Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Chemistry

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Profiles of bisphenols and benzophenone-type UV filters concentrations in sediment from Trondheimsfjorden: Associations with PAHs and trace elements

Master's thesis in Environmental Chemistry
Supervisor: Alexandros Asimakopoulos
May 2019

Trondheimsfjorden, 16th of June 2018. Photo by the author.

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NTNU
Kunnskap for en bedre verden

Preface

This Master's thesis is written as part of the two year Master's degree study programme of Environmental Toxicology and Chemistry (MSENVITOX), within the specialization of Environmental Chemistry, at the Department of Chemistry in NTNU Gløshaugen. Target readers include scientists and academic personnel with key knowledge in the areas of environmental sciences and analytical chemistry. Other readers interested in these areas may also find this thesis worthy of reading. The work has been done solely in cooperation with different kinds of personnel at the Department of Chemistry. All materials and experiments have been funded by said department. Work with the thesis, including writing, collection of samples and material, practical work in laboratories and computer related work, was carried out during the period of Autumn 2017 to Spring 2019.

Trondheim, May 15, 2019

Olav Leiros Bakkerud

Acknowledgements

The first person I would like to give my very special thanks to is Alexandros Asimakopoulos for being my main supervisor in this Master's project. He was available at all times and was always willing to share his knowledge with me through the many conversations we had. This project would not have been a reality without him.

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The support of my family have also helped my motivation stay high during the time I have worked on my thesis.

Finally, a personal thanks goes to my friend Karoline Sofie Gjelstad who also did work on the sediment samples for her Master's project. Her contribution in joining the work for PAH determination was invaluable, and it was great to have a labpartner who also enjoyed listening to 80s music on the radio during long hours in the lab as much as I did.

- OLB.

Abstract

In this Master's project, 64 unique marine sediment samples from various locations in Trondheimsfjorden, Norway were acquired by sampling from a research vessel the 31st of May 2018. The aim of the project has been to determine occurrences and evaluate correlations between several organic and inorganic chemical contaminants in the sediment. The target chemicals of interest included nine bisphenol analogues, five benzophenone analogues, sixteen EPA priority polycyclic aromatic hydrocarbons, and nine trace elements. Total organic matter content was also evaluated.

Sediment samples were treated with various techniques and methods for extracting target chemicals, including liquid-solid extraction (LSE), solid phase extraction (SPE), accelerated solvent extraction (ASE) and microwave-assisted acid digestion. After treatment, the samples were analysed using liquid chromatography-tandem mass spectrometry (LC-MS/MS), high performance liquid chromatography with ultraviolet and fluorescence detection (HPLC-UV/FLD), and inductively coupled plasma mass spectrometry (ICP-MS).

The total concentration sum of bisphenols ($\sum$BPs), benzophenones ($\sum$BzPs) and polycyclic aromatic hydrocarbons ($\sum$PAHs) in sediment samples ranged from 0.67 - 12.1 ng/g dry weight (d.w.), 0.25 - 34.7 ng/g d.w. and 33.4 - 548 ng/g d.w., respectively. The concentration of elements ranged from 0.018 - 525 µg/g d.w. Percentage total organic matter content ranged from 1.97 - 4.80 %. BPA and BPF were the predominant bisphenol analogues and accounted for 52.5% and 41.9% of the total bisphenol concentration respectively. BzP-3 accounted for 57.6% of the total benzophenone concentration.

Correlations among sediment samples were evaluated by the use of principal component analysis (PCA). Results from PCA analysis indicated variations between samples from different sampling locations based on levels of trace elements and polycyclic aromatic hydrocarbons, as well as correlations between some target analytes including BPA and BzP-3. To the authors knowledge, this is the first time occurrences of bisphenols, benzophenones, polycyclic aromatic hydrocarbons and trace elements in marine sediment have been reported.

Sammendrag

I dette masterprosjektet har 64 marine sedimentprøver fra diverse lokaliseringer i Trondheimsfjorden blitt hentet med forskningsfartøy den 31. mai 2018. Formålet med prosjektet har vært å fastslå forekomster og evaluere korrelasjoner av samtlige organiske og uorganiske kjemiske stoffer i sedimentprøvene. Dette har inkludert ni bisfenolanaloger, fem benzofenonanaloger, seksten prioriterte polysyskliske aromatiske hydrokarboner og ni sporgrunnstoffer. Innhold av totalt organisk materiale i sedimentprøvene er også blitt evaluert.

Sedimentprøvene er blitt behandlet med en rekke teknikker og metoder for å ekstrahere stoffer fra prøvematrisene, inkludert flytende-fast ekstraksjon (LSE), fast-fase ekstrasjon (SPE), aksellerert løsemiddelekstrasjon (ASE) og mikrobølge-assistert syrefordøyning. Etter prøvebehandling er prøvene blitt analysert med væskekromatografi tandem massespektrometri (LC-MS/MS), høypresisjonsvæskekromatografi med ultrafiolett og fluoresens deteksjon (HPLC-UV/FLD) og induktivt koblet plasma massespektrometri (ICP-MS).

De totale konsentrasjonssummene av henholdsvis bisfenoler ($\sum$BPs), benzofenoner ($\sum$BzPs) og polysykliske aromatiske hydrokarboner ($\sum$-PAHs) i sedimentprøvene varierte fra 0.67 - 12.1 ng/g tørrvekt (d.w.), 0.25 - 34.7 ng/g d.w. og 33.4 - 548 ng/g d.w. Konsentrasjoner av grunnstoffer varierte fra 0.018 - 525 µg/g d.w. Prosentvis totalt organisk materiale varierte fra 1.97 - 4.80%. BPA og BPF var de mest dominerende bisfenolanalogene og stod for henholdsvis 52.5% og 41.9% av den totale konsentrasjonssummen av bisfenoler. BzP-3 stod for 57.6% av den totale benzofenonkonsentrasjonen.

Korrelasjoner mellom sedimentprøver ble evaluert med bruk av prinsipal komponentanalyse (PCA) basert på forekomster av kjemiske stoffer og prøveinnsamlingsdata. PCA-plottene indikerte at korrelasjoner mellom prøvetakingsposisjoner og nivåer av sporgrunnstoffer og polysyskliske aromatiske hydrokarboner var tilstede, samt korrelasjoner mellom visse kjemiske stoffer, deriblant BPA og BzP-3. Til forfatterens kjennskap er dette første gang forekomster av bisfenoler, benzofenoner, polysykliske aromatiske hydrokarboner og sporgrunnstoffer i marine sedimenter er rapportert.

Contents

Chapter 1

Introduction

1.1 Background and motivation

There are many types of organic and inorganic chemical compounds present in nature. Some of them have properties making them act as chemical pollutants that are of concern to the environmental health. This project is dedicated towards the use of different methods for the extraction and analysis of real environmental samples to determine the occurrences of such pollutants. Two specific emerging pollutant classes, namely bisphenols (BPs) and benzophenone-type UV filters or benzophenones (BzPs), are of special focus in this project. Other pollutants of interest include polycyclic aromatic hydrocarbons (PAHs) and trace elements. The concentrations of all of these pollutants are to be determined in sediment samples of marine origin in a Norwegian fjord by using different techniques. Motivation to do experiments for such a project stems from a desire to acquire new knowledge about current pollutant levels and to evaluate possible correlations between them. This will be done by employing advanced equipment for laboratory work with a chemometric approach to statistically analyse data from experiments. To the authors knowledge, this is the first time occurrences of bisphenols, benzophenones, polycyclic aromatic hydrocarbons and trace elements in sediment have been determined simultaneously.

1.2 Trondheimsfjorden

Trondheimsfjorden is the third longest fjord of Norway (approx. 130 km), which reaches from Agdenes kommune, southeast to Trondheim, and further northeast up to the regions of Steinkjer [1]. It is an important fjord for industry, tourism and other related activities, with ships being frequently used for transportation in and out of harbor areas. Figure 1.1 shows a map of the fjord with some commonly used ship routes for commuting and tourism.

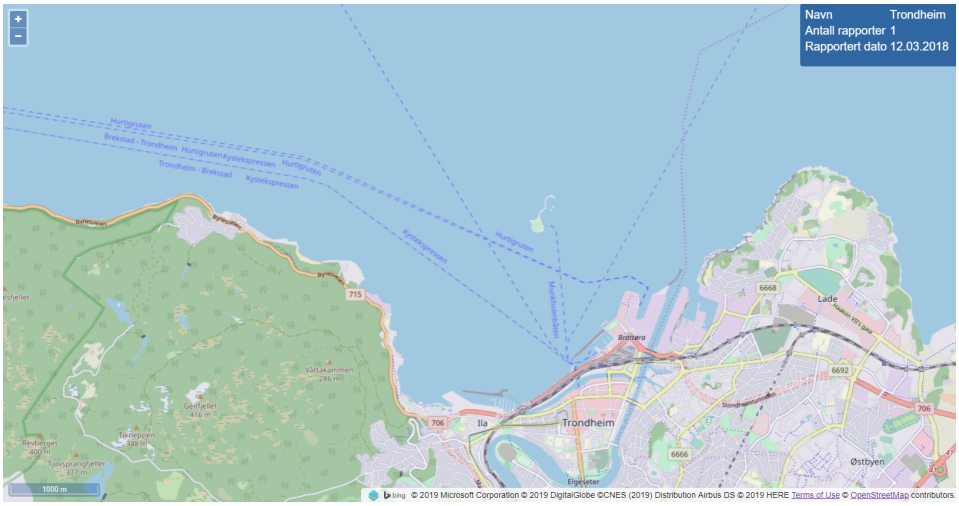

Figure 1.1: Map of Trondheimsfjorden. Common ship routes are marked with blue dotted lines.

1.2.1 Project Renere havn

In 2015, Trondheim municipality initiated a project named "Renere havn", with the goal of minimizing levels of contamination in the fjord by dredging large masses of seafloor material and covering the exposed deeper levels with clean sediment masses [2]. Trondheimsfjorden had historically been heavily contaminated with heavy metals and organic pollutants due to local inputs from mining and other industrial activities. There was a concern of the pollutants settled at the seafloor being able to diffuse from the sediments back into the water column, where they could potentially harm the marine biota in the fjord. The project was mainly concerned with the alleviation of organic and inorganic pollutants, of which included PAHs, polychlorinated biphenyls, tributyltin, and trace metal elements such as As, Hg, Pb, Cu and Cr among others [3]. Although laboratory investigations were conducted prior to the project to analyse the sediments for these analytes, no investigations on the levels of BPs or BzPs specifically were conducted to the authors knowledge. Based on continued tourism and other activities in and around the fjord, it can be estimated that new inputs likely will have increased concentrations of different pollutants of interest in different areas. This will depend on the sources of pollution, as well as the properties of the pollutants that are to be retained in sediments.

1.3 Objectives

The scientific objectives of this project are to:
(a) Determine concentrations and profiles of occurrence of selected bisphenols, benzophenones, polycyclic aromatic hydrocarbons and trace elements in marine sediment from the sub-Arctic region of Trondheim, Norway; and
(b) Establish possible correlations among the target chemicals.

1.4 Approach

1.4.1 Acquisition of environmental samples

The samples to be collected for this project consists of sediment material from the seafloor of various locations around Trondheimsfjorden in Trondheim, Norway. Sediment will be collected close to the Høvringen wastewater treatment plant in the inner-western part of the fjord close to the city, and also from a location further out in the fjord using a marine research vessel supplied by NTNU. Figure 1.2 shows a map of Trondheim with the sampling locations, hereby referred to as sampling stations, in Trondheimsfjorden.

1.4.2 Acquisition of chemicals for laboratory work

Chemicals, solvents and other reagents (see chapter 3) will be acquired through online purchases from various suppliers. The choice of chemicals and suppliers will depend on the availability, quantities and price ranges.

1.4.3 Sample storage, preparation and analysis

After sediment samples are gathered from different sampling locations, they will be transported to a storage location and be stored in such a way that they represent the original sample at the time of sampling as close as possible. Clean sampling equipment will be employed as samples are collected and transported, and the samples will be stored at appropriate temperatures away from sunlight exposure. This will ensure the lowest possible degradation of any chemical analytes present in the samples.

The analytical equipment that will be used for analysing samples often requires samples to be in a suitable form before injection or application. Therefore, portions of sediment samples to be analysed will first be treated so that they consist of suitable extracts that have analytes in a dissolved phase using one or more solvent(s). This will be achieved

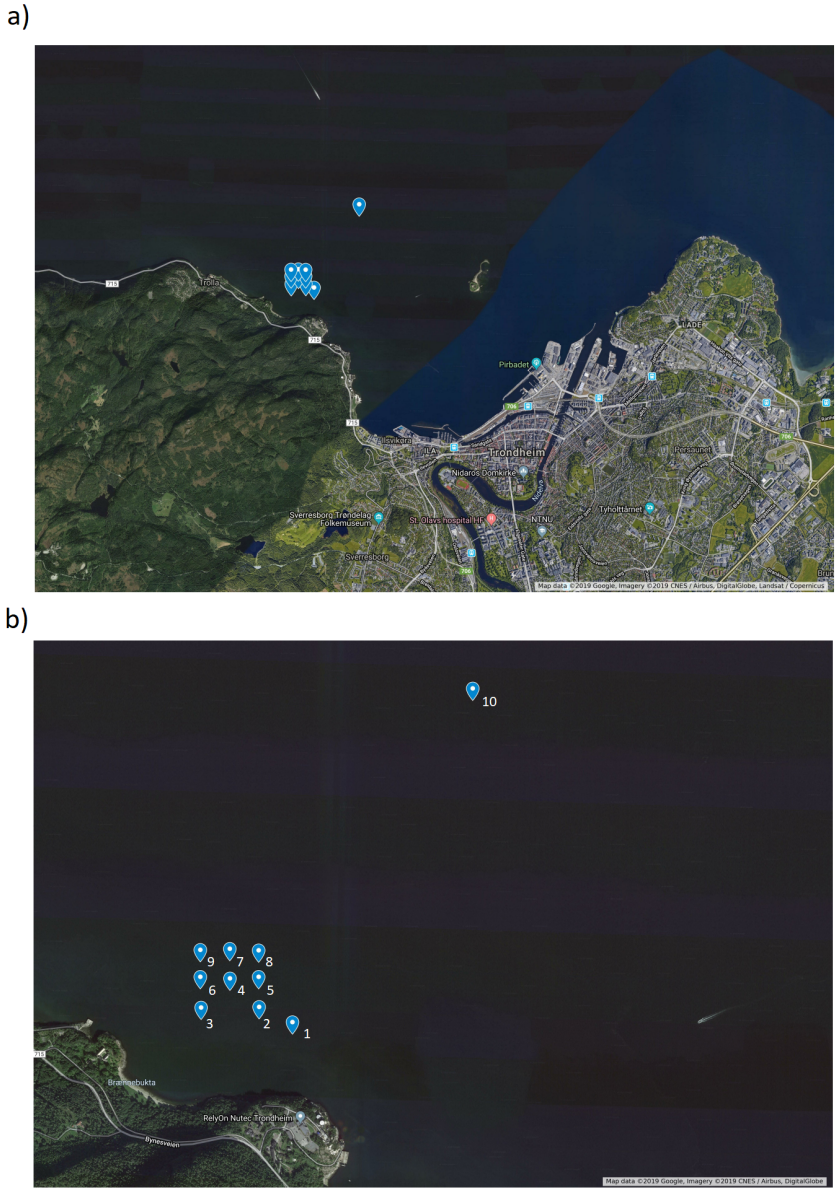

Figure 1.2: Map of Trondheimsfjorden. Image a) shows the inner-part of the fjord with Trondheim city to the south. The Munkholmen islet can be seen close to the middle of the image. Image b) shows the sampling station numbers with corresponding points of sampling near Høvringen.

by using a number of sample preparation methods and procedures in laboratories, where the choice of method will depend upon the chemical compound(s) of focus and the analytical equipment to be used (see chapter 4).

1.4.4 Data processing

When samples have been analysed and raw data can be visualized on a computer e.g. in the form of chromatograms, the processing of the data will be performed by employing various computer software. Different software exists from suppliers that can be directly linked through the computer to the analytical hardware. The processing will be carried out in order to acquire accurate data which can be further exported as numerical computer files. The data will undergo different mathematical calculations and also statistical analysis so that results can be obtained.

1.5 Outline

The remainder of this thesis is structured in a way similar to scientific reports written at NTNU in general, and also follows styles of scientific journal articles found elsewhere. Chapter 2 contains background theory regarding the different pollutants of interest, and also of the aspects relevant for determination of them. Chapter 3 lists the chemicals, material equipment and computer software used in this project. Chapter 4 describes the experimental protocols that were conducted in detail. Chapter 5 presents all results from the experiments. Chapter 6 involves a discussion of the presented results. The final chapter summarizes with conclusions of the results and the discussion of them, and also gives recommendations for further work.

Chapter 2

Theoretical background

2.1 Bisphenols and benzophenones

Bisphenols (BPs) are organic chemical compounds with molecular weights generally in the range of 200 - 350 g/mol. The most well-known analogue is bisphenol A, BPA, which has been used as a monomer for plastic and epoxy resin production with a high demand over the years [4]. Other BP analogues also exist and are used for similar applications such as plastic polymer manufacturing [5–7]. Bisphenols have different IUPAC- and trivial names according to their chemical structures. Common for all BPs is the presence of at least two phenol moieties, which are linked through different functional groups. Figure 2.1 shows the structure of BPA, or 2,2-bis(4-hydroxyphenyl)propane, containing two adjacent phenolic groups bonded through a -CH_2-hydrocarbon in the center of the molecule.

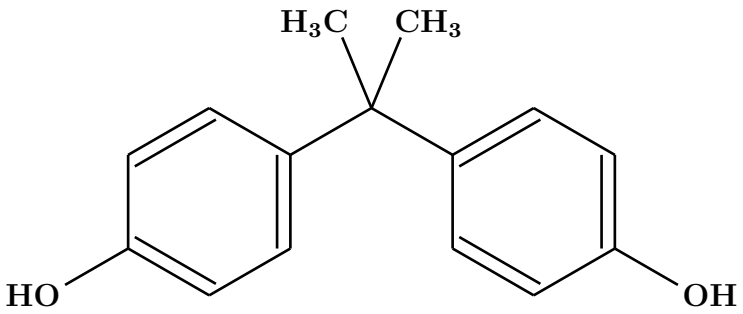

Figure 2.1: Bisphenol A, 2,2-bis(4-hydroxyphenyl)propane.

Table 2.1: Properties of bisphenol target analytes in this project.

Full name	Abbreviation	Molecular weight (g/mol)	Reported log Kow (n-octanol/water)	CAS-number
2,2-bis(4-hydroxyphenyl)propane	BPA	228.29	3.60[a]	80-05-7
4,4'-(hexafluoroisopropylidene)diphenol	BPAF	336.24	5.50[a]	1478-61-1
4,4′-(1-phenylethylidene)bisphenol	BPAP	290.36	4.86[b]	1571-75-1
2,2-bis(4-hydroxyphenyl)butane	BPB	242.31	4.20[a]	77-40-7
4,4′-dihydroxydiphenylmethane	BPF	200.23	2.90[a]	620-92-8
1,3-bis(2-(4-hydroxyphenyl)-2-propyl)benzene	BPM	346.46	n.a.	13595-25-0
4,4′-sulfonyldiphenol	BPS	250.27	1.70[c]	80-09-1
4,4′-cyclohexylidenebisphenol	BPZ	268.35	5.00[b]	843-55-0
4,4′-(1,4-phenylenediisopropylidene)bisphenol	BPP	346.46	n.a.	2167-51-3

[a] From [8].
[b] From [9].
[c] From [10].

Bisphenols are generally somewhat non-polar organic molecules, owing to the number of aliphatic and aromatic functions present. Partition coefficients of selected BPs of interest are listed in table 2.1. Most are in the range of 3-5, with one notable exception being the relatively polar BPS, which has a value below 2. Some degree of polarity of the BPs may be expected from the hydroxyl groups on either of the phenolic groups.

Benzophenones (BzPs) are organic compounds with molecular structures and masses similar to BPs. Due to their physicochemical properties making them capable of absorbing UV-radiation, they are employed as ingredients in sunscreen products for topical application, and also as additives in other personal care products for photostabilization

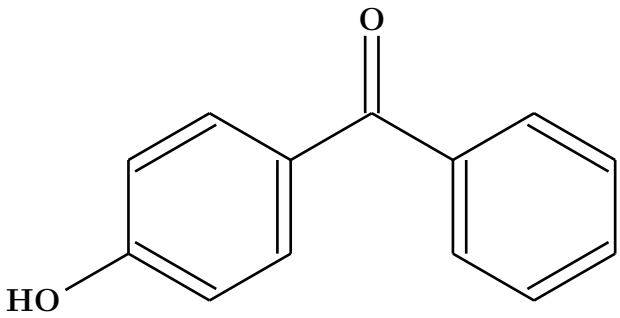

Figure 2.2: 4-OH-BzP, 4-Hydroxybenzophenone.

[11]. BzPs consists of two aromatic rings linked through a ketone functional group in the center. The aromatic rings may have substituents attached in different positions (*-ortho, -meta, -para*) with respect to the ketone group. These substituents usually consists of OH-groups; ether functions are also possible. Figure 2.2 shows the structure of a simple BzP, namely 4-OH-BP, or 4-hydroxybenzophenone.

Table 2.2: Properties of benzophenone target analytes in the present study.

Full name	Abbreviation	Molecular weight (g/mol)	Reported log Kow (n-octanol/water)	CAS-number
2,4-dihydroxybenzophenone	BzP-1	214.22	3.17[a]	131-56-6
2,2',4,4'-tetrahydroxybenzophenone	BzP-2	246.22	3.16[b]	131-55-5
2-hydroxy-4-methoxybenzophenone	BzP-3	228.24	3.79[a]	131-57-7
4-Hydroxybenzophenone	4-OH-BzP	198.22	3.07[c]	1137-42-4
2,2'-dihydroxy-4-methoxybenzophenone	BzP-8	244.24	3.93[a]	131-53-3

[a] Values from [11].
[b] Values from [12].
[c] Values from [13].

As is the case for BPs, BzPs are somewhat non-polar in nature. The partition coefficient of BzPs of interest in this project lies in the range of 3-4, as shown in table 2.2.

2.1.1 Toxicity of bisphenols and benzophenones

Due to their widespread use and occurrence in the environment, there has been a concern of the potential toxic effects of BPs and BzPs on different organisms including humans. Both BPs and BzPs have been shown to exhibit toxic effects on organisms by numerous studies [7, 11]. Particularly, their potential endocrine disrupting capabilities have been of concern. It has been shown that BPA can have damaging effects on male and female reproductive systems in humans [14]. Despite being used as replacements for BPA in recent years, other BP derivatives have also been shown by several studies to induce similar adverse biochemical changes in organisms [4, 5, 7, 9]. In the case of BzPs, these compounds have been tested in laboratory studies and found to exhibit estrogenic-like effects [13]. Such effects have been shown for fish exposed to BzP-1, BzP-2, BzP-3 and BzP-4, and BzP-8 have been considered a genotoxic compound [11].

The pathways of exposure to organisms by BPs and BzPs can vary, depending on the organism of interest and the environment in which the organism inhabits. Human exposure can be expected to come from contact with products that are based on plastic, given that both BPs and BzPs are used as plasticizers.

2.1.2 Sources of marine input

BPs and BzPs may be released to marine environments through wastewater, and the increasing issue with marine plastic littering may also play a role in the global input of these chemicals into the oceans [11, 15]. Once in the marine environment, if not absorbed by biota in the water column or degraded through different pathways, BPs and BzPs have the potential to accumulate in sea floor sediments. Thus far, there have been several studies devoted to the analysis of sediments for different BPs and BzPs, where compounds have been quantified at various concentrations at different sediment depths worldwide. It has been of interest to assess and monitor the concentrations in these environmental matrices over time, given that sediments can potentially serve as sinks for BPs and BzPs [5, 16, 17]. It can also be estimated

that some BPs and BzPs may diffuse through the sediments back into the water column, where they can be further absorbed by biota and induce biochemical changes, as described earlier. This will depend on the specific compound in question with regards to its various chemical properties.

2.1.3 Bisphenols and benzophenones in Norway and the Norwegian environment

Although BPA is not produced in Norway, it is still used as raw material for plastics production and in chemical products such as paint and glues [18]. The data for the usage of other BP analogues however, as well as the emissions of bisphenols in Norway, is limited. To the authors knowledge, no specific data on the usage or emissions of BzPs in Norway has been compiled.

Studies on BP and BzP occurences in the norwegian environment are scarce. In 2013, the Norwegian Environment Agency (Miljødirektoratet) performed a screening programme for the determination of selected BPs and other contaminants in various parts of Oslofjorden as well as Lake Mjøsa [19]. Wastewater effluents and leachates, sediments, and biota were examined for the occurence of these chemicals, as well as other emerging contaminants of concern. It was found that the two bisphenols BPF and BPA could be detected in sediment samples from Oslofjord. Bisphenols were only found in two out of five samples however, one contained BPF at a concentration of 47 ng/g d.w., while the other contained BPA at a concentration of 44 ng/g d.w. All other samples had BP concentrations below LOD values. In two out of five samples from Lake Mjøsa, all BPs examined in the study could be detected, with concentrations ranging from 0.06 - 47 ng/g d.w.

BzP-3 was one of the organic UV-filters which were also analysed in various samples in the screening programme. It was found in marine biota samples and treated wastewater, but was not detected in any of the sediment samples.

2.2 Solid-phase extraction

Solid-phase extraction (SPE) is a concentration and clean-up sample preparation technique used for retaining analytes from complex samples while removing contaminants. During an SPE procedure, analytes of interest are retained in a cartridge containing a sorbent packing material with chemical properties favorable for interactions with the analytes. Figure 2.3 shows a typical SPE procedure with the associated steps involved. Cartridges are first conditioned by passage of a solvent or mix of solvents through the cartridge. Samples in the form of extracts (from one or more previously performed sample preparation procedures) are therafter loaded onto the cartridges and the sample solvent is allowed to pass through the packing material. During this step, analytes will be retained by the cartridge packing material, while the sample solvent with unwanted contaminants passes through. After loading is finished, the cartridge is washed with solvent(s) that are chosen for eluting contaminants that may also have been retained by the packing material while minimizing target analyte elution. The procedure is finished by a drying step of the cartridge using a vacuum pump, followed by the elution of target analytes with a solvent that have a sufficiently high affinity for the target analytes. The solvents used during the conditioning, loading, washing and elution steps must be chosen so that analyte loss will be minimized at any stage .

SPE has been used previously to extract BP analogues simultaneously from dust-based samples [6]. SPE has also been used for extracting several BzP derivatives from different complex matrices, including dust samples, as well as raw water and wastewater samples [11, 21]. Wang et al. found that the octadecyl carbon chain (C18) based column type has shown to be the most suitable for simultaneous extraction of several bisphenol derivatives from lake surface sediment samples [22]. Based on the previous use of SPE for both BP and BzP analogue extractions, the experiments in this project will also be performed using SPE as a method for simultaneous extraction of BPs and BzPs.

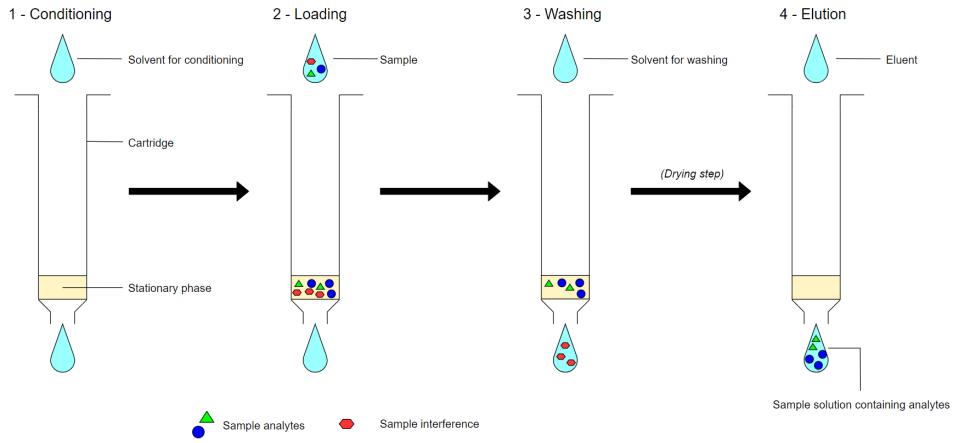

Figure 2.3: Typical solid-phase extraction procedure with different steps involved. Custom-made illustration based on [20].

2.3 Liquid Chromatography

Liquid Chromatography (LC) has proven to be a highly effective alternative for separating various analytes in environmental samples before detection using dedicated instruments [23]. Using a packed reversed-phase (RP) column with a packing material that has farily non-polar functional groups such as C18 chains, target analytes of the different organic pollutant classes including BPs, BzPs and PAHs can be separated based on their affinity to the packing material when run through the column. The retention of each target analyte will vary depending on various chemical properties, mainly the polarities of the compounds (see tables 2.1, 2.2 and 2.3). Solvents used as MPs can be chosen for optimal separation of compounds with acceptable run times and consumption of solvents as well.

2.3.1 Standards

An important part both in the qualitative and quantitative interpretation of chromatograms is the use of standards. Standards are pure compounds of the target analytes usually in solid form. By using standards, standard solutions can be prepared with known concentrations

of one or more chemical compound(s) of interest in a suitable solvent. When analysed under a given set of conditions in a chromatographic scheme, the retention time (RT) of the peak for the chemical compound in the standard can be compared to that of a peak in a more complex sample, such as an environmental sample. If the retention times of the peaks from the standard solution and the sample solution match (when both solutions are analysed under the same conditions), then the peak in the sample can be concluded to be from the same chemical compound as that from the standard solution. Standards are also used to calibrate the detector so that unknown concentrations of analytes in sample solutions can be quantified.

2.3.2 Internal standards

One way of extracting quantitative information from chromatograms is to analyse several solutions made from standards of target analytes at different concentrations and make a calibration curve through linear regression. This is referred to as external standard calibration, where the detector signal of the standards is plotted as a function of their known concentrations. However, to compensate for analytical errors such as amount of sample injected in the analytical instruments, a specific type of standard spiked in all samples and standard solutions at a constant concentration can be employed as well [24]. Such a standard is called an internal standard (IS). The IS has similar chemical properties to the analyte(s) of interest. Examples of internal standard types include deuterated or ^{13}C-containing isotopes of the compound that the internal standard is an analogue of (see figures B.3 for examples). There are a number of requirements that must be met for an internal standard to be used for quantification. The IS must have similar retention times to the target analyte(s), behave in a similar manner during samples preparation and extraction, not be present in the sample beforehand and also be sufficiently stable and pure, among others [25]. The use of isotopically labelled internal standards is especially advantageous when performing mass spectrometric analysis, given that these compounds in addition can be separated from their normal target analyte analogues by mass differences.

2.4 Mass spectrometry and tandem mass spectrometry

Mass spectrometry (MS) have been used to large extents in conjunction with LC in recent years, given its reputation as a sensitive and specific type of detection for many kinds of analytes. In a mass spectrometer, dissolved analytes in solution are ionized by an ion source to create positive or negative ions, depending on the selection of positive or negative modes for the MS-apparatus [26]. By selecting negative mode through computer software, so-called precursor ions of the form $[M\text{-}H]^-$ are created, where M is the original molecule or analyte of interest and -H refers to the loss of a proton particle with positive charge. Due to the loss of the proton, the precursor ion will have a mass close to the original mass of analyte minus 1.

