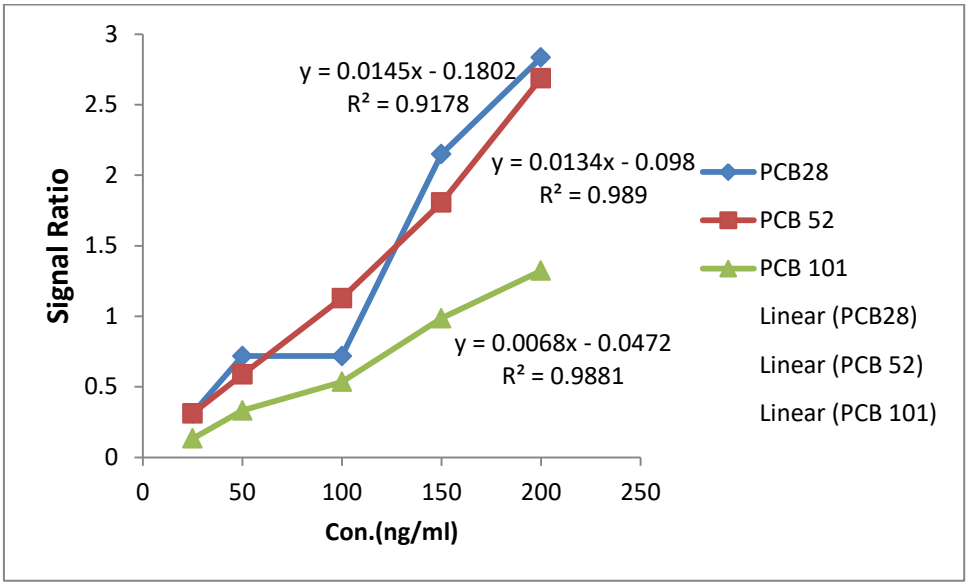
CON. ng/ml	PCB 138						
	Aisd	Arstd	fr(reseponse factor	%R(recovery	Average	STDV	
0	71762	17859	0.373	98.92	98.88333	0.081445	
5	34026	19541	0.861	98.94			
10	Nf	27367					Con. Isd
50	Nf	11361					15ng
100	Nf	15028					Con. Rstd
150	Nf	27496					10ng
200	20335	19154	1.41	98.79			

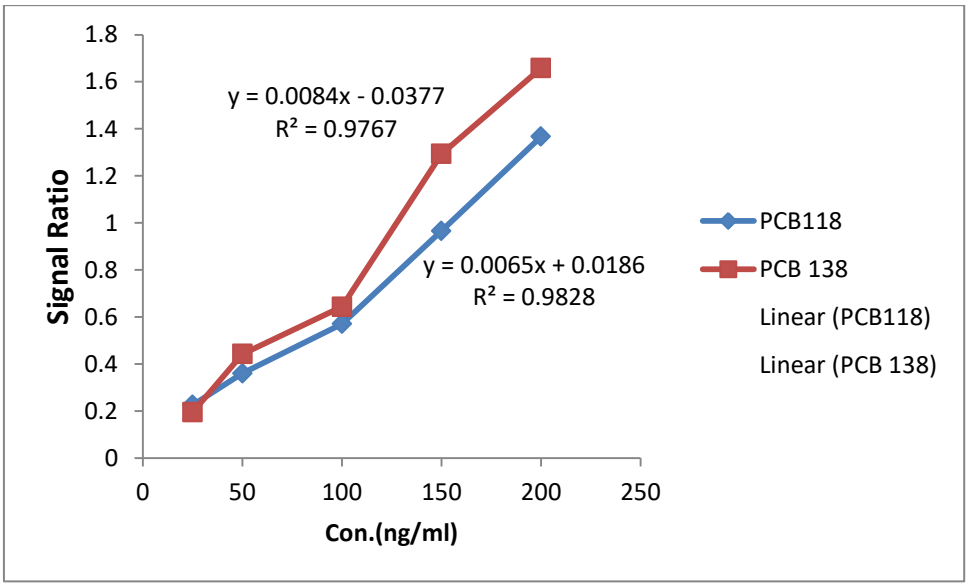
CON. ng/ml	PCB 153						
	Aisd	Arstd	fr(reseponse factor	%R(recovery	Average	STDV	
0	76468	17859	0.3503	98.99	98.89	0.193391	
5	32820	19541	0.893	98.98			
10	Nf	27367					Con. Isd
50	19334	11361	0.8814	98.99			15ng
100	Nf	15028					
150	Nf	27496					Con. Rstd
200	24885	19154	1.15	98.6			10ng

