Master's thesis

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Profiles of Benzothiazoles and Benzotriazoles in Marine Sediments from Trondheimsfjorden: The Associations with Trace Elements and Polycyclic Aromatic Hydrocarbons

Master's thesis in Environmental Chemistry
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Abstract

Sediments can act as a sink for environmental pollutants where contaminants can be stored, causing secondary emissions in aquatic systems. This is especially true for lipophilic contaminants, such as polycyclic aromatic hydrocarbons (PAHs), which are rapidly adsorbed to particulate matter. However, despite the frequent detection of benzothiazoles (BTHs) and benzotriazoles (BTRs) in surface waters and ground waters, knowledge about their occurrence and profiles in sediments is still scarce.

In this study, liquid chromatography tandem mass spectrometry (LC-MS^2) was used for the simultaneous determination of nine BTHs and seven BTRs in sediment samples from Trondheimsfjorden. The target benzothiazoles were benzothiazole, 2-chlorobenzothiazole, 2-hydroxybenzothiazole, 2-mercaptobenzothiazole, 2-methylthiobenzothiazole, 2-thiocyanomethylthiobenzothiazole, 2-methylbenzothiazole, 2-aminobenzothiazole, and 2-morpholin-4-yl-benzothiazole, while the target benzotriazoles were benzotriazole, 4-methyl-1-H-benzotriazole (tolyltriazole), 5,6-dimethyl-1-H-benzotriazole (xylyltriazole), benzotriazole-5-carboxylic acid, 5-chloro-1-H-benzotriazole, 5-amino-1-H-benzotriazole and 1-hydroxybenzotriazole. In addition, 16 PAHs were measured in the sediment samples by high-performance liquid chromatography with fluorescence detection (HPLC-FLD) in order to establish differences in occurrence profiles for these three groups of organic contaminants. The analysis of trace elements in the

samples was done by inductively coupled plasma mass spectrometry (ICP-MS), to establish possible associations between organic contaminants and trace elements.

This study demonstrated the occurrence of BTHs, BTRs, and PAHs in sediment samples, which were found to range from 9.32-152 $ng\,g^{-1}$ for $\sum$(9)BTHs (median: 62.3 $ng\,g^{-1}$), while the concentrations ranged from 3.48-67.9 $ng\,g^{-1}$ for $\sum$(7)BTRs (median: 7.73 $ng\,g^{-1}$). $\sum$(13)PAHs was found to range from 29.1-548 $ng\,g^{-1}$ (median: 351 $ng\,g^{-1}$). This is the first time concentration profiles between these three groups of organic contaminants are reported together, and the higher abundance of PAHs in sediments is thought to be a result of differences in physicochemical properties.

Making use of principal component analysis (PCA) it was possible to see some associations between the different contaminants, and the sampling sites. The PCA indicated associations between the PAHs and TTR and XTR, while BTR were found to be associated with several trace metals. The PCA also indicated that BTR were found in highest abundance at the site furthest away from the shoreline, and it was proposed that this might be due to commercial ship traffic in this area or due to different properties of the sediments.

Sammendrag

Sedimenter kan fungere som en sink for miljøgifter der kontaminanter kan bli lagret, noe som fører til sekundære utslipp i akvatiske systemer. Dette gjelder spesielt for lipofile forurensninger, som polysykliske aromatiske hydrokarboner (PAHer) som raskt adsorberes til partikler. Selv om benzothiazoler (BTHer) og benzotriazoler (BTRer) ofte detekteres i overflatevann og grunnvann, er det derimot lite kunnskap om forekomsten av disse forbindelsene i sedimenter.

I dette studiet ble væskekromatografi tandem massespektrometri (LC-MS^2) brukt for simultan bestemmelse av ni BTHer og syv BTRer i sedimentprøver fra Trondheimsfjorden. De analyserte benzothiazolene inkluderer benzothiazole, 2-chlorobenzothiazole, 2-hydroxybenzothiazole, 2-mercaptobenzothiazole, 2-methylthiobenzothiazole, 2-thiocyanomethyl-thiobenzothiazole, 2-methylbenzothiazole, 2-aminobenzothiazole, og 2-morpholin-4-yl-benzothiazole, mens de anlyserte benzotriazolene inkulderer benzotriazole, 4-methyl-1-H-benzotriazole (tolyltriazole), 5,6-dimethyl-1-H-benzotriazole (xylyltriazole), benzotriazole-5-carboxylic acid, 5-chloro-1-H-benzotriazole, 5-amino-1-H-benzotriazole og 1-hydroxybenzotriazole. I tillegg ble 16 PAHer analysert i sedimentene ved hjep høypresisjonsvæskekromatografi med fluorescensdeteksjon (HPLC-FLD). Dette ble gjort for å etablere forskjeller i konsentrasjonsprofiler mellom de tre gruppene av organiske forurensninger. Analyse av sporelementer i prøvene ble gjort med

induktivt koblet plasma massespektrometri for å se på assosiasjoner mellom organiske forurensninger og sporelementer. Analyse of sporelementer i prøvene ble gjort ved hjelp av induktivt koblet plasma massespektrometri (ICP-MS), for å etablere mulige sammenhenger mellom organiske forurensninger og sporelemeter.

Dette studiet demonstrerte forekomsten av BTHer, BTRer og PAHer i sedimentprøver, som ble funnet til å variere fra 9,32-152 $\mathrm{ng\,g^{-1}}$ for $\sum$(9)BTHer (median: 62,3 $\mathrm{ng\,g^{-1}}$, mens konsentrasjonen varierte fra 3,48-67,9 $\mathrm{ng\,g^{-1}}$ for $\sum$(7)BTRer (median: 7,73 $\mathrm{ng\,g^{-1}}$). $\sum$(13)PAHer ble funnet til å spenne fra 29,1-548 $\mathrm{ng\,g^{-1}}$ (median: 351 $\mathrm{ng\,g^{-1}}$). Dette er første gang konsentrasjonsprofiler mellom disse tre gruppene av organiske miljøgifter er rapportert sammen, og den høyere forekomsten av PAHer i sedimentene er mest sannsynlig et resultat av ulike fysisk-kjemiske egenskaper.

Ved hjelp av prinsipalkomponentanalyse (PCA) var det mulig å se noen assosiasjoner mellom de ulike kontaminantene og prøvetakingssted. PCAen indikerte en assosiasjon mellom PAHer og TTR og XTR, mens det ble funnet at BTR var korrelert med flere ulike spormetaller. PCAen indikerte også at BTR ble funnet mest ved stasjonen lengst fra kystlinjen, og det ble foreslått at dette kan ha en sammenheng med båttrafikk i dette området. Det kan også skyldes ulike egenskaper hos sedimentene i dette området.

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Abbreviations

ACN	Acetonitrile
ASE	Accelerated solvent extraction
BTHs	Benzothiazoles
BTRs	Benzotriazoles
CE	Collision energy
CECs	Contaminants of emerging concern
CID	Collision-induced dissociation
CV	Cone voltage
DAD	Diode array detector
DCM	Dichloromethane
ECs	Emerging contaminants
ESI	Electrospray ionization
FLD	Fluorescence detector
F-PAH	Fluorinated polycyclic aromatic hydrocarbon

GC	Gas chromatography
HCl	Hydrochloric acid
H_2O	Water
HPLC	High-performance liquid chromatography
IR	Ion ratio
IS	Internal standard
LC	Liquid chromatography
LC-MS	Liquid chromatography mass spectrometry
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LLOQ	Lower limit of quantification
LOD	Limit of detection
LSE	Liquid-solid extraction
ME	Matrix effect
MeOH	Methanol
MF	Matrix factor
MP	Mobile phase
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
m/z	Mass to charge ratio
N_2 (g)	Nitrogen gas

PAHs	Polycyclic aromatic hydrocarbons
PCA	Principal component analysis
PCB	Polychlorinated biphenyls
PLE	Pressurized liquid extraction
POPs	Persistent organic pollutants
ppb	part per billion
PTFE	Polytetrafluoroethylene
QC	Quality control
QqQ	Triple-quadrupole
RP	Reversed phase
RR	Relative response
RRT	Relative retention time
RSD	Relative standard deviation
RT	Retention time
SIM	Selected ion monitoring
SPE	Solid-phase extraction
SRM	Selected reaction monitoring
STD	Standard deviation
TBT	Tributylytin
UHPLC	Ultra-high performance liquid chromatography
USAE	Ultrasound-assisted solvent extraction

US EPA	The United States Environmental Protection Agency
UV	Ultraviolet
WWTPs	Wastewater treatment plants

Abbreviations of Target Analytes

BTH	Benzothiazole
2-Cl-BTH	2-chlorobenzothiazole
2-M-BTH	2-morpholin-4-yl-benzothiazole
2-Me-BTH	2-methylbenzothiazole
2-Me-S-BTH	2-methylthiobenzothiazole
2-NH_2-BTH	2-aminobenzothiazole
2-OH-BTH	2-hydroxybenzothiazole
2-S-BTH	2-mercaptobenzothiazole
2-SCNMeS-BTH	2-thiocyano-methylthiobenzothiazole
BTR	Benzotriazole
BTR-COOH	Benzotriazole-5-carboxylic acid
TTR	4-methyl-1-H-benzotriazole or tolyltiazole
XTR	5,6-dimethyl-1-H-benzotriazole or xylyltriazole
1-OH-BTR	1-hydroxybenzotriazole

5-Cl-BTR	5-chloro-1-H-benzotriazole
5-NH_2-BTR	5-amino-1-H-benzotriazole
NAP	Naphthalene
ACY	Acenaphthylene
ACE	Acenaphthene
FLU	Fluorene
PHE	Phenanthrene
ANT	Anthracene
PYR	Pyrene
FLT	Fluoranthene
CHR	Chrysene
BaA	Benzo[a]anthracene
BkF	Benzo[k]fluoranthene
BbF	Benzo[b]fluoranthene
BaP	Benzo[a]pyrene
BgP	Benzo[ghi]perylene
IND	Indeno[1,2,3-cd]pyrene
DBA	Dibenz[a,c]anthracene

Chapter 1

Introduction

Environmental pollutants are ubiquitous in the environment, and pollution of aquatic environments has been intensively studied for many decades due to the possible impacts of pollutants on wildlife and human health. The sources of these contaminants to the environment are diverse, from fossil fuel burning to industrial activity and sewage discharge. Once pollutants are released into aquatic systems they can undergo a variety of processes, such as photolysis, chemical oxidation, and microbial degradation, which may alter their behaviour and fate in the environment [1]. Trace contaminants that are removed from the water column can be adsorbed to particular matter and eventually deposit onto bottom sediments [2]. In this way fine-grained sediments can act as a sink for contaminants, where temporal changes in contamination can be recorded [3]. The contaminants present in sediments are of interest due to the possibility of secondary emissions, where accumulated contaminants leach out, causing "new" emissions even though the primary emissions are reduced [1].