Tandem mass spectrometry (MS/MS) refers to mass spectrometry methods where atleast two stages of mass analysis occurs [27]. The number of steps can be labelled as MS^n, where n refers to the number of steps where some ion is being analysed. There are several different so-called scan modes available when using MS/MS that can be controlled with related computer software. One such scan mode is the selected reaction monitoring (SRM). With this scan mode, two analysers coupled in series, such as the Triple Quadrupole analyser (see further below), are set to focus on selected masses of precursor ions and product ions during a fragmentation step. Only ions that have these specific masses will ultimately be detected by a detector. Due to the way SRM operates, an increase in both selectivity and sensitivity for analytes is expected [26]. In cases where two or more product ion masses are selected for further detection, the mode is referred to as multiple reaction monitoring (MRM) [28].

2.4.1 Ionization

The ion source component of an MS-instrument is a device responsible for creating ions from analytes of interest, namely precursor ions. With LC, the compatible ion source types include Electrospray Ion-

ization (ESI), Atmospheric-Pressure Chemical Ionization (APCI) and Atmospheric-Pressure Photoionization (APPI). For this project, ESI will be used as the ionization method, given its availability and its ease of use. ESI work with an incoming liquid eluent containing analytes from a chromatographic system being forced through a capillary into an electric field. Fine droplets are created in the field, where analytes and other components from the sample acquires a charge depending on the voltage set between the capillary and a counter-electrode [29].

A so-called modifier (commonly a salt) can be added in small amounts to the mobile phase used during chromatographic separation to enhance various parameters during the MS ionization and fragmentation steps. One such modifier, ammonium hydroxide, has shown to improve sensitivities when analysing BPA and several analogues, including BPS, BPF, BPE, BPB, BPAF, BPAP and BPZ in the negative mode [30]. For this project, the same salt will be applied as modifier during LC-MS/MS analysis procedures.

2.4.2 Triple quadrupole analysers

For the tandem MS procedure used as a part of bisphenol and benzophenone determination in this project (see Chapter 4), a triple quadrupole (TQ) analyser is a well suited analyser to carry out such operations. A normal quadrupole analyser is a device made up of four parallell steel rods, which separates ions that will have different trajectories in an electric field based on the m/z ratios of the ions [26]. A triple quadrupole analyser is composed of three sets of rods, making it capable of making precursor ions, as well as smaller fragmented ions from the precursor ions through fragmentation in the second set of rods. Figure 2.4 shows a schematic drawing of a triple quadrupole analyser. The fragmented ions are referred to as product ions. Ultimately, the TQ analyser will allow for separation of ions created in the MS-ion source component before detection. The detector in an MS apparatus can be tuned to only detect certain ions of a specific mass per charge (m/z). Depending on what specie is associated with the analyte of interest (e.g. hydrogen in $[M\text{-}H]^-$), the m/z may deviate significantly from the original mass of the analyte. With both precursor and prod-

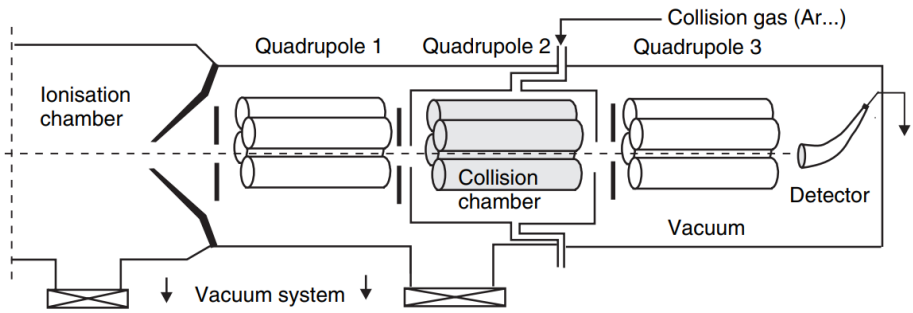

Figure 2.4: Schematic drawing of a triple quadrupole analyser. Fragmentation of precursor ions to form product ions occur in the collision chamber. Reproduced with permission from [31]. ©John Wiley & Sons.

uct ions available, the detector can be chosen to be highly specific with detection of only certain ions at a known retention time. This yields highly sensitive data for specific analytes, and allows for the choice of ions as both qualifiers and quantifiers.

2.5 Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) belongs to a class of organic compounds that are ubiquitous and present at various concentrations throughout nature [32]. PAHs consists of atleast two or more aromatic rings fused together, allowing for several different derivatives to exist. Figure E.1 in Appendix E shows the structures of PAH target analytes of interest in this project. Given their predominant non-polar aromatic structures, PAHs are highly hydrophobic and have low solubility in water. As such, they can be dissolved using different organic solvents, and may be adsorbed to particulates that can undergo sedimentation. Table 2.3 lists names and properties of PAH target analytes included this project.

Table 2.3: Names and chemical properties of polycyclic aromatic hydrocarbon target analytes included in this project.

Full name	Abbreviation	Molecular weight (g/mol)	Reported log Kow (n-octanol/water)*	CAS-number
Naphtalene	NAP	128.17	3.34	91-20-3
Acenaphtylene	ACY	152.20	3.62	208-96-8
Acenaphtene	ACE	154.21	4.00	83-32-9
Fluorene	FLU	166.22	4.22	86-73-7
Phenanthrene	PHE	178.23	4.57	85-01-8
Anthracene	ANT	178.23	4.68	120-12-7
Fluoranthene	FLT	202.26	5.20	206-44-0
Pyrene	PYR	202.26	4.98	129-00-0
Benzo[a]anthracene	BaA	228.29	5.91	56-55-3
Chrysene	CHR	228.29	5.81	218-01-9
Benzo[b]fluoranthene	BbF	252.32	6.12	205-99-2
Benzo[k]fluoranthene	BkF	252.32	6.11	207-08-9
Benzo[a]pyrene	BaP	252.32	6.13	50-32-8
Dibenzo[a.c]anthracene	DBA	278.35	6.50	215-58-7
Benzo[ghi]perylene	BGP	276.34	6.22	191-24-2
Indeno[1.2.3-cd]pyrene	IND	276.34	6.58	193-39-5

*Values from [33]

Toxicities of PAHs have been of concern, as several are known to be human carcinogens, including benzo[a]anthracene (BaA), chrysene (CHR), benzo[b]fluoranthene (BbF), benzo[a]pyrene (BaP) and benzo-[ghi]perylene (BGP) [34]. Some important sources of PAHs into the marine environment include natural processes such as forest fires and volcanic eruptions. Anthropogenic sources such as wastewater runoff and oil spills among others also contribute to the input of PAHs into the environment [35, 36].

2.5.1 Accelerated solvent extraction

Accelerated solvent extraction (ASE) is a type of sample preparation technique that can be employed for solid and semi-solid samples. The technique is suitable for extracting semi-volatile organic compounds such as PAHs by using appropriate solvents for extraction. A specialized instrument capable of delivering solvents from a reservoir to an

oven compartment with elevated temperatures and high pressures is used to flush cells containing samples with soluble analytes. Solvent composition, temperature, pressure and extraction times can be tailored for optimal extraction of specific compounds in different sample matrices. The cells are assembled as stainless steel casings with samples (freeze-dried or otherwise) filled on top of cellulose filters placed at the bottom of the casings. Diatomaceous earth is mixed with the sample to fill the cells. To increase extraction output while retaining impurities, the cells can additionally be filled with aluminium and copper powder in between the samples and cellulose filters. Aluminium acts to remove lipids, while copper will retain sulfur-containing impurities [37].

ASE has previously been shown to be an effective method for extracting PAHs from soil samples [38, 39]. Methods for extraction have previously been developed at NTNU for different projects. For this project, ASE will be the method of choice for extracting PAHs in marine sediments collected from Trondheimsfjorden, which will further be analysed by using liquid chromatography coupled with UV-detection.

2.5.2 Detection of polycyclic aromatic hydrocarbons using UV absorbtion and fluorescence detectors

Among different spectrophotometric detectors that can be used in conjunction with LC, the UV-visible absorption detector such as the diode array detector (DAD) and the fluorescence detector (FLD) are considered among the most important [40]. UV absorbtion detectors are applicable for most organic compounds that to some extent absorb UV light. Fluorescence detectors are more sensitive, but also selective in that they will work only for organic compounds that are fluorescent. In the case of PAHs, fluorescence detectors are well suited given that several PAH compounds have the ability to fluoresce with high detection sensitivity [41]. This is also the case for fluorinated polycyclic aromatic hydrocarbon (F-PAH) internal standards included in this project, which have similar detectability as their non-fluorinated analogues using FLD [42]. ACY is the exception among PAH com-

pounds in that it does not fluoresce. For this reason, detection of PAHs in the sediment samples collected for this project will also be conducted by employing a DAD detector for UV absorbtion.

2.5.3 Classification of polycyclic aromatic hydrocarbon content in sediments

A classification system where sediment status can be characterized based on concentrations of different PAHs have been proposed by the Norwegian Pollution Control Authority earlier [43]. Table 2.4 shows the classification of sediment associated with concentration levels of individual PAHs as well as the sum of these PAHs. The system can be used to assess the current status of sediment samples by comparing the quantified levels of PAHs found in new sediment samples with values from the table.

Table 2.4: Classification of sediment status by concentration of PAHs in sediments.

PAH	Class I Background (µg/kg)	Class II Good (µg/kg)	Class III Moderate (µg/kg)	Class IV Bad (µg/kg)	Class V Very bad (µg/kg)
NAP	<2	2-290	290-1000	1000-2000	>2000
ACY	<1.6	1.6-33	33-85	85-850	>850
ACE	<4.8	4.8-160	160-360	360-3600	>3600
FLU	<6.8	6.8-500	500-1200	1200-2300	>2300
PHE	<6.8	6.8-500	500-1200	1200-2300	>2300
ANT	<1.2	1.2-31	31-100	100-1000	>1000
FLT	<8	8-170	170-1300	1300-2600	>2600
PYR	<5.2	5.2-280	280-2800	2800-5600	>5600
BaA	<3.6	3.6-60	60-90	90-900	>900
CHR	<4.4	4.4-280	280-280	280-560	>560
BbF	<46	46-240	240-490	490-4900	>4900
BkF		<210	210-480	480-4800	>4800
BaP	<6	6-420	420-830	830-4200	>4200
DBA	<12	12-590	590-1200	1200-12000	>12000
BGP	<18	18-21	21-31	31-310	>310
IND	<20	20-47	47-70	70-700	>700
$\sum$PAH-16	<300	300-2000	2000-6000	6000-20000	>20000

2.6 Trace elements in sediments

Inorganic analysis of environmental samples usually involve the measurement of elemental composition by appropriate analytical techniques. Trace elements and metals have the capability to accumulate in sediments due to the affinity of elements to the sediments and also due to sediment adsorption capacities [44]. The species in which elements are present will however play a role in their distribution profiles as well as potential toxicity for organisms. Input sources of trace elements into aquatic systems and marine sediment include natural processes such as wind dust deposition, volcanic eruptions and forest fires, as well as anthropogenic activities such as river discharge and dumping [45].

There are several elements that have been considered especially problematic when present in aquatic systems due to their toxicity for organisms in different types of marine ecosystems [46]. Some of these include copper, zinc, cadmium, mercury, lead, chromium, selenium and arsenic. As several of these elements were also of focus during sediment characterisation through the "Renere havn" project, they are given more room in this project for detailed study.

2.6.1 Determination of elements using inductively coupled plasma mass spectrometry

Analysis of elements in different sample matrices can be performed by employing several different analytical techniques. One such powerful technique is inductively coupled plasma mass spectrometry (ICP-MS). ICP-MS has many advantages, including high sensitivities and good precision. The technique has many applications, including analysis of environmental samples such as sediments for trace elements [47, 48]. Any sample that are to be analysed using ICP-MS will have to undergo some form of sample preparation to obtain extracts that can be injected into the ICP-MS apparatus. One such method is based on utilizing microwave assisted digestion with nitric acid [49]. This method ensures that elements are extracted from the solid sediment matrix to liquid form while retaining unwanted sample components.

2.7 Loss-on-ignition for total organic matter determination

Total organic carbon constitutes an important parameter in the characterisation of sediment material [50], as the carbon content available in sediment can influence the behaviour of chemicals present in the matrix. Several methods have been proposed for the determination of organic carbon in soil and sediment. The organic carbon can roughly be estimated as the total organic matter content found by using a method known as loss-on-ignition (LOI) [51]. During a loss-on-ignition procedure, heated destruction of all organic matter in a sample is performed using a furnace in conjunction with gravimetric determination of sample weight loss after combustion. Prior to combustion, crucibles containing samples are dried to remove water and moisture present in the sample. Percentage organic matter content can then be calculated based on the formula:

$$\%Organic\ matter = \frac{Mass_{CD}\ (g] - Mass_{CI}\ (g)}{Mass_{CD}\ (g) - Mass_{CE}\ (g)} \times 100 \qquad (2.1)$$

where CD = crucible with sample after oven drying, CI = crucible with sample after ignition, and CE = empty crucible.

2.8 Quality assurance and quality control

To ensure that the experimental data from determination of analytes are of an acceptable standard, several measures must be made to make sure the results are accurate, precise and reliable. These measures constitute what is referred to as quality assurance and quality control (QA/QC) [52, 53].

The steps used for quality control of the different analytical procedures in this project are described more detailed in chapter 4. For constructing calibration curves with high linearity and precision for quantification, internal standards are employed in conjunction with external

standards. The use of internal standards will account for variations in an analysis with respect to e.g. differences in injected volumes to the analytical equipment and can also be used to control for losses of analyte during the procedures. Control of potential sample carryover and cross contamination during chemical analysis is achieved by analysing solvent blanks (only pure solvent) in between samples at regular intervals. Method blank samples, or reagent blank samples (blanks that are prepared in the same way as samples through the whole sample preparation procedure) are also to be employed for analysis. Any signals that are measured during analysis of reagent blanks can be considered sources of contamination from the equipment and/ or sample treatment steps. This contamination will be estimated to have occurred for an entire batch where samples and one or more reagent blank(s) sample(s) have been prepared in conjunction. Any signal measured for a normal sample can therefore be subtracted with the signal measured from a reagent blank sample to account for contamination. To check for potential drift in instrumental sensitivity, calibration standard solutions are run at regular intervals during the whole chemical analysis to compare signals measured with one another. They should ideally remain constant during the anaysis.

In cases where it is available, a standard reference material may be employed for analysis as well. The reference material is a sample from a third party supplier with known concentrations of analytes in a matrix similar to real samples acquired for study. By analysing and quantifying analytes from the reference material, the accuracy of a method can be controlled with regards to deviations from certified values.

2.9 Data processing and quantification

Following an analytical scheme, post processing of data will be carried out in order to quantify analytes or other parameters related to the sediment sample.

After analysis have been conducted for some analyte(s) using instrumental equipment for compound detection, the resulting data will be in the form of numerical values related to the properties of peaks in vari-

ous chromatograms. For this project, the relationship between detector signal and measured peak areas will be used for analyte quantification. Peak areas are integrated by using appropriate settings with computer software that is connected to the detector. Depending on how well peaks are integrated, manual corrections may have to be done afterwards to correct for inconsistent integration.

By using internal standards spiked in samples for analytical error corrections, the calibration curves are constructed from calibration standard solutions by plotting the area of target analyte divided by the area of the internal standard as a function of the concentration of target analyte divided by the concentration of internal standard in the solutions [25]. It is then the ratio of target analyte to internal standard which will be used for concentration calculations, and this ratio will reflect the losses of target analyte occurring during sample preparation and analysis. In cases where the concentration of internal standard in spiked sample solutions and standard solutions are prepared at the same level, only the concentration of target analyte is needed for the x-axis.

In the case of BPs and BzPs to be determined using LC-MS/MS, isotopically labelled internal standards will not be available as direct analogues for each target analyte. Therefore, quantification will be performed using internal standard calibration as well as what is known as matrix-match calibration. Matrix-match calibration is performed by using one or more solutions containing the sample matrix which has been spiked with target analytes and internal standards prior to the extraction procedure [54]. Here, such solutions correspond to what is referred to as spiked samples. These solutions are used to construct the calibration curve, and will compensate for the different matrix effects experienced by the target analyte(s) and the internal standard.

2.9.1 Recoveries and matrix effects

During any steps of an analytical scheme, some loss of analyte is to be expected and may significantly affect the results acquired from raw data. Loss of analyte may occur during steps related to sampling,

sample transport, storage, sample preparation and from introduction of sample into dedicated instruments for analysis.

The loss of analyte during sample preparation steps before analysis, such as extraction, solvent change, clean-up and concentration can be quantitatively estimated by fortifying, or spiking special types of samples with a given amount of analyte(s) at appropriate concentration levels. Samples that are spiked with analyte before any extraction steps takes place are hereby referred to as spiked samples, while samples that are spiked after all extractions steps have been conducted before analysis will be referred to as matrix match samples. Two types of analyte recoveries, namely absolute recovery and relative recovery can be calculated for a given analyte based on the following formulas [55]:

$$\%Absolute\ recovery = \frac{Area_{A,\ SP} - Area_{A,\ S\ or\ B}}{Area_{A,\ MM} - Area_{A,\ S\ or\ B}} \times 100 \qquad (2.2)$$

$$\%Relative\ recovery = \frac{\left(\frac{Area_{A,\ SP}}{Area_{IS,\ SP}} - \frac{Area_{A,\ S\ or\ B}}{Area_{IS,\ S\ or\ B}}\right)}{\left(\frac{Area_{A,\ MM}}{Area_{IS,\ MM}} - \frac{Area_{A,\ S\ or\ B}}{Area_{IS,\ S\ or\ B}}\right)} \times 100 \qquad (2.3)$$

where A = analyte, S = sample, SP = spiked sample, B = blank sample and MM = matrix match sample.

The use of areas of samples or blanks will compensate for analyte that may already be in the sample used to make spiked samples and/ or matrix match samples. The choice of either will depend upon which yields a (higher) signal.

Analyte signal suppression or enhancement may occur in the presence of interferences such as proteins and lipids from sample matrices. Especially when employing ESI as an ion source during MS-detection, one can expect matrix effects to occur in complex samples [56]. This results from a change in the charge distribution between analytes and the matrix interferences in the droplets, and will be especially important when analyzing sediment sample material for the presence of

bisphenols and benzophenones using LC-MS/MS. Calculations of matrix effects has been described elsewhere and can be calculated using the following formula [55]:

$$\%ME = MF - 1 \times 100 \tag{2.4}$$

where %ME is percentage matrix effect and MF is known as the matrix factor. For the calculation of MFs, matrix match samples are employed in conjunction with standard solutions containing target analyte(s) at the same level as the matrix match samples. MFs are calculated based on the formula:

$$MF = \frac{Area_{A,\ MM} - Area_{A,\ S}}{Area_{A,\ STD\ SOL} - Area_{A,\ STD\ SOL(0ppb)}} \tag{2.5}$$

where $STD\ SOL$ = standard solution at a corresponding concentration level to the matrix match sample.

2.9.2 Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) describes the lowest concentration limit of an analyte that can be said to be distinguishable from baseline noise, and the lowest concentration limit of analyte that can be reliably quantified, respectively. A number of methods and formulas have been proposed for the calculations of LOD and LOQ, depending on the purpose [57].

For the purpose of this study, the lower limit of quantitation (LLOQ) will be estimated as the lowest concentration level that can be reliably quantified based on the lowest acceptable value from a calibration standard solution [5, 58]. The relationship between the LLOQ and the LOD can then be expressed as:

$$LOD = \frac{LLOQ}{3} \tag{2.6}$$

where LLOQ is the concentration of an analyte in the standard solution with units ng/mL. From the LLOQ and LOD, lower limits of quantitation and limits of detection in theoretical samples on a weight/weight basis can be estimated through similar calculations as used for quantifying analytes in real samples. This is achieved by using a theoretical sample amount representative for the amount of all samples used during a sample preparation procedure. Using the known volume of the standard solution prepared as well, equation 2.6 can be rewritten as:

$$LOD\ (ng/g) = \frac{LLOQ\ (ng/mL) \times \frac{Volume_{STD\ SOL}\ (mL)}{Theoretical\ sample\ amount\ (g)}}{3} \quad (2.7)$$

2.9.3 Relative retention time

The retention time (RT) of an analyte will depend on a number of factors related to the chromatographic system, and may not always remain the same between sequence runs. Factors that may affect the retention time includes variations in mobile phase flow rates, column temperatures and chemical changes in the column packing material over time. For more accurate determination of different peaks in a chromatogram where peak identification only relies on the RT, the relative retention time (RRT) can be evaluated by comparing the RT of an analyte to the RT of another compound [59]. In this project, the RT of the analyte(s) will be evaluated against the RT of a selected IS. Calculation of the RRT is given in equation 2.8:

$$RRT = \frac{RT_A}{RT_{IS}} \quad (2.8)$$

The relative retention time of an analyte to the IS should remain more or less constant, as the IS will experience similar retention fluctuations as the analyte (given that the IS has similar chemical properties to the analyte(s)).

2.10 Principal Component Analysis

Principal component analysis (PCA) is a widely used multivariate statistical method for comparing data. In simple form, PCA can be described as a mathematical technique for finding patterns and relationships in a dataset [60]. This is done by creating plots from multidimensional data which is scaled down to only contain a few dimensions that keeps the values in the original dataset intact.

By using PCA, X,Y-plots can be created where clusters of data points, or observations, are visualized together. In this case, observations correspond to individual sediment samples. Principal components (PCs) are the axes on the plot which will describe most of the variation in the data. The first axis is abbreviated PC1, or Dim1, and the second is abbreviated PC2, or Dim2. Data point values that are plotted on the graphs are calculated using mathematical formulas which take into account the weights of variables on the variations between PCs. In this case, variables correspond to target analyte concentrations, sediment sampling locations, and similar. Data points that are spread along the first axis have more variation from each other than data points spread along the second axis. If data points are clustered together, this implies that similarities between the samples may exists as given by the sample variables. The variables will to different degrees influence variation among samples, and therefore the variation as indicated by the principal components. The variables relative impact on the PCs can be visualised as so-called loadings.

A PCA biplot consists of a plot of sample data points, as well as loadings which are positioned according to the variables impact on the variations in the principal components. By examining such plots visually, they can give additional information about possible relationships between the observations and the variables [61].

Chapter 3

Materials and equipment

3.1 Chemicals

Analytical standards for bisphenol and benzophenone target analytes of interest were obtained from the supplier Sigma-Aldrich (St. Louis MO, USA). Of BPs, these included nine compounds:

- 2,2-bis(4-hydroxyphenyl) propane [Bisphenol A, BPA], $\geq$ 99%, 50g
- 4,4′-(hexafluoroisopropylidene)diphenol [Bisphenol AF, BPAF], $\geq$ 99.0%, 100mg
- 4,4′-(1-phenylethylidene)bisphenol [Bisphenol AP, BPAP], $\geq$ 99.0%, 100mg
- 4,4′-sulfonyldiphenol [Bisphenol S, BPS], $\geq$ 98%, 100mg
- 4,4′-dihydroxydiphenylmethane [Bisphenol F, BPF], $\geq$ 98.0%, 100mg
- 4,4′-(1,4-phenylenediisopropylidene)bisphenol [Bisphenol P, BPP], $\geq$ 99.0%, 100mg
- 4,4′-cyclohexylidenebisphenol [Bisphenol Z, BPZ], $\geq$ 99.0%, 100mg

- 2,2-bis(4-hydroxyphenyl)butane [Bisphenol B, BPB], $\geq$ 98.0%, 100mg
- 1,3-bis(2-(4-hydroxyphenyl)-2-propyl)benzene [Bisphenol M, BPM], $\geq$ 99.0%, 100mg

Of BzPs, these include six compounds:

- 2-hydroxy-4-methoxybenzophenone [Benzophenone 3, BzP-3], $\geq$ 98%, 50mg
- 2,4-dihydroxybenzophenone [Benzophenone-1, BzP-1], 99%, 100g
- 2,2'-dihydroxy-4-methoxybenzophenone [Benzophenone-8, BzP-8], $\geq$ 98%, 100mg
- 2,2',4,4'- tetrahydroxybenzophenone [Benzophenone-2, BzP-2], 97%, 25g
- 4-hydroxybenzophenone [4-OH-BzP], 98%, 25g

Six isotopically labeled internal standards were purchased from Cambdridge Isotope Laboratories (Andover MA, USA), and consisted of the following: Bisphenol A (Ring-$^{13}C_{12}$, 99%), Bisphenol AF (Ring-$^{13}C_{12}$, 99%), Bisphenol B (Ring-$^{13}C_{12}$, 99%), Bisphenol F (Ring-$^{13}C_{12}$, 99%) and Bisphenol S (Ring-$^{13}C_{12}$, 98%). All standards had a concentration of 100 µg/mL in acetonitrile. Two deuterated internal standards, Bisphenol A-d_{16} [BPA-d16], 98% atom D, 250mg and Benzophenone-d_{10} [BzP-d10], 99% atom D, 1g were also acquired from Sigma-Aldrich (St. Louis MO, USA).

Methanol (MeOH) (hypergrade for LC-MS) was acquired from Merck (Billerica MA, USA) and was employed for preparation of stock solutions from standards.

Milli-Q ultrapure distilled water (Millipore, Burlington MA, USA), MeOH (hypergrade for LC-MS) from Merck (Billerica MA, USA) and acetonitrile (ACN) (gradient grade for liquid chromatography) from VWR Chemicals (Rue Carnot, Fontenay-sous-Bois, France) from were used as solvents for procedures related to bisphenols and benzophenones sample preparation and extraction

For the MS infusion procedures (see Chapter 4), a 25% ammonium hydroxide solution (Sigma-Aldrich, St. Louis MO, USA) with water (LC-MS grade) from VWR Chemicals (Rue Carnot, Fontenay-sous-Bois, France) and MeOH (hypergrade for LC-MS) from Merck (Billerica MA, USA) was employed.

A mixture of 16 U.S. EPA priority pollutant PAHs at a concentration of 100 µg/mL each in toluene was purchased from Chiron AS (Trondheim, Norway), and consisted of the following compounds: Naphtalene, acenaphthylene, acenaphtene, fluorene, phenanthrene, pyrene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, fluoranthene, anthracene, benzo[a]pyrene, dibenz[a,h]anthracene, benzo[ghi]perylene and indeno[1,2,3-cd]pyrene.

A mixture of 5 fluorinated PAHs "F-PAHs All in One Cocktail, Window 1-4" at a concentration of 200 µg/mL each was purchased from Chiron AS (Trondheim, Norway), and consisted of the following compounds: 1-Fluoronaphtalene, 4-Fluorobiphenyl, 3-Fluorophenanthrene, 1-Fluoropyrene and 3-Fluorochrysene.

Distilled water and acetone (technical grade) from VWR Chemicals (Rue Carnot, Fontenay-sous-Bois, France) were used for washing glassware and other equipment before use.

Copper powder (<425 µm, 99,5% trace metals basis) and aluminium oxide powder (0,05-0,15 mm, activated, neutral, Brockmann I) were purchased from Sigma-Aldrich (St. Louis MO, USA).

Acetone (HPLC Isocratic grade), dichloromethane (DCM) (GC capillary grade) and acetonitrile (HPLC isocratic grade) were purchased from VWR Chemicals (Rue Carnot, Fontenay-sous-Bois, France) to be used for Accelerated Solvent Extraction procedures. Acetonitrile (HPLC isocratic grade, VWR Chemicals) and Milli-Q ultrapure distilled water (Millipore, Burlington MA, USA) was used for HPLC analysis of extracts.

Concentrated HNO_3 (Ultra-Pure grade, distilled by Milestone SubPur unit) and Milli-Q ultrapure water (18.2 MΩ) were used for the different steps in elemental analysis. The certified reference material Soil GBW 07408(GSS-8) (Chinese National Center for Standard Materials) was employed for method validation.

3.2 Instrumental equipment

Freeze-drying of sediment samples were done by using a Christ Alpha 1-4 LD plus laboratory freeze dryer (Martin Christ, Osterode am Harz, Germany).

A Fermaks TS 8056 oven (Bergen, Norway) and Carbolite ELF 11/6 (201) furnace (Carbolite Gero, Hope Valley, UK) were used for removal of water and for combustion of organic matter during total organic matter content determination of sediment samples, respectively.

A high-pressure Milestone UltraCLAVE digestion unit (Milestone, Sorisole, Italy) was used for digesting sediment samples prior to elemental composition determination.

A Thermo Scientific ELEMENT 2 high resolution ICP-MS instrument (Thermo Fisher Scientific, Waltham MA, USA) was used for determining elemental concentrations in sediment samples.

A Thermo Scientific Dionex ASE 150 Accelerated Solvent Extractor (Thermo Fisher Scientific, Waltham MA, USA) with stainless steel extraction cells was used for performing accelerated solvent extraction (ASE) of sediment samples.

A Biotage TurboVap Classic LV Concentration Evaporator Workstation (Biotage, Charlotte NC, USA) was used for concentrating extracts during different experimental procedures using a heated water bath and a nitrogen (N_2) gas supply.

A Branson Model 3510-DTH Ultrasonic Cleaner (Branson, Danbury CT, USA) was used for ultrasonication of samples.

An Agilent 1260 Infinity II LC system (Agilent Technologies, Santa Clara CA, USA) with a Diode-Array detector (DAD) and a Fluorescence Detector (FLD) was used for analysis of sediment samples for PAH determination. The column used was a Supelcosil LC-PAH HPLC column (4.6 mm x 250 mm x 5 µm) with a Supelcosil LC-18 Supelguard Cartridge guard column (4 mm x 20 mm x 5µm).

SPE-cartridges to be used were Strata X-CW (33µm, polymeric weak cation, 60 mg/3 cm3) and Strata X-RP (33µm, polymeric reversed

phase, 200 mg/3 cm3) cartridges, purchased from Phenomenex (Torrance CA, USA). The cartridges were assembled on a Supelcosil Visiprep 24 SPE-manifold (Sigma-Aldrich, St. Louis MO, USA). A vacuum pump was used for adjusting elution speeds through the column during the different SPE-steps.

Determination of BPs and BzPs was performed using a Waters Acquity™ Ultra Performance Liquid Chromatography (UPLC) I-class system from Waters Corporation (Milford MA, USA). The column used for method testing and for BPs and BzPs analysis of all sediment samples was a Kinetex C18-column (30 x 2.1 mm, 1.3 µm, 100Å) from Phenomenex (Torrance CA, USA), with a Phenomenex C18-guard column (recommended for 2.1mm inner diameter columns).

A Waters Xevo™ TQ-S Triple Quadrupole Mass Spectrometer system from Waters Corporation (Milford MA, USA) with an ESI-ion source was used for all tandem MS analysis of solutions.

3.3 Computer software

MassLynx v.4.1 and TargetLynx v.4.1 (Waters Corporation, Milford MA, USA) were used for preparing MS-settings, initializing sample runs and for processing data during LC-MS/MS analysis.

Agilent OpenLab Chromatography Data System ChemStation version C.01.07 (Agilent Technologies, SantaClara CA, USA) was used for preparing HPLC-settings, initializing sample runs and for acquiring chromatogram data from the HPLC analyses.

Microsoft Excel 2016 and the statistical computing software R [62] were used for general data processing and for principal component analysis, respectively.