CON. ng/ml	PCB 180						
	Aisd	Arstd	fr(reseponse factor	%R(recovery	Average	STDV	
0	82738	17859	0.323	98.76	98.732	0.246008	
5	38815	19541	0.75	98.32			
10	Nf	27367					Con. Isd
50	Nf	11361					15ng
100	26601	15028	0.847	98.94			Con. Rstd
150	22982	27496	1.79	98.74			10ng
200	31063	19154	0.924	98.9			

Appendix C

A. Calibration curves of each the Dutch Seven PCBs.





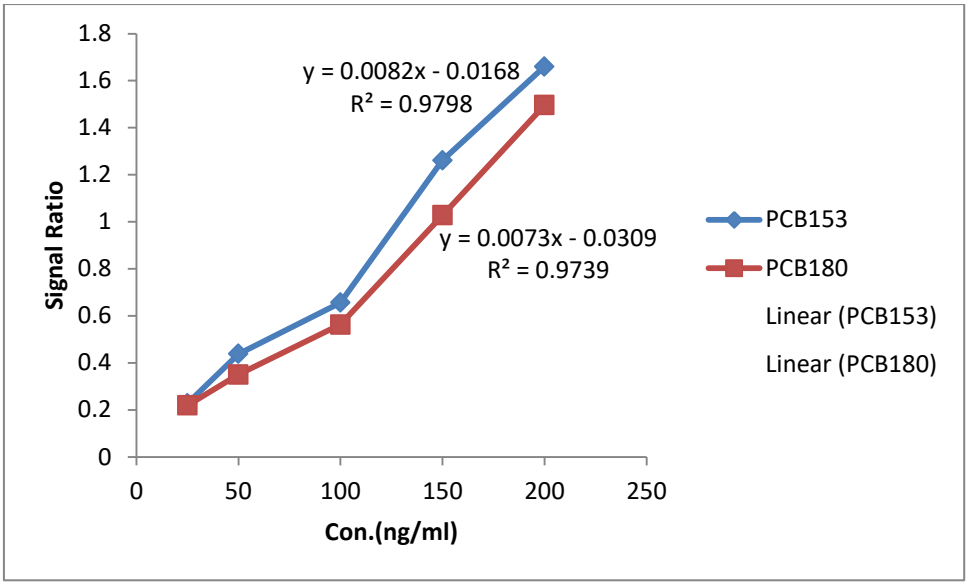


Table C.1. Signal area of each the Dutch seven PCB with their internal standard and signal area ratio. It is used to construct the calibration curves(signal ratio Vs concentration).

Con.	PCB28	PCB28*	Ratio		PCB52	PCB52*	Ratio		PCB101	PCB101*	Ratio
5	Nf	109342	-		Nf	40265	-		nf	60493	-
25	44935	144255	0.311		17496	56209	0.311		10789	81414	0.133
50	120201	167355	0.718		39628	67465	0.587		31407	94560	0.332
100	197281	170329	0.718		71691	63495	1.129		53288	99658	0.535
150	304995	141891	2.15		105825	58602	1.806		86083	87287	0.986
200	436677	15406	2.834		152493	56763	2.687		130754	98934	1.322

*=Internal standard , Con.= concentration in ng/ml, nf= not found

Con.	PCB118	PCB118*	Ratio		PCB138	PCB138*	Ratio
5	-	61983			3017	40635	0.074
25	17511	77475	0.226		10651	54550	0.195
50	36986	102871	0.360		31459	71142	0.442
100	66565	116770	0.570		48636	75624	0.643
150	95410	98717	0.966		86441	66846	1.293
200	144938	105990	1.367		116725	70412	1.658

*=Internal standard , Con.= concentration in ng/ml

Con.	PCB 153	PCB153*	Ratio		PCB180	PCB180*	Ratio
5	4117	46101	0.089		-	47011	-
25	13747	60513	0.227		13093	59723	0.219

50	35673	81512	0.438		28060	80167	0.350
100	58821	89604	0.656		47948	85185	0.563
150	95508	75736	1.261		85130	82807	1.028
200	133379	80349	1.660		123149	82312	1.496