The first aim of this study was to determine the presence of selected organic chemicals in sediments from Trondheimsfjorden. The main chemi-

cals of interest in this study was benzothiazoles (BTHs) and benzotriazoles (BTRs), as both groups of compounds have been detected in high concentrations in wastewater samples, indicating that they are not efficiently removed during wastewater treatment processes [4–6]. However, since these compounds are relatively polar and hydrophilic, they are not expected to be found in sediments, and their presence in this matrix is not well documented [7]. In this thesis the occurrence of 16 different benzothiazoles and benzotriazoles were determined in sediments, where several compounds were investigated in this matrix for the first time.

The sediments were also analyzed for polycyclic aromatic hydrocarbons (PAHs), which are considered to be persistent environmental pollutants. Due to their high lipophilicity they are swiftly sorbed onto particles and subsequently deposited onto sediments in aquatic environments [8]. The aim of analyzing the PAHs in addition to the other organic contaminants was to establish if there could be any associations between these three groups of organic contaminants; two of those being emerging pollutants. The final aim was of this thesis was to investigate if there were any associations between the three groups of organic contaminants and a selection of trace elements, since it is known that several of the BTHs and BTRs are used as corrosion inhibitors [9, 10].

Chapter 2

Background

2.1 Emerging Contaminants

Emerging contaminants (ECs) is a term that is commonly used to define chemicals that are not commonly monitored in the environment [11–13]. In general, there is limited information about these compounds, from their production volumes to their physicochemical properties. There is also little information about their effects on humans and the environment [14]. The definition of what emerging contaminants are may differ, but as a general approach, ECs are compounds that have recently been found to appear in the environment [15]. The United States Environmental Protection Agency (US EPA) defines emerging pollutants as new chemicals without regulatory status and which impact on the environment and human health is poorly understood [12]. Another group of compounds that have a similar definition is contaminants of emerging concern (CECs), which are chemicals that have been observed in the environment for some time, but where new concerns such as occurrence, levels, fate, and toxicity have

been raised [14]. Many of the emerging contaminants are not new compounds, but have gained a new interest in scientific communities due to their omnipresence in the environment [16].

There have been an increased awareness regarding the presence of ECs in the environment, since several studies have found that these chemicals occur at significant levels [11–13]. Among chemicals that are classified as emerging contaminants are; industrial, agricultural and household chemicals (e.g. flame retardants, plasticides, fluorinated compounds and nanomaterials), as well as different pharmaceutical and personal care products (e.g. antibiotics, drugs and sunscreen agents) [17]. These compounds can enter the environment through different means[14, 18], where their physicochemical properties (e.g. water solubility, polarity and vapour pressure) ultimately will determine their behaviour and fate in the environment [15]. Some of the compounds that are classified as ECs and CECs can have natural sources, but due to anthropogenic emissions, elevated concentrations can be observed in the environment. Since there is a lack of regulation for the use and release of most ECs, these compounds are continuously released to the environment. Once released they can accumulate and cause adverse effects on ecosystems and biota [14].

Contaminants may end up in the environment in different ways, such as through industrial waste, incomplete removal in sewage treatment plants, and atmospheric deposition. The way that they enter the environment will depend on their pattern of usage. The sources for contaminants to end up in the environment can be divided into two groups; point sources and non-point sources [13]. Point-source pollution originates from discrete locations whose input into aquatic systems can often be defined in a spatially discrete manner. Examples of point sources are industrial effluents, municipal sewage treatment plants, and waste disposal sites [15]. Non-point sources, also referred to as diffuse sources, originates from indistinct sources that typically occur over large areas. Typical non-point sources include agricultural runoff from bio-solids and manure, urban runoff, diffuse

aerial deposition, and rain overflow in urban areas [13, 15]. For diffusive sources it is hard to link the pollution back to its original source, and as a consequence it is difficult to control and measure their effect on the environment [13].

There is still a knowledge gap regarding ECs, and the need to understand the different processes controlling their transport, fate and effects on humans and wildlife has increased the necessity to study their occurrence in the environment. One of the biggest challenges is to identify chemicals which potentially will present a risk to different receptors. Many of these chemicals are present at trace levels in the environment, and there is therefore a need for developing analytical methods that can identify and quantify ECs in different matrices down to part per billion (ppb) levels in concentration [11]. Without proper identification, their sources and transportation pathways to the environment cannot be determined [13]. The presence of emerging contaminants in the environment is concerning since they do not appear as individual components, but as a complex mixture, which could lead to synergistic effects. The ubiquity of a number of potentially toxic emerging contaminants in the environment underlines the need to better understand their occurrence, fate and ecological impact [19].

2.2 Benzothiazoles and Benzotriazoles

Benzothiazoles (BTHs) and benzotriazoles (BTRs) are two groups of high production volume organic compounds that have been classified as emerging contaminants [4, 20]. BTHs are heterocyclic aromatic compounds that consists of a 1,3-thiazole ring fused with a benzene ring. BTRs are another group of heterocyclic aromatic compounds, that consists of two fused rings with three nitrogen atoms. The general structure of BTH and BTR is given in Figure 2.1. Both groups of compounds are commonly used in

a variety of industrial and household products, such as flame and corrosion inhibitors, fungicides, pesticides, dishwasher detergents, anti-fogging fluids and vulcanization accelerators in rubber [20, 21]. In addition to anthropogenic sources, it has been found that certain BTHs can be derived from natural sources. For instance, BTH and 2-methylbenzothiazole (2-Me-BTH) are known constituents in tea leaves, while BTH has been found in tobacco smoke [22, 23].

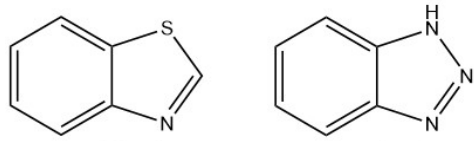

Figure 2.1: General structure of benzothiazole and benzotriazole

Due to the widespread use of both BTHs and BTRs they can easily reach different environmental media, such as surface waters, groundwater, soils and sediments [4]. Detectable concentrations of BTH- and BTR derivatives have been reported in indoor air [24], indoor dust [25], tap water [26, 27], wastewater [4, 6, 28], textile [29, 30], and seafood [31], among others. A few studies have also detected BTR and a few of its derivatives in sediments [32–34] and soil [33]. Several studies have found that these compounds are present in high concentrations in wastewater due to insufficient removal during the treatment process [4]. Since they are not removed sufficiently during this treatment, relatively high concentrations have also found in receiving water bodies [21, 35, 36]. However, their occurrence in sediments is scarcely reported since these compounds are not expected to be found in high concentrations in this matrix [7]. Previous detected levels of BTHs and BTRs in selected, relevant matrices are presented in Table 2.1. The ubiquity of these compounds provides potential routes for human exposure through inhalation, ingestion, and adsorption through textiles [29]. Studies done on human exposure to BTHs and BTRs have detected both

groups of compounds in urine from humans [10, 22, 37], and their occurrence in human tissues have also been documented [38]. A study have also reported on benzotriazole ultraviolet stabilizers and 1-H-BTR in breast milk and amniotic fluids from pregnant women [39], highlighting the risk for direct fetal exposure.

Even though these compounds are commonly found in the environment, there is little knowledge about the toxicity of these compounds. Studies done on the toxicity of benzothiazole and 2-mercaptobenzothiazole (2-S-BTH) indicated that they may pose a risk at sufficient exposure, which may result in central nervous system breakdown, and liver and kidney damage [50]. Another study also found that BTH and 2-S-BTH show acute toxicity in various aquatic test systems [51]. Moreover, BTHs have been found to cause eye, skin, and respiratory irritation [30]. 2-thiocyanomethylthiobenzothiazole (2-SCNMeS-BTH) is one of the BTH derivatives which have been found to act as an endocrine disruptor [52].

Toxicity studies done on BTR and its derivatives have revealed that BTRs are phytotoxic, and that they can have carcinogenic, mutagenic, and esterogenic effects on marine animals [53, 54]. It has also been found that benzotriazole (BTR) is toxic to aquatic organisms, and that it can cause long-term adverse effects on aquatic ecosystems [55]. This compound has also been classified as a suspected carcinogen by The Dutch Expert Committee on Occupational Standards [56]. Developmental toxicity of BTRs have been reported in marine animals, when zebrafish (*Danio rerio*) embryos were treated with 4-methyl-1-H-benzotriazole (4-Me-BTR) [57]. It has also been reported that BTR derivatives containing more methyl groups could be more toxic towards bacteria [58]. One study found that if BTR and copper (Cu) are co-present in soil, BTR could reduce the toxicity of Cu towards terrestrial invertebrates [59]. This is probably due to the formation of a complex between BTR and Cu, which will decrease the bioavailability of Cu to organisms exposed to the contaminated soil [58].