Chapter 4

Experimental setup

4.1 Sampling of marine sediment from Trondheimsfjorden

Sediment samples were sampled using a stainless steel box corer onboard the marine research vessel R/V Gunnerus employed by NTNU. Sediments were brought up as blocks to the main deck on a platform previously washed with sea water. Block sections were cut using a stainless steel knife for sampling into aluminium boxes with lid, and a teflon plate for sampling into polystyrene cups. The samples in aluminium boxes would be further analysed for organic pollutants (BPs, BzPs, PAHs) and total organic matter content, while samples collected in polystyrene cups would be analysed for trace element pollutants. An equal number of samples were collected in aluminium boxes and polystyrene cups respectively (n=64x2). Nitrile gloves were worn during the whole sampling procedure.

Sediment blocks were sampled from three sides, for sampling triplicates of top, middle and bottom parts of the sides. In cases where the sediment blocks were of smaller size, the sides were divided into top and bottom parts for sampling. In cases where only smaller residual sediment material was brought up to deck without having a block-like structure, sampling was done in bulk (See table J.1 for details.). Figure

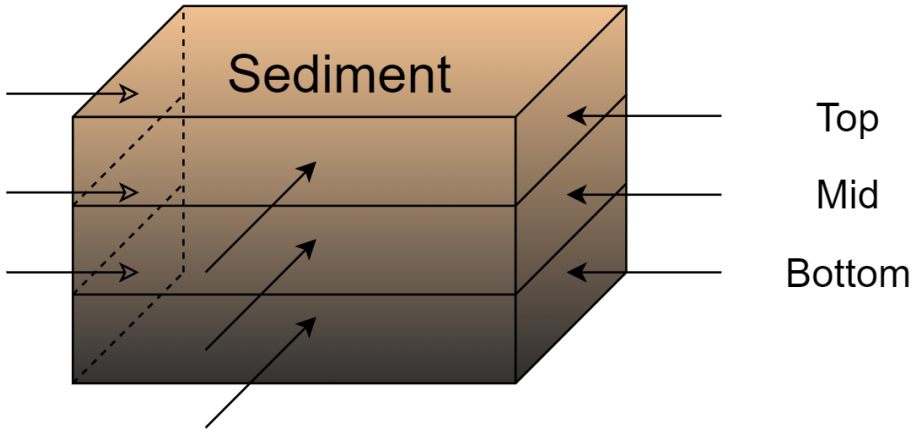

Figure 4.1: Illustration of a typical sediment block collected using a box corer from the research vessel R/V Gunnerus during sampling in Trondheimsfjorden. Sampling points are indicated with arrows.

4.1 shows a schematic of a typical sediment block with sampling points for top, middle and bottom parts.

Sediment samples were brought to NTNU Gløshaugen the same day as sampling took place. All sediment samples were brought to a freezer (-20 °C) for further storage.

4.1.1 Freeze drying

Sediment samples were freeze dried for at least 24 hours in each freeze-dryer run until no moisture could be observed in the samples. The majority of the samples were freeze dried within three weeks of sampling. Selected sample containers were weighed before and after freeze drying to control for the complete evaporation of moisture. After drying, the containers were closed with aluminium lids, wrapped in aluminium foil and brought to a freezer for storage.

4.2 Bisphenols and benzophenones analysis

4.2.1 General procedure for preparation of bisphenols and benzophenones stock solutions for mass spectrometry infusions

Portions of chemical standards were weighed on an analytical weight to approximately 0.0100 grams ± 0.0001 g and diluted with MeOH to 10 mL to achieve concentrations of 1000 ppm. From the 1000 ppm solutions, an equal amount of 10 ppm and 1 ppm solutions were prepared by diluting appropriate aliquots of the 1000 ppm stock solutions with MeOH as solvent. All stock solutions were capped, wrapped in parafilm and kept in a freezer (-22 °C) for storage until further use.

4.2.2 Calibration standard solutions containing bisphenol and benzophenone target analytes and $^{13}C_{12}$ isotopically labelled internal standards

Table D.1 shows weights of target analyte standards for preparation of stock solutions. The steps involved in preparation of standard solutions used during determination of BPs and BzPs are many, and will therefore not be described in detail. Briefly, instead of preparing each BP and BzP target analyte as individual 1ppm solutions described earlier, two 100ppb mixtures of all BP and BzP target analytes at 1 mL in MeOH were prepared. A standard solution mixture of $^{13}C_{12}$ isotopically labelled internal standards was prepared at 1ppm by mixing appropriate aliquots of individual $^{13}C_{12}$ standards (see chapter 3) and diluting to 1 mL using MeOH.

Then, calibration standard solutions containing target analytes and internal standards were prepared using the two 100ppb target analyte solutions and the 1ppm IS mix to achieve the following concentrations of target analytes: 0.1, 0.2, 0.5, 1, 2, 5, 10, 20 and 50 ng/mL. All solutions were prepared with concentrations of internal standards at 20 ng/mL. Each calibration standard solution was diluted to 1 mL by using MeOH, ACN and ultrapure Milli-Q water to achieve a solvent

ratio of 1:1:1,33. This was the closest achieveable ratio to that of the sample extracts prepared by using the method adapted after Asheim et al [58] (see chapter 4.2.3). One blank solution at 0 ng/mL not containing target analytes or internal standards was prepared in conjunction with the calibration standard solutions using the same solvent ratio as the calibration standard solutions.

4.2.3 Method testing for bisphenols and benzophenones determination

For the purpose of this project, two different methods were tested for the extraction efficiencies of BPs and BzPs from sediment samples. For both methods, portions of sample 6 (see table J.1) were used to prepare the following subset of samples for testing: One normal sample (only internal standard added before extraction), three spiked samples (internal standard and target analytes added before extraction) and two matrix match samples (internal standard and target analytes added after extraction). One blank sample (only solvent without sediment, with internal standard added before extraction) was also prepared for each method to check for contamination during testing.

The first tested method was adapted after a method by Yu et al. [63], and can be briefly described as follows: Freeze-dried samples were weighed (approximately 0.1 g, see table C.1) into 15 mL Eppendorf tubes and spiked with 20 ng of BPA-d16 (200 µL of a 100 ppb solution). The samples were extracted with 5 mL of MeOH/Milli-Q water mixture (5:3, v/v) by shaking in an orbital shaker at 250 oscillations/min for 30 minutes. After centrifugation at 4800 × g for 5 minutes, the supernatants were transferred into 15 mL glass tubes. The extraction was repeated twice for all samples before the extracts for each sample were combined and concentrated to approximately 4 mL under a gentle stream of nitrogen. After dilution to 10 mL with 0.2 % formic acid (pH 2.5), the extracts were purified by using Strata X-CW cartridges (33 µm, polymeric weak cation, 60 mg/3 cm^3). The cartridges were conditioned with 5 mL of MeOH and 5 mL of Milli- Q water. After loading the samples, the cartridges were washed with 15 mL of MeOH/Milli-Q water (1:3, v/v) and 5 mL of Milli-Q water, and then

eluted with 5 mL of MeOH. The eluates were transferred into vials and evaporated to approximately 0.5 mL under a gentle stream of nitrogen gas. After evaporation, the samples were diluted to 1 mL using MeOH and finally vortex mixed before being transferred for LC-MS/MS analysis.

The second tested method was adapted after a method by Asheim et al. [58]. Briefly, freeze-dried samples (approximately 0.1 g, see table C.1) were transferred into 15 mL Eppendorf tubes. 5 mL of acidified MeOH/Milli-Q water (1:1 v/v, pH adjusted to 1-3 with HCl solution) and internal standard/ target analytes (20 µL of 1 ppm solutions) were added, then liquid-solid extraction (LSE) was performed by vortex mixing for 1 min. Following mixing, the mixtures were ultra-sonicated for 45 min. at 35 °C. The mixtures were centrifuged for 5 min and the supernatants were collected into 50 mL PP tubes. 50 mL of acidified Milli-Q water (pH adjusted to <3 with HCl solution) were added to the tubes, and the solutions were extracted using Strata X-RP cartridges (33 µm, polymeric reversed phase, 200 mg/3 cm^3). Prior to extraction, all samples were vortex mixed for 1 min. The cartridges were conditioned with 10 mL of MeOH and equilibriated with 10 mL of acidified Milli-Q water (pH adjusted to <3 with HCl solution). After loading of the samples, the cartridges were washed with 10 mL of acidified Milli-Q water (pH adjusted to <3 with HCl solution) and then dried under vacuum for 1 min. The compounds were eluted using 10 mL of MeOH/ACN (1:1 v/v) and collected into 15 mL Eppendorf tubes. The tubes were placed on a heated tray (40 °C) and eluents were evaporated to approximately 250 µL under a gentle stream of nitrogen gas. After evaporation, the eluents were diluted to 1 mL with final in-vial solvent composition of MeOH/ACN/Milli-Q water (1:1:2) and transferred for LC-MS/MS analysis.

The extraction of BPs and BzPs from all sediment samples listed in table J.1 was ultimately performed by employing the procedure based on the method by Asheim et al. See chapter 6 for details.

4.2.4 Determination of bisphenols and benzophenones in sediment samples using liquid chromatography - tandem mass spectrometry

Transitions for analytes in individual 1 ppm standard solutions were found based on fragmentation in negative mode (ESI-). Conditions for the transition procedures are given in tables C.2 and D.3 during method testing and during determination of BPs/ BzPs in all sediment samples listed in table J.1, respectively.

Before analysis of all sediment samples, the cone and system was cleaned by running 1 hour of acetone blanks. 20 injections of standard solutions at 10 ng/mL were run to saturate the cone.

Analysis was performed in negative mode (ESI-). Mobile phases consisted of MeOH (hypergrade for LC-MS, Merck) as the organic phase (position A) and 0.1% v/v ammonium hydroxide solution in water (LC-MS grade, VWR Chemicals) (position B). The injection volume was set at 4 µL with a flow rate of 300 µL/min.

The capillary was set at 1.5 kV, the cone at 50 V and the source offset at 30 V. Source temperature was set at 150 °C and the desolvation temperature at 350 °C. The cone gas flow was set at 150 L/hour and the desolvation gas flow at 650 L/hour. Collision gas flow was at 0.15 mL/min and the nebulizer gas flow was at 7.0 bar. These settings were applied during the method testing and the determination of BPs/BzPs in all sediment samples.

The mobile phase gradients used during analysis can be viewed in Appendix D, table D.6.

4.3 Polycyclic aromatic hydrocarbon analysis

Sediment samples that had been previously freeze dried were left to defrost for 30 min. at room temperature before being mixed with a glass rod. A cellulose filter was placed in an extraction cell, which was further filled with approximately 1 g of activated Cu and 2 g of activated Al. A portion of sediment sample (5 g ± 0,1 g, see table F.1) was mixed with activated diatomaceous earth (approximately 2 mL) in a clean beaker. Internal standards (200 µL of a 1 µg/mL mix) were added to the beaker, mixed thoroughly with the sample and transferred to the extraction cell. The cell was placed in the Accelerated Solvent Extractor system which was set at conditions given in table F.2 as an already pre-setup method for PAHs. Prior to extractions, the ASE system was rinsed by running approximately 5 mL of solvent three times through an empty rinse cell.

After extraction, the extracts were further concentrated to a volume of approximately 1 mL using the Biotage TurboVap evaporator which was set at 40 °C and 5 psi pressure. The concentrated samples were added acetonitrile (10 mL, HPLC analytical grade) before being filtered with 0,45 µm PTFE syringe filters using disposable syringes. Following filtration, sample extracts were concentrated again using the Biotage TurboVap evaporator at 45 °C and 5 psi pressure to a volume of approximately 1 mL. The extracts were transferred to LC vials which were made up to the 1,5 mL mark with acetonitrile before being stored in a refrigerator (5 °C). The samples were filtered again using 0,45 µm PTFE syringe filters on the day of HPLC analysis.

Standard solutions for calibration of the HPLC-instrument were prepared by diluting aliquots of the 16 PAHs mixture (see chapter 3) and the 5 F-PAHs mixture with acetonitrile. The following concentrations of target analytes were prepared: 50, 100, 200, 400, 1000 and 1500 ng/mL. Internal standards were prepared at a concentration of 200 ng/mL in all standard solutions.

4.4 Elemental analysis

Dried sediment samples were digested and analysed for elemental composition as described by Halbach et al. [49]. Briefly, approximately 250-350 mg of freeze dried sediments from CC-cups were weighed into perfluoroalkoxy (PFA) vessels (18 mL volume). Then 9 mL of 50 % concentrated HNO_3 (Ultra-Pure grade, distilled by Milestone SubPur unit) was added to the samples and samples were digested with the use of a high-pressure digestion unit UltraCLAVE (Milestone, Sorisole (BG)). After digestion, the samples were diluted to a total volume of 108 mL prior to analysis.

Elemental composition was determined by ICP-MS using an ELEMENT 2 high resolution ICP-MS instrument (Thermo Finnigan model, Bremen, Germany). The analysis of different elements was performed using different resolutions: low, medium, and high to avoid interferences. The instrument was calibrated using 0.6 M HNO_3 solutions of matrix matched multi-element standards which were run after every 10 samples. A calibration curve consisting of five different concentration levels was made from multi-element standards and used for quantifying elements in samples. Detection limits and limits of quantification were based on the instrument detection limits (IDL), given in table 5.6. The IDL results from the concentration yielding 25 % of relative standard deviation at n=3 scans for a given element, which were calculated in dry weight for the used sample amount.

4.5 Total organic matter content analysis

The total organic matter was determined using a standard loss-on-ignition procedure [64]. A portion of sediment from those collected in aluminium boxes in May were transferred to 50 mL PP tubes and left to defrost at room temperature for 2 - 4 hours. After defrosting, sediment samples were homogenized using a spatula. Approximately 10 grams (see table H.1) of the defrosted sediment samples were transferred to pre-weighed crucibles. Crucibles with samples were left to dry in an

oven (Fermaks TS 8056) at 105 °C overnight and then re-weighed before being put into a combustion furnace (Carbolite ELF 11/6 (201)) for 3 hours at 550 °C. After combustion, crucibles were weighed to estimate the total loss of organic matter.

Reproducibility of the method was estimated based on percentage total organic matter content in three replicates of an extra sediment sample (see table J.1), which were compared to find % RSD.

4.6 Quality control

4.6.1 Bisphenols and benzophenones analysis

Glassware equipment and other equipment to be used for preparation of stock solutions and general sample preparation was rinsed using soap and tap water, distilled water and MeOH (gradient grade for liquid chromatography, VWR Chemicals) and set to dry for at least 24 hours.

One reagent blank sample was prepared for every batch of samples that could be prepared simultaneously during one method run. Signals found in blank samples were subtracted from regular samples to compensate for contamination. Solvent blank solutions prepared of either acetone or MeOH were run periodically to control for possible carryover of analytes between sample injections. Calibration standard solutions were run after every 20 injections to check for potential drift in instrument sensitivity. To estimate recoveries and matrix effects of target analytes and internal standards, a total of 9 samples were prepared as spiked samples: 3 with a conc. of 10 ng/mL, 3 with a conc. of 25 ng/mL, and 3 with a conc. of 50 ng/mL, and a total of 6 samples were prepared as matrix match samples: 2 with a conc. of 10 ng/mL, 2 with a conc. of 25 ng/mL, and 2 with a conc. of 50 ng/mL. All spiked samples and matrix match samples were made by using sample 64 (see table J.1).

4.6.2 Polycyclic aromatic hydrocarbon analysis

Glassware equipment and other equipment to be used during ASE extractions was rinsed using soap and tap water, distilled water and acetone (technical grade, VWR Chemicals) and set to dry for at least 24 hours.

One reagent blank was prepared for every batch of samples prepared during one day, and was analysed in conjunction with samples to check for possible contamination during sample extraction and analysis. One sample (sample 38) was run at the start, middle and the end during one HPLC run to check for potential degradation of analytes. Reagent blanks were prepared with acetone and run periodically during analysis to control for sample carryover in the HPLC instrument. Reproducibility of the method was controlled by extracting one sample (sample 64) three times, which was run consequtively during an analysis. Recoveries were estimated by preparing a subset of spiked samples (n = 3) and matrix match samples (n = 3) from sediment sample 64 (see table F.1). Spiked samples and matrix match samples were spiked with 300 ng of of PAH target analytes and F-PAH internal standards from a standard solution mix.

For the confirmation of exact retention times belonging to the different analytes, 80 µL aliquots of extracts prepared from samples 15, 25, 30, 40, 50, 60 and MB18 (see table F.1) were spiked with 20 µL of the 1000 ng/mL standard solution and analysed in one run. Relative retention times were calculated in all samples, standard solutions and reagent blank samples using F-PHE as the standard compound.

4.6.3 Elemental analysis

To assess possible contamination during sample preparation, reagent blank samples of HNO_3 and Milli-Q ultrapure water (18.2 MΩ) were prepared using the same procedure as for the samples. For each of a total of two sample batches digested on two consecutive days, two blank samples were prepared and analysed in conjunction with the sample batches. Results were corrected for reagent blank samples values. The certified reference material Soil GBW 07408 (GSS-8) (Chinese National

Center for Standard Materials) was used to validate the accuracy of the method through frequent analysis. The precision of the method was controlled by the standard deviation of three parallel analysis of a sample.

4.6.4 Total organic matter content analysis

One sample (sample 17 collected as extra sample, see table J.1 and 5.10) was analysed in triplicate to validate the precision of the LOI-method.

Chapter 5

Results

5.1 Method testing for bisphenols and benzophenones determination

Figures 5.1 and 5.2 show the recoveries of bisphenols and benzophenones found during method testing, respectively. Figure 5.3 shows the matrix effects calculated for both target pollutant classes.

The transitions for each analyte from the method testing experiments can be viewed in table C.2, Appendix C.

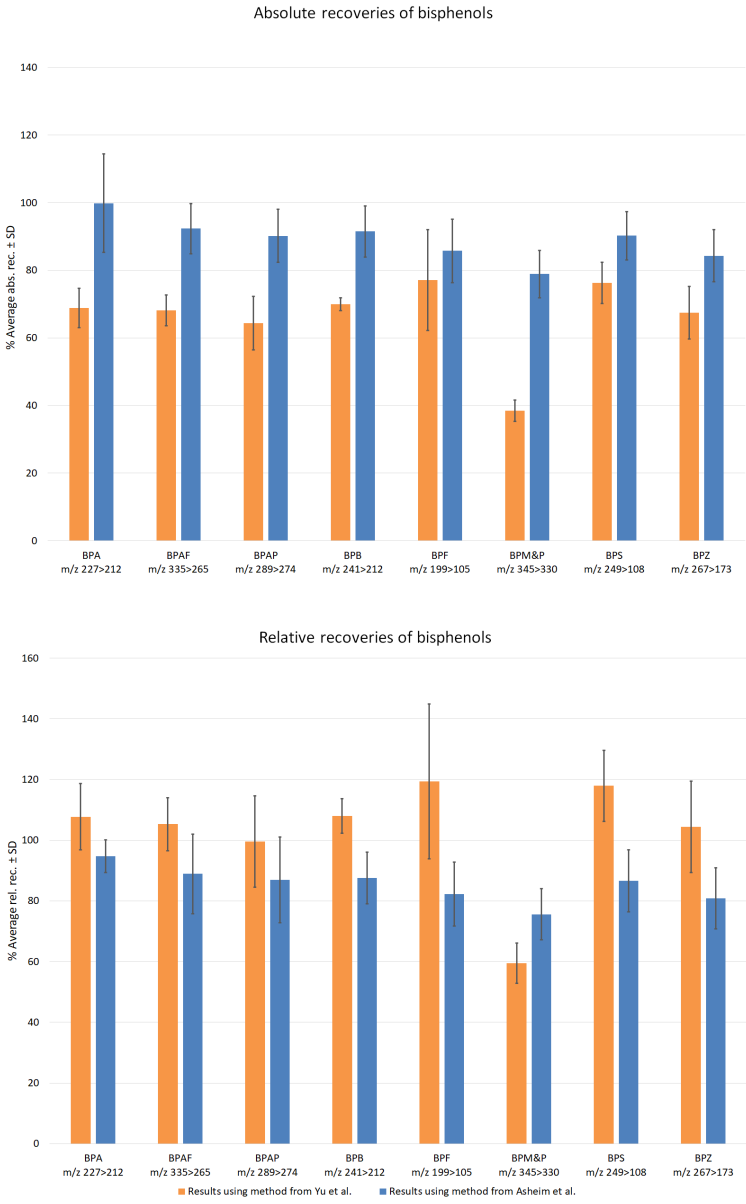

Figure 5.1: Percentage average recoveries of bisphenols (± SD) for both methods during testing. Recoveries were calculated using spiked (n = 3) and matrix match samples fortified at 20 ng/mL.

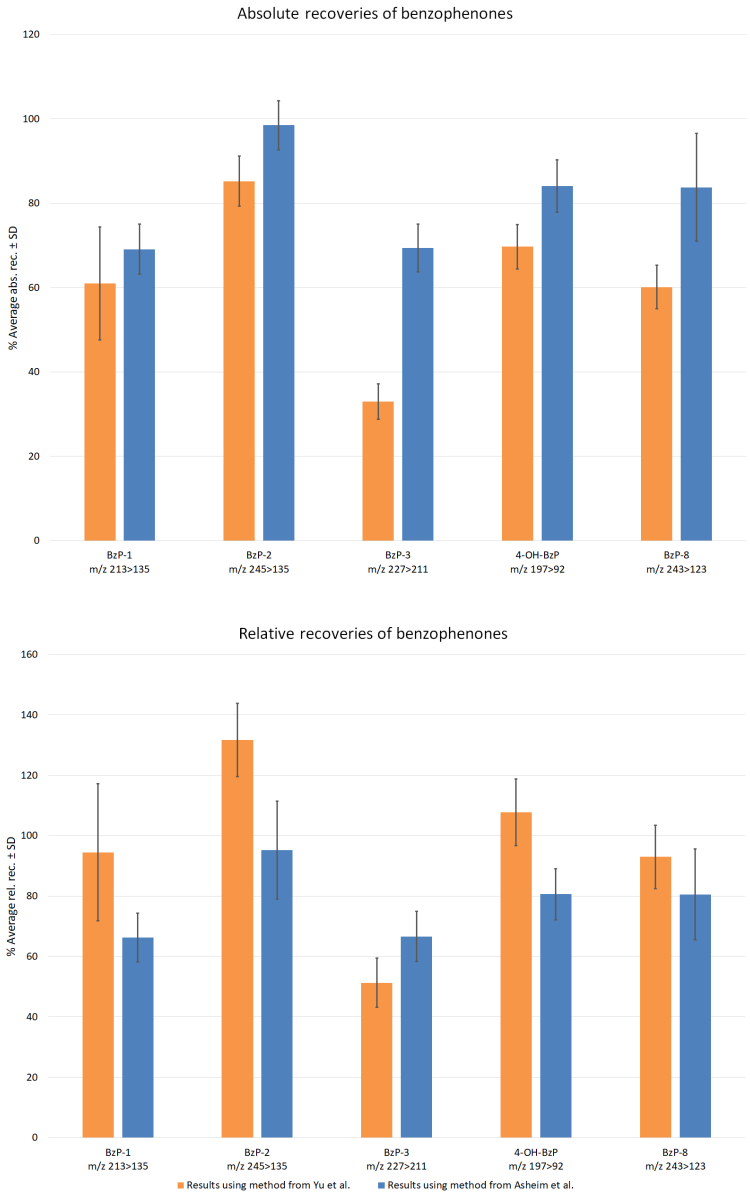

Figure 5.2: Percentage average recoveries of benzophenones (± SD) for both methods during testing. Recoveries were calculated using spiked (n = 3) and matrix match samples fortified at 20 ng/mL.

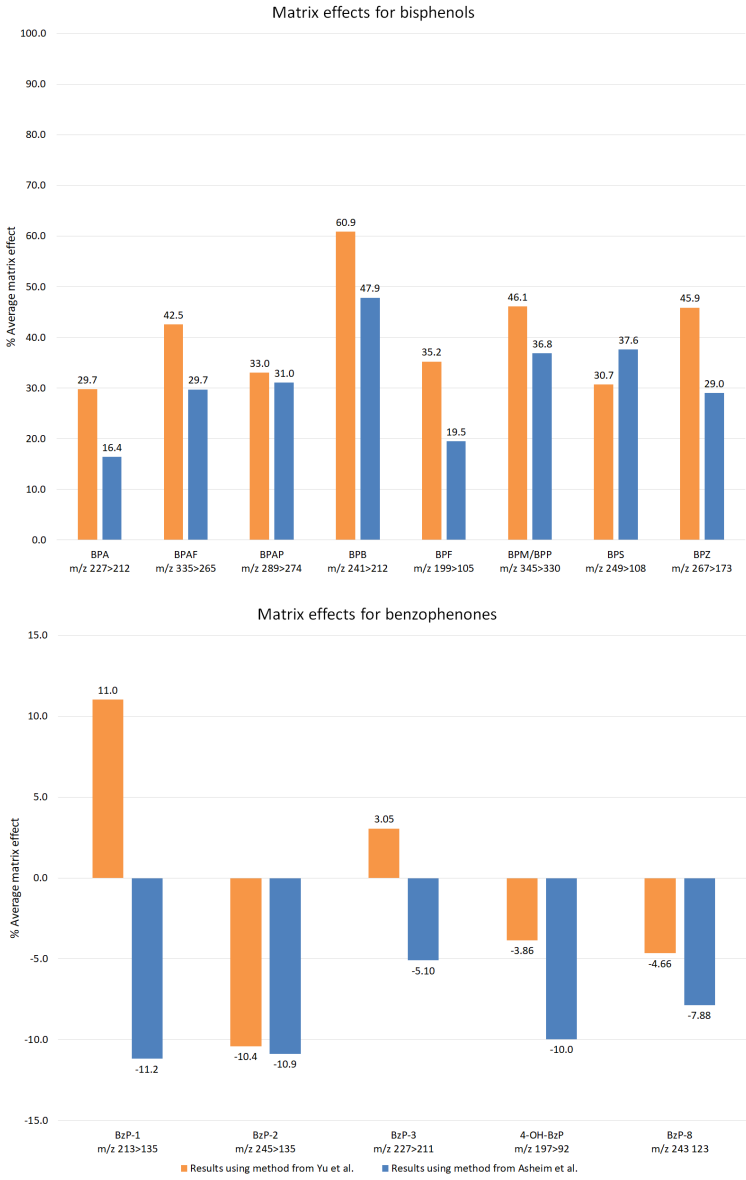

Figure 5.3: Percentage average matrix effects for bisphenols and benzophenones found with both methods during testing. Matrix effects were calculated using matrix match samples (n = 2) fortified at 20 ng/mL.

5.2 Bisphenols and benzophenones in sediment samples

Table 5.1 shows the LLOQ and LOD for each target analyte. Values are determined from calibration standard solutions and a theoretical sample amount of 0.5 g.

Table 5.1: Limits of detection (LOD) and lower limits of quantitation (LLOQ) for bisphenol and benzophenone target analytes. Units are in ng/g dry weight.

Target analyte	LOD	LLOQ
BPA	0.13	0.40
BPAF	0.067	0.20
BPAP	0.067	0.20
BPB	0.067	0.20
BPF	0.13	0.40
BPM&BPP	0.067	0.20
BPS	0.067	0.20
BPZ	0.067	0.20
BzP-1	0.067	0.20
BzP-2	0.067	0.20
BzP-3	0.067	0.20
4-OH-BzP	0.067	0.20
BzP-8	0.067	0.20

Reagent blank samples contained quantifiable amounts of BPA (0.83 - 3.89 ng/g), BPB (nd - 0.27 ng/g), BPF (1.19 - 2.87 ng/g), BPS (nd - 0.26 ng/g), BPZ (nd - 0.11 ng/g), BzP-1 (0.13 - 0.89 ng/g), BzP-2 (nd - 0.43 ng/g), BzP-3 (0.32 - 0.57 ng/g), 4-OH-BzP (nd - 0.47 ng/g) and BzP-8 (nd - 0.12 ng/g). The compounds BPAF, BPAP and BPM & BPP could not be detected in any reagent blank samples.

Table 5.2 shows concentrations of BPs with descriptive statistical values for all sediment samples collected from Trondheimsfjorden. Concentrations below LOD were not included when doing calculations.

Table 5.2: Concentrations with percentage detection rates of bisphenol target analytes in sediment samples from Trondheimsfjorden. Concentration units are in ng/g dry weight.

	BPA	BPAF	BPAP	BPB	BPF	BPM&BPP	BPS	BPZ	∑BPs
Trondheimsfjorden (n = 64)									
Mean	2.42	0.10	0.00	0.00	1.66	0.00	0.14	0.27	3.62
Median	1.82	0.088	0.00	0.00	1.42	0.00	0.099	0.19	3.15
Max	8.62	0.20	0.075*	nd	6.36	0.074*	0.57	1.06	12.1
Min	0.26	0.069	0.00	nd	0.18	0.00	0.072	0.070	0.67
% Detection rate	82.8	9.38	1.56	0.00	79.7	1.56	31.3	46.9	96.9

nd = not detected.
*BPAP and BPM&BPP were quantified in one sample.

Table 5.3 shows concentrations of BzPs with descriptive statistical values for all sediment samples collected from Trondheimsfjorden. Concentrations below LOD were not included when doing calculations.

Table 5.3: Concentrations with percentage detection rates of benzophenone target analytes in sediment samples from Trondheimsfjorden. Concentration units are in ng/g dry weight.

	BzP-1	BzP-2	BzP-3	4-OH-BzP	BzP-8	∑BzPs
Trondheimsfjorden (n = 64)						
Mean	0.47	0.86	0.84	0.17	0.16	1.95
Median	0.29	0.22	0.61	0.16	0.17	1.27
Max	4.60	29.6	3.04	0.40	0.24	34.7
Min	0.096	0.067	0.13	0.075	0.068	0.25
% Detection rate	73.4	78.1	98.4	37.5	9.38	98.4

Figure 5.4 and 5.5 shows the distributions of the sum of BPs and selected BP analogues in sediment samples from the different sampling stations, respectively. Concentration values below LOD for each target analyte were removed prior to doing calculations. No samples were acquired from station 7.

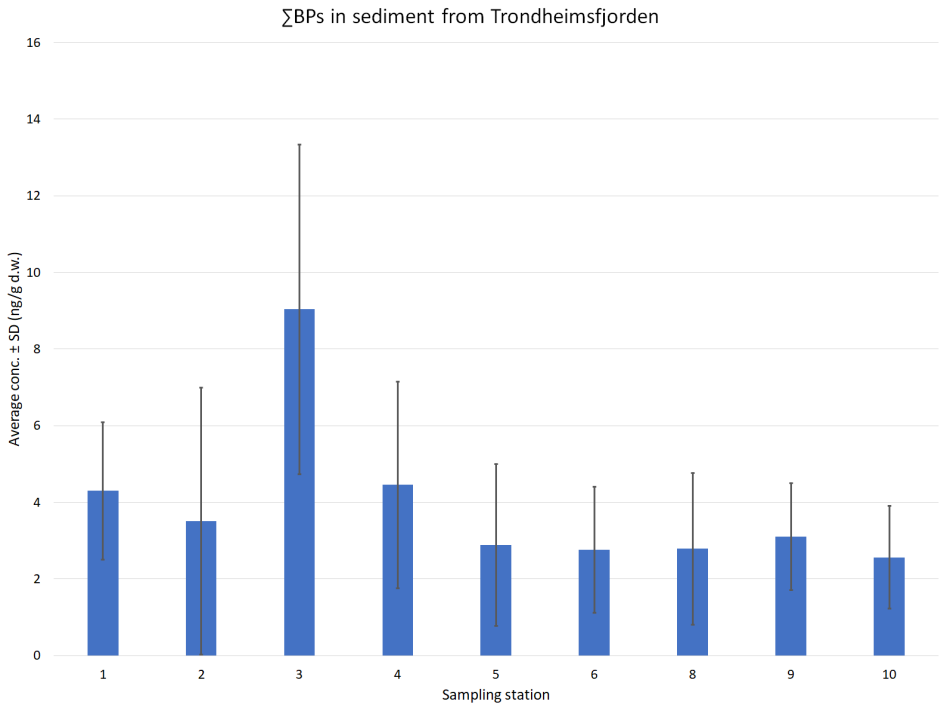

Figure 5.4: Average distributions (± SD) of the sum of bisphenol analogues in sediment samples from the different sampling stations.