*=Internal standard , Con.= concentration in ng/ml

D. Representative GCMS Chromatograms.

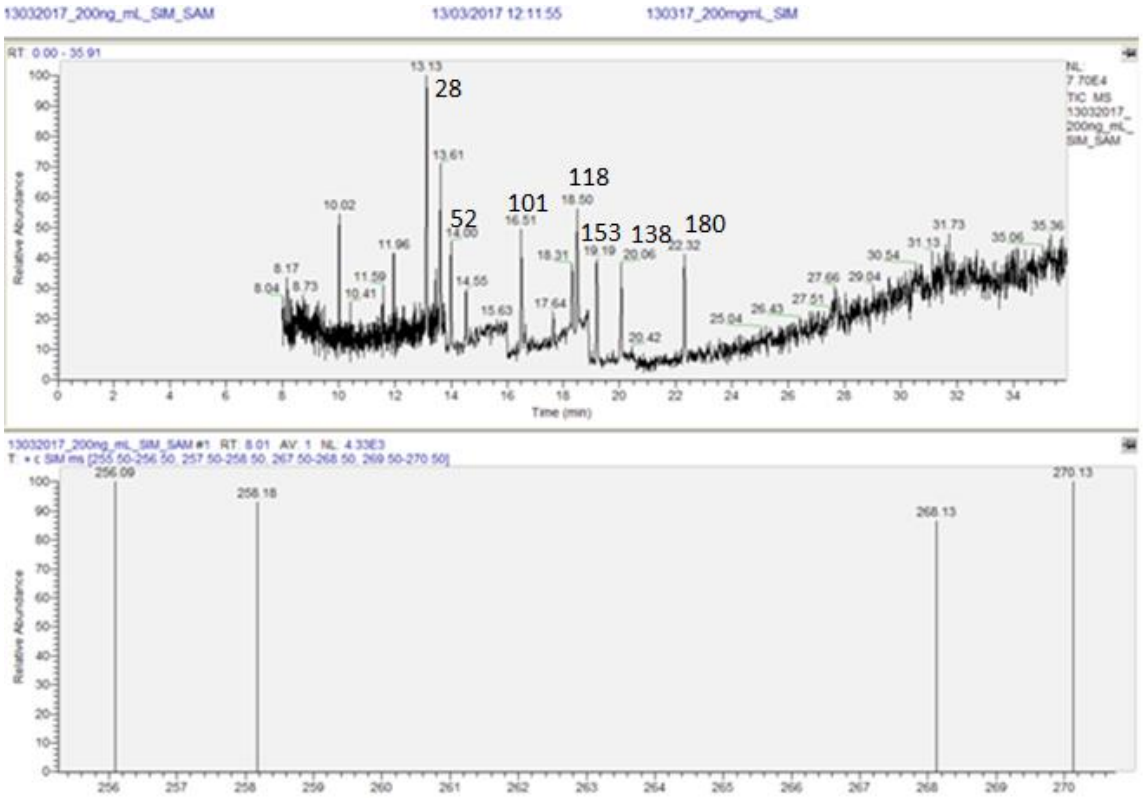


Figure D.1. GC-MS Chromatogram of 200ng/mL calibration solution. The first chromatogram is GC chromatogram while the second is mass spectroscopy chromatogram. The Dutch Seven PCBs IUPAC numbers (PCB 28, 52, 101, 118, 138, 153, 180) are shown in GC chromatogram.