Table 2.1: Occurrence of benzothiazoles and benzotriazoles in relevant sample matrices

Compound	Matrix	Unit	Concentration	Country	Reference
Benzotriazoles					
BTR	Ground water	ng/L	280	Australia	[35]
	Sediment	ng/g	10-13	United States	[40]
	Sediment	ng/g	0.385	China	[32]
	Sediment	ng/g	0.424-33.4	United States	[32]
	Sediment	ng/g	14.5-630	Brazil	[41]
	Sediment	ng/g	<LOQ-231	Spain	[42]
	Sludge	ng/g	<LOQ-27.1	Spain	[43]
4-Me-BTR	Sludge	ng/g	3.4-82.3	Spain	[43]
5-Me-BTR	Ground water	ng/L	154	Australia	[35]
	Sediment	ng/g	1.59-165	United States	[32]
	Sediment	ng/g	<LOQ	Brazil	[41]
	Sediment	ng/g	n.d. - <LOQ	Spain	[42]
	Sludge	ng/g	<LOQ-30.4	Spain	[43]
TTR	Sediment	ng/g	11	United States	[40]
	Sediment	ng/g	278	Germany	[44]
XTR	Ground water	ng/L	114	Australia	[35]
5-Cl-BTR	Ground water	ng/L	97.5	Australia	[35]
	Sludge	ng/g	n.d.-2.3	Spain	[43]
Benzothiazoles					
BTH	Sludge	ng/g	265	Germany	[45]
2-S-BTH	Sediment	ng/g	n.d.-70.0	Sweden	[46]
2-Me-S-BTH	Sludge	ng/g	n.d.-93.3	Spain	[43]
	Sludge	ng/g	326	Germany	[45]
2-OH-BTH	Sediment	ng/g	n.d.-31.0	United States	[47]
	Sludge	ng/g	<LOQ-181.2	Spain	[43]
	Sludge	ng/g	307	Germany	[45]
2-M-BTH	Sediment	ng/g	2.5-5.1	Japan	[48]
	Muddy sands	ng/g	49-360	United States	[49]

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2.2.1 Benzothiazoles

Benzothiazoles (BTHs), with the general formula C_7H_5NS, are a group of aromatic heterocyclic compounds that consist of a 1,3-thiazole ring fused to a benzene ring [60]. 2-substituted benzothiazoles are a group of commonly used industrial chemicals, that are primarily used as vulcanization accelerators in rubber and tire production [51, 52, 61]. Common vulcanization accelerators include 2-mercaptobenzothiazole (2-S-BTH), and other derivatives derived from this compound [51, 61]. BTHs are also commonly used as corrosion inhibitors, fungicides in leather and paper manufacturing, ultraviolet light stabilizers in plastics and textiles, food flavouring agents and as additives in pharmaceuticals [7, 61, 62]. Both BTH and 2-S-BTH are also used as corrosion inhibitors in antifreeze fluids [51], while 2-SCNMeS-BTH can be used as a fungicide [61]. 2-morpholin-4-yl-benzothiazole (2-M-BTH) is used as a vulcanization accelerator in rubber [63], and it has also been documented that this compound is used in antifreeze liquids in cars [47]. Furthermore, 2-aminobenzothiazole (2-NH_2-BTH) is a known constituent of several azo disperse dyes [52]. The different derivatives of BTHs included in this study are presented in Figure 2.2.

The greatest source for introduction of BTHs to the environment is thought to be through wastewater discharge. Benzothiazoles are not completely removed during the treatment process, since municipal wastewater treatment plants are not optimized for the removal of organic micropollutants [4, 64]. BTHs can enter wastewater through different sources, such as landfill leachate and street run-offs, as well as through industrial and household wastewater. The main sources for BTHs in street run-offs are assumed to be tire wear and antifreeze agents [52]. BTHs have been reported in many environmental matrices due to their wide applicability, and it was estimated that the production of BTH in the United States in 1993 was 4.5-450 tons [60]. Exact production information for BTH and its derivatives

are not readily available, but since BTH can be derivatized to yield a wide range of biologically active compounds [30, 65], it is expected that these chemicals are produced in high volumes [60].

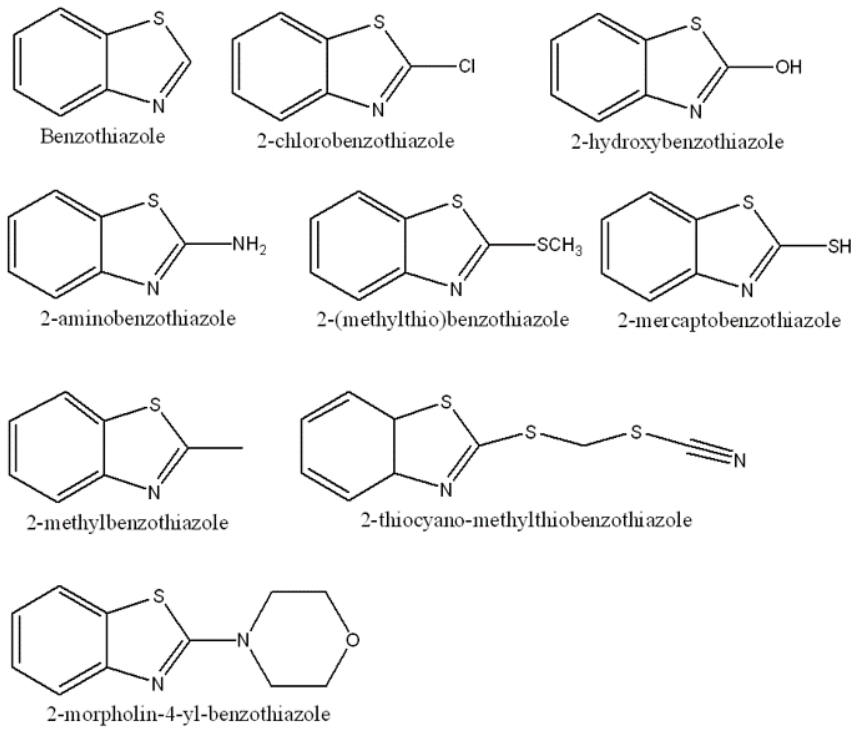


Figure 2.2: Derivatives of benzothiazoles analyzed for in this study

2.2.2 Benzotriazoles

Benzotriazoles (BTRs), with the chemical formula $C_6H_5N_3$, are a class of organic compounds that consists of a benzene ring fused to a five-membered ring that contains three nitrogen atoms [66]. Commonly used BTRs include 1-H-benzotriazole (BTR), as well as different derivatives such as xylyltriazole (XTR, or 5,6-dimethyl-1-H-benzo-triazole), tolyltriazole (TTR, a mixture of the two isomers 4-methyl-1-H-benzotriazole and 5-methyl-1-H-benzotriazole), 5-chlorobenzotriazole (5-Cl-BTR) and 1-hydroxybenzotriazole (1-OH-BTR). Other less common derivatives of BTR include benzotriazole-5-carboxylic acid (BTR-COOH) and 5-amino-benzotriazole (5-NH_2-BTR) [36]. The structures of the benzotriazoles included in this study are given in Figure 2.3. BTRs have been classified as high-production volume substances, where the production of BTR in the United States was reported to be 850 tons in 2012 [67]. It has been reported that BTR and 5-Me-BTR are the most used BTRs in industrial processes and household detergents, and they are therefore also the most detected BTRs globally within the environment [66]. One of the properties of BTRs is that they can form stable coordination compounds with some metals, making them suitable as corrosion inhibitors. Both BTR and 5-Me-BTR have these properties and are consequently commonly used as anticorrosive additives [67]. BTRs are added to many formulations that comes in contact with metals, such as aircraft de-icing fluids, automotive antifreeze formulations, brake fluids, metal-cutting fluids and dishwasher detergents [68]. Other common application of BTRs are as ultraviolet light stabilizers in plastics, as anti-fogging agents in photography, and as additives in some pesticides and herbicides [7, 20, 36].

The greatest source for introduction of BTRs to the environment is thought to be through discharge of treated wastewater, since several studies have reported that these compounds are only partially removed during the treatment process [4, 35]. Benzotriazoles are in general characterized by their

low vapor pressure, high polarity, high water solubility and low octanol-water partition coefficients (log K_{OW}) [69], making them mobile in aqueous environments. Due to their low volatility, they may persist in aquatic environments [36]. Moreover, they display resistance to oxidation under environmental conditions, and are stable under moderate UV irradiation [66].

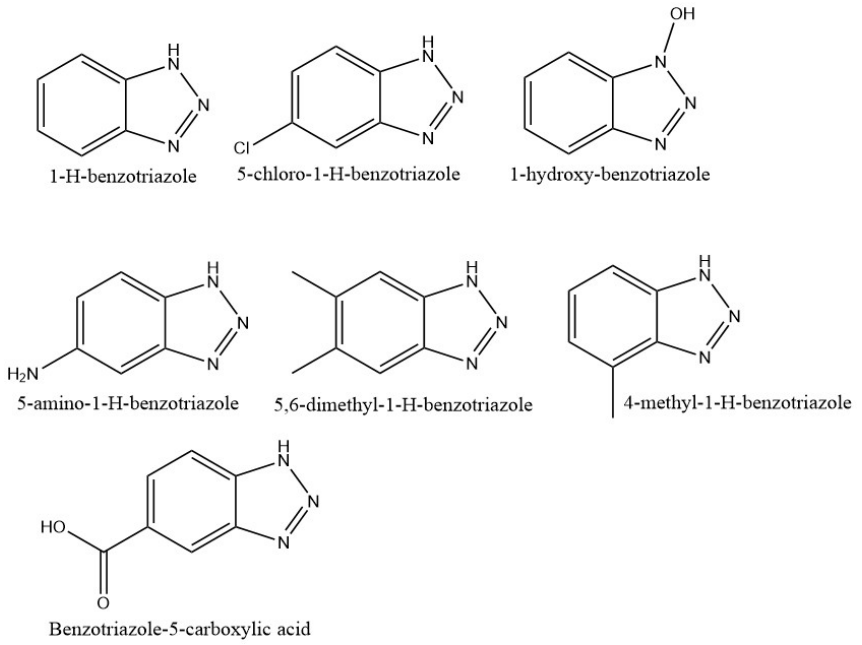


Figure 2.3: Structures of the seven BTRs analyzed for in this study

2.2.3 Environmental Transformation of BTHs and BTRs

Emerging contaminants will to different extents undergo transformation in the environment. Some substances are rather inert and will therefore persist and barely degrade, while others are quite rapidly biodegraded [55]. Different natural processes can dilute, transform, or eliminate contaminants in the environment, which may result in a mixture of parent compounds and transformation products. Dispersion and dilution play a key role in the removal of pollutants in the environment by decreasing their average concentrations. Immobilization through sorption onto sediments and suspended materials is also an important process [70]. Chemical transformation of ECs can be influenced by biotic or abiotic factors. Abiotic transformation includes photochemical degradation, where the photolytic reactions often are quite complex, yielding several reaction products. ECs can also be removed or transformed by microorganisms that utilize the compounds as an energy source [17].