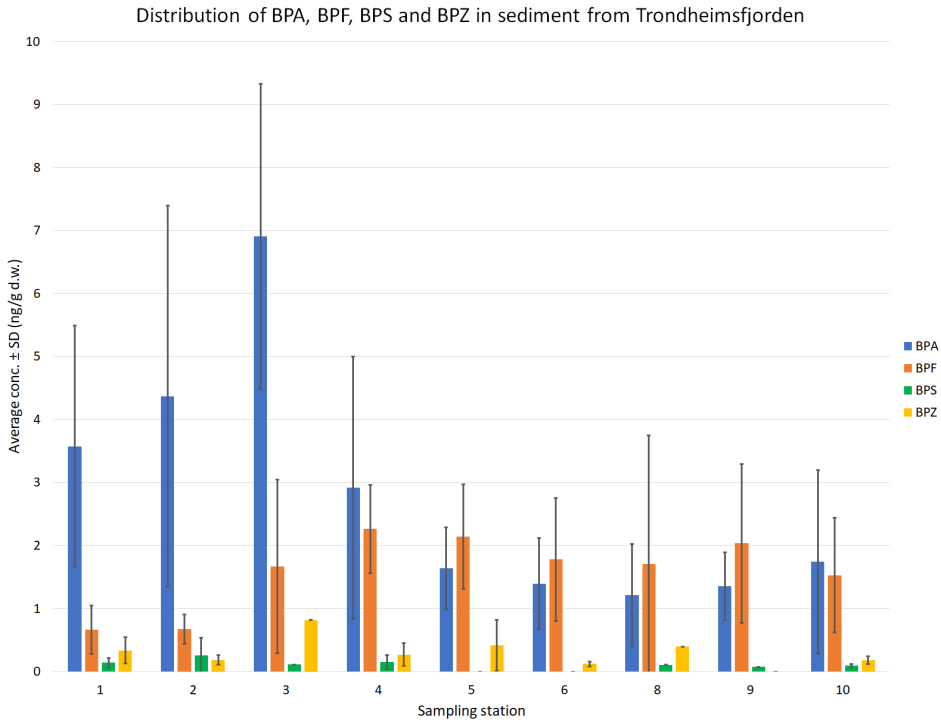

Figure 5.5: Average distributions (± SD) of individual bisphenol analogues in sediment samples from the different sampling stations.

Figure 5.6 and 5.7 show the distributions of the sum of BzPs and each BzP analogue in sediment samples from the different sampling stations, respectively. Concentration values below LOD for each target analyte were removed prior to doing calculations.

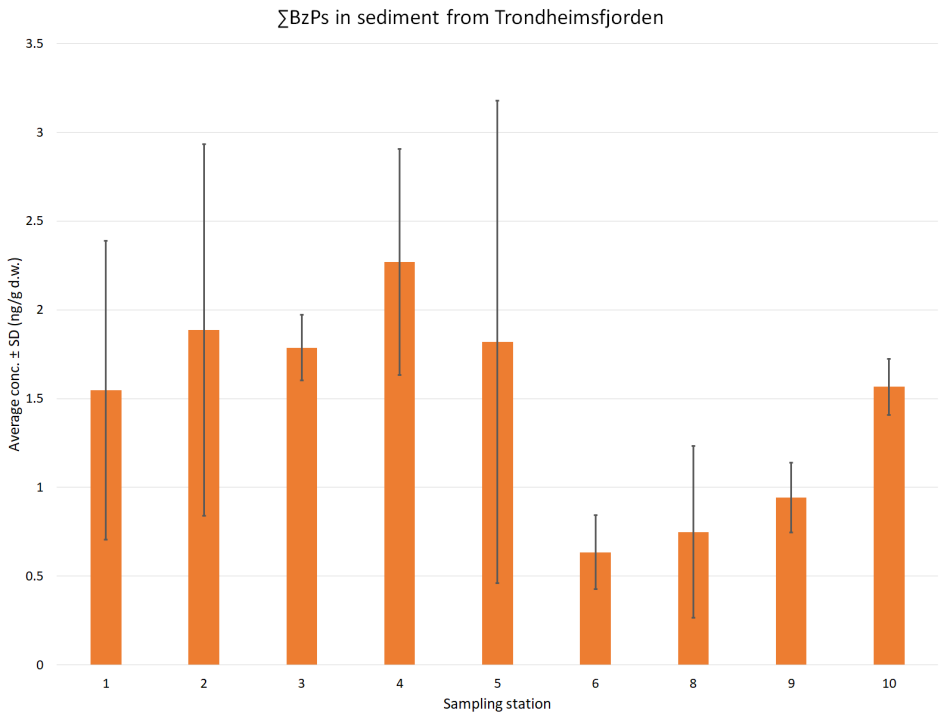

Figure 5.6: Average distributions (± SD) of the sum of benzophenone analogues in sediment samples from the different sampling stations.

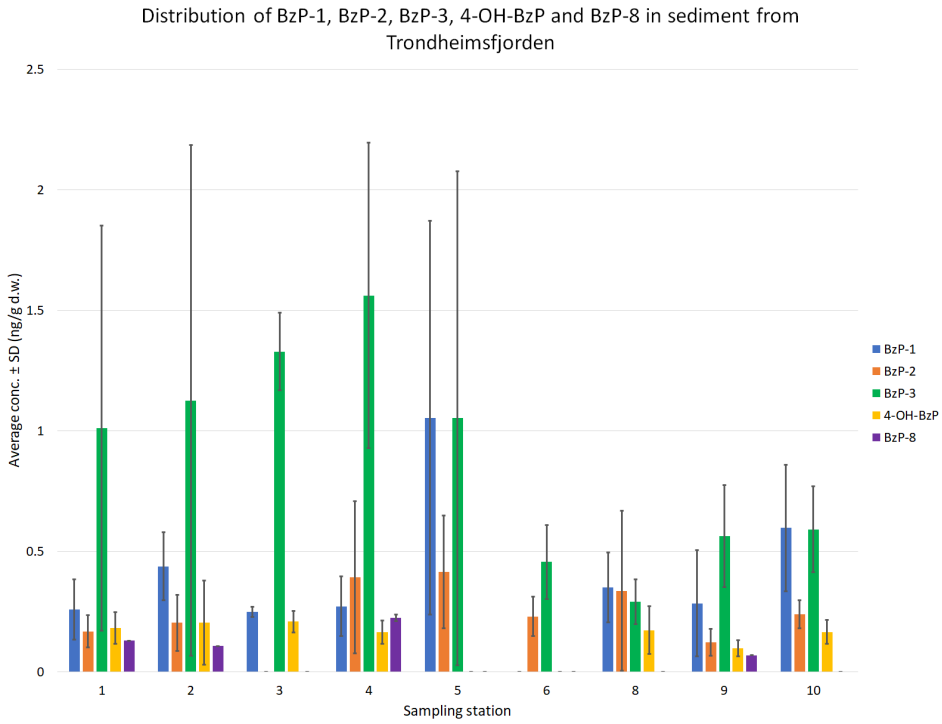

Figure 5.7: Average distributions (± SD) of individual benzophenone analogues in sediment samples from the different sampling stations.

Figure 5.8 show the composition profiles of individual BP and BzP analogues in sediments based on fractions of the total sum of analogues (∑BPs and ∑BzPs), respectively. Concentration values below LOD for each target analyte were removed prior to doing calculations.

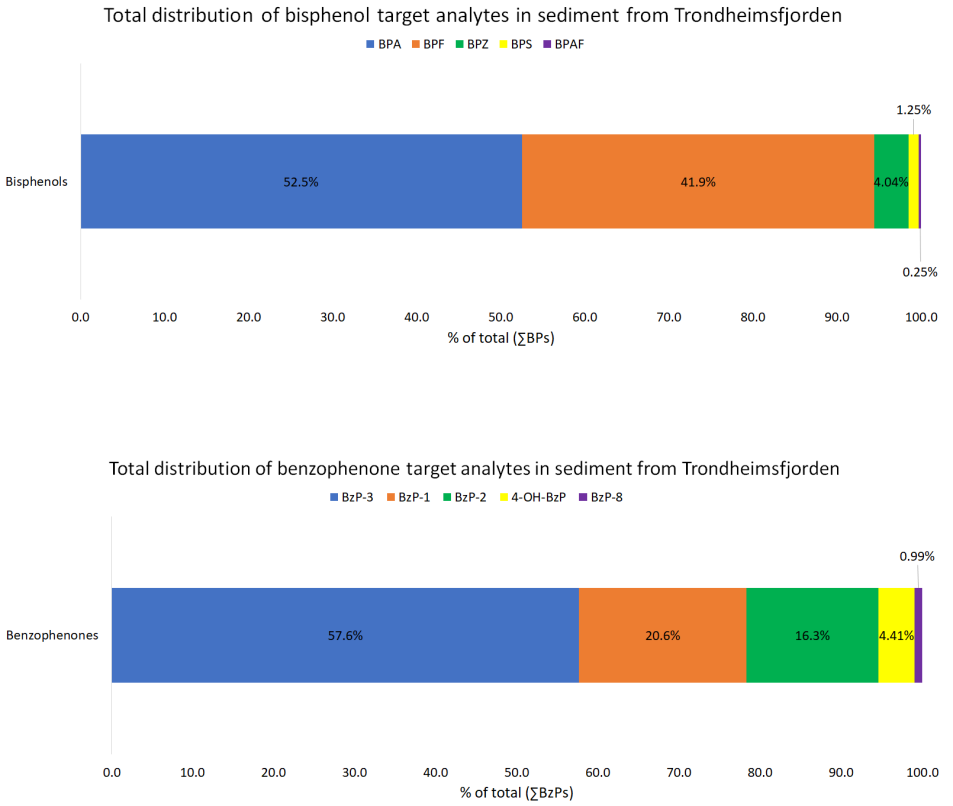

Figure 5.8: Percentage distribution of bisphenol and benzophenone target analytes in sediment from Trondheimsfjorden.

Figure 5.9 and figure 5.10 show the absolute and relative recoveries of bisphenols and benzophenones, respectively. Blank sample subtraction was used for calculating the recoveries (see equation 2.2 and 2.3). Figure 5.11 shows calculated matrix effects for both bisphenols and benzophenones.

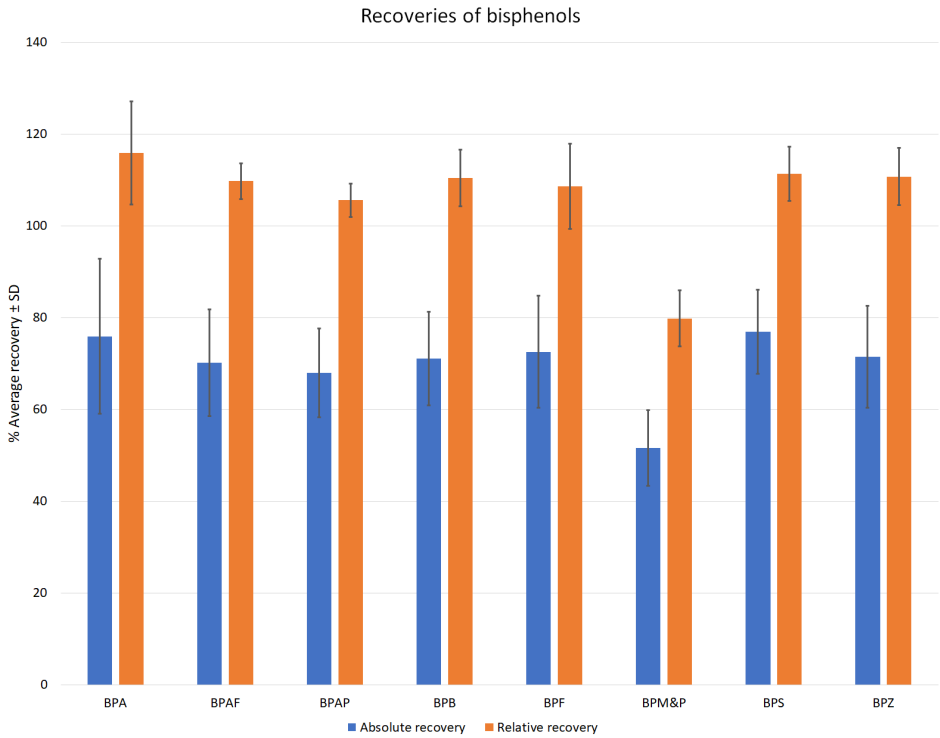

Figure 5.9: Percentage average recoveries (± SD) of bisphenol target analytes. Values are presented as averages of spiked samples (n=9) and matrix match samples (n=6) fortified at 10, 25 and 50 ng/mL.

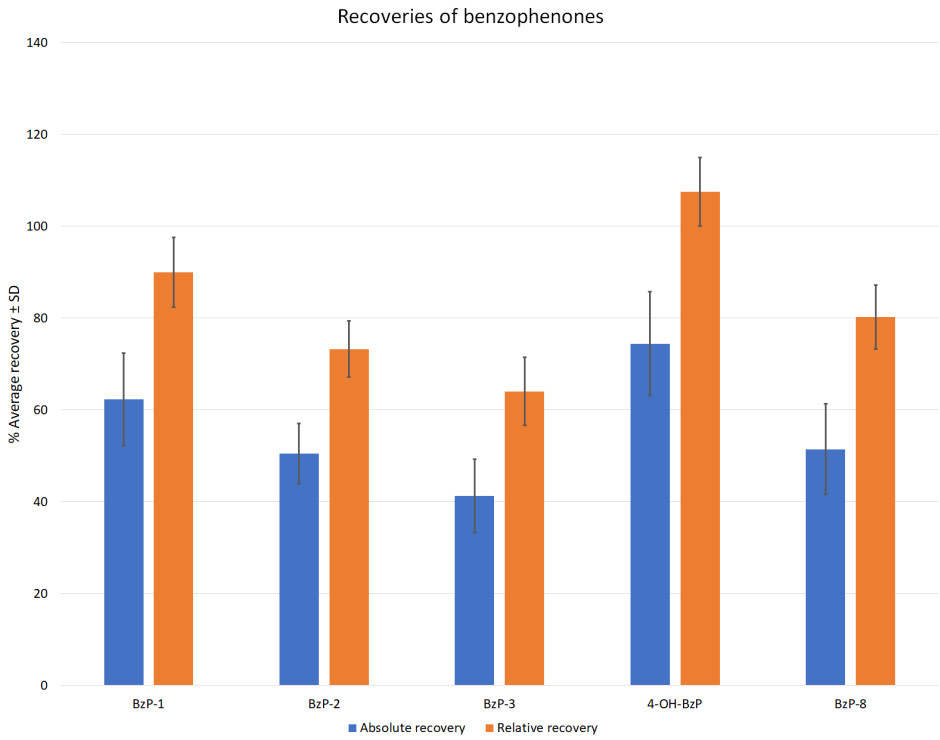

Figure 5.10: Percentage average recoveries (± SD) of benzophenone target analytes. Values are presented as averages of spiked samples (n=9) and matrix match samples (n=6) fortified at 10, 25 and 50 ng/mL.

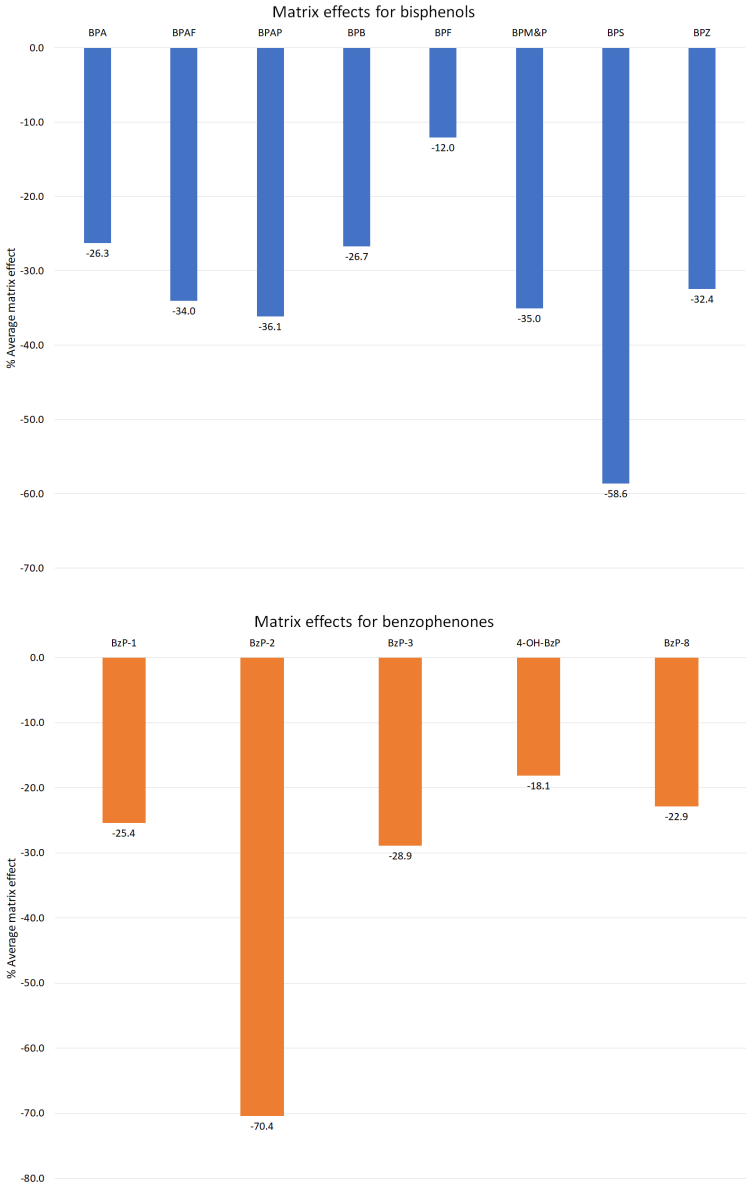

Figure 5.11: Percentage average matrix effects for bisphenols and benzophenones. Matrix effects were calculated using matrix match samples (n=2) and standard solution fortified at 10 ng/mL of target analytes.

Retention times in chronological order with relative retention times of BPs and BzPs can be viewed in table D.4, Appendix D. RTs and RRTs were calculated from calibration standard solutions at 10, 25 and 50 ng/mL. $^{13}C_{12}$ BPAF was used as the internal standard for RRT calculation.

Transitions for the target analytes and internal standards can be viewed in table D.3, Appendix D.

Calibration curves made using different $^{13}C_{12}$ BP internal standards for bisphenol and benzophenone target analyte quantification are given in Appendix D, figure D.1 and D.2.

5.3 Polycyclic aromatic hydrocarbons in sediment samples

Table 5.4 shows the LLOQ and LOD of each PAH target analyte. Values are estimated from calibration standard solutions used during HPLC analysis and a theoretical sample amount of 5 g. See equation 2.7.

Table 5.4: Limit of detection (LOD) and lower limit of quantitation (LLOQ) of PAH target analytes. Units are in ng/g dry weight.

Compound	LOD	LLOQ
NAP	5.00	15.0
ACE	5.00	15.0
FLU	5.00	15.0
PHE	5.00	15.0
ANT	5.00	15.0
FLT	5.00	15.0
PYR	5.00	15.0
BaA	5.00	15.0
CHR	5.00	15.0
BbF	5.00	15.0
BKF	5.00	15.0
BaP	5.00	15.0
DBA	5.00	15.0

No PAH target analytes could be detected in any reagent blank samples analysed during HPLC-runs.

Table 5.5 shows concentrations of PAHs with descriptive statistical values for all sediment samples collected from Trondheimsfjorden. Concentrations below LOD were not included when doing calculations.

Table 5.5: Concentrations with percentage detection rates of polycyclic aromatic hydrocarbons in sediment samples from Trondheimsfjorden. Concentration units are in ng/g dry weight.

Trondheimsfjorden	(n=64)				
PAH	Mean	Median	Max	Min	% Detection rate
NAP	10.5	9.15	46.1	5.98	90.6
ACE	0.00	0.00	12.5*	0.00	1.56
FLU	9.99	9.99	14.1	5.89	3.13
PHE	22.0	20.1	58.8	6.44	96.9
ANT	9.13	7.78	27.2	5.56	78.1
FLT	58.0	59.0	110	10.0	100
PYR	56.4	57.3	111	7.11	100
BaA	31.4	32.0	56.0	2.63	100
CHR	37.7	39.3	56.2	5.13	98.4
BbF	45.4	47.0	71.4	5.32	100
BKF	21.8	22.2	31.7	8.81	96.9
BaP	41.3	41.2	59.3	7.83	96.9
DBA	8.83	8.59	12.2	6.00	84.4
$\sum$PAHs	335	351	548	33.4	100

* ACE was quantified in one sample.

Figure 5.12 shows the total concentrations of PAH target analytes (sum of each PAH target analyte: ∑PAHs) at the different sampling stations. Values are calculated as averages for all sediment samples at the different stations. Concentrations below LOD for each target analyte were removed prior to calculations.

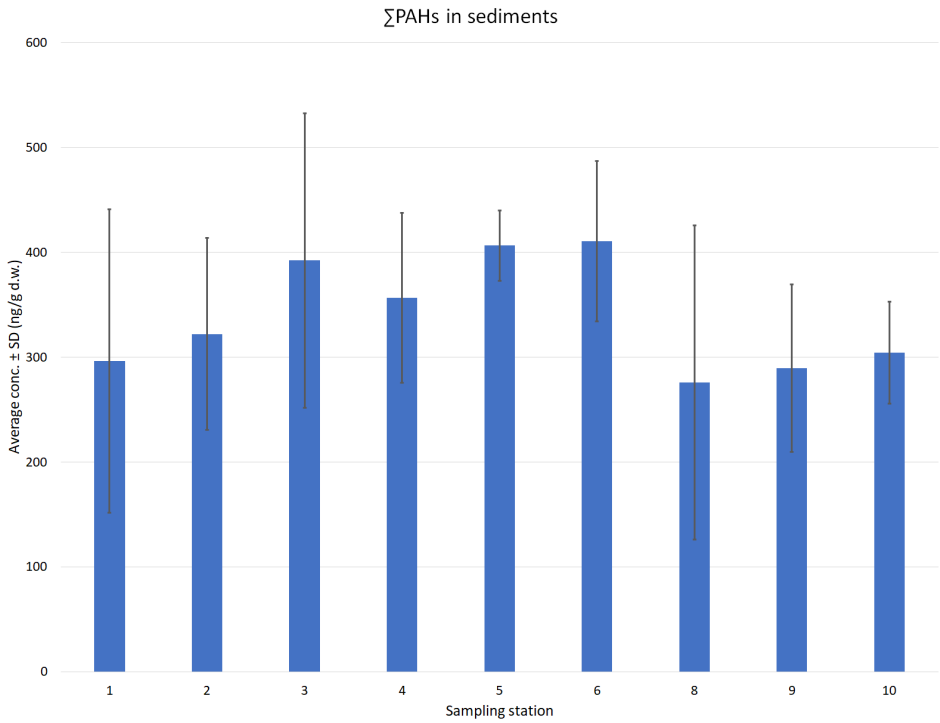

Figure 5.12: Average concentrations (± SD) of the sum of polycyclic aromatic hydrocarbon target analytes at each sampling station.

Figure 5.13 shows distributions of individual PAH target analytes of the total ($\sum$PAHs). Values are calculated as average concentration fractions of PAH target analytes for all sediment samples. Concentrations below LOD for each target analyte were removed prior to calculations.

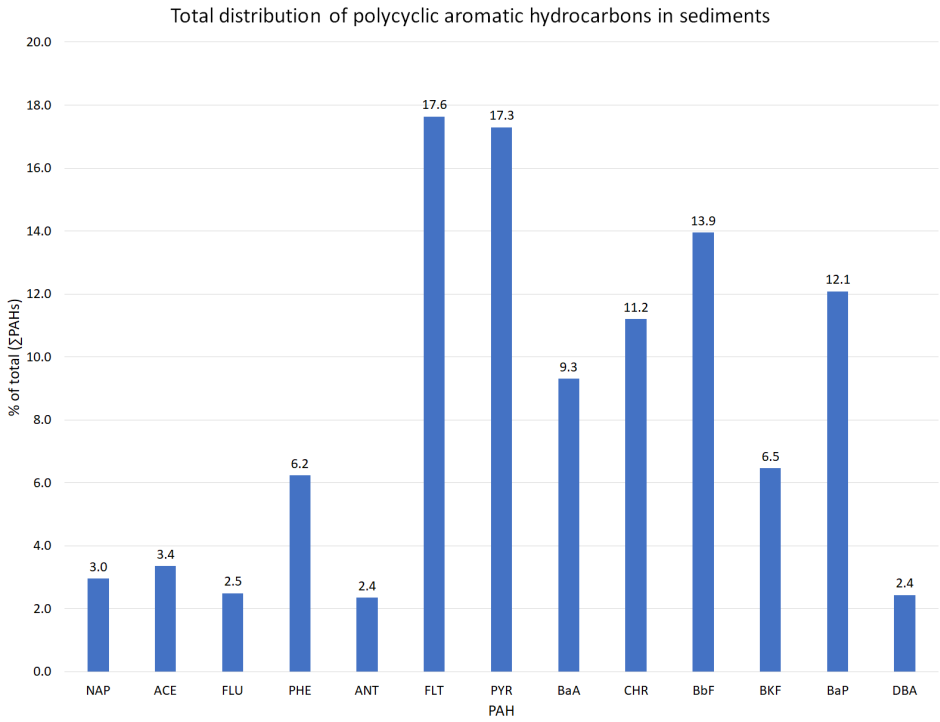

Figure 5.13: Percentage distribution of individual polycyclic aromatic hydrocarbon compounds in sediment from Trondheimsfjorden.

Figure 5.14 shows the percentage average recoveries of PAH target analytes. Calculations were done using sample subtraction given that no target analytes were detected in blank samples (see equation 2.2). F-PHE was the internal standard used for relative recovery calculations.

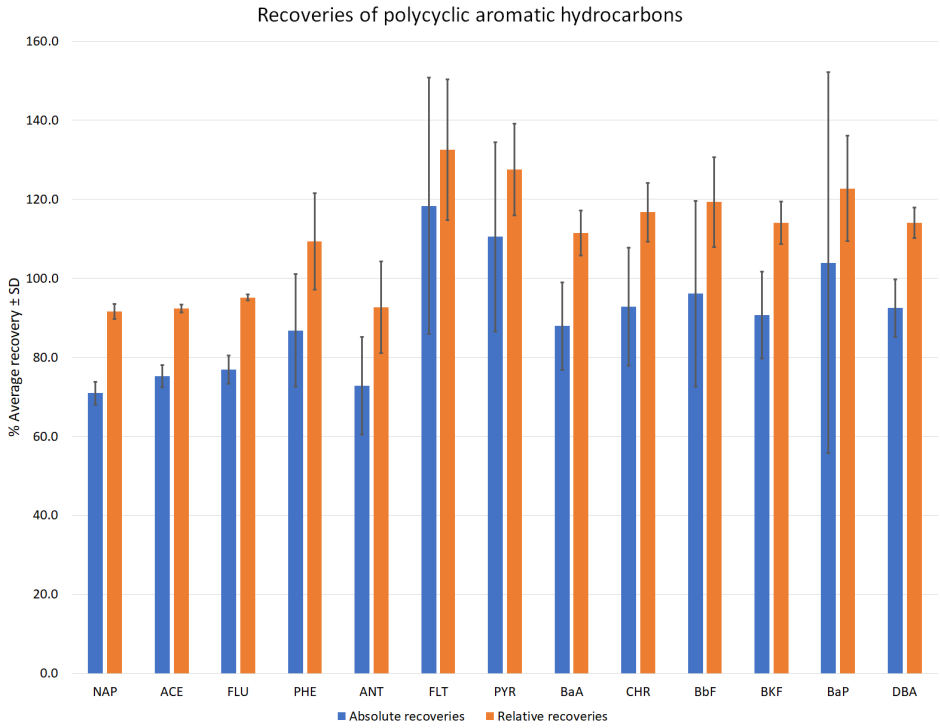

Figure 5.14: Percentage average recoveries (± SD) of polycyclic aromatic hydrocarbon target analytes. Values were calculated as averages from spiked samples (n = 3) and matrix match samples (n = 3) fortified with 300 ng of target analytes and F-PAHs.

Three representative chromatograms from the analysis of sediment samples for PAHs can be viewed in figure F.1, Appendix F.

Retention times and relative retention times of the different PAH compounds were found from one sample analysed during the RT confirmation check and can be viewed in table F.5, Appendix F. F-PHE was used as the IS for RRT calculations.

Calibration curves made using F-PHE as internal standard for PAH target analyte quantification are given in figure F.2, Appendix F.

5.4 Elements in sediment samples

Table 5.6 shows detection limits and limits of quantification calculated for each element.

Table 5.6: Instrumental limits of detection (IDL) and limits of quantitation (LOQ) for elements in sediments. Limits are estimated based on theoretical concentrations in sediments with associated uncertainty levels of RSD 25%. Concentration units are in µg/g dry weight.

Element	IDL	LOQ	Element	IDL	LOQ	Element	IDL	LOQ
Ag*	0.035	0.10	Hf	0.0029	0.0086	S	57.6	173
Al	0.58	1.73	Hg	0.0058	0.017	Sb*	0.0094	0.028
As*	1.56	4.67	Ho	0.00058	0.0017	Sc	0.012	0.035
Au*	0.0021	0.0064	Ir	0.0014	0.0043	Se*	0.12	0.36
B	0.23	0.69	K	2.88	8.64	Si	28.8	86.4
Ba	0.037	0.11	La	0.0058	0.017	Sm	0.0014	0.0043
Be*	0.20	0.60	Li	0.086	0.26	Sn	0.029	0.086
Bi	0.0029	0.0086	Lu	0.00058	0.0017	Sr	0.072	0.22
Ca	5.76	17.3	Mg	1.44	4.32	Tb	0.00058	0.0017
Cd*	0.026	0.077	Mn	0.017	0.052	Th	0.0014	0.0043
Ce	0.00058	0.0017	Mo*	0.096	0.29	Ti	0.058	0.17
Co	0.012	0.035	Na	28.8	86.4	Tl	0.00072	0.0022
Cr	0.058	0.17	Nb*	0.028	0.083	Tm	0.0014	0.0043
Cs	0.0014	0.0043	Nd	0.00058	0.0017	U	0.00072	0.0022
Cu	0.086	0.26	Ni	0.043	0.13	V	0.0086	0.026
Dy	0.0023	0.0069	P	1.15	3.46	W	0.0029	0.0086
Er	0.00086	0.0026	Pb	0.0058	0.017	Y	0.0012	0.0035
Fe	0.058	0.17	Pr	0.00086	0.0026	Yb	0.0012	0.0035
Ga	0.020	0.060	Pt*	1.47E-06	4.41E-06	Zn	0.12	0.35
Ge*	0.058	0.18	Rb	0.035	0.10	Zr	0.072	0.22

*IDL and LOQ estimated using real sediment samples in this study, for cases where samples had concentration levels with RSD values close to or higher than 25%.