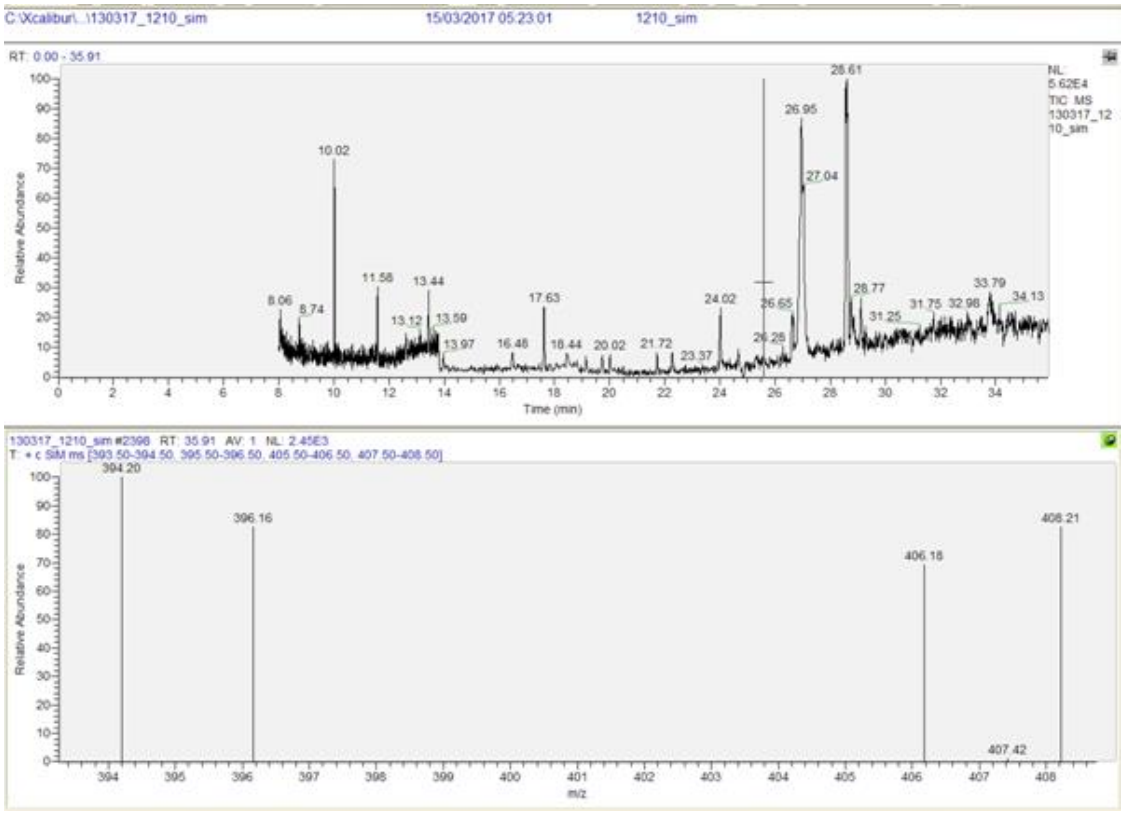


Figure D.2. GC-MS Chromatogram of Verdal sample with sample code of 1210. The first chromatogram is GC chromatogram while the second is mass spectroscopy chromatogram.

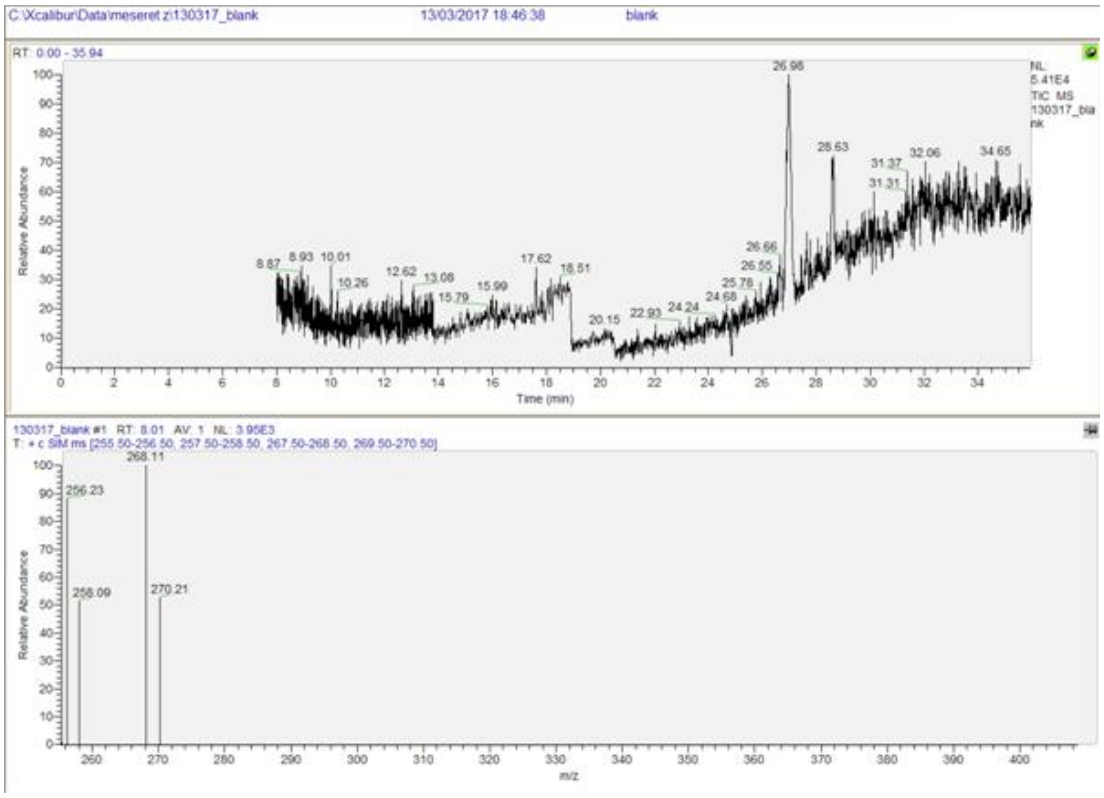


Figure D.3. GC-MS Chromatogram of the Blank extract. The first chromatogram is GC chromatogram while the second is mass spectroscopy chromatogram.