Studies have indicated that the different derivatives of BTH and BTR will undergo various degradation and transformation processes in the environment [23, 47, 60, 64, 71]. There have been a few studies on the microbial transformation of 2-substituted BTHs, where it has been found that BTH is rapidly biodegraded under aerobic conditions [60]. 2-OH-BTH also appear to be totally biodegradeable [23, 47]. It is thought that BTH and 2-OH-BTH are biodegradeable through enzymatic transformations by different microorganisms. De Wever et al. found that certain microorganisms can transform BTH to 2-S-BTH which is rapidly transformed to a more stable, methylated product (e.g. 2-Me-S-BT) [23]. 2-S-BTH can also be transformed to 2-OH-BTH or BTH by photolysis [47]. Studies done on the degradation of 2-SCNMeS-BTH has indicated that it can be hydrolyzed to 2-S-BTH, which again is methylated. 2-SCNMeS-BTH can also be transformed directly to 2-Me-S-BTH through photolysis. It has also been suggested that 2-SCNMeS-BTH can be degraded to BTH under

anaerobic conditions [23]. In chlorine containing waters it has been found that BTH can be oxidized by hydrochloride to 2-OH-BTH. It has also been proposed that 2-M-BTH can be photodegradaded to BTH following a similar mechanism as 2-S-BTH [60]. Different transformation pathways for benzothiazoles in the environment are demonstrated in Figure 2.4

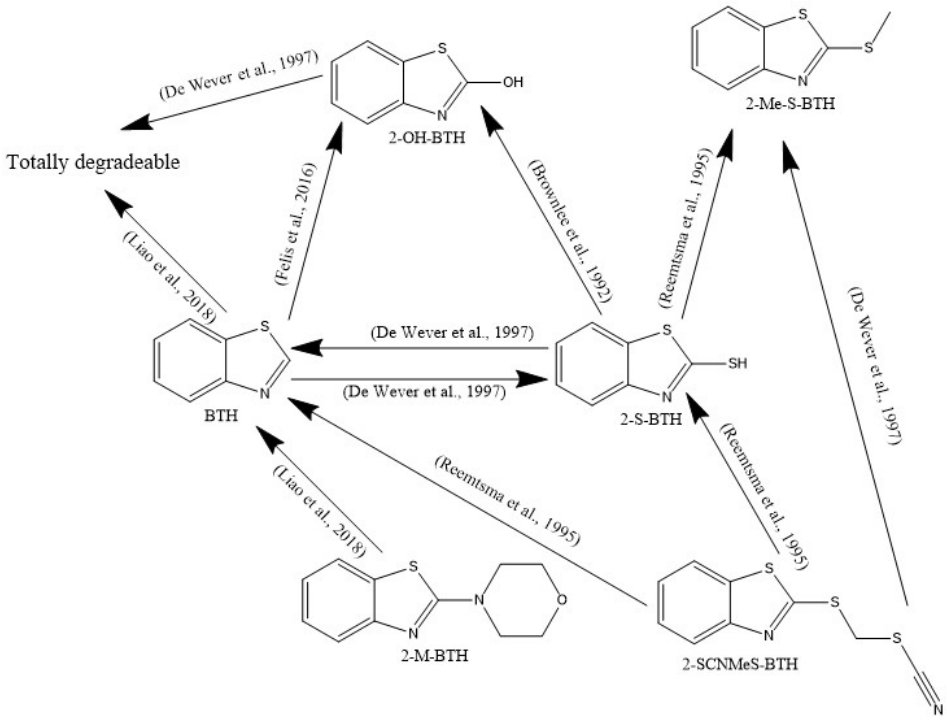


Figure 2.4: Overview of transformation pathways for BTHs in the environment

Benzotriazoles will also go through different transformation processes in the environment. It has been found that BTR can undergo direct photolysis to produce a variety of byproducts in the environment. XTR, a methylated derivative of BTR, is thought to be a possible transformation product from BTR [71]. Other transformation products from BTR includes 1-methylbenzotriazole (1-Me-BTR) [71], 1-OH-BTR, and 4-hydroxybenzotriazole (4-OH-BTR) [64]. It has also been proposed that BTR-COOH is a transformation product of 5-Me-BTR [71], and it can also be a transformation product of BTR [64]. An overview of different transformation products of BTR and some of its derivatives is given in Figure 2.5.

A study done on the biodegradation of certain benzotriazoles, demonstrated that BTR, XTR, TTR, and 5-Cl-BTR can undergo microbial degradation under both aerobic and anaerobic conditions [58, 72]. BTR was found to be slowly degradeable under both aerobic and anaerobic conditions, where the degradation was fastest under aerobic conditions [58]. It has been found that different reducing conditions (nitrate, sulfate, and Fe (III) reducing) affected their biodegradation, indicating that the redox conditions have an influence on the microbial degradation of BTR and its derivatives. 5-Me-BTR is completely removed through biodegradation under aerobic conditions. Under both anaerobic and aerobic conditions demethylation of 5-Me-BTR to BTR occurs, before BTR is transformed further to other degradation products. The degradation of 5-Cl-BTR was fastest under anaerobic conditions, where it is dechlorinated to produce BTR [72].

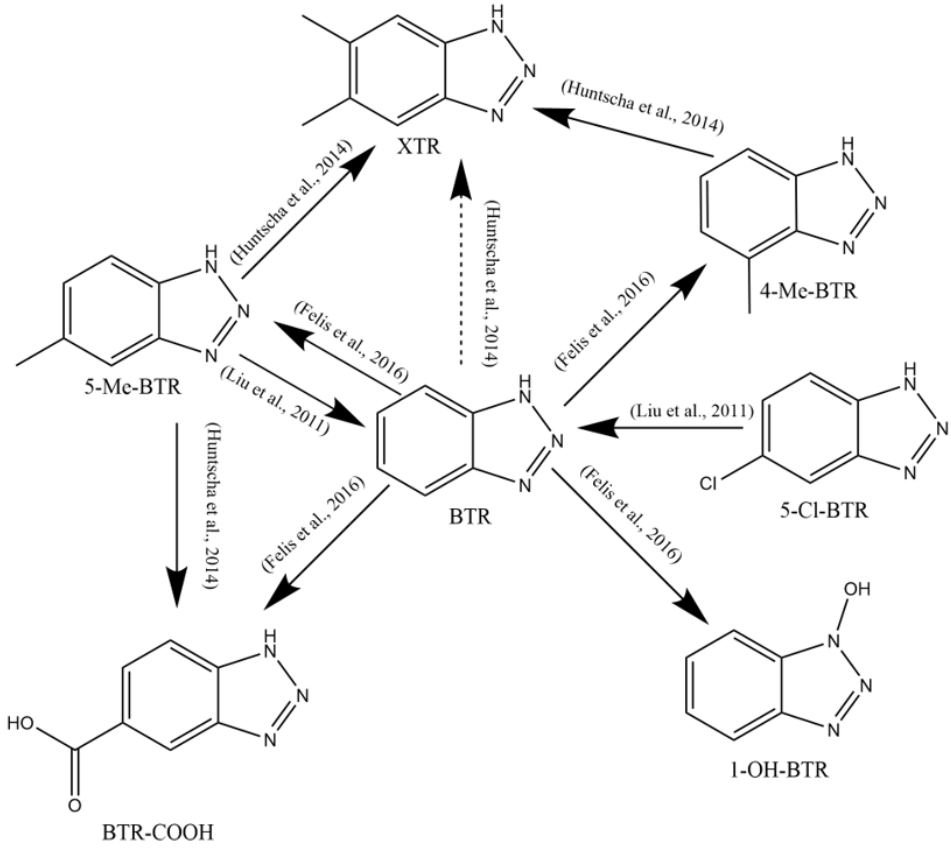


Figure 2.5: Overview of different transformation products of BTR and some of its derivatives.

2.2.4 Adsorption of BTHs and BTRs to Sediments

The presence of benzothiazoles and benzotriazoles in the environment is well documented, but most studies have focused on their occurrence in various aqueous compartments [21, 73, 74]. There have also been several studies on their occurrence in sludge and water from wastewater treatment plants (WWTPs), to investigate their removal efficiency [4, 6, 28]. Information about their presence in soil and sediments, on the other hand, is limited [32, 40–42], and the few studies that have looked into this have analyzed quite few derivatives. It would, however, be reasonable to assume that BTHs and BTRs can reach sediments through sewage sludge [75], since high concentrations of these compounds are found in wastewater [4, 6].

In general, BTHs and BTRs have relatively low octanol-water partition coefficients (K_{OW}), indicating that these compounds are hydrophilic as they have a high affinity to the water phase. Experimental values for the log K_{OW} values for these compounds are given in Table B.3 (Appendix B) [47]. They are therefore expected to be present in the water phase, but it has been suggested that different mechanisms can contribute to the adsorption of both BTHs and BTRs to sediments. Mechanisms that are believed to contribute to the adsorption of BTHs and BTRs are hydrophobic interactions of the non-polar part of the molecules with organic matter in the sediments, charge transfer, and sorption through intraparticle diffusion [66]. In soil it has also been found that the sorption of BTRs to soil varied as a function of organic carbon in the soil [69]. It would be reasonable to assume that the same would be true for sediments, and a few studies have detected trace levels of some BTH and BTR derivatives in this matrix [32, 34, 40–42]. Previous research have also suggested that as the contact time between the contaminants and sediments increase, compounds will diffuse to less accessible sorption sites, making it more difficult for them to be desorbed again, thus making sediments a sink for contaminants [76].

2.2.5 Choice of Method

The choice of method for the analysis of BTHs and BTRs may vary, partially depending on the sample matrix. Like other emerging contaminants, BTHs and BTRs will be found in trace levels, and an efficient extraction procedure followed by analysis with a sensitive instrumental technique is required in order to detect these levels [7]. For solid matrices (e.g. sediments and soil) several different extraction techniques have been reported, such as ultrasound-assisted solvent extraction (USAE) [30], pressurized liquid extraction (PLE) [35, 77], and liquid-solid extraction (LSE) [43], often followed by sample clean-up by liquid-solid extraction (SPE) [7]. Furthermore, sample pre-treatment is necessary before the extraction, which will usually include freeze-drying of the samples to remove water which could distort the response in the detector [31].