Table 5.7 shows concentrations of elements with descriptive statistical values for all sediment samples collected from Trondheimsfjorden. Concentrations below LOD were not included when doing calculations.

Table 5.7: Concentrations with percentage detection rates of elements in sediment from Trondheimsfjorden. Concentration units are in µg/g dry weight.

Trondheimsfjorden	(n = 64)										
Element	Mean	Median	Max	Min	%Detection rate	Element	Mean	Median	Max	Min	%Detection rate
Ag	0.35	0.34	1.09	0.041	95.3	Mo	0.31	0.25	0.91	0.098	85.9
Al	2.99E+4	2.95E+4	3.96E+4	1.95E+4	95.3	Na	7199	6796	1.26E+4	5100	95.3
As	12.9	5.31	90.5	3.41	95.3	Nb	0.091	0.090	0.14	0.046	95.3
Au	0.0085	0.0069	0.048	0.0023	92.2	Nd	24.5	24.3	29.2	17.4	95.3
B	26.3	24.3	59.0	15.5	95.3	Ni	40.6	40.3	52.9	29.6	95.3
Ba	167	154	778	91.7	95.3	P	654	626	1277	491	95.3
Be	0.79	0.74	2.42	0.45	95.3	Pb	31.7	24.9	113	9.84	95.3
Bi	0.60	0.44	2.57	0.17	95.3	Pr	6.37	6.33	7.57	4.57	95.3
Ca	1.61E+4	1.53E+4	5.07E+4	1.26E+4	95.3	Pt	3.00E-04	2.32E-04	0.0017	4.41E-06	48.4
Cd	0.16	0.079	1.08	0.041	93.8	Rb	52.0	50.9	75.8	28.5	95.3
Ce	54.9	54.2	66.0	39.1	95.3	S	2303	1009	1.62E+4	716	95.3
Co	13.1	11.6	28.0	9.00	95.3	Sb	0.047	0.036	0.13	0.017	95.3
Cr	95.7	96.1	113	70.0	95.3	Sc	5.92	5.98	6.93	4.48	95.3
Cs	2.65	2.59	3.95	1.41	95.3	Se	0.63	0.35	3.40	0.14	90.6
Cu	47.3	32.3	220	15.7	95.3	Si	1970	1932	2666	1541	95.3
Dy	3.84	3.86	4.43	2.92	95.3	Sm	4.86	4.77	5.93	3.53	95.3
Er	2.10	2.11	2.34	1.65	95.3	Sn	0.61	0.59	1.04	0.26	93.8
Fe	3.68E+4	3.40E+4	7.05E+4	2.54E+4	95.3	Sr	80.5	77.0	184	67.4	95.3
Ga	8.83	8.71	11.5	6.79	95.3	Tb	0.64	0.64	0.75	0.49	95.3
Ge	0.41	0.37	1.62	0.16	95.3	Th	6.90	6.86	9.12	4.44	95.3
Hf	0.30	0.30	0.38	0.22	95.3	Ti	645	641	782	512	95.3
Hg	0.099	0.097	0.16	0.018	95.3	Tl	0.48	0.42	1.28	0.26	95.3
Ho	0.74	0.75	0.81	0.58	95.3	Tm	0.30	0.30	0.33	0.23	95.3
Ir	nd	nd	0	0	0.00	U	1.48	1.48	1.77	1.11	95.3
K	9720	9510	1.47E+4	5566	95.3	V	77.5	75.2	124	56.7	95.3
La	25.7	25.7	30.8	18.2	95.3	W	0.013	0.012	0.031	0.0059	95.3
Li	25.8	25.6	36.5	16.0	95.3	Y	20.8	21.0	23.5	16.5	95.3
Lu	0.26	0.26	0.30	0.20	95.3	Yb	1.94	1.90	5.26	1.49	95.3
Mg	1.50E+4	1.47E+4	2.02E+4	1.03E+4	95.3	Zn	129	95.2	525	52.5	95.3
Mn	441	439	617	320	95.3	Zr	11.9	12.0	18.1	5.02	95.3

Table 5.8 shows selected elements from table 5.7 with concentrations and percentage detection rates in sediment samples.

Table 5.8: Concentrations with percentage detection rates of selected elements in sediment samples from Trondheimsfjorden. Concentration units are in µg/g dry weight.

Trondheimsfjorden	(n = 64)				
Element	Mean	Median	Max	Min	%Detection rate
As	12.9	5.31	90.5	3.41	95.3
Cd	0.16	0.079	1.08	0.041	93.8
Cr	95.7	96.1	113	70.0	95.3
Cu	47.3	32.3	220	15.7	95.3
Hg	0.099	0.097	0.16	0.018	95.3
Ni	40.6	40.3	52.9	29.6	95.3
Pb	31.7	24.9	113	9.84	95.3
Se	0.63	0.35	3.40	0.14	90.6
Zn	129	95.2	525	52.5	95.3

Figures 5.15 and 5.16 show the distributions of the selected elements in sediment samples from the different sampling stations. Values below LOD for each element target analyte were removed prior to calculations.

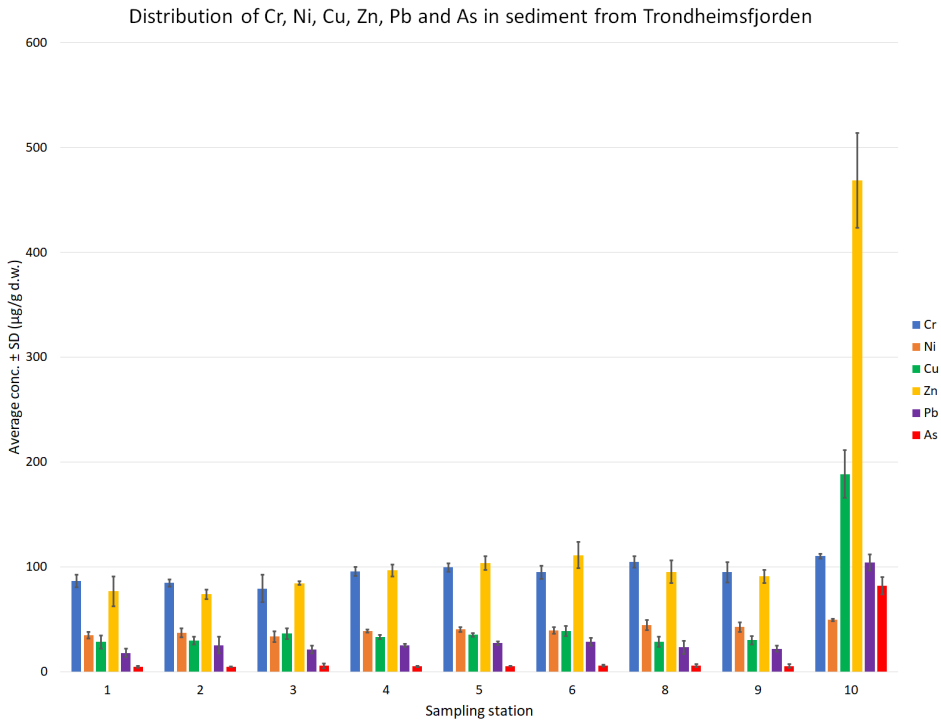

Figure 5.15: Distribution of chromium, nickel, copper, zinc, lead and arsenic in sediment samples from different stations in Trondheimsfjorden.

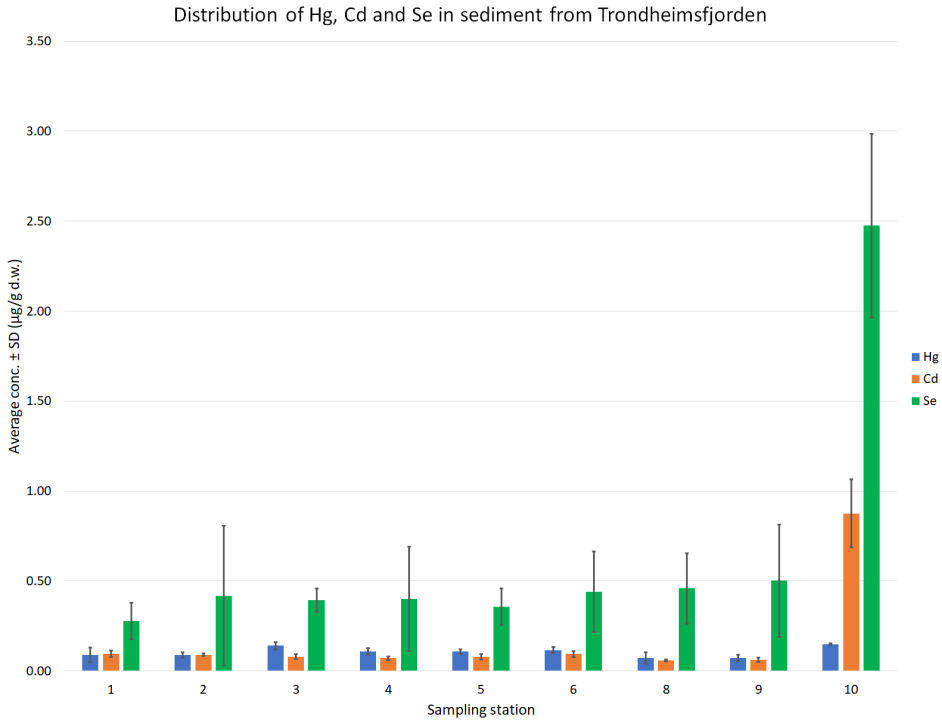

Figure 5.16: Distribution of mercury, cadmium and selenium in sediment samples from different stations in Trondheimsfjorden.

5.5 Total organic matter content in sediment samples

Table 5.9 shows data acquired from LOI procedures performed on the 64 sediment samples collected from Trondheimsfjorden. Three replicates of one extra sample yielded loss of organic matter which yielded a % RSD of 2.56, as shown in table 5.10.

Table 5.9: Percentage total organic matter content for all sediment samples from Trondheimsfjorden.

Sample no.	% Organic matter content	Sample no.	% Organic matter content	Sample no.	% Organic matter content	Sample no.	% Organic matter content
1	2.63	17	2.81	33	2.67	49	2.73
2	2.06	18	3.09	34	2.43	50	2.26
3	2.24	19	2.94	35	2.56	51	2.22
4	2.00	20	2.55	36	2.47	52	2.29
5	1.97	21	2.72	37	2.46	53	2.77
6	2.96	22	4.80	38	2.73	54	2.71
7	2.38	23	2.62	39	3.06	55	2.71
8	2.18	24	2.62	40	2.70	56	2.66
9	2.69	25	2.73	41	2.66	57	2.80
10	2.23	26	2.80	42	2.69	58	2.42
11	2.82	27	2.89	43	2.90	59	3.23
12	3.05	28	2.72	44	3.14	60	3.46
13	3.05	29	3.68	45	2.99	61	3.27
14	2.71	30	2.88	46	3.00	62	3.57
15	3.23	31	3.21	47	3.71	63	2.96
16	3.58	32	2.84	48	2.92	64	2.90

Table 5.10: Data from loss on ignition for extra samples.

Sample no.	% Organic matter content
17 (extra sample).1	2.70
17 (extra sample).2	2.77
17 (extra sample).3	2.84
	%RSD = 2.56

Figure 5.17 shows the percentage total organic matter content in sediment samples at different sampling stations.

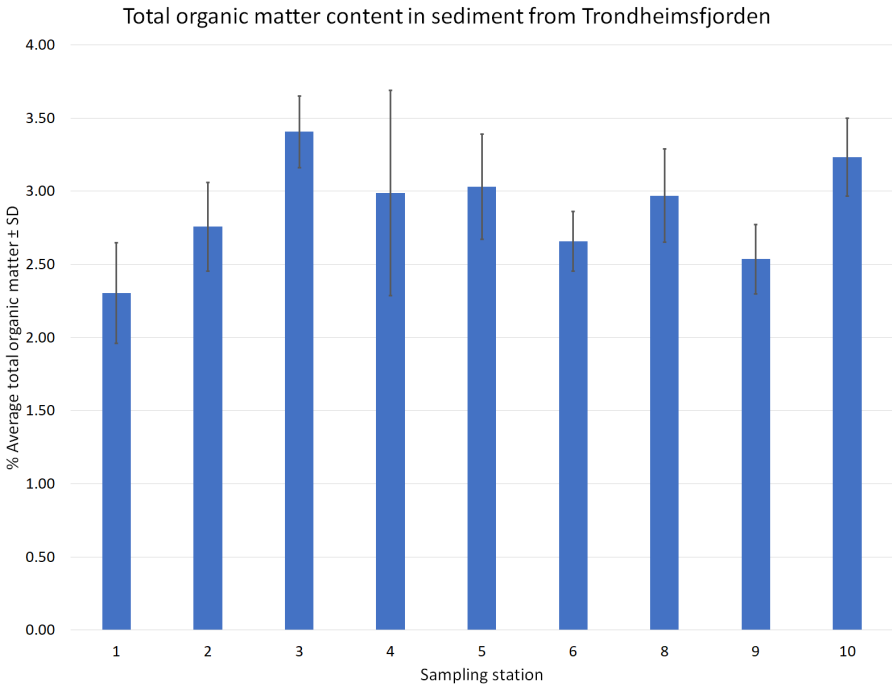

Figure 5.17: Total organic matter found in sediment samples at different sampling stations from Trondheimsfjorden.

5.6 Principal component analysis

Principal component analysis biplots and correlation plots for all organic and inorganic target analytes are given in Appendix I. Plots were constructed based on calculated target analyte concentrations, percentage total organic matter, sampling station number, sampling depth, and the size and placement (top/middle/bottom) of sediment sample sections. All concentration values used were scaled to units of ng/g, and values below LOD for each target analyte were removed prior to constructing the plots.

Chapter 6

Discussion

6.1 Method testing for bisphenol and benzophenone determination

Table C.2 shows the percursor and product ions found for bisphenols and benzophenones during method testing. Bisphenol M and bisphenol P, being two structural isomers, were not able to be separated in the chromatograms, as the same split peaks appeared for both compounds at their respective product ions with m/z 330.2 and 133.0 (see e.g. figure D.5). Given that these bisphenols were not expected to be found in large amounts in sediments, it was chosen to integrate the split peaks for the two compounds together as one using m/z 330.2 for further determination of bisphenols in all sediment samples (hence the naming BPM&BPP).

The choice of either method for BPs/BzPs determination in all sediment samples would be based on the recoveries and matrix effects associated with the method. A recovery closer to 100 percent and a matrix effect closer to 0 percent for a given target analyte would indicate the method being more optimal for the extraction of the analyte. Figure 5.1 and figure 5.2 both indicate that absolute recoveries of BPs and BzPs are generally higher when using the method from Asheim et al. On the other hand, the same figures show that relative recover-

ies of BPs and BzPs mostly were higher when employing the method adapted after Yu et al. The higher absolute recoveries associated with the method from Asheim et al. implies better extraction efficiencies of BP and BzP analytes, but the relative recoveries were lower than for the method by Yu et al. This implies the latter method showing a better compensation of losses when employing the BPA d16 internal standard. However, given that the BPA-d16 standard was also ultimately omitted in favor of using $^{13}C_{12}$ isotopically labelled bisphenol standards for further determination of BPs and BzPs in sediment samples, further discussion will not be made here.

The matrix effects for BPs as seen in figure 5.3 show that all BPs, with the exception of the compound BPS, had lower calculated matrix effects with the method adapted after Asheim et al. Matrix effects for BzPs were more varying, but generally showed a lower magnitude for BzPs with the method by Yu et al. The results here are however not reported with standard deviations. The real ME values may therefore be significantly different from the ones calculated with the available data.

Based on the results from the method testing, the method by Asheim et al. was ultimately chosen for further work on BPs and BzPs determination in sediment samples. This choice was based on the high absolute recoveries, as well as the acceptable MEs observed for the target analytes.

During testing of both methods, it was discovered that signals associated with different target analytes in the regular samples were relatively low or in some cases non-existent when compared to signals measured for blank samples. For this reason, it was also decided to increase the mass of sample portions from 0.1 to 0.5 grams for further BPs and BzPs determination in all sediment samples. It was estimated that this would sufficiently increase the analyte signals while not decreasing recoveries or increasing the magnitude of matrix effects (due to the incorporation of more sample material) too much when employing the same LC-MS/MS method as used during method testing.

6.2 Determination of bisphenols and benzophenones in sediment from Trondheimsfjorden

Recoveries of BPs are shown in figure 5.9. The results show that most BPs had absolute recoveries between 60 - 80%, which is lower than than what was found during testing of the same method. The range can still be considered within acceptable range for the purpose of this project. Relative recoveries were mostly between 100 - 120%, which reflects the compensation of loss using the $^{13}C_{12}$ isotopically labelled bisphenol standards. Recoveries of BzPs are shown in figure 5.10. Absolute recoveries appears to be more in the 40 - 80% region, while relative recoveries are in the 60 - 110% range.

The results indicate that absolute recoveries of both BPs and BzPs were lower than what was found during method testing of the method adapted after Asheim et al. Relative recoveries remained stable or increased for the analytes however, especially for BPs and also for BzP-1 and 4-OH-BzP. The increase of sample size from 0.1 to 0.5 grams is a possible cause for lowered absolute recoveries of both BPs and BzPs. On the other hand, these losses seem to be well compensated by the internal standards as reflected by the relative recoveries.

The results also show that recoveries of BzPs were generally lower than for BPs with the method adapted after Asheim et al. for sediment sample extraction. Loss of target analyte during sample preparation procedures can occur at all steps involved, such as the vortex-mixing, ultrasonication, solid phase extraction and N_2-gas evaporation. Higher affinity to sediment sample matrix, more retention in the SPE-packing material and/ or a larger degree of evaporation can all be possible causes for the lower recoveries of BzPs compared to BPs. For future work, a method more suited for the extraction of benzophenones should be considered through more focus on method development and optimization, but this is beyond the scope of the current project.

Matrix effects for BPs and BzPs are shown in figure 5.11. For BPs, most MEs were in the range of -26% to -36%. The negative MEs stand as opposite to the positive ones found during method testing. The

true reason remains unknown, although the increase of sample size or other causes related to preparation of matrix match samples could have had an effect. Two notable exceptions occurred, being BPF with ME of -12.0% and BPS with ME of -58.6%. The lack of functional groups in the BPF molecule is a propable cause of the relatively low matrix effects associated with this compound. On the other hand, the presence of the relatively reactive sulfonyl group in BPS could be the cause of this compound experiencing a higher matrix effect than the other bisphenol target analytes [58]. For benzophenones, MEs were generally in the approximate range of 20 - 30% with one clear exception being benzophenone-2, which experienced the ME of largest magnitude among all target analytes with a value of -70.4%. This is likely due to BzP-2 being the only target analyte with four hydroxyl groups present, which then suggests it is the most reactive towards sample matrix components. The MEs found for benzophenones during method testing were of markedly lower magnitude. The smaller sample sizes as well as the higher spiked concentrations in the matrix match samples could have contributed to the larger MEs observed during testing.

Quantification of BPs and BzPs was done by employing $^{13}C_{12}$ isotopically labelled bisphenol standards. In cases where such isotopically labelled standards were not available as direct analogues of target analytes, the internal standards were chosen to closest match the retention time of the target analytes. Spiked samples were also employed for constructing calibration curves in these cases, as described in chapter 2. Ideally, isotopically labelled standards should be available for all the different target analyte analogues. Due to cost and availability however, this was not the case for this project. Quantification using the calibration curves were performed by setting the intercept equal to 0 when quantifying all BP and BzP target analytes. This was done as slight variations in calibration points with higher concentrations may significantly alter the intercept of the curve along the Y-axis, thereby influencing lower concentration levels.

Table 5.1 show that most target analytes had similar detection and quantification limits, which were found to be at the lowest concentration levels in the calibration standard solutions. This suggests that the use of MS as means of detection during analysis proved to be well

suited for the target analytes. The two exceptions were BPA and BPF which had LODs and LLOQs at the second lowest calibration standard solution concentration levels. This suggests a somewhat lower sensitivity for these compounds, although the limits can still be considered relatively low.

Results showed that most bisphenol and benzophenone target analytes could be detected in blank samples. Blank sample contamination has been reported and recognized in previous studies [8, 10, 55]. The ubiquitous presence of these compounds in plastic products, presumably also in various plastic-based laboratory equipment could explain their presence in blank sample extracts. Contamination could also have occurred from cross-contamination during steps such as N_2 evaporation [55]. The use of blank sample signal subtraction to compensate for contamination in samples serves to yield more accurate results and is routinely done in studies where BPs and BzPs are detected in blank samples. However, contamination should ideally be avoided as much as possible through different measures such as using clean laboratory equipment.

Mean concentrations of BPs and BzPs are shown in table 5.2 and 5.3, respectively. The concentrations found for benzophenones in sediment from Trondheimsfjorden are similar to what has been found for the same target analytes in sediment samples from U.S. rivers by Zhang et al. [13]. In that study, mean concentrations between BzP-1, BzP-2, BzP-3, 4-OH-BzP and BzP-8 were in the range of 0.424 - 2.65 ng/g dry weight. Baron et al. also found BzP-3 in the range of nd - 2.96 and nd - 5.38 ng/g dry weight in sediments from Chile and Colombia, respectively, which is similar in range for BzP-3 found in this project [16]. On the other hand, Tsui et al. found BzP-1, BzP-3 and BzP-8 in the median ranges of 1.6 - 2.3, 2.5 - 8.6 and 8.4 - 16.2 ng/g dry weight, respectively, for samples from Hong Kong and Tokyo Bay [17]. This is considerably higher than the median values found for these target analytes here.

The concentrations of bisphenols in this project can also be compared with previous studies. Liao et al. have reported the occurrences of BPA, BPAF, BPAP, BPB, BPF, BPP, BPS and BPZ in sediment from US, Japan and Korea earlier [5]. The authors found levels of

BPA in U.S. sediments at mean and median concentrations of 5.14 and 1.49 ng/g dry weight, respectively. BPF was found in the same U.S. sediments at mean and median concentrations of 3.24 and 1.44 ng/g dry weight. BPS was found at a mean concentration of 0.21 ng/g dry weight. This is similar to what has been found of BPA, BPF and BPS in this project (table 5.2). The absence of BPAF, BPAP, BPB and BPP as seen in this project was also reported by Liao et al. with similar trends for the sediment samples from U.S., Japan and Korea. However, the levels of BPs in sediment from Japan and Korea in the same study were also found at higher levels overall with larger variations in concentrations. It is also interesting to note that the samples from U.S., Japan and Korea had mostly non-detectable levels of BPZ, with the highest % detection rate being 2.9% for Korean sediment samples. This is contrary to what has been found for sediment from Trondheimsfjorden in this project, where BPZ could be detected in 46.9% of the samples analysed.

With respect to the Norwegian screening programme, previously reported concentrations of BPA and BPF in Oslofjorden was considerably higher than what was found in Trondheimsfjorden in this project [19]. BzP-3 could not be detected in the screening programme either, which is contrary to current results. The bisphenols were only quantified in two out of five samples however, and while the findings could suggest a difference in the occurrence of the target pollutants between norwegian regions, further studies should be conducted to produce more comparable findings here.

Of all BPs, BPA and BPF were the dominating analogues in sediment from Trondheimsfjorden, each being detected in approximately 80% of sediment samples at the highest mean and median concentrations. BPA and BPF also accounted for more than 90 percent of the total distribution of BPs (figure 5.8). The predominance of these two compounds among bisphenol target analytes was also found for sediment from U.S., Japan and Korea by Liao et al. [5]. In the case of BzPs, benzophenone-1, benzophenone-2 and benzophenone-3 were found in most samples from Trondheimsfjorden, with BzP-3 having a detection rate of 98.4%. BzP-3 was also found to be the most predominant compound among target BzPs. This can be explained based on BzP-3 being one of the most commonly used derivatives among benzophe-

nones for different applications.

It is interesting to note that all benzophenone target analyte compounds could be detected in the sediment samples from Trondheimsfjorden. Given that the samples were acquired in late May, which is within the Norwegian summer season, a plausible contributing factor in the presence of these compounds could be the seasonally increased used of sunscreen products and similar where BzPs are employed as ingredients or additives. This correlation would however have to be evaluated further by the study of consumer product ingredients and the usage patterns of these in Norway throughout the year. The acquisition of new sediment samples in the same sampling locations as used in this project during different seasons such as the winter season should also be considered for further studies here.

The comparison of BPs and BzPs occurences above suggests that similar patterns of occurrence between Trondheimsfjorden and some countries for the compounds that are reported exists. This similarity between sediment from Trondheimsfjorden and the U.S. can especially be noted, as both BP and BzP target analytes were found at similar concentration ranges.

6.3 Determination of polycyclic aromatic hydrocarbons in sediment from Trondheimsfjorden

Recoveries of PAHs are shown in figure 5.14. Absolute recoveries ranged from 70.9 - 118%, while relative recoveries using F-PHE as internal standard ranged from 91.6 - 133%. The recovery values for the PAH target analytes are considerably high, and suggests that the use of ASE for extracting PAHs from the sediment samples proved to be effective. No PAH target analytes could be detected in reagent blank samples prepared. This also suggests that background contamination of samples during laboratory work was kept to a minimum or that background levels of PAHs are relatively low, atleast in comparison to the concentration levels found in sediment samples.

Figure F.1 shows three representative raw chromatograms (without post-integration performed manually) from the analysis of sediment sample extracts using HPLC-FLD. Both chromatogram a) and b) shows the recurring pattern observed of baseline drift and peak overlap for several peaks. This complicated peak integration and was the reason for which manual post-integration adjustments were made in most sample chromatograms. The use of manual integration is generally not recommended and should be avoided by using optimized software integration parameters. However, by utilizing manual correction, peaks could in most cases be integrated in a similar manner. The overlapping peaks and drifting baselines show that the analysis of sediment sample extracts using HPLC-DAD/FLD could potentially be improved through more focus om method development and optimization. This is especially the case for the baseline drift observed, as the use of many excitation and emittance wavelengths with FLD could be a possible cause [65].

F-PHE was chosen as the internal standard both for constructing calibration curves for PAH target analyte quantification as well as for RRT calculations. This was due to the relatively stable and non-overlapping peak seen for this compound in both sample and standard solution chromatograms. Areas for the peak were also relatively high, which suggests a high detector sensitivity for the compound. Calculations using the calibration curves were performed with the intercept set equal to 0. This was done for similar reasons as described with quantification of BPs and BzPs in sediment samples (see section 6.5).

Limits of detection and quantification for PAHs are shown in table 5.4. For all target analytes listed, the limits were found to be at the lowest concentration of the calibration standard solutions. This suggests that the FLD detector employed for detection of the target analytes was well suited, which is in accordance with its reported sensitivity for PAHs [41]. Of all PAH target analytes, 11 out of the 16 compounds were quantified in sediment samples, with the six exclusions being ACE, FLU, ACY, BGP and IND. ACE and FLU could only be detected in one and two samples respectively, while ACY, which is not detectable with FLD, could not be found in any sample DAD-chromatograms. The two compounds BGP and IND could not be found during recovery studies of PAHs in spiked samples and matrix match samples and was

on this basis excluded from further data processing.

The percentage distribution of individual PAHs in sediment based on the total sum of PAHs is shown in figure 5.13. There appears to be a trend where, with the exception of DBA, heavier PAH analogues make up a larger fraction than other analogues of lower mass. The compounds FLT, PYR, BaA, CHR, BbF, BKF and BaP that contribute to this trend also have higher reported octanol-water partition coefficients as shown in table 2.3. This suggests that these PAHs are bound to the sediment masses to a larger extent than the other PAHs with lower hydrophobicity, which will probably be more soluble in the surrounding water column.

By comparing values from table 5.5 with the PAH classification system given in table 2.4, most individual PAH concentration levels found in the sediment samples correspond to Class II (good) levels. This is also seen for the sum of PAHs compared to the $\sum$PAH-16 classification values. The comparison shows that current contamination of PAHs in Trondheimsfjorden is at a relatively low level compared to historical values where Class IV and V values have been reported [3]. This apparent decrease is also consistent with the reported general decrease of PAH emissions in Norway [66]. It should however be noted that the presence of BGP and IND could not be assessed in this project due to inconclusive recovery studies for these compounds. A full evaluation of the 16 EPA PAH compound occurrences may therefore not be carried out here, and comparing the sum of PAHs from table 5.5 with classification values may strictly speaking not be done directly.

6.4 Determination of elements in sediment from Trondheimsfjorden

Concentrations of all elements determined in the sediment samples are listed in 5.7. Almost every element could be detected in the samples, with one clear exception being iridium, which was not detected in any samples. The absence of iridium can be explained by it being one of the most rare elements occurring in nature [67]. Given the historical pollution in Trondheimsfjorden as well as the persistence of elements

in sediment, the high detection rates of elements in the samples may be considered as expected.

Figure 5.15 and 5.16 shows the average concentrations of selected elements at the different sampling stations. A notable trend can be observed where several elements have higher concentrations in sediment samples from station 10 compared to all other stations. The PCA biplots also show this pattern, with elements clustering closer to samples from station 10 along the PC1 axis (see figure I.1). This is however not as evident for all elements, such as Cr and Ni. A possible explanation for the general increased accumulation of elements at station 10 could be a higher degree of ship traffic and related activities near this station [68]. The causes of the different degrees of increased concentration at station 10 between the elements remains unknown however, which suggests this could be an area worthy of further studies.

The current concentration levels as given by mean and median values in table 5.7 appear to be similar to what has been reported in 2011 for the Høvringen area [69]. This applies to the elements As, Pb, Cd, Cu, Hg and Zn which were examined and found at similar concentrations. Levels of Ni and Cr appear to have been somewhat increased based on current values. However, this could be due to variations in sampling locations, means of analysis or similar. It should be mentioned that the concentration levels of elements reported in 2011 in the harbour areas close to the cities were considerably higher than in the Høvringen area, and that these study areas laid the foundation for the "Renere havn" project initiated in the following years [2, 3].

6.5 Determination of total organic matter content in sediment from Trondheimsfjorden

Percentage total organic matter content in sediment samples from Trondheimsfjorden is shown in table 5.9. Organic matter content were in the range of 1.97% - 4.80 %. Although total organic matter will give a rough estimate of the total organic carbon present, the values found

in Trondheimsfjorden are similar to previous finds in another fjord in northern parts of Norway, where Sauer et al. found the average C_{org} to be 2.54% (weight%) in upper layer sediments [70]. With regards to the reproducibility of the loss-on-egnition method (see table 5.10), the use of loss-on-ignition for total organic matter content determination in sediment samples is considered satisfactory for the purpose of this project.