The separation of the different BTH and BTR derivatives is usually performed by either gas chromatography (GC) or liquid chromatography (LC) techniques coupled with mass or tandem mass (MS or MS/MS) detection [10]. Since both groups of compounds consists of aromatic ring systems, detection can also be done by UV-vis or fluorescence detection [7]. The preferential use of LC or GC coupled with MS/MS is due to the high selectivity and sensitivity of these methods. Due to the low volatility of BTHs and BTRs, LC-MS/MS is usually used for their analysis [7, 31, 37]. Since these compounds are highly polar, electrospray ionization (ESI) is the most common interface between the LC and MS, since it provides good ionization efficiency in positive mode for most of the different derivatives [7]. Despite the matrix effect being a major problem, ESI is preferred when working with LC. To overcome this disadvantage, atmospheric pressure chemical ionization (APCI) is sometimes used, since it is less vulnerable to matrix effects [31]. The most common analyzer used for MS/MS detection is the triple-quadrupole mass analyzer (QqQ), but the use of other analyzers have also been reported [7, 74].

Several studies have used GC for the analysis of both BTHs and BTRs, and it is possible to do so without doing any prior derivatization steps [20, 31, 35, 73]. However, GC is not commonly used since it is difficult to find a column that can separate a large number of these compounds simultaneously. If GC is used for their analysis, electron ionization (EI) is usually used as the interface. Since EI is considered a hard ionization technique, low mass fragments of the target analytes are obtained, and the sensitivity of these methods are thus decreased [7].

2.3 Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are a diverse group of organic compounds that are commonly studied in the environment. The group consists of homogeneous π-electron systems of hydrogen atoms and sp^2-hybridized carbon atoms, and are composed of two or more fused aromatic rings [78]. Several hundred different PAHs have been identified, but the most commonly PAHs consists of two to seven fused aromatic rings. The different compounds have varying characteristics and will have different interactions with and effects on biological systems [79]. PAHs can be decomposed through different chemical and biological processes that can occur in both the atmosphere and in aquatic environments [80]. The tendency for PAHs to bind to particulate matter allows them to be transported by air and water, where they ultimately settle out in sediments [81]. In this way sediments works as a sink, where the contaminants can accumulate and persist for a long time [79]. Mechanisms affecting the fate of selected PAHs in the environment is given in Figure 2.6.

Anthropogenic activities is an important factor in the increased levels of these compounds in the environment, even though they also exist as natural products. PAHs are formed during incomplete combustion of organic

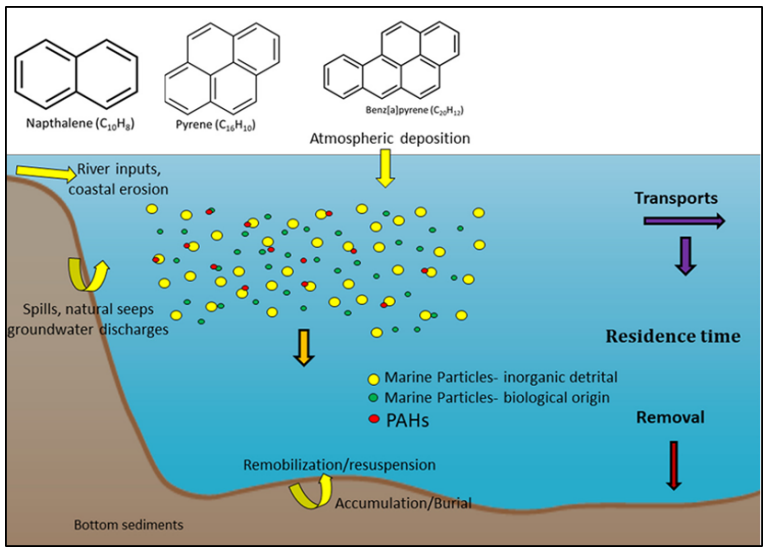


Figure 2.6: Fate of selected PAHs in aquatic environments. The figure is adapted with permission from Adhikari et al

material, and are also present in fossil fuel such as oil, gas and coal [79]. Natural sources for PAHs include forest fires, oil seeps, and volcanic eruptions, while anthropogenic sources will include fossil fuel burning, municipal solid waste incineration and incomplete combustion from automobiles [82]. Due to the previous widespread use and consequently emissions of these compounds, PAHs are frequently detected in air, soil, water and sediments, and are considered to be ubiquitous in the environment [81, 83].

Some PAHs are especially important to consider from an environmental point of view, since they have been found to be carcinogenic and mutagenic [80]. The US Environmental Protection Agency (USEPA) have identified 16 PAHs as toxic pollutants due to their effect on mammals and aquatic organisms, and these PAHs are therefore the most studied ones in the environment [81]. The priority PAHs include naphtalene (NAP), acenapthy-

lene (ACY), acenaphthene (ACE), fluorene (FLU), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLT), pyrene (PYR), benzo[a]anthracene (BaA), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a] pyrene (BaP), chrysene (CHR), benzo[g,h,i]perylene (BgP), indeno[1,2,3-cd]pyrene (IND), and dibenz[a,c]anthracene (DBA). The structures of these compounds are presented in Figure 2.7

PAHs are semivolatile compounds under environmental conditions, making them quite mobile in the environment [80]. Most PAHs, especially the larger ones, are hydrophobic and will therefore be insoluble in water [84]. On the other hand, they will have high affinity to phase transitions, such as water surfaces and the surface of particles. Since most PAHs will be lipophilic they can easily sorb to fine-grained organic rich sediments. This strong affinity to the organic phase is of importance for the bioavailability of the compounds [79]. Two ringed PAHs, and to a lesser degree three-ringed PAHs, can dissolve in water, making them more bioavailable than the larger PAHs. These compounds will be quite volatile, and will therefore predominantly be found in the vapour phase in the atmosphere [8]. In contrast, PAHs with five or more rings have very low solubility in the water phase, and will mainly be found in a solid state bound to particulate air pollution, soils, or sediments. Once they have adsorbed on to particulate matter they will have low mobility and be less accessible for biological uptake and degradation, increasing their persistence in the environment [84].

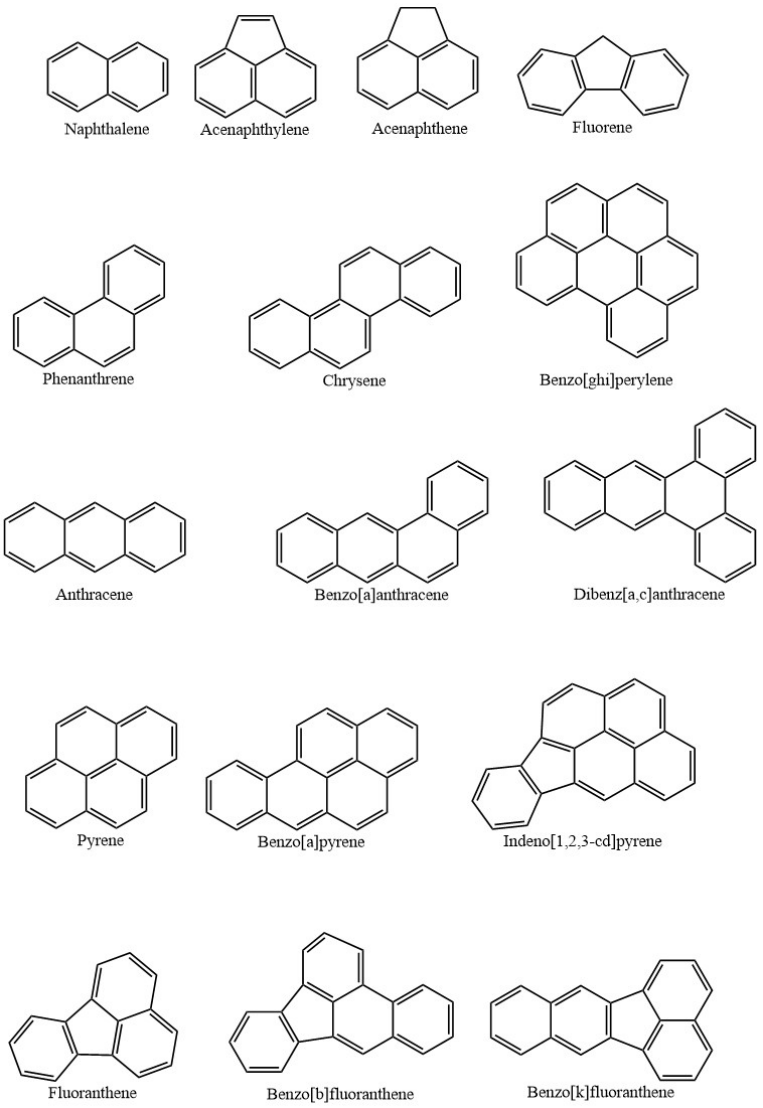


Figure 2.7: Structures of the 16 PAHs included in this study

2.4 Sediment Classification System

A classification system for the environmental quality of contaminated marine sediments have been in force in Norway since 1997. The classification is based on analysis of sediment, where the purpose is to establish environmental aims, prioritize sites where improvements can be made, and assess the success of measurements taken relative to the acceptance criteria [85]. In 2015 new limits were classified for prioritized environmental contaminants by the Norwegian Environmental Agency. In the classification system there are defined five concentration intervals, where each class limit represent an expected increase of damage towards ecological communities in the sediments. The limits are based on available information from laboratory tests, where the definition of class borders are based on ecotoxicological data [85, 86]. A description of the different classification classes is given in Figure 2.8. There has been made a classification system for a variety of organic contaminants, and the classification levels for PAHs in sediments can be found in Table C.6 in Appendix C.

Class I Background	Class II Good	Class III Moderate	Class IV Bad	Class V Very bad
Background levels	No toxic effects	Chronic effects of long-term exposure	Acute toxic effects of short-term exposure	Comprehensive toxic effects

Figure 2.8: Principles of the Norwegian environmental quality classification system for contaminants in sediments.

2.5 Trondheimsfjorden

Trondheimsfjorden is situated at the coast of central Norway, and stretches from Ørland in the west to Steinkjer in the north. The fjord is about 130 km long, which makes it the third-longest fjord in Norway [87]. In the Trondheim area, many decades with heavy industry and harbour activity, dumping of waste directly into the fjord, and open sewer outlets have lead to considerable contamination of the fjord. Contamination has also occurred through leaching from landfills and accidental spills from private and public companies [88].

Several studies of sediments from Trondheimsfjorden have detected large quantities of environmental pollutants such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), tributyltin (TBT), and heavy metals, making the seafloor heavily polluted [88]. Several projects have focused on cleaning the seafloor in harbors near Trondheim in order to reduce the contamination levels in seawater and sediments. A survey performed in 2010 showed that the level of pollutants in aquatic compartments are decreasing, and this is thought to be mainly due to the reduction of contamination sources on land. Thus, the seabed is now one of the biggest sources for pollution in the harbor area [89].

The latest project that focused on cleaning the seabed in Trondheimsfjorden, Renere havn (Cleaner harbor), finished in 2016, and was one of the biggest clean ups of the harbor as of yet. The project was done as a joint project between Trondheim harbor and Trondheim municipality, where the aim was to clean the harbor basin in Trondheim to prevent further spread of contaminated sediments [89]. Coastal traffic cause a risk of erosion, and thereby resuspension of contaminated sediments to the water column [88]. The areas that were in focus for this project was Kanalen, Brattørbassenget, Nyhavna, and Ilsvika. In these areas the seafloor was covered in clean masses to avoid further spread of the contaminated seafloor. Dredged areas

were covered with a layer of gravel to avoid spreading of residual contamination to these areas [89]. In this study sediments from the fjord were analyzed for PAHs, PCBs and a variety of trace elements, but the occurrence of benzothiazoles and benzotriazoles was not measured. Therefore, these two groups of organic pollutants were in focus for this study.

2.6 Sample Preparation

The basic concept of sample preparation is to convert the real sample matrix into a sample suitable for analysis [90]. Trace analysis of organic contaminants is a challenging process due to the complexity of the different sample matrices. The effect of the matrix may affect crucial method parameters such as limit of detection (LOD), lower limit of quantification (LLOQ), linearity, precision and accuracy. A sample preparation that involves isolation of target analytes, purification of the extract and preconcentration is therefore required [91]. During the sample preparation step target analytes are isolated from the complex sample matrix, where the removal of interfering matrix compounds is an important aspect. Since the compounds will be present in trace levels, a pre-concentration step will be important in order to improve the instrumental sensitivity [11].

2.6.1 Solid-Liquid Extraction

Solid-liquid extraction (SLE) is an extraction technique that is commonly used to isolate organic compounds from a solid sample matrix [92]. Separation of a compound from a solid matrix is achieved by dissolving the sample in a solvent that the target analytes are soluble in, but where other constituents of the sample are not. There are multiple factors that determine the efficiency of the extraction, where the type of solvent used is

considered the most important one. Other factors that will have an effect on the recovery is the ratio between solvent and solid sample, time of contact, temperature, and particle size of the solid matrix [93]. The efficiency of solid-liquid extraction can be increased with ultrasonication. Ultrasound is then used to speed up the extraction of both organic and inorganic compounds from the sample matrix. Ultrasound-assisted solid-liquid extraction is an effective and time-efficient method for the extraction of organic molecules from a solid matrix. The sonication accelerates the mass transfer between the solid phase and the liquid phase, thus making the extraction process more efficient [94].

2.6.2 Solid-Phase Extraction

Solid-phase extraction (SPE) is commonly used for clean-up, solvent exchange, extraction, class fractionation and concentration of trace pollutants from environmental samples [95, 96]. The clean up of a sample can be done in two different ways. Impurities that are present in the samples can be sorbed onto an appropriate solid phase contained in a disposable plastic cartridge while the analytes are eluted with a suitable eluent. Clean-up can also be done by using a solid phase that sorbs the analytes of interest, while the rest of the sample passes through the cartridge [97]. SPE is commonly used in trace level analysis, due to its low consumption of organic solvents and its ability to perform several steps at the same time [96, 98]. Other advantages of SPE is that it improves the selectivity and reproducibility of the extraction, and will generally result in high recoveries of the target analytes [98].

A SPE method consists of four main steps (see Figure 2.9). The cartridges that contains the sorbent are first conditioned by passage of a water-miscible organic solvent (e.g. methanol). This is followed by an equilibration step where water is passed through the column to increase the

effective surface area and remove interferences that may reside inside the column [95]. The aqueous sample is loaded at the top of the cartridge, and is passed through the cartridge by vacuum, with a flow rate of about 1-2 drops per second. When the samples are fully loaded, a washing step is performed to remove interfering compounds present in the samples. The cartridges are washed with a nonpolar solvent for polar analytes, and with a polar solvent for nonpolar analytes. A drying step is often performed after the washing step to prevent water from being in the final sample. Finally the target analytes are eluted with a suitable organic solvent or mix of organic solvents [98].

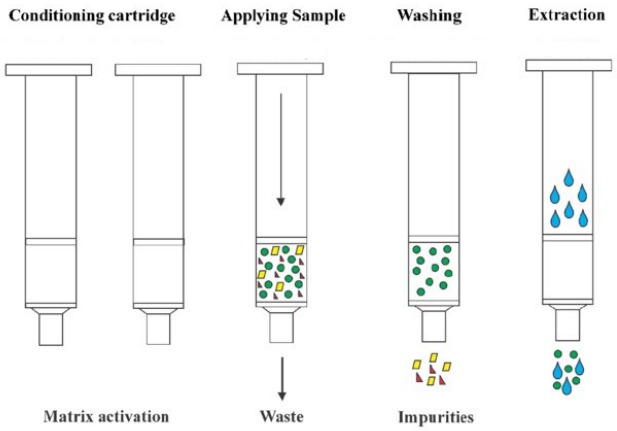


Figure 2.9: The main steps in solid-phase extraction. The figure is adapted from Abo et. al [99].

The choice of sorbent is a key element in SPE, since it can control crucial parameters such as selectivity and affinity. This choice will strongly depend on the target analytes, and the types of interactions desired between the analytes and the chosen sorbent. The matrix that is to be analyzed

should also be considered when choosing a sorbent, since different compounds in the matrix may interact with both the target analytes and the sorbent [90]. A wide variety of adsorbents are available for different applications, which makes SPE an applicable technique for the extraction of large specter of analytes [92].

2.6.3 Accelerated Solvent Extraction

Accelerated solvent extraction (ASE) is a solid-liquid extraction process that can be used for the extraction of different organic and inorganic compounds from a complex solid or semisolid sample matrix [100, 101]. The process exploits high temperature and pressure to accelerate the extraction procedure, saving both time and solvent compared to traditional extraction techniques [102]. The use of higher temperatures increases the capacity of solvents to solubilize the analytes and penetrate the sample matrix, which will increase the extraction efficiency. If sufficient pressure is then exerted on the solvent during the extraction it will be kept in a liquid state as the temperature is increased above the solvents boiling point [101]. The efficiency of the extraction will depend on the nature of the sample matrix, the analytes to be extracted and the location of the analytes within the sample matrix [100].

2.7 Instrumentation

When analysis of complex samples are required, a separation technique such as liquid chromatography (LC) can be coupled to a mass spectrometer (LC-MS). The combination of these two techniques is common for trace level analysis due to its robustness and high performance [103]. The increased use of LC-MS for these purposes is due to its ability to analyze polar organic compounds without having to perform a derivatization step beforehand [104]. When a mixture of compounds in a complex matrix needs to be analyzed, the ability to separate the individual compounds with chromatographic techniques is an essential step. The efficient separation of target analytes from each other, and from the different matrix components is important to reduce the background noise which will result in improved signals for the target analytes [97].

2.7.1 Liquid Chromatography

Liquid chromatography encompasses a variety of separation techniques with the common feature that they use a liquid as the mobile phase. It allows for great versatility regarding the separation of compounds, since both the stationary and mobile phase can interact with the analytes, thus influencing the separation of them [97]. Separation of compounds is achieved by injection of a dissolved sample into a stream of mobile phase that is pumped into a column packed with a solid separation material. Separation occurs since the compounds dissolved in the MP can either stay in the solvent or adhere to the packing material in the column, where the time spent between the two phases will vary between compounds based on their physicochemical characteristics [105].

The solvent delivery system distributes pressurized mobile phase with desired composition and flow rate through the injector and to the start of the

column, where it equilibrates with the stationary phase. The sample is generally dissolved in the mobile phase, and if that is not possible, a solvent with equal or lower eluent strength than the mobile phase can be used [97]. The sample is first loaded into a sample loop, before it is injected into the pressurized mobile phase stream. Solvent is pumped through the injector sample loop to wash the sample through the column, which is where the separation of the different compounds occur based on their polarity [105]. Since separation of compounds is determined by their retention, the composition of the mobile phase and its combination with a stationary phase is important, since these are the main factors responsible for retention of analytes [104]. The separated compounds will pass through the column exit line where they are transferred to the detector flow cell. The detector will interpret changes in concentration as changes in signal voltage. Since the separation in liquid chromatography occur in a dynamic manner, it requires a detection system that can work on-line and produce an instantaneous spectrum [97, 104].

The determination of organic contaminants in environmental samples is a challenge due to low concentrations and a large amount of interfering substances that may be co-extracted with the target analytes. Chromatographic separation will therefore be a crucial step in the analysis of emerging contaminants in complex sample matrices, since an efficient separation step will reduce the background noise, and thus improve the signal for target analytes [106].

2.7.2 Liquid Chromatography Mass Spectrometry

For qualitative analysis, mass spectrometry (MS) is commonly used in combination with a HPLC system to quantify emerging pollutants in environmental samples [107]. Today it is common to perform multi-residue analysis, which allows for the determination of numerous of compounds in

the same run, which is both time and cost efficient. Simultaneous analysis of compounds with different physicochemical characteristics often impose compromises between the different performance parameters [104]. When performing multi-residue analysis, it is therefore common to use ultra-high performance liquid chromatography (UHPLC), which uses columns with small particles. These columns will elute the sample components in narrow, concentrated bands, which will result in better chromatographic resolution and increased peak capacity. The use of small particles results in higher plate numbers (N), and shorter separation time, making this technique popular for multi-residue analysis [108–110].

Electrospray Ionization

In order to be able to couple a chromatographic system with a mass spectrometer, an interface is required. The function of the interface is to allow the system to function at near optimum conditions by solving the incompatibility problems between the two systems. Problems can arise due to the difference in material flow requirements, pressure gradients, and the presence of nonvolatile materials essential for the separation [111]. After chromatographic separation of the target analytes, they are transferred to the mass spectrometer for quantification. Everything passing through the column will go into the mass spectrometer; that includes target analytes, elution solvents, matrix compounds, and volatile reagents [111]. The solvent and contaminants will have to be evaporated, while the target analytes are ionized in the interface before they are transferred to the mass analyzer. The majority of current LC-MS applications employ atmospheric pressure ionization sources based on electrospray or chemical ionization [104].