6.6 Principal component analysis

Principal component biplots where sediment samples were grouped according to their sampling stations can be seen in figures I.1 and I.2. Samples from station 1 and 2 appear to be somewhat more clustered together to the left in the plot, while samples from station 8 and 9 appear to be more clustered around the low-mid area. This could suggest that samples from these stations are somewhat different from each other in areas close to Høvringen. The differences between samples from these stations are however somewhat subtle and not as evident as the difference between samples from station 10 and all other stations. All data points for station 10 are clustered together to the top-right of the plot, which strongly suggests that samples from this station have more unique expression profiles.

For all biplots, the loadings yielded a PC1 (Dim1) variation of 36.5% and a PC2 (Dim2) variation of 16.1%. As seen in figure I.1, several elements appear to be grouped to the right along PC1. This suggests that these elements are contributing most to sample variations along the first PC. A few elements, namely Ag, Sn, Mb, Hg and W appear to be grouped together further out along PC2, which indicates that these elements are contributing more to variations on the second PC. Among organic target analyte loadings seen in figure I.2, PAHs appear to be influencing PC2 the most, while BP and BzP target analytes are mostly centered in the middle of the plot and do not appear to be influencing variations considerably among any of the principal components.

Both biplots show that sediment samples within stations are spread along PC1 and PC2 with the exception of station 5 samples and pos-

sibly station 3 samples (only two samples were acquired). This spread indicates that variations between samples from the same station is mostly influenced by the presence of elements and PAHs at different levels in the samples. On the other hand, sediment samples from station 10 are more clustered together to the right along the PC1 axis as noted earlier. This suggests that sediment samples from this station are different from samples acquired at other stations, which based on the loadings is mostly due to the presence of elements. Indeed, both figure 5.15 and 5.16 show that several elements with large influence on PC1 variations are at higher concentration levels at this station compared to other stations.

Figures I.5 and I.6 show biplots where sediment samples are grouped based on the section of sediment block from which they were sampled (see figure 4.1). The data points show the same spread along both axes on the graph as in other biplots. However, as the samples from the top, middle and bottom sediment sections appear to mostly be grouped together across PC1 and PC2 in the middle of the plot (except for samples at station 10), there appears to be little to no variations between sediment samples that were taken from different sediment block sections regardless of sampling stations. This suggests that although concentrations of elements and PAHs are mostly influencing the variations along the principal components, and therefore the samples, the samples along the depth of sediment material are mostly similar to one another between stations. It should however be noted here that the sediment sections were roughly estimated with a ruler during the sampling campaign, and that the actual sizes of the sediment sections along with their vertical depth were quite varying, as seen in table J.1.

Figures I.3 and I.4 shows the biplots where sediment samples are grouped according to the depth from which they originated. It can be noted that the depth increase seems to go from left to right along PC1 along with data points for samples from the different stations. However, as the sampling station depth are not evaluated with any other sediment sample variables in this project, these biplots are not considered highly informative and will not be discussed further.

Figure I.7 shows the correlation plot containing all elements which were included in this project. The plot show that most of the elements selected for further study appear to be positively correlating with one another. This can be shown for the elements Ni, Cr, Se, Cd, As, Zn, Cu and Pb. In the case of mercury however, this element appears to possibly be somewhat negatively correlated with nickel and chromium, but the correlation uncertainty undermines this observation. The plot seem to suggest that Ni, Cr, Se, Cd, As, Zn, Cu and Pb could have similar sources of input into Trondheimsfjorden, as their positive correlations are significant.

Figure I.8 shows the second correlation plot with organic target analytes. Aside from the more evident positive correlations between target analytes of the same pollutant classes (BPs, BzPs, PAHs), most correlations between different target analytes appear to be non-significant as noted with the X-marks. There does however appear to be some correlations worth noting. This includes the somewhat positive correlations seen between BPA and BzP-3, as well as BzP-3+4-OH-BzP. These correlations could potentially indicate that these BP and BzP analogues have a similar source of input into Trondheimsfjorden. The slight positive correlations between total organic matter (abbreviated POM in the PCA plots) and several of the PAHs can also be noted, and this correlation would be expected based on PAHs having a mostly lipophilic character. They should therefore be more bound to sample material containing a larger fraction of organic matter [71].

It is to be mentioned that while correlation patterns of various significance can be noted from both the biplots and the correlation plots, the combined variations of PC1 and PC2 account for 52.6% of total variations in the dataset. The two principal components should ideally account for as much of the variation in the dataset as possible. Fully conclusive remarks based on the PCA analysis are therefore difficult to make here, and the correlations that have been discussed so far should be interpreted with care.

6.7 Samples acquisition and locations in Trondheimsfjorden

The choice of sampling locations for station 1-9 was due to their close priximities to a wastewater treatment plant in the Høvringen area. This would provide samples with characteristics that could be described by nearby wastewater treatment processes close to the fjord. Sampling was also done further out in the fjord at station 10 with the intention of obtaining background data that could be used to compare levels with other samples from the other stations. However, although station 10 is located further out from the treatment plant area, it is located in more open water where boats may be travelling more frequently. Indeed, figure 1.1 shows that several ship routes are marked very close in proximity to this station. As has been discussed earlier, ship related activity could have had an impact on the local environment around this location when considering the higher trace element levels. On the other hand, such a correlation cannot be seen when considering levels of organic target analytes at this station. The choice of station 10 as location to acquire sediment may still not have been the most optimal choice, and as such, new location(s) for acquiring sediment samples with background contamination levels should be reconsidered for any future work with new sampling campaigns.

Figure 1.2 shows that most samples collected for this project were centered around one area of Trondheimsfjorden. It would also be of interest to carry out new sampling in other areas around the fjord. In particular, sampling locations close to the Ladehammeren wastewater treatment plant located on the other side of the fjord would be of interest for further study. This treatment plant utilizes chemical treatment of incoming wastewater through flocculation processes [72]. A comparison of target pollutant levels between sediment close to the Høvringen treatment plant and the Ladehammeren treatment plant could potentially give more insight into the input sources of the different target chemicals.

Chapter 7

Conclusions

Marine sediment samples were successfully acquired from Trondheimsfjorden and characterized based on various parameters related to sampling locations and physical sediment sample properties. Methods for sample extraction and the determination of selected bisphenols, benzophenones, polycyclic aromatic hydrocarbons and trace elements were further characterized based on recoveries, matrix effects and limits of detection. The extraction and determination of target analytes proved satisfactory based on acceptable recoveries and matrix effects, as well as adequately low limits of detection and quantification. However, the protocols for benzophenone extraction and PAH determination did show potential for improvement. Further method testing should be considered for more accurate determination of these compounds using LC-MS/MS and HPLC-FLD.

Results showed that several target analytes could be detected and quantified in the sediment samples. Occurrences of bisphenols, benzophenones, polycyclic aromatic hydrocarbons, trace elements and total organic matter were comparable with previous studies. Principal component analysis biplots indicated that variations between samples collected from different sampling locations were mostly influenced by the presence of trace elements and polycyclic aromatic hydrocarbons. No clear differences could be observed between samples collected from different sediment block sections. Possible correlations between organic target analytes in sediment samples were observed from correlation

plots. Further studies should be conducted to evaluate these correlations. New sampling locations along with current ones, other seasons for sampling through the year and the use of equipment for more accurate sampling of the sediment material should also be considered for new sampling campaigns in Trondheimsfjorden.

Appendix A

Acronyms

APCI Atmospheric Pressure Chemical Ionization

APPI Atmospheric Pressure Photoionization

ASE Accelerated solvent extraction

d.w. dry weight

ESI Electrospray ionization

FLD Fluorescence detection

HPLC High Performance Liquid Chromatography

IS Internal standard

LC Liquid chromatography

LSE Liquid-solid extraction

LC-MS/MS Liquid chromatography-tandem mass spectrometry

LOD Limit of detection

LOI Loss on ignition

LOQ Limit of quantification

LLOQ Lower limit of quantitation

ME Matrix effect

MeOH Methanol

MF Matrix factor

MM Matrix match

MP Mobile phase

MRM Multiple reaction monitoring

MS Mass spectrometry

m/z Mass-per-charge ratio

nd Not detected

RP Reversed-phase

RRT Relative retention time

RT Retention time

SPE Solid-phase extraction

SRM Selected reaction Monitoring

TQ Triple Quadrupole

UPLC Ultra Performance Liquid Chromatography

UV Ultraviolet

v/v volume/volume

Appendix B

Molecular structures of bisphenol target analytes, benzophenone target analytes and bisphenol internal standards

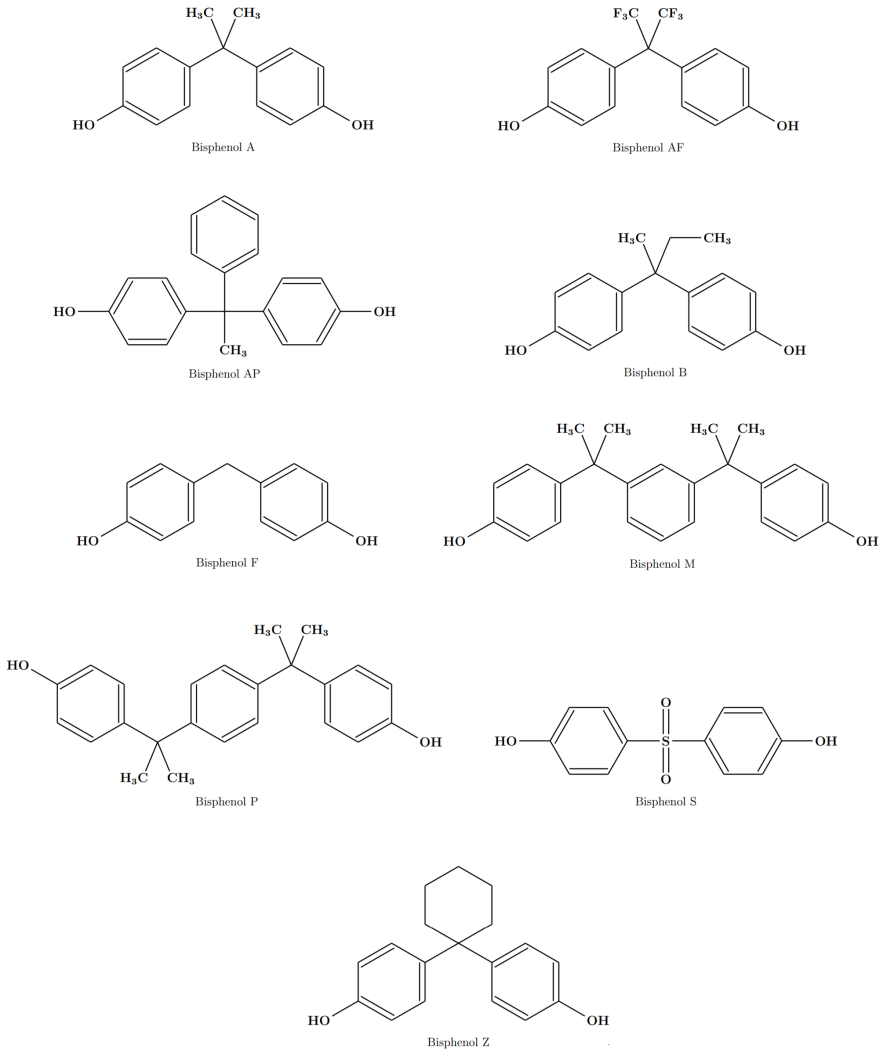

Figure B.1: Bisphenol target analytes included in this project.

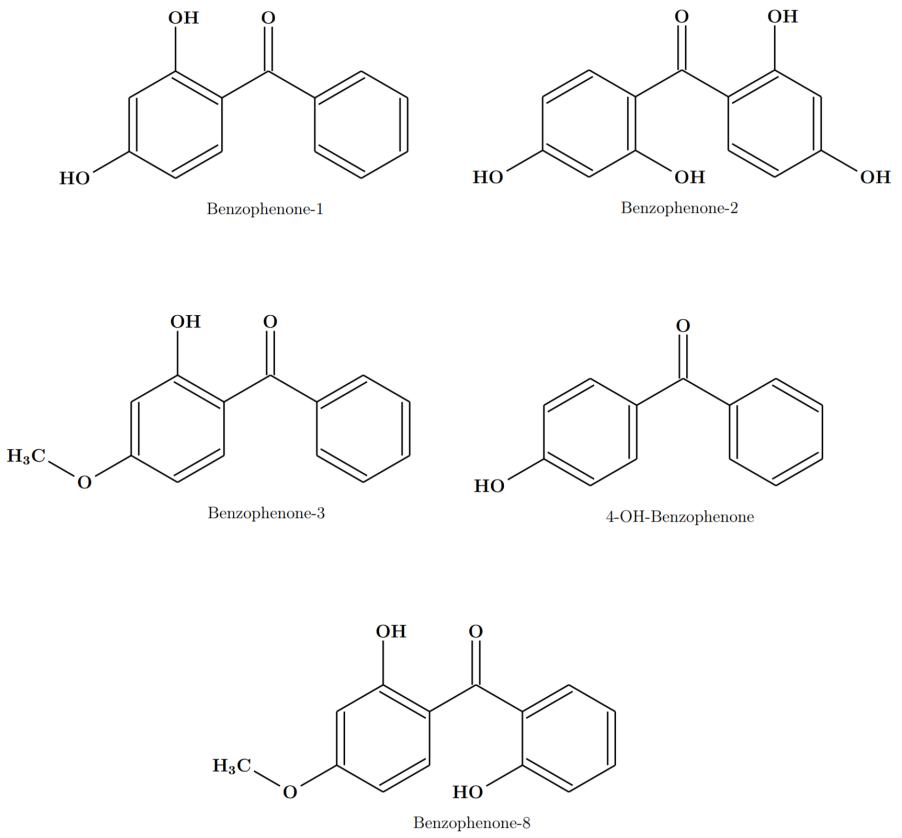

Figure B.2: Benzophenone target analytes included in this project.

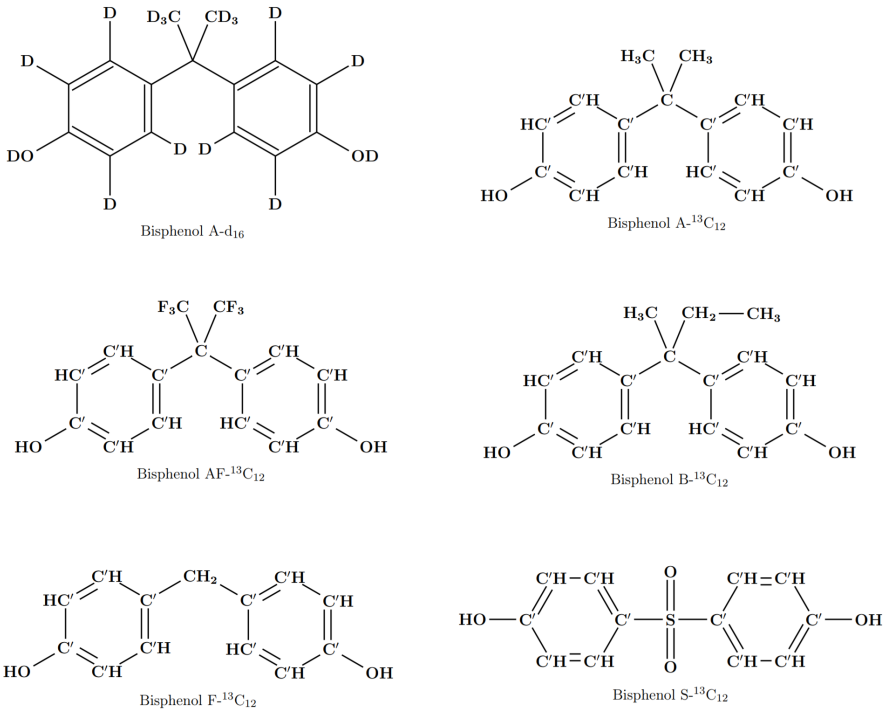

Figure B.3: Bisphenol internal standard compounds included in this project. ^{13}C labeled atoms are indicated with apostrophe signs.

Appendix C

Conditions for bisphenols and benzophenones method testing

Table C.1: Masses weighed of sediment sub-samples portions for bisphenols and benzophenones method testing. All sub-samples were prepared from sample 6 in table J.1.

Adapted method	Sample	Mass weighed (g)
Yu et al.	1, IS spike only	0.1027
	2, Spike 1, 20 ppb	0.1000
	3, Spike 2, 20 ppb	0.1005
	4, Spike 3, 20 ppb	0.1004
	5, Matrix Match 1, 20 ppb	0.1013
	6, Matrix Match 2, 20 ppb	0.1009
Asheim et al.	7, IS spike only	0.1034
	8, Spike 4, 20 ppb	0.1037
	9, Spike 5, 20 ppb	0.1038
	10, Spike 6, 20 ppb	0.0999
	11, Matrix Match 3, 20 ppb	0.1033
	12, Matrix Match 4, 20 ppb	0.1010
	Total sample amount:	12 + two blank samples (no sediment added)

Table C.2: Data from tandem mass spectrometry transitions during method testing.

Compound	Formula/ mass	Transition no.	Precursor ion (m/z)	Product ion(s) found (m/z)	Cone voltage (V)	Collision energy (eV)	Ion mode	Quantifier (m/z)	Qualifier (m/z)
Bisphenol A	C15H16O2	1	227.1	212.1	50.0	18.0	ES-	212.1	133.1
		2	227.1	133.1	50.0	24.0	ES-		
Bisphenol AF	C15H10O2F6	1	335.0	265.1	40.0	24.0	ES-	265.1	177.0
		2	335.0	177.0	40.0	42.0	ES-		
Bisphenol AP	C20H18O2	1	289.1	274.1	20.0	18.0	ES-	274.1	195.1
		2	289.1	195.1	20.0	28.0	ES-		
Bisphenol B	C16H18O2	1	241.1	212.2	20.0	18.0	ES-	212.2	212.2
Bisphenol F	C13H12O2	1	199.1	105.0	46.0	20.0	ES-	105.0	93.0
		2	199.1	93.0	46.0	20.0	ES-		
Bisphenol M&P	C24H26O2	1	345.1	330.2	8.0	46.0	ES-	330.2	133.0
		2	345.1	133.0	14.0	26.0	ES-		
Bisphenol S	C12H10SO4	1	249.0	156.0	40.0	22.0	ES-	108.0	156.0
		2	249.0	108.0	40.0	26.0	ES-		
Bisphenol Z	C18H20O2	1	267.1	173.1	40.0	24.0	ES-	173.1	145.1
		2	267.1	145.1	40.0	36.0	ES-		
Bisphenol A-d16	242	1	241.2	223.2	34.0	20.0	ES-	142.1	223.2
		2	241.2	142.1	34.0	24.0	ES-		
Benzophenone-1	C13H10O3	1	213.0	135.0	46.0	18.0	ES-	135.0	91.0
		2	213.0	91.0	46.0	28.0	ES-		
Benzophenone-2	C13H10O5	1	245.0	135.0	40.0	14.0	ES-	135.0	109.0
		2	245.0	109.0	40.0	16.0	ES-		
Benzophenone-3	C14H12O3	1	227.1	211.1	40.0	20.0	ES-	211.1	183.0
		2	227.1	183.0	40.0	34.0	ES-		
4-OH-BzP	C13H10O2	1	197.0	120.0	60.0	22.0	ES-	92.0	120.0
		2	197.0	92.0	60.0	28.0	ES-		
Benzophenone-8	C14H12O4	1	243.0	123.0	46.0	16.0	ES-	123.0	93.0
		2	243.0	93.0	46.0	18.0	ES-		

Appendix D

Conditions for bisphenols and benzophenones determination in all sediment samples

Table D.1: Masses of bisphenol and benzophenone chemical standard portions weighed for stock solution preparations.

Compound	Mass weighed (g)
Bisphenol A	0.0101
Bisphenol AF	0.0100
Bisphenol AP	0.0100
Bisphenol B	0.0100
Bisphenol F	0.0101
Bisphenol M	0.0103
Bisphenol P	0.0102
Bisphenol S	0.0100
Bisphenol Z	0.0102
Benzophenone-1	0.0102
Benzophenone-2	0.0103
Benzophenone-3	0.0100
4-hydroxybenzophenone	0.0101
Benzophenone-8	0.0100

Table D.4: Retention times and relative retention times of bisphenols and benzophenones.

Compound	RT (min)	RRT
BPS	0.24	0.15
$^{13}C_{12}$ BPS	0.24	0.15
BzP-2	0.24	0.15
BzP-1	0.30	0.19
4-OH-BzP	0.30	0.19
BPF	1.34	0.84
$^{13}C_{12}$ BPF	1.35	0.84
BzP-8	1.43	0.89
BPAF	1.60	1.00
$^{13}C_{12}$ BPAF	1.60	1.00
BPA	1.84	1.15
$^{13}C_{12}$ BPA	1.84	1.15
BPB	2.15	1.34
$^{13}C_{12}$ BPB	2.15	1.34
BPAP	2.30	1.44
BPZ	2.50	1.56
BzP-3	2.53	1.58
BPM&P	3.20	2.00

Table D.2: Masses weighed of sediment sample portions for bisphenols and benzophenones extraction and analysis. Sample numbers are corresponding to the sediment samples listed in table J.1.

Sample	Mass weighed (g)	Sample	Mass weighed (g)	Sample	Mass weighed (g)	Sample	Mass weighed (g)
1	0.5004	23	0.5009	44	0.5022	64B, 10 ppb	0.5002
2	0.5003	24	0.5005	45	0.5001	64A, 25 ppb	0.5010
3	0.5013	25	0.5002	46	0.5004	64B, 25 ppb	0.5001
4	0.5028	26	0.5000	47	0.5021	64A, 50 ppb	0.5013
5	0.5013	27	0.5008	48	0.5019	64B, 50 ppb	0.5009
6	0.5017	28	0.5002	49	0.5008	64 Spike.1, 10 ppb	0.5005
7	0.5019	29	0.5009	50	0.5014	64 Spike.2, 10 ppb	0.5016
8	0.5009	30	0.5012	51	0.5020	64 Spike.3, 10 ppb	0.5006
9	0.5010	31	0.5007	52	0.5019	64 MM.1, 10ppb	0.5010
10	0.5006	32	0.5016	53	0.5016	64 MM.2, 10 ppb	0.5001
11	0.5001	33	0.5009	54	0.5008	64 Spike.1, 25 ppb	0.5004
12	0.5019	34	0.5007	55	0.5007	64 Spike.2, 25 ppb	0.5016
13	0.5011	35	0.5004	56	0.5007	64 Spike.3, 25 ppb	0.5015
14	0.5001	36	0.5003	57	0.5016	64 MM.1, 25 ppb	0.5002
15	0.5012	37	0.5020	58	0.5013	64 MM.2, 25 ppb	0.5006
16	0.5003	38	0.5019	59	0.5001	64 Spike.1, 50 ppb	0.5014
17	0.5008	39	0.5016	60	0.5012	64 Spike.2, 50 ppb	0.5003
18A	0.5010	40	0.5001	61	0.5018	64 Spike.3, 50 ppb	0.5018
18B	0.5001	38	0.5002	59	0.5015	64 MM.1, 50 ppb	0.5014
18C	0.5002	39	0.5017	60	0.5012	64 MM.2, 50 ppb	0.5017
19	0.5011	40	0.5018	61	0.5018		
20	0.5015	41	0.5002	62	0.5015		
21	0.5015	42	0.5017	63	0.5012		
22	0.5003	43	0.5018	64A, 10 ppb	0.5019	Total sample amount:	87 + 6 blank samples (no sediment added)

Table D.3: Data from tandem mass spectrometry transitions of sediment samples for bisphenols and benzophenones determination.

Compound	Formula/ mass (g/mol)	Transition no.	Precursor ion (m/z)	Product ion(s) (m/z)	Cone voltage (V)	Collision energy (eV)	Ion mode	Quantifier (m/z)	Qualifier (m/z)
Bisphenol A	C15H16O2	1	227.1	212.1	50.0	18.0	ES-	212.1	133.1
		2	227.1	133.1	50.0	24.0	ES-		
Bisphenol AF	C15H10O2F6	1	335.0	265.1	40.0	24.0	ES-	265.1	177.0
		2	335.0	177.0	40.0	42.0	ES-		
Bisphenol AP	C20H18O2	1	289.1	274.1	20.0	18.0	ES-	274.1	195.1
		2	289.1	195.1	20.0	28.0	ES-		
Bisphenol B	C16H18O2	1	241.1	212.1	20.0	18.0	ES-	212.1	212.1
Bisphenol F	C13H12O2	1	199.1	105.0	46.0	20.0	ES-	105.0	93.0
		2	199.1	93.0	46.0	20.0	ES-		
Bisphenol M&P	C24H26O2	1	345.1	133.0	8.0	46.0	ES-	330.2	133.0
		2	345.1	330.2	14.0	26.0	ES-		
Bisphenol S	C12H10SO4	1	249.0	156.0	40.0	22.0	ES-	108.0	156.0
		2	249.0	108.0	40.0	26.0	ES-		
Bisphenol Z	C18H20O2	1	267.1	173.1	40.0	24.0	ES-	173.1	145.1
		2	267.1	145.1	40.0	36.0	ES-		
$^{13}C_{12}$ Bisphenol A	240	1	238.8	224.0	30.0	20.0	ES-	224.0	224.0
$^{13}C_{12}$ Bisphenol AF	348	1	346.7	276.9	20.0	24.0	ES-	276.9	207.9
		2	346.7	207.9	20.0	36.0	ES-		
		3	346.7	187.9	20.0	44.0	ES-		
$^{13}C_{12}$ Bisphenol B	254	1	252.9	223.9	16.0	18.0	ES-	223.9	216.7
		2	252.9	216.7	16.0	20.0	ES-		
$^{13}C_{12}$ Bisphenol F	212	1	210.8	110.9	22.0	26.0	ES-	98.9	110.9
		2	210.8	98.9	22.0	24.0	ES-		
		3	210.8	81.9	22.0	28.0	ES-		
$^{13}C_{12}$ Bisphenol S	262	1	260.8	161.8	50.0	20.0	ES-	161.8	113.9
		2	260.8	113.9	50.0	28.0	ES-		
		3	260.8	97.9	50.0	36.0	ES-		
Benzophenone-1	C13H10O3	1	213.0	135.0	46.0	18.0	ES-	135.0	91.0
		2	213.0	91.0	46.0	28.0	ES-		
Benzophenone-2	C13H10O5	1	245.0	135.0	40.0	14.0	ES-	135.0	109.0
		2	245.0	109.0	40.0	16.0	ES-		
Benzophenone-3	C14H12O3	1	227.1	211.1	40.0	20.0	ES-	211.1	183.0
		2	227.1	183.0	40.0	34.0	ES-		
4-OH-BzP	C13H10O2	1	197.0	120.0	60.0	22.0	ES-	92.0	120.0
		2	197.0	92.0	60.0	28.0	ES-		
Benzophenone-8	C14H12O4	1	243.0	123.0	46.0	16.0	ES-	123.0	93.0
		2	243.0	93.0	46.0	18.0	ES-		

Table D.5: Bisphenol internal standards used for constructing calibration curves in conjunction with bisphenol and benzophenone target analytes.

Target analyte compound	IS compound chosen for quantification
Bisphenol A	$^{13}C_{12}$ Bisphenol A
Bisphenol AF	$^{13}C_{12}$ Bisphenol AF
Bisphenol AP	$^{13}C_{12}$ Bisphenol B
Bisphenol B	$^{13}C_{12}$ Bipshenol B
Bisphenol F	$^{13}C_{12}$ Bisphenol F
Bisphenol M&P	$^{13}C_{12}$ Bisphenol B
Bisphenol S	$^{13}C_{12}$ Bisphenol S
Bisphenol Z	$^{13}C_{12}$ Bisphenol B
Benzophenone-1	$^{13}C_{12}$ Bisphenol S
Benzophenone-2	$^{13}C_{12}$ Bisphenol S
Benzophenone-3	$^{13}C_{12}$ Bisphenol B
4-Hydroxybenzophenone	$^{13}C_{12}$ Bisphenol S
Benzophenone-8	$^{13}C_{12}$ Bisphenol AF

Table D.6: Mobile phase gradients used for analysis of sediment samples during LC-MS/MS procedures. A is the organic phase (MeOH) and B is the water phase (0.1% ammonium hydroxide solution in Milli-Q water).

Time (min)	Flow (mL)	%A	%B	Step
Initialized	0.3	75	25	Initialized
0.1	0.3	75	25	6
3.4	0.3	25	75	5
3.5	0.3	1	99	6
3.8	0.3	1	99	1
3.9	0.3	75	25	3
4.0	0.3	75	25	6

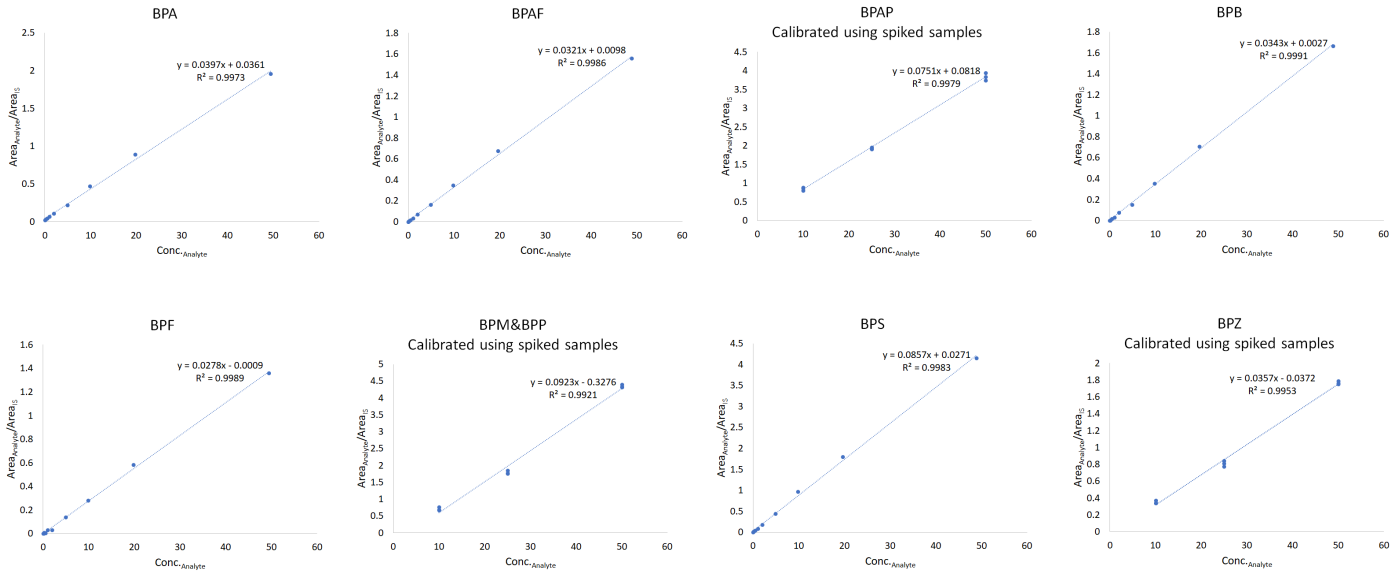

Figure D.1: Calibration curves for bisphenol target analytes. In cases where corresponding internal standard analogues for target analytes were not available, calibration was conducted by using internal standards with closely matching retention times and spiked samples.

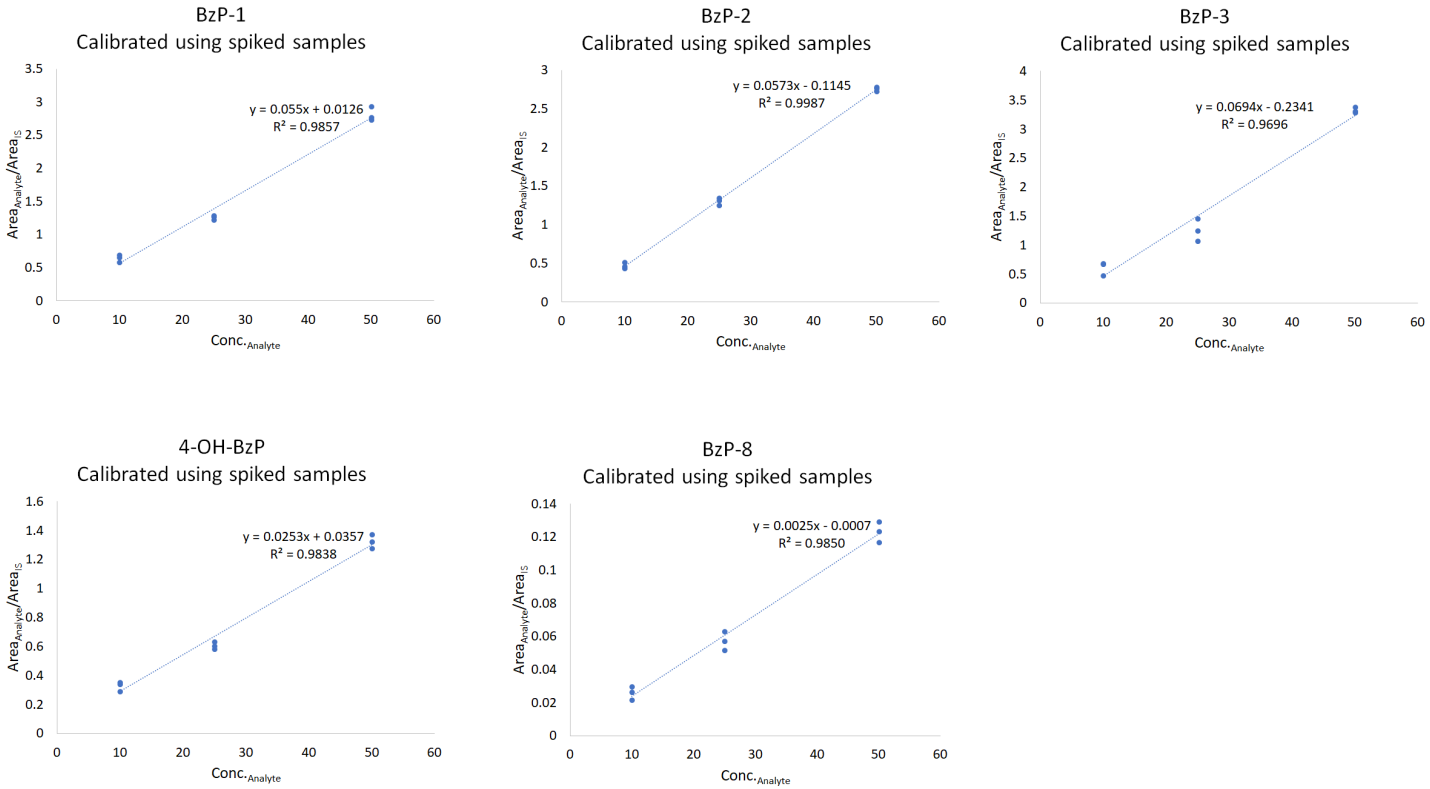

Figure D.2: Calibration curves for benzophenone target analytes.

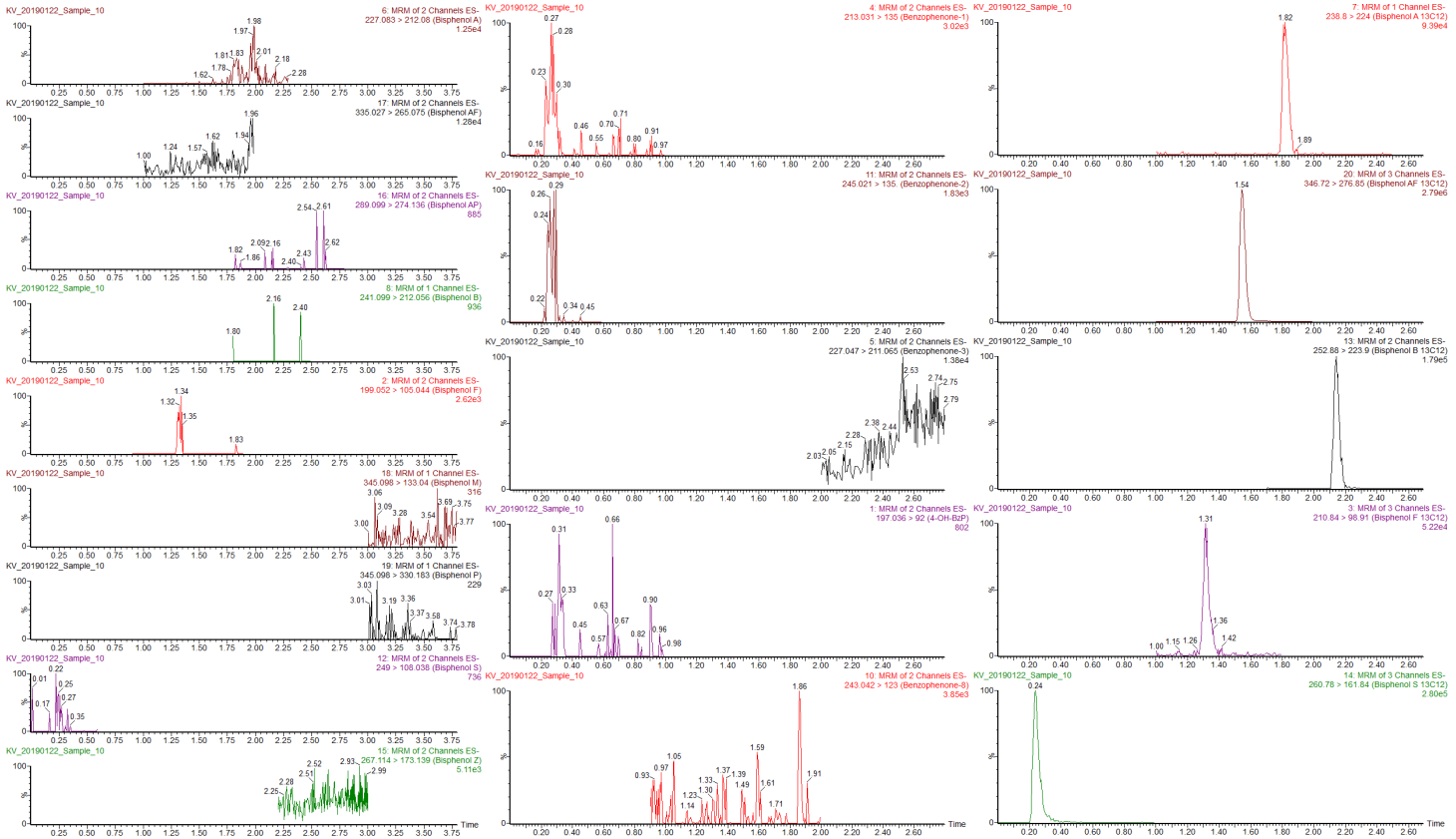

Figure D.3: LC-MS/MS chromatograms obtained of bisphenol target analytes (left), benzophenone target analytes (middle) and isotopically labelled bisphenol standards (right) in sample 10.

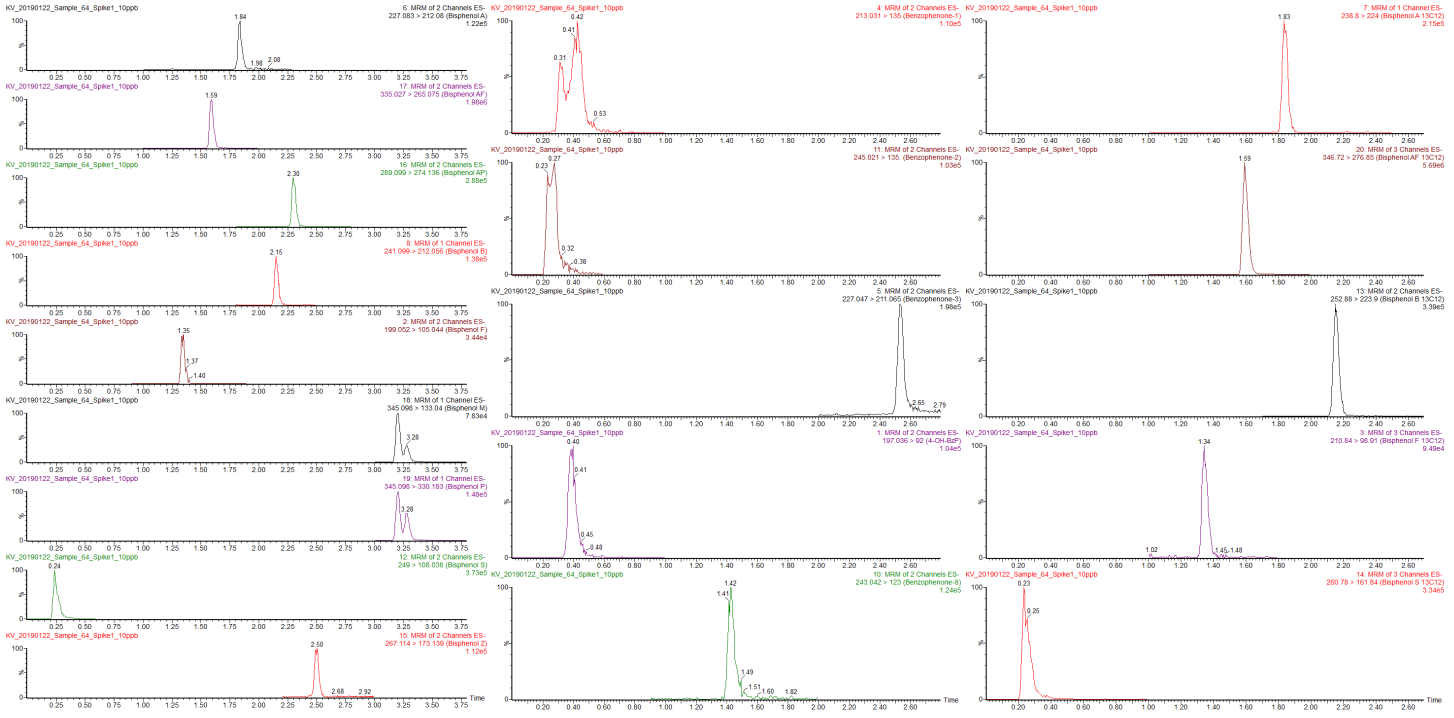

Figure D.4: LC-MS/MS chromatograms obtained of bisphenol target analytes (left), benzophenone target analytes (middle) and isotopically labelled bisphenol standards (right) in sample 64 spiked at 10 ng/mL (Sample64Spike.1, 10 ppb).

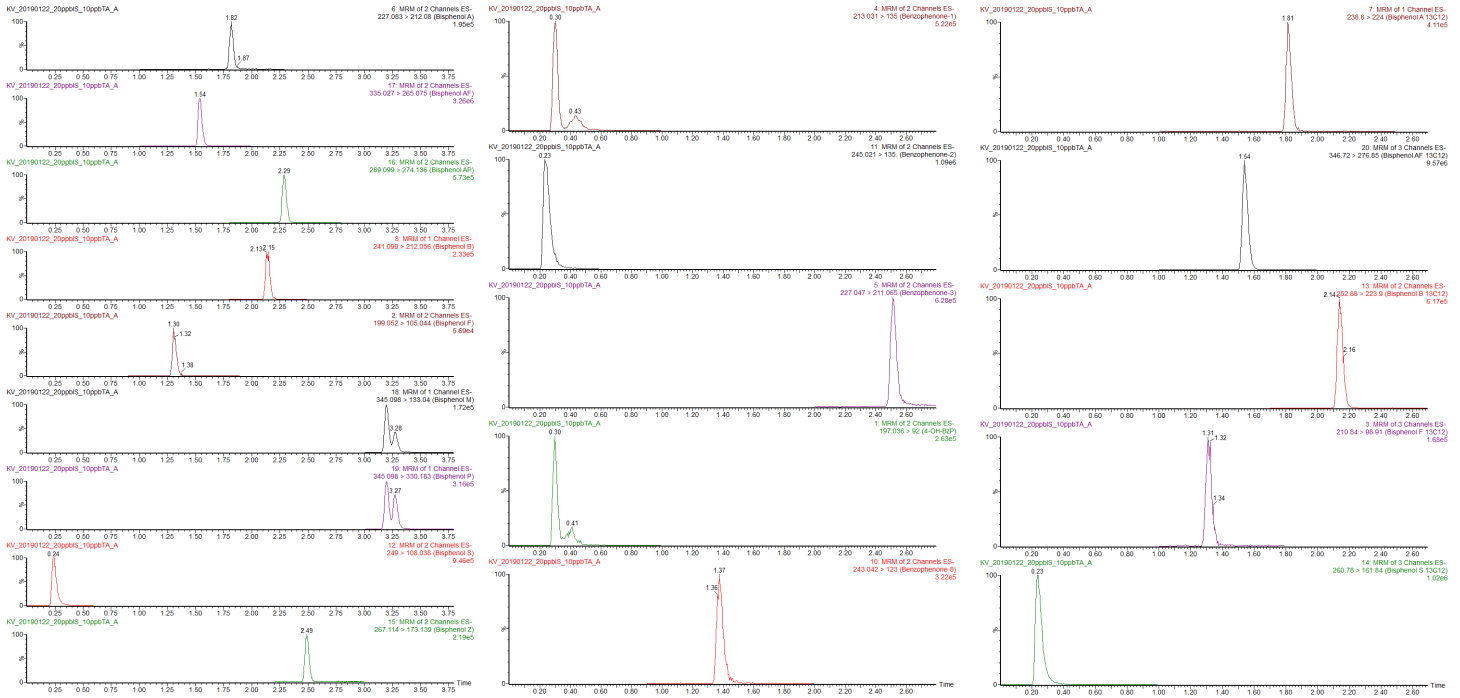

Figure D.5: LC-MS/MS chromatograms obtained of bisphenol target analytes (left), benzophenone target analytes (middle) and isotopically labelled bisphenol standards (right) in calibration standard solution fortified at 10 ng/mL.

Appendix E

Molecular structures of polycyclic aromatic hydrocarbon target analytes and internal standards

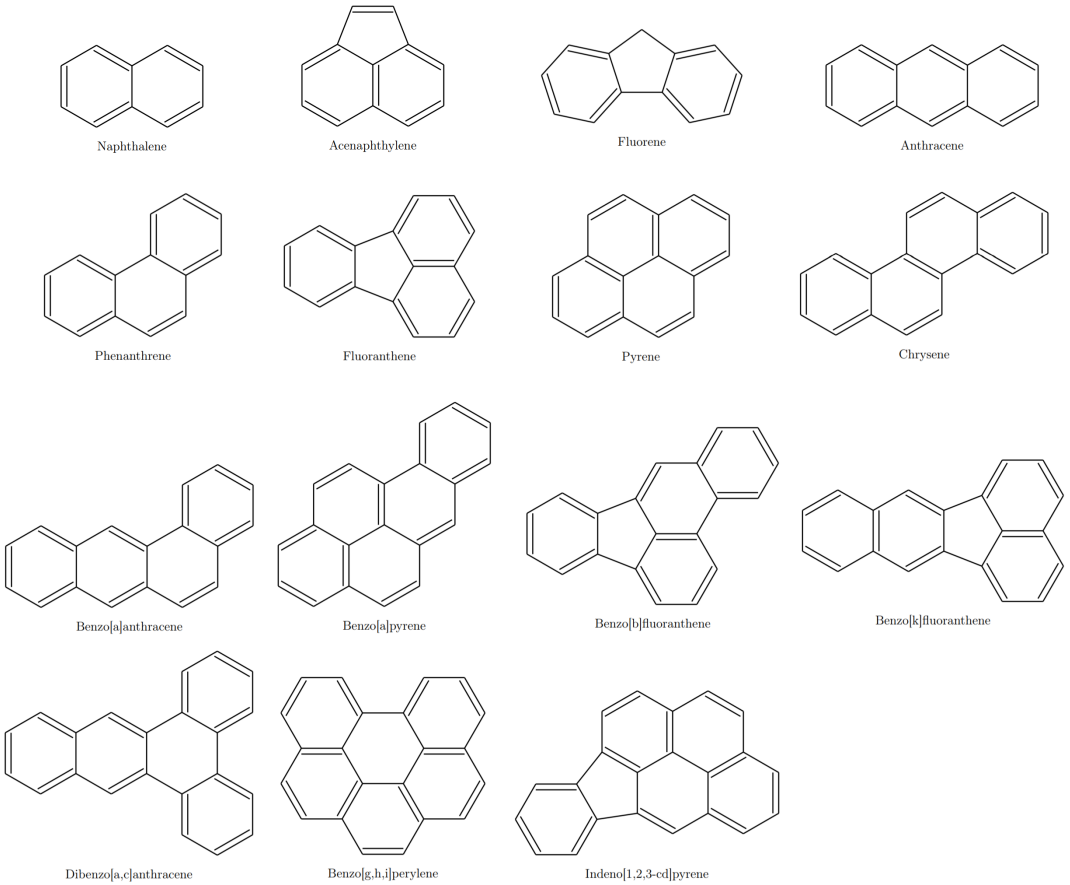

Figure E.1: PAH target analytes included in this project.

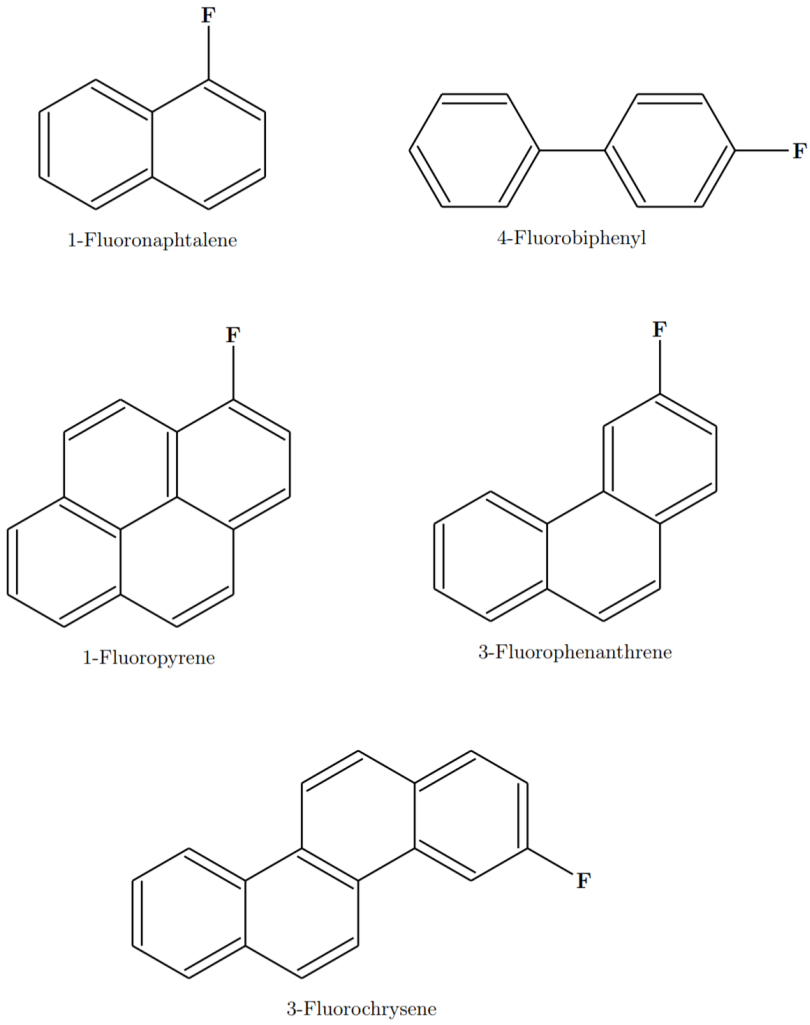

Figure E.2: Fluorinated PAH internal standards included in this project.

Appendix F

Conditions for polycyclic aromatic hydrocarbon analysis

Table F.1: Masses weighed of sediment sample portions for polycyclic aromatic hydrocarbon extraction and analysis. Sample numbers are corresponding to the sediment samples listed in table J.1.

Sample	Mass weighed (g)	Sample	Mass weighed (g)	Sample	Mass weighed (g)
1	5.0463	26	5.0144	51	5.0751
2	5.0160	27	4.9650	52	5.0734
3	5.0110	28	5.0583	53	5.0512
4	5.0567	29	5.0172	54	4.9827
5	5.0416	30	5.0540	55	5.0527
6	5.0385	31	5.0189	56	5.0688
7	4.9969	32	5.0365	57	4.9968
8	5.0057	33	4.9240	58	5.0461
9	5.0027	34	4.9587	59	4.9309
10	5.0358	35	5.0156	60	5.0319
11	4.9907	36	5.0355	61	5.0162
12	4.9389	37	5.0134	62	4.9251
13	5.0767	38	5.0060	63	5.0537
14	5.0330	39	5.0130	64.1	5.0202
15	4.9231	40	4.9747	64.2	5.0021
16	5.0385	41	5.0256	64.3	5.0231
17	5.0524	42	4.9020	64Spike.1	5.0147
18	5.0423	43	4.9700	64Spike.2	5.0472
19	5.0426	44	4.9635	64Spike.3	4.9995
20	5.0233	45	5.0181	64MM.1	5.0154
21	4.9226	46	4.9432	64MM.2	5.0420
22	5.0699	47	5.0845	64MM.3	5.0400
23	4.9646	48	5.0574		
24	5.0222	49	5.0741	Total sample amount:	72 + 14 blanks
25	4.9195	50	5.0141		(no sediment added)

Table F.2: Dionex 150 Accelerated solvent extractor settings used during extraction of PAHs from sediment samples.

System information	Value
Solvent	Acetone - Dichloromethane
System pressure (psi)	1500
Oven temperature (°C)	100
Cell size	10
Sample size (g)	5
Static extraction time (min)	5
Rinse volume (mL)	6
Nitrogen purge (s)	90
Extraction time (min)	19

Table F.3: Mobile phase gradients used during HPLC analysis of sediment samples.

Time (min)	%Acetonitrile	%Milli-Q water
0 - 5	40	60
5 - 30	100	0
30 - 45	100	0

Table F.4: Fluorescence excitation and emission wavelength settings used during HPLC analysis of sediment samples.

Time (min)	Excitation wavelength (nm)	Emission wavelength (nm)
0 - 19.5	270	333
19.5 - 21,0	245	350
21.0 - 26.5	260	420
26.5 - 29.5	265	380
29.5 - 37	290	430
37 - 45	300	500

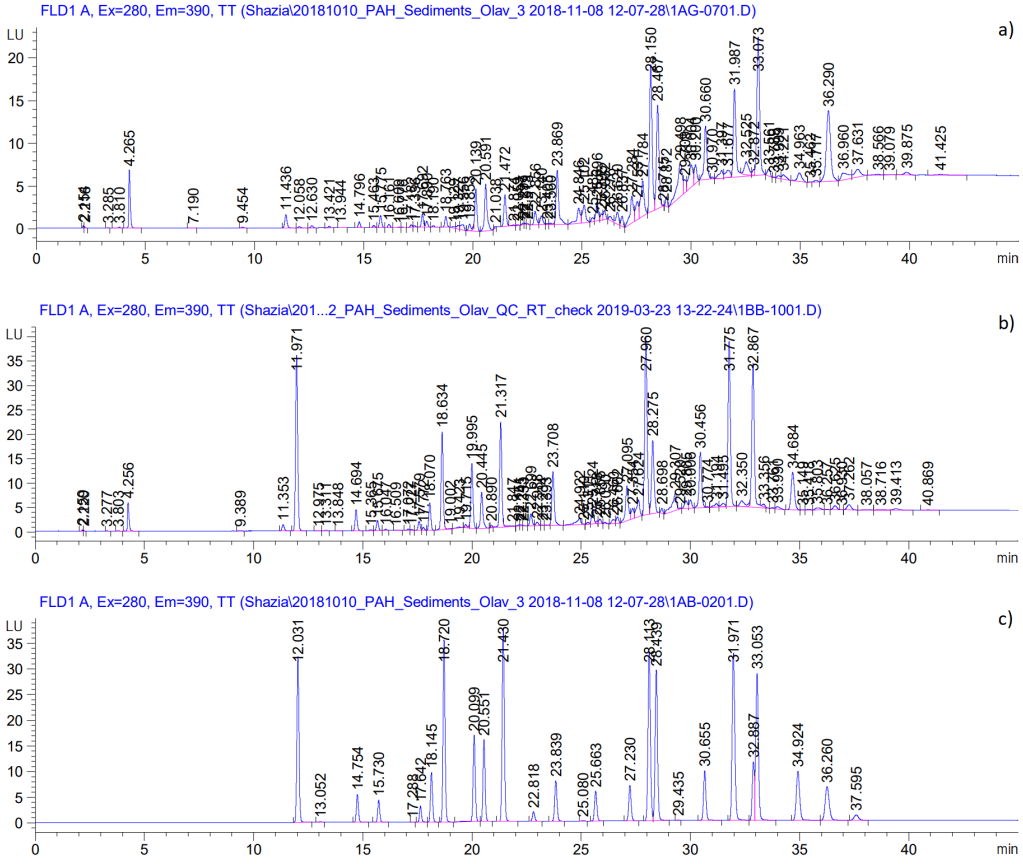

Figure F.1: Raw chromatograms obtained during HPLC-FLD analysis of sediment samples. Chromatogram a) shows sample 25 after extraction. Chromatogram b) shows sample 25 spiked with 20 ng of target analytes and internal standards to confirm retention times and relative retention times. Chromatogram c) shows standard solution at 200 ng/mL analyzed with the same batch as sample 25.

Table F.5: PAH compounds with corresponding RTs and RRTs for sample 40, analysed during RT confirmation check.

Compound	RT [min]	RRT
NAP	14.70	0.72
F-NAP	15.67	0.77
F-BIP	17.57	0.86
ACE	18.06	0.88
FLU	18.63	0.91
PHE	12.00	0.98
F-PHE	20.45	1.00
ANT	21.32	1.04
FLT	22.70	1.11
PYR	23.71	1.16
F-PYR	25.53	1.25
BaA	27.10	1.33
CHR	27.97	1.37
F-CHR	28.28	1.38
BbF	30.46	1.49
BKF	31.77	1.55
BaP	32.86	1.61
DBA	34.68	1.70

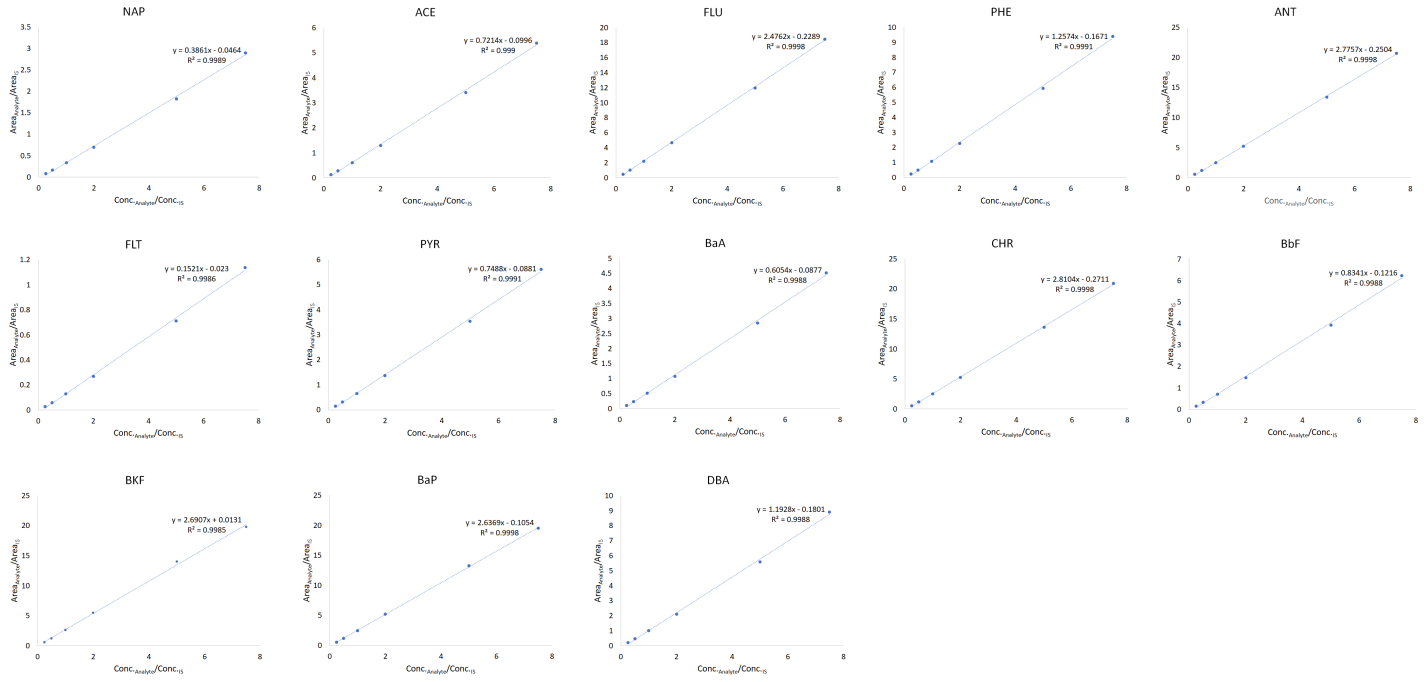

Figure F.2: Calibration curves for polycyclic aromatic hydrocarbon target analytes using linear regression. F-PHE was used as internal standard for instrument calibration.

Appendix G

Conditions for elemental analysis

Table G.1: Masses weighed of sediment sample portions for digestion and ICP-MS analysis. Sample numbers are corresponding to the sediment samples listed in table J.1.

Sample	Mass weighed (mg)	Sample	Mass weighed (mg)	Sample	Mass weighed (mg)
1	314.9	25	326.5	49	282.0
2	269.3	26	262.4	50	331.4
3	299.7	27	322.6	51	333.7
4	302.9	28	290.4	52	259.0
5	310.5	29	288.8	53	255.1
6	324.5	30	302.2	54	327.5
7	322.2	31	266.0	55	296.0
8	295.1	32	289.7	56	319.5
9	306.6	33	261.1	57	257.5
10	265.2	34	301.1	58	296.2
11	336.0	35	302.6	59	342.2
12	294.9	36	261.1	60	339.4
13	310.0	37	283.4	61	337.7
14	286.7	38	304.9	62	342.0
15	280.8	39	268.8	63	310.4
16	319.1	40	347.4	64	311.0
17	317.2	41	316.3		
18	285.3	42	269.3		
19	290.2	43	302.9		
20	319.4	44	344.9		
21	264.1	45	336.9		
22	284.3	46	310.4		
23	256.3	47	342.1	Total sample amount:	67 + 4 blanks
24	280.5	48	336.3		(no sediment added)

Appendix H

Conditions for total organic matter determination

Table H.1: Masses weighed of sediment sample portions for total organic matter determination using loss-on-ignition. Sample numbers are corresponding to the sediment samples listed in table J.1.