Electrospray ionization (ESI) is a common interface in LC-MS due to its wide range of applications. ESI is used under atmospheric pressure, and is viewed as the most versatile ionization technique for neutral compounds

and ions in a solution. It is also recommended to be used for highly polar compounds [111]. Ions are produced in the solution by either accepting or donating a proton to produce positive or negative ions. Ionization is achieved by passing the sample solution through a heated metal capillary, where a potential of 3-6 kV is applied, forming a spray of fine droplets directed towards a counter electrode. A positive (+) potential is applied to generate positive ions, while a negative (-) potential is applied for negative ions [104].

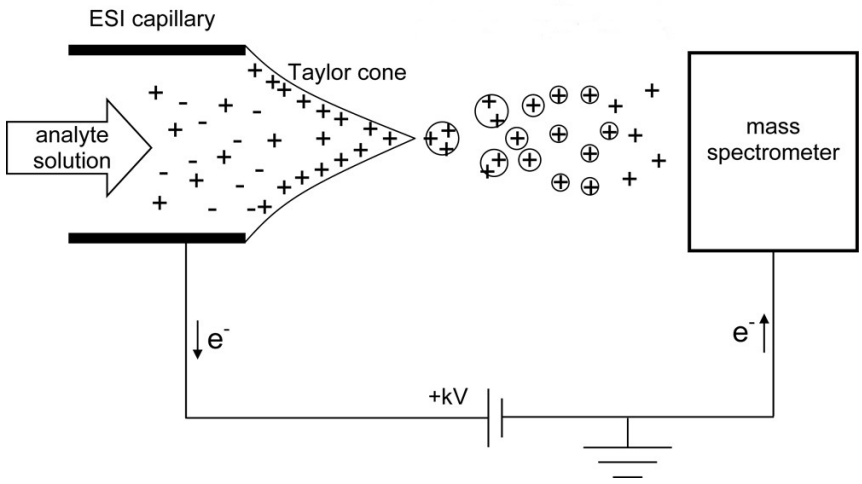


Figure 2.10: Mechanisms for electrospray ionization. The figure is adapted with permission from Konermann et al. [112]. Copyright©2012 American Chemical Society

There are three main steps in the production of gas phase ions by ESI: formation of charged droplets at the capillary tip (Taylor Cone), shrinkage of the charged droplets by solvent evaporation, and production of gas phase ions from the very small and highly charged ions (see Figure 2.10) [104, 112]. A nebulizing gas (usually N_2) is mixed with the mobile phase at the

outlet of the capillary, to promote the formation of charged droplets that undergoes size reduction by solvent evaporation as they move towards the sampling orifice. When the Coulomb repulsion forces between the charges exceeds the surface tension of the droplets, the resulting instability tears the droplets apart, producing smaller, charged droplets. These droplets will undergo further solvent evaporation, and when the charge density becomes too high, ions will desorb from the droplet into the gas phase. Solvent evaporation from these fine particles result in the yield of ions in the gas phase, which are then transferred to the mass analyzer [113].

Mass Analyzers

Once the gas-phase ions have been produced, they need to be separated according to their mass-to-charge (*m/z*) ratio prior to detection. Separation of ions according to their *m/z* ratio can be achieved by imposing external electric or magnetic fields on the ion beam. The five main characteristics for measuring the performance of a mass analyzer are the mass range limit, the scan speed, the transmission, the mass accuracy and the resolution [92]. The mass range determines the limit of *m/z* over which the mass analyzer can measure ions. The scan speed is the rate at which the analyzer measures over a particular mass range. The transmission is an expression of the ratio of the number of ions reaching the detector and the number of ions entering the mass analyzer. The mass accuracy is an indication of the accuracy of the *m/z* provided by the analyzer. The resolving power of a mass spectrometer is a measure of its ability to yield two distinct signals for two ions with neighbouring masses [103].

Linear Quadrupole

One of the most common mass analyzers for trace organic analysis is the linear quadrupole mass analyzer. The quadrupole mass analyzer consists of four parallel hyperbolic rods in a square array. Opposite rods are applied voltages of opposite charge, but of equal magnitude. Ions entering the mass analyzer will oscillate in the electric field produced by the rods [103]. The motion of an ion in the electric field will depend on its *m/z* ratio, and the voltage applied to the rods. At any given moment, only ions with a particular *m/z* value will have stable trajectories through the quadrupole mass filter. All other ions will oscillate with greater amplitudes, causing them to become unstable, and neutralized by collision with one of the rods. In this manner, only ions with selected *m/z* ratios will pass through the analyzer and reach the detector [104].

Tandem Mass Spectrometry

When structure elucidation or trace level quantification of target analytes are required, tandem mass spectrometry (MS/MS) is commonly used, since greater sensitivities are required [111]. Tandem mass spectrometers are instruments with a single ion source and two mass analyzers separated by a reaction region [104]. LC-MS/MS instruments, mainly triple quadrupole, are today commonly used for reliable determination of emerging contaminants in the environment [91, 114].

The triple quadrupole (QqQ) mass spectrometer consists of a linear combination of three quadrupole mass analyzers in series, that are designed to cleave molecular ions into fragments called product ions. Only the first and third quadrupole (Q1 and Q3) have scan abilities, while the second quadrupole (Q2) acts as a reaction region and holding cell, and is commonly called the collision cell. In the collision cell, fragments that have

been separated in Q1 interacts with an inert collision gas (He, Ar, Xe) to produce fragments that can be separated in the third quadrupole [111]. Q1 can be operated in either full-scan mode or selected ion monitoring (SIM) mode in order to select ions that can pass on through to the other analyzers in the system. In the second quadrupole, Q2, collision-induced dissociation (CID) occurs when fragments from Q1 collides with an inert gas present in the collision cell, producing fragments of the ions passed on from the first quadrupole. The last analyzer, Q3, can also be operated in either full-scan or SIM mode, resulting in four different modes of application [104].

In the first mode, called product ion scan, selected precursor ions with a specific *m/z* ratio are selected and isolated in Q1. Collision-induced dissociation results in fragmentation in the collision cell (Q2), and these product ions are scanned for and determined in the last quadrupole [104]. Another mode of operation is called precursor ion scan. In this scan mode, Q1 is set to scan over a mass range of interest, while Q3 is set to transmit a specific m/z ratio, namely a product ion of interest. Thus, all the precursor ions that produce ions with the selected mass through fragmentation in Q2 are detected. In neutral loss scan, both Q1 and Q3 scan over a selected mass range. A neutral fragment is selected, and fragmentation that leads to a loss of that neutral mass is detected [103].

For targeted trace organic analysis the most common approach is to operate with both the mass analyzers in SIM mode, which is called multiple reaction monitoring (MRM) [111]. In Q1 specific ions are transmitted to the collision cell where they undergo fragmentation. In Q3 only ions that produce a given fragment is detected. Compounds that are analyzed in this manner must be known and characterized beforehand, since the masses scanned for in the two analyzers must be known [104]. The absence of scanning allows one to focus on the precursor and fragment ions over longer times, increasing the sensitivity and thus get an increase in selectivity [103]. Another advantage with MRM is that it is designed to analyze for specific components of impure mixtures, without having to completely

purify the samples, which will make the extraction process less complex [111].

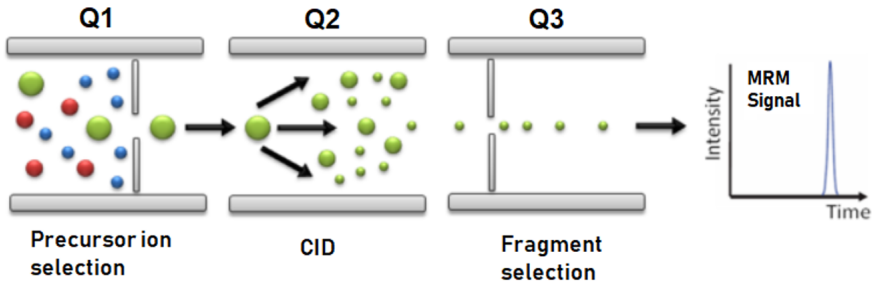


Figure 2.11: Schematic representation of mass spectrometry analysis in MRM mode (triple quadrupole mass spectrometer). In MRM, the first (Q1) and third (Q3) quadrupole serve as mass filters, while the second quadrupole (Q2) acts as a collision cell. Q1 allows selected precursor ions to pass through which are subsequently fragmented by collision induced dissociation (CID) in Q2. Product ions enter Q3 where only ions used for quantification are selected and passed on to the detector [115]. The figure is adapted with permission from Schmidt et al. (2008) [116].

2.8 Quantification and quality control

Quality control is defined as the operational techniques and activities that are used to fulfill the requirements for quality [117]. In order to make sure that the target analytes can be reliably quantified in the analyzed samples, several steps have to be performed. These steps are done to make sure that the quality of the performed work is good enough, and is an important part of the analytical work.

2.8.1 Relative Response

The relative response (RR) is a factor that can be used to compensate for variations in the signal intensity for a target analyte. The observed fluctuations in the response of an analyte can be due to differences in the sample preparation (e.g. loss of analyte) or variations in the chromatographic system. To compensate for these variations, the ratio between the signal intensity for an analyte and the internal standard can be calculated as shown in Equation 2.1 [118].

$$RR = \frac{\text{Response of analyte}}{\text{Response of internal standard}} \tag{2.1}$$

2.8.2 Retention Time and Relative Retention Time

The retention time (RT) of a compound is a measure of the time it takes for a compound to pass through the chromatographic column. The retention time for a compound is measured as the time from injection to when it is detected. This parameter is, however, not a fixed matter and will be dependent on the chromatographic system and conditions. The retention time of a selected compound can also fluctuate between two consecutive injections. Factors that can alter the RT of a compound includes column degradation, changes in the mobile phase flow rate, instability in the column temperature, and air bubbles in the mobile phase [119]. These factors can make it difficult to compare retention times, since different injections can show small differences in the retention time. Relative retention times (*RRT*) can be used to reduce the effect of some of these variabilities. The *RRT* is an expression of the retention time of an analyte relative to the retention time of the internal standard (Equation 2.2). The observed fluctuations in retention times for individual compounds will also be observed for

the internal standard, thus the ratio should remain the same. The *RRT* will therefore be more reliable for the quantification of target analytes [120].

$$RRT = \frac{\text{RT analyte}}{\text{RT internal standard}} \tag{2.2}$$

2.8.3 Ion Ratio

Another confirmation parameter that can be used is the ion ratio (*IR%*) for the individual target analytes. The ion ratio is calculated by dividing the area of the confirmation ion by the area of the quantification ion. This ratio will be unique for each analyte in a sample matrix and can therefore be used to quantify the individual analytes [121].

$$IR\% = \frac{\text{Area confirmation ion}}{\text{Area quantification ion}} \times 100\% \tag{2.3}$$

2.8.4 Absolute Recovery and Relative Recovery

The recovery of the analytes is a measure of the efficiency of an analytical method, especially the efficiency of the sample work-up steps. The recovery of an analyte can be measured by adding equal and known amounts of target analytes to an individual sample matrix prior to and after the extraction. This is done to investigate for possible loss of analyte during the extraction procedure, and will give information about the quality of the work-up step [117]. The absolute recovery for each target analyte, at a specific fortification level, was calculated according to Equation 2.4, while

the relative recovery was calculated according to Equation 2.5.

$$\text{Absolute recovery} = \frac{\text{Area pre-ext spiked matrix}}{\text{Area post-ext spiked matrix}} \tag{2.4}$$

$$\text{Relative recovery} = \frac{\frac{\text{Area of pre-ext spiked matrix}}{\text{Area IS}}}{\frac{\text{Area post-ext spiked matrix}}{\text{Area IS}}} \tag{2.5}$$

From the areas of the extracts, the area of the reagent blank or the area of the corresponding sample is subtracted, depending on which area is higher. This is done to compensate for the level of analyte that is already present in the sample, or to compensate for contamination occurring during the sample preparation step [92].

The absolute recovery will be the actual recovery of the analytes, but will generally characterized by higher uncertainty than the corresponding relative recovery. In calculations of the relative recovery all areas are divided by the area of the internal standard, and this recovery will therefore compensate for loss of analytes during the sample preparation. The relative recovery values obtained will depend strongly on the surrogate internal standard added to the sample [122].

2.8.5 Matrix Effect

When developing a method for LC-MS/MS analysis it is crucial to consider the matrix effect. LC-MS/MS suffer from some disadvantages like ionic suppression or enhancement due to coelution of matrix compounds. Most times matrix components will affect the analytical measurement of the the target analytes negatively [123]. The ionization efficiency of an analyte may also be affected when matrix compounds enter the ionization source

at the same time as the analytes. Thus, the matrix effect can affect both the accuracy and the reproducibility of the chromatographic method [124] The effect on the response from matrix intereferences on the target analytes, is called the matrix factor and can be expressed as

$$MF = \frac{\text{(Area of post-ext spiked matrix)-(Area of reagent blank)}}{\text{Area of standard solvent solution}} \quad (2.6)$$

where the area of the post-extracted matrix is referred to as the matrix match, and the area of a standard solvent solution is given at the same concentration of target analytes as the matrix match. From Equation 2.6 the matrix effect percentage can be expressed as follows

$$ME\% = (\text{MF-1}) \times 100\% \quad (2.7)$$

The matrix effect has been expressed according to Equation 2.7 in previous studies to report on the magnitude of the matrix effect on target analytes [125]. The matrix effect will usually be higher on the early-eluting peaks, since hydrophilic compounds present in the sample matrix are not well retained on reversed-phase columns, and they will therefore usually elute within the first minutes [126].

2.8.6 Instrumental Level of Detection and Lower Level of Quantification

The limit of detection (LOD) is the lowest concentration of an analyte that can be detected, but not necessarily quantified under the stated condition of the test [127]. The lower limit of quantification (LLOQ), often called the limit of quantification, is the lowest concentration of an analyte that

can be determined with acceptable precision and accuracy under the stated conditions[97].

There are many different ways to estimate the these parameters, and the choice of method will vary depending on the matrix and the analytical method used. In this study the LLOQ was set to the lowest concentration that could be detected in the calibration curve [125, 128], and from this the LOD was determined as follows

$$\mathrm{LOD} = \frac{\mathrm{LLOQ}}{3} \tag{2.8}$$

2.8.7 Internal Standard Method

The internal standard method is commonly used for quantitative analysis, and is useful when the instrumental response varies from run to run. The standard that is chosen for this method cannot be a component that is already present in the sample, and it must meet several criteria. It should elute near the peaks of interest, but it must be well resolved from them, and it should also be chemically similar to the analytes of interest, so that they behave similarly during the extraction process and through the analysis [119]. This last criteria has made the internal standard method very popular for chromatographic techniques coupled to mass spectroscopy, since isotopes of the target analytes can be used as internal standards. The method consists of making calibration solutions made from pure samples of the analytes. A known amount of internal standard is added to the calibration mixtures and to the samples. The signal that is produced by the analyte can be compared with the signal of the internal standard to determine the amount of analyte present in the samples. A calibration curve is constructed for each target analyte, based on the ratio of the response for the analyte and the internal standard response in the standard mixtures, plotted

against the concentration of the target analytes [97].

2.8.8 Reproducibility and Repeatability

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple samplings of the same homogeneous sample under the set conditions. Precision may be considered at three levels: repeatability, intermediate precision, and reproducibility [97]. Reproducibility refers to the variation in measurements made on a subject under changing conditions. The changing conditions may be due to measurements being made over a period of time, under which the system can undergo non-negligible changes. A measurement is said to be repeatable when the variation between different analyses is smaller than a pre-determined acceptance criteria. Repeatability, on the other hand, refers to the variation in repeat measurements made on the same subject under identical conditions. The measurements are then made by the same instrument or method, the same operator, and the measurements are performed under a short period of time [129].

Repeatability can be calculated by using the standard deviation (Equation 2.10) or the relative standard deviation (Equation 2.11). The average value can be calculated with Equation 2.9.

$$\bar{x} = \frac{\sum_{i=1}^{n} x_{\mathrm{i}}}{n} \tag{2.9}$$

$$STD = \sqrt{\frac{\sum_{i=1}^{n} (x_{\mathrm{i}} - \bar{x})^2}{n-1}} \tag{2.10}$$

where x_1, x_2,..., x_n are the observed values for the individual tests, $\bar{x}$ is the average value of the data sample, n is the number of samples and n-1 is the

degree of freedom. Standard deviation is used to measure the precision, and measures the amount of variation in a data set [130].

The relative standard deviation (RSD%) is given by Equation 2.11, and will often give a clearer picture of the data quality than the standard deviation [130].

$$RSD\% = \frac{STD}{\bar{x}} \times 100\% \tag{2.11}$$

2.9 Statistics

2.9.1 Principal Component Analysis

Principal component analysis (PCA) forms the basis for multivariate data analysis, and is used to analyze a data table representing observations described by several dependent variables, which in general are inter-correlated [131]. The goal of the PCA is to extract the important information from the data table and express this extracted data as a set of orthogonal variables called principal components [132].

The PCA computes new variables, called principal components, that are obtained from linear combinations of the original variables. The first principal component should have the largest possible variance of the variables, and will therefore describe the majority of the variance in the data table. The second principal component should also have the largest possible variance, under the constraint of being orthogonal to the first component. Other principal components are calculated in the same way, and will describe less of the data compared to the first principal component. [132].

PCA is used to identify patterns within a data set, where the aim is to clus-

ter similar observations. The desired issue is to project and visualize the data set in a two-dimensional space where minimal information is lost. It will therefore be necessary to decrease the number of variables to a limited amount of linear combinations that can describe the data set. Each linear combination will correspond to a specific principal component. A loading plot will show which variables are influential for the PCA model, and how these variables are correlated to each other. Points that are close to each other in the loading plot indicate a similar data profile, and these compounds will be closely related [133].

Chapter 3

Materials and methods

3.1 Sample Collection

In this study samples were collected from the Høvringen area, and in the area where the samples were taken, Høvringen treatment plant is located. This treatment plant accepts ⅔ of the wastewater from Trondheim. When the wastewater has gone through several treatment processed, it is discharged into the fjord fjord at a depth of 48 to 65 meters in two discharge pipes [134]. A map of the treatment plant area is given in Figure A.1, where it is illustrated where the wastewater treated in the plant originates from. In the area where the samples were taken there is also some commercial ship lines, and an overview over boat lines in Trondheimsfjorden is given in Figure 3.1.

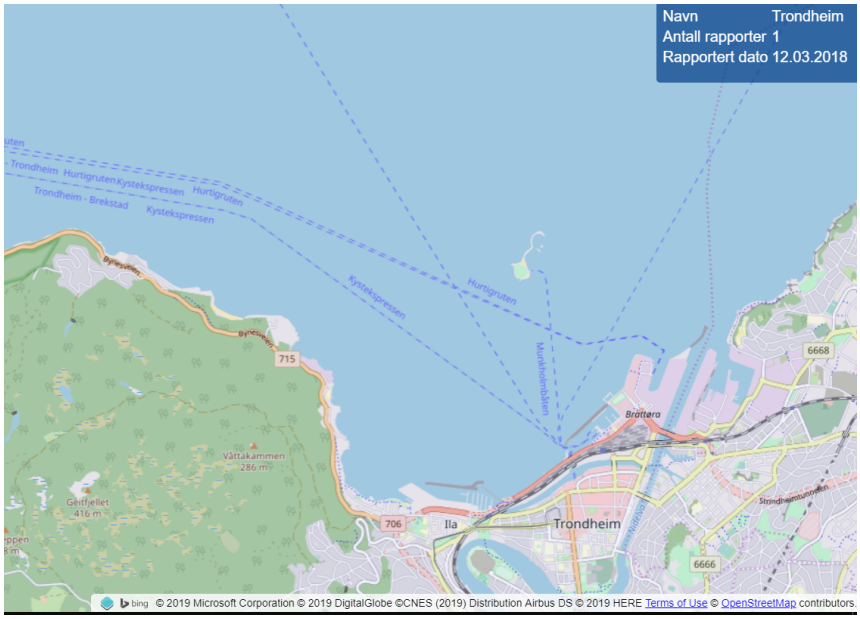