Sample	Mass weighed [g]	Sample	Mass weighed [g]	Sample	Mass weighed [g]
1	7.9120	25	9.2526	49	10.7420
2	8.1409	26	15.0242	50	10.0622
3	8.2243	27	8.2863	51	8.6094
4	8.3991	28	11.9488	52	7.8415
5	9.4223	29	11.3836	53	11.7506
6	7.2702	30	11.0503	54	8.8272
7	8.5152	31	10.2299	55	9.0048
8	7.5760	32	9.1040	56	11.0184
9	7.7836	33	10.3915	57	9.4143
10	10.6338	34	9.2645	58	9.3292
11	12.5192	35	10.4706	59	6.2994
12	9.8097	36	13.6176	60	6.7719
13	10.5695	37	11.5616	61	10.7977
14	8.8182	38	8.8231	62	8.1085
15	8.9601	39	8.8928	63	7.6902
16	11.7500	40	10.7704	64	9.2419
17	9.5079	41	10.6389	17 (extra sample).1	8.7614
18	9.1296	42	9.1083	17 (extra sample).2	8.8561
19	10.7472	43	12.9582	17 (extra sample).3	8.4259
20	9.7250	44	11.0156		
21	8.4753	45	11.6516		
22	10.4997	46	12.3915		
23	8.8241	47	12.6304		
24	9.2608	48	10.5542	Total sample amount:	67

Appendix I

Principal component analysis plots

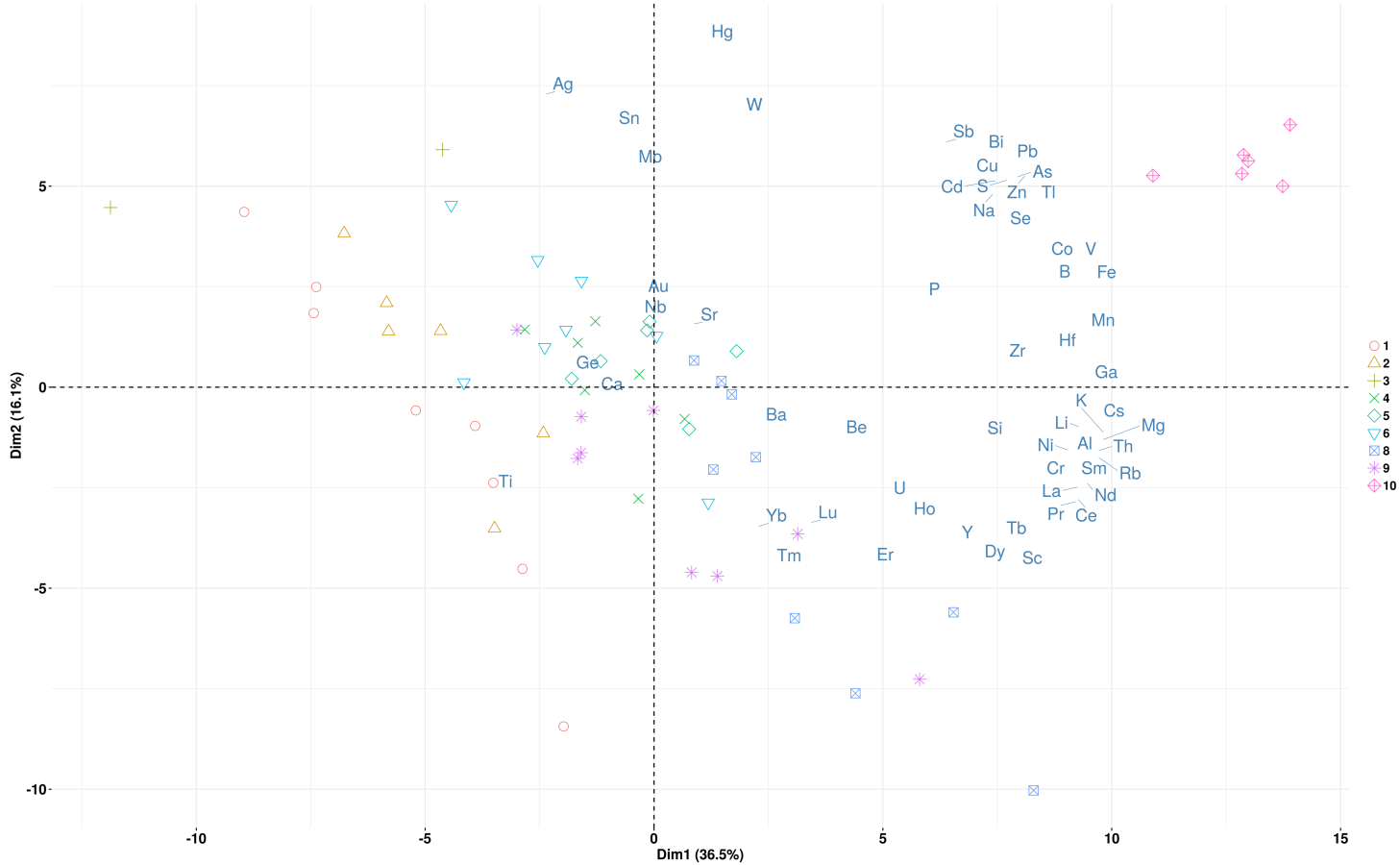

Figure I.1: Principal component analysis biplot, showing elements grouped with sampling stations.

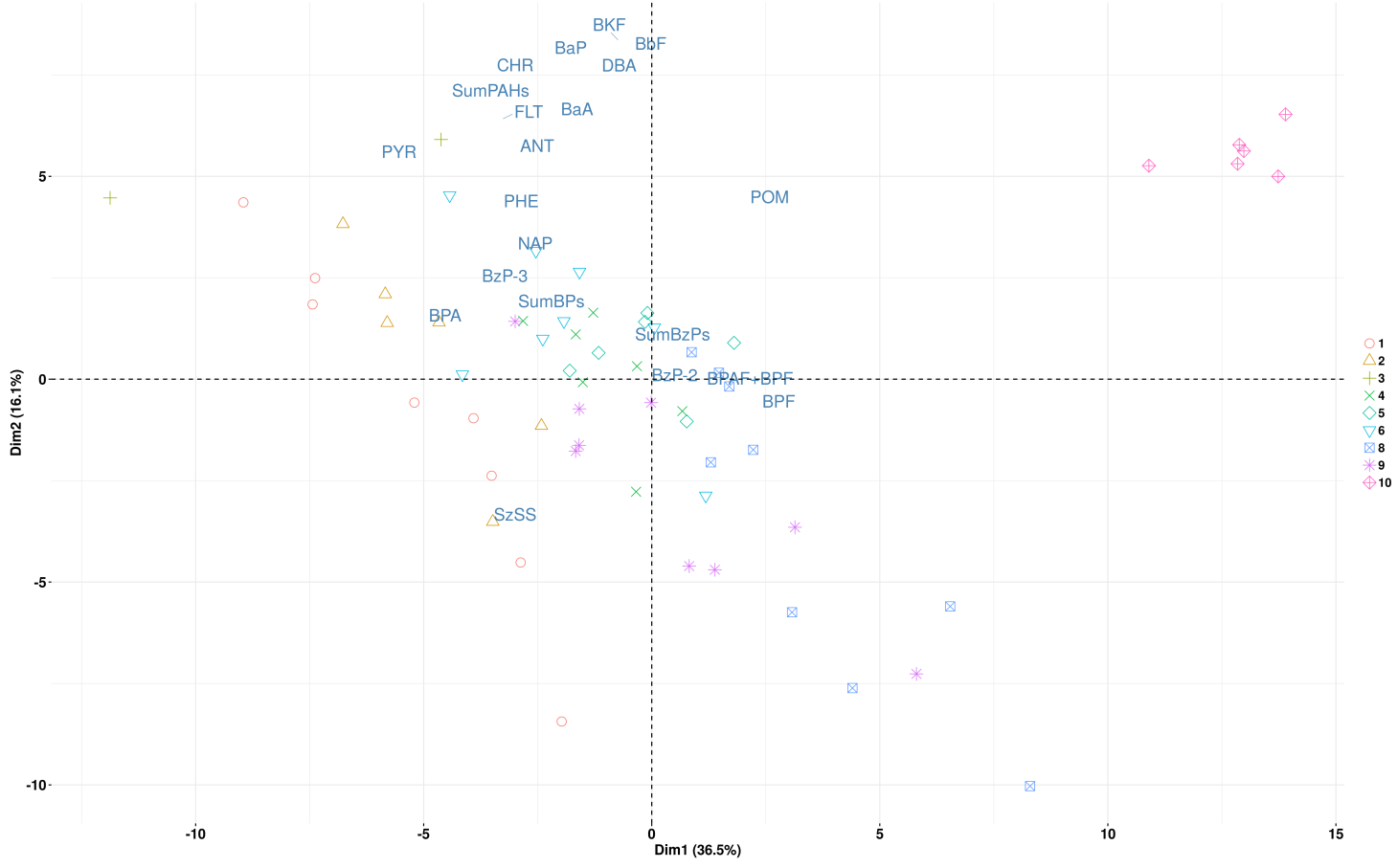

Figure I.2: Principal component analysis biplot, showing organic target analytes grouped with sampling stations.

Figure I.3: Principal component analysis biplot, showing elements grouped with sampling location seafloor depth.

Figure I.4: Principal component analysis biplot, showing organic target analytes grouped with sampling location seafloor depth.

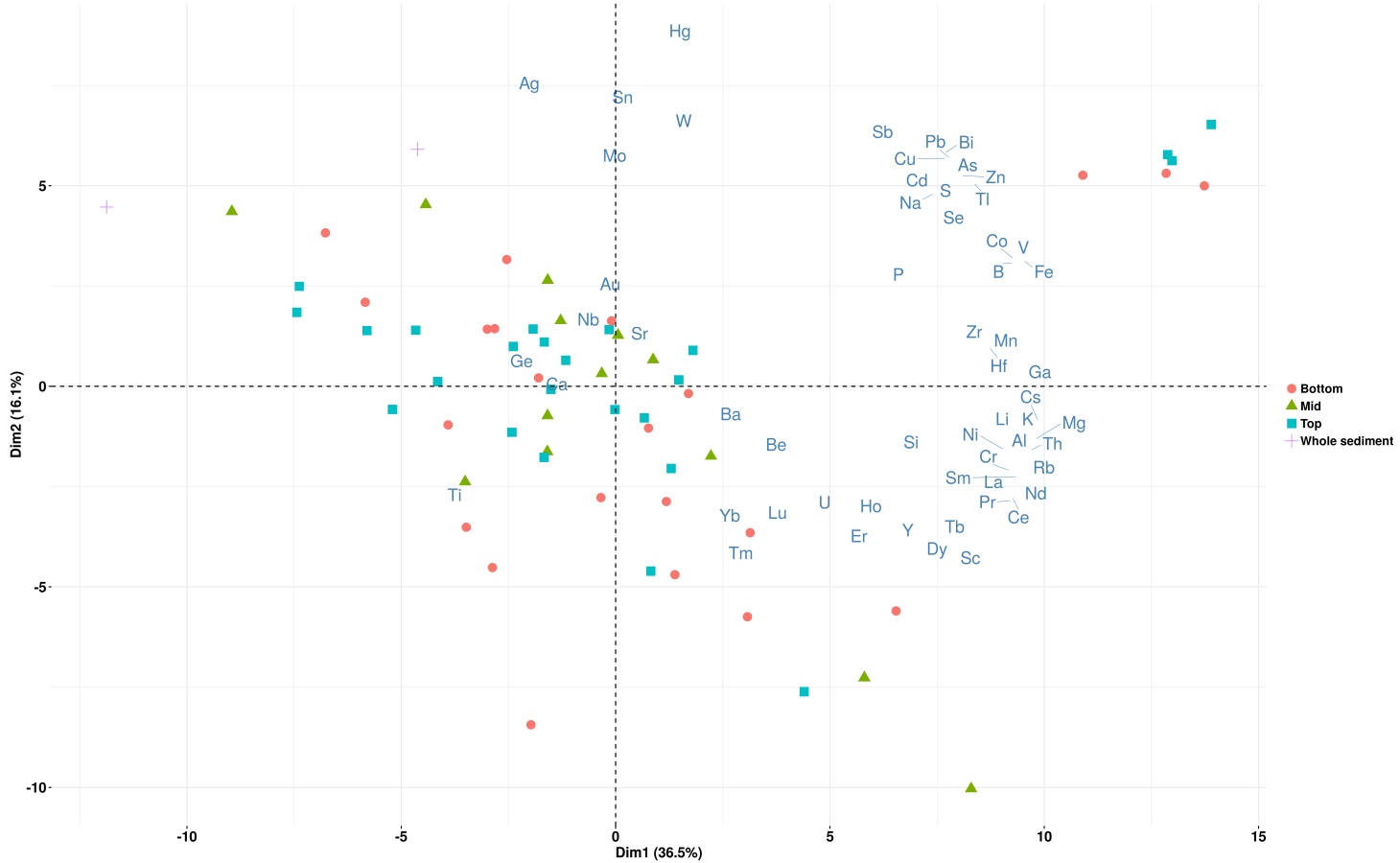

Figure I.5: Principal component analysis biplot, showing elements grouped with sediment block sampling sections.

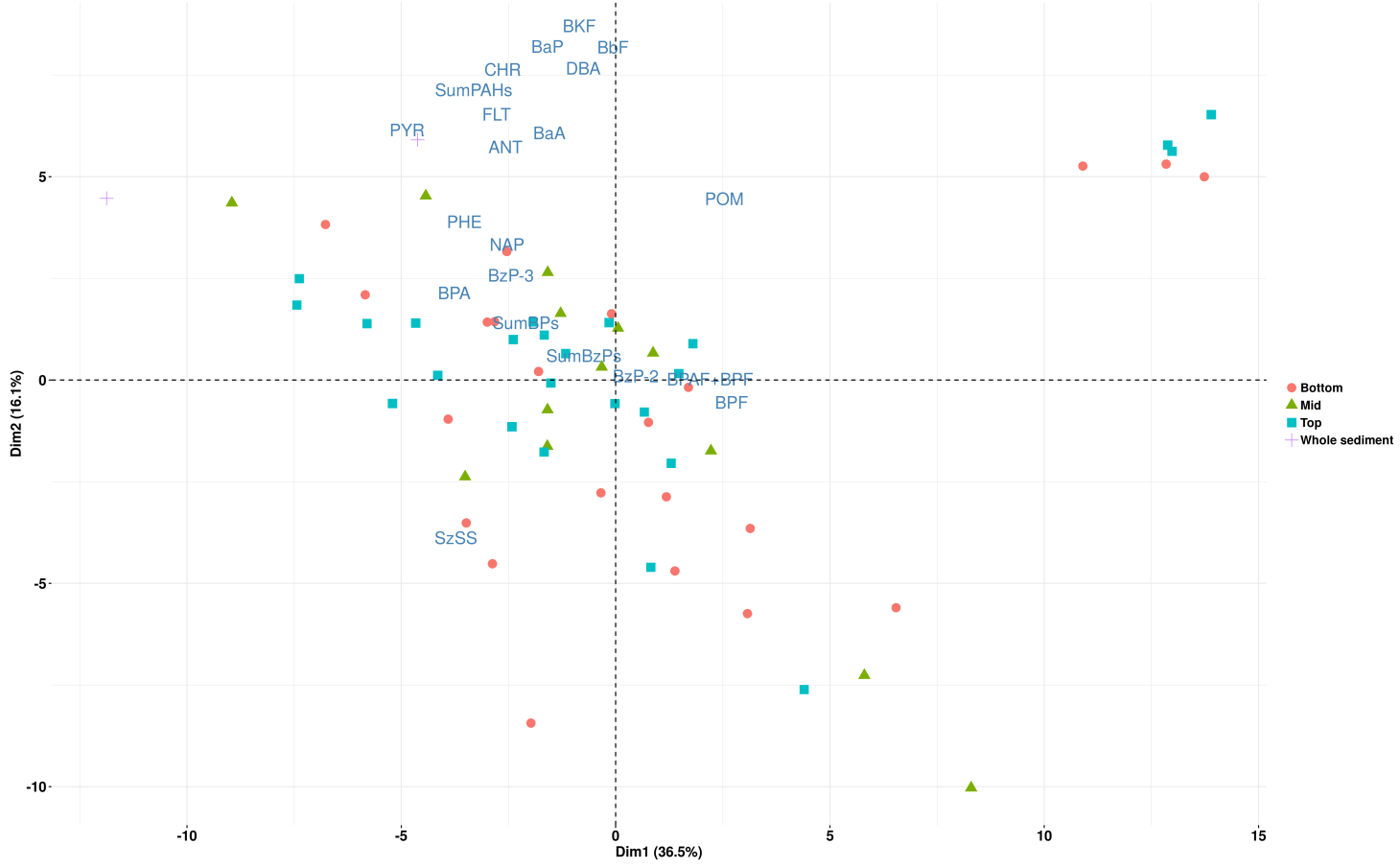

Figure I.6: Principal component analysis biplot, showing organics grouped with sediment block sampling sections.

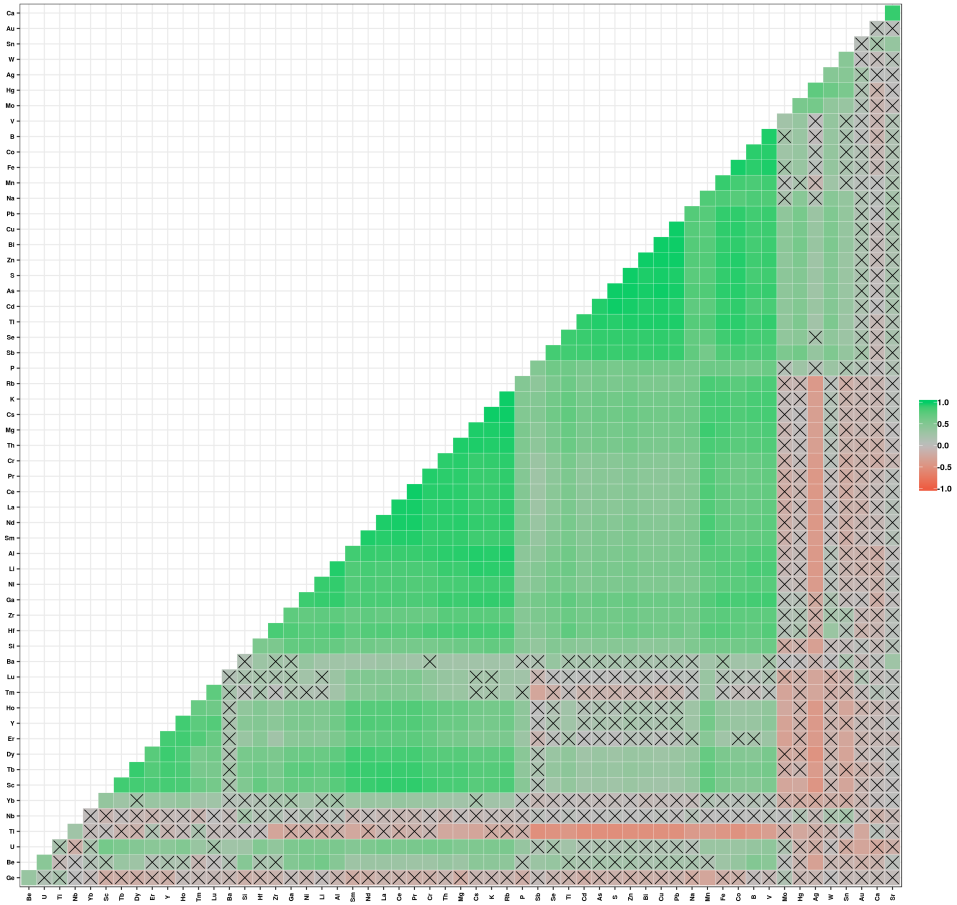

Figure I.7: Principal component analysis correlation plot, showing correlations among elements.

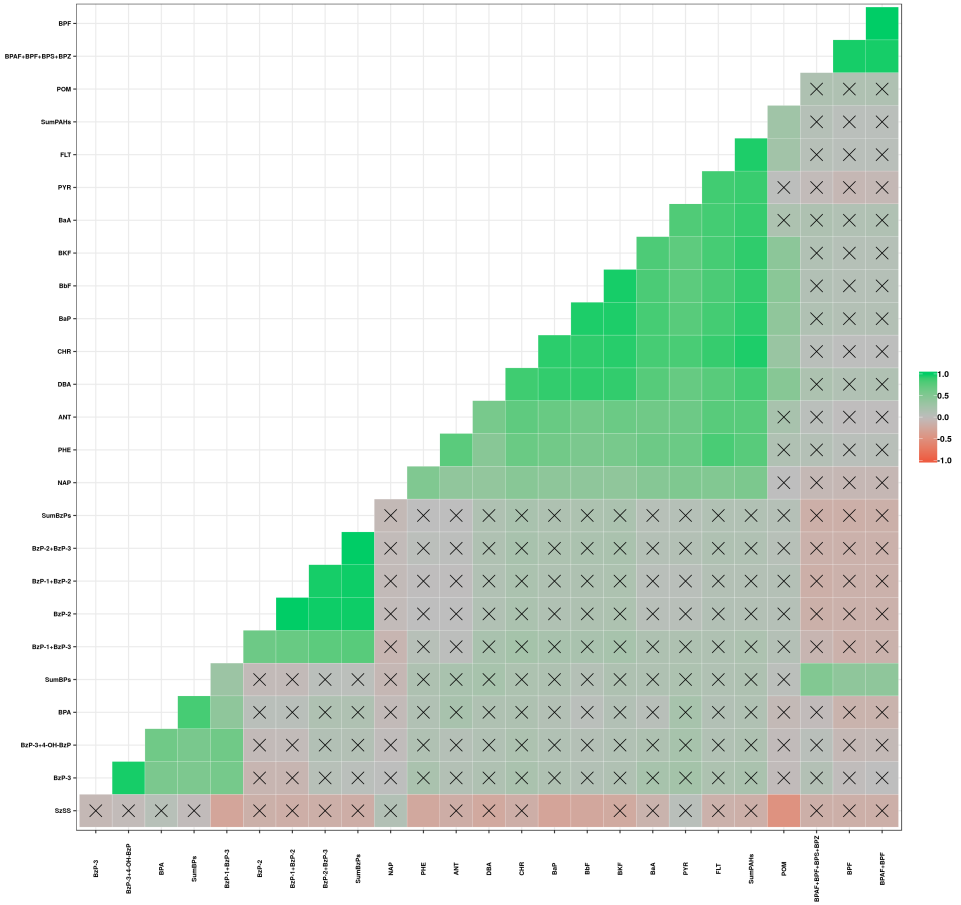

Figure I.8: Principal component analysis correlation plot, showing correlations among organic target analytes.

Appendix J

Data from sediment sampling campaign

Table J.1: Dimensions, coordinates, time of sampling, seafloor depth and sediment block section depths for sediment samples collected from the research vessel R/V Gunnerus during the sampling campaign on the 31st of May 2018.

Sample no.	Station no.	Time	Latitude	Longitude	Seafloor depth	No. of replicates	Depth of sediment section	Sediment section size	Position of sediment section	Extra sample collected in Al box (for LOI analysis)	Sediment dimensions Length	Width	Height	General observations
1	1	9,20	N 63° 26,961'	E 10° 20,340'	93	1	0-8	8	Top		36	23	23	Sediment was intact, very few rocks present, rocks were generally at the bottom
2							8-16	8	Bottom					Three replicates from the sediments
3						2	0-6	6	Top					
4							6-14	8	Mid					
5							14-23	9	Bottom					
6						3	0-5	5	Top					
7							5-11	6	Mid					
8							11-17	6	Bottom					
9	2	9,55	N 63° 26,989'	E 10° 20,202'	109	1	0-6	6	Top		36	23	10	Quite a few rocks at the bottom of sediments
10							6-12	6	Bottom					Two replicates from the sediments were collected
11						2	0-3	3	Top					from two sides of sediment, while third one
12							3-6	3	Bottom					was taken from by cutting the sediment in the middle
13						3	0-4	4	Top					
14							4-8	4	Bottom					
15	3	10,10	N 63° 26,988'	E 10° 19,961'	88	1	0-2	2	Whole sediment				2	Sediment was only 2 cm in height, and had quite a lot of rocks
16						1	0-2	2	Whole sediment					Three replicates from the sediments were collected from three sides of sediment
17	4	10,30	N 63° 27,042'	E 10° 20,081'	136	1	0-7	7	Top	✓	36	34	23	Sediment had few rocks at the bottom
18							7-12	5	Mid					Three replicates from the sediments were
19							12-18	6	Bottom					collected from three sides of sediment
20						2	0-4	4	Top					
21							4-7	3	Mid					
22							7-12	5	Bottom					
23						3	0-5	5	Top					
24							5-8	3	Mid					
25							8-13	5	Bottom					
26	5	12,20	N 63° 27,045'	E 10° 20,200'	154	1	0-3	3	Top		36	24	6	Sediment was thin and had rocks at the bottom
27							3-6	3	Bottom					Two replicates from the sediments were collected
28						2	0-3	3	Top					from two sides of sediment, while third one
29							3-6	3	Bottom					was taken from by cutting the sediment in the middle
30						3	0-2.5	2.5	Top					
31							2.5-5	2.5	Bottom					
32	6	12,40	N 63° 27,045'	E 10° 19,958'	127	1	0-7	7	Top		31	32	20	Three replicates from the sediments were
33							7-14	7	Mid					collected from three sides of sediment
34							14-20	6	Bottom					
35						2	0-7	7	Top					
36							7-12	5	Mid					
37							12-18	6	Bottom					
38						3	0-6	6	Top					
39							6-12	6	Mid					
40							12-20	8	Bottom					
	7	13,00	N 63°27,097'	E 10° 20,080'	163	n/a	n/a	n/a	n/a		n/a	n/a	n/a	No sediment acquired
41	8	13,10	N 63° 27,094'	E 10° 20,200'	180		0-7	7	Top		38	33	19	Two replicates from the sediments were collected
42							7-12	5	Mid					from two sides of sediment, while third one
43							12-18	6	Bottom					was taken from by cutting the sediment from inside
44							0-4	4	Top					closer to the third side of the sediment
45							4-6	2	Mid					
46							6-10	4	Bottom					
47							0-4	4	Top					
48							4-7	3	Mid					
49							7-12	5	Bottom					
50	9	13,30	N 63° 27,095'	E 10° 19,958'	131		0-3	3	Top		38	32	10	There were rocks at the bottom
51							3-6	3	Mid					Two replicates from the sediments were collected
52							6-8	2	Bottom					from two sides of sediment, while third one
53							0-3	3	Top					was taken from by cutting the sediment from inside
54							3-5	2	Mid					closer to the third side of the sediment
55							5-8	3	Bottom					
56							0-2	2	Top					
57							2-5	3	Mid					
58							5-7	2	Bottom					
59	10	14,10	N 63° 27,580'	E 10° 221,088'	332		0-1.5	1.5	Top		33	32	6	Sediment from Mid Fjord, for background readings
60							1.5-3	1.5	Bottom					Two replicates from the sediments were collected
61							0-3	3	Top					from two sides of sediment, while third one
62							3-6	3	Bottom					was taken from by cutting the sediment from inside
63							0-2.5	2.5	Top					closer to the third side of the sediment
64							2.5-5	2.5	Bottom					

Literature

[1] Haugen MO. Trondheimsfjorden; 2018. Available from: https://snl.no/Trondheimsfjorden.

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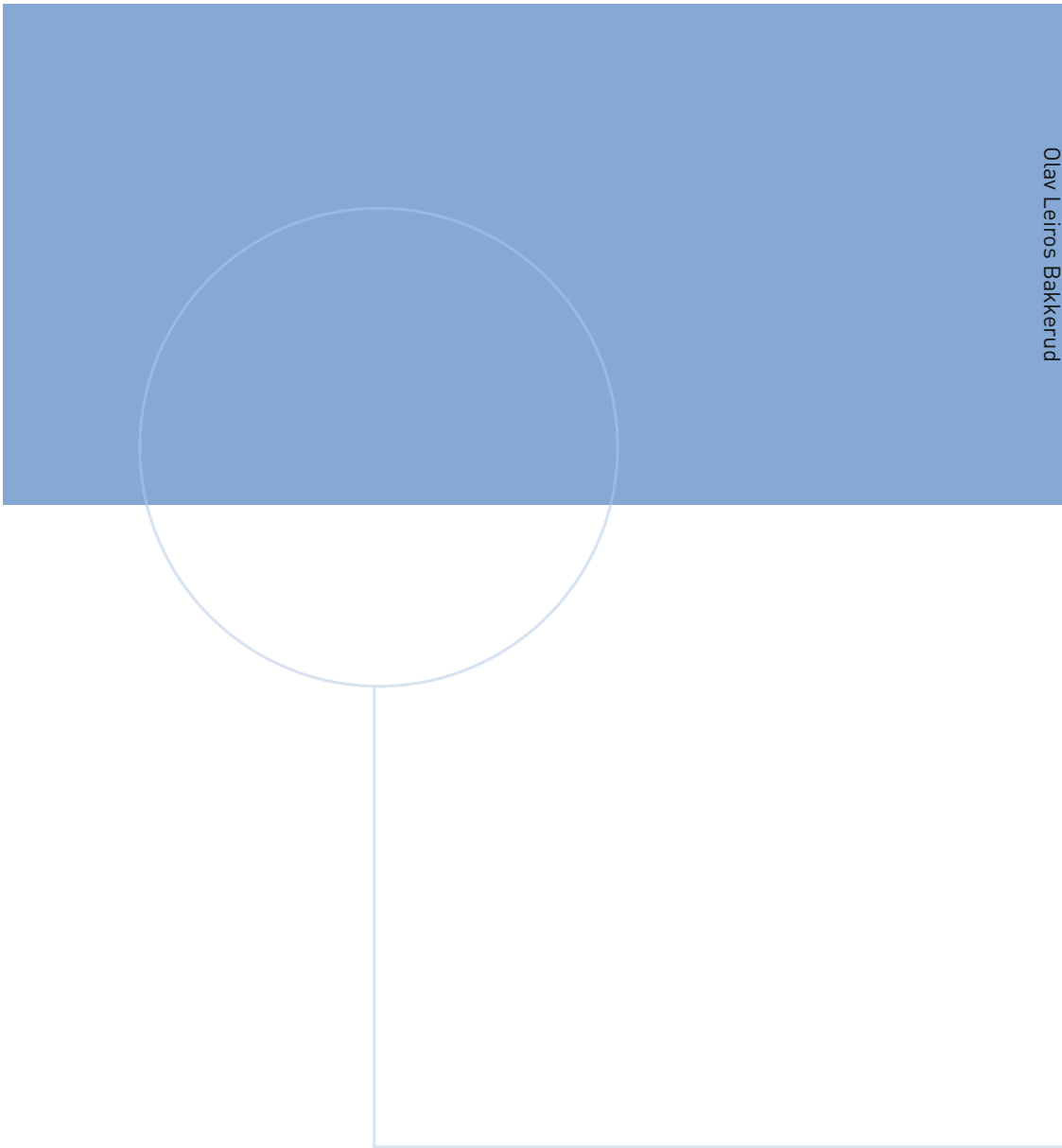

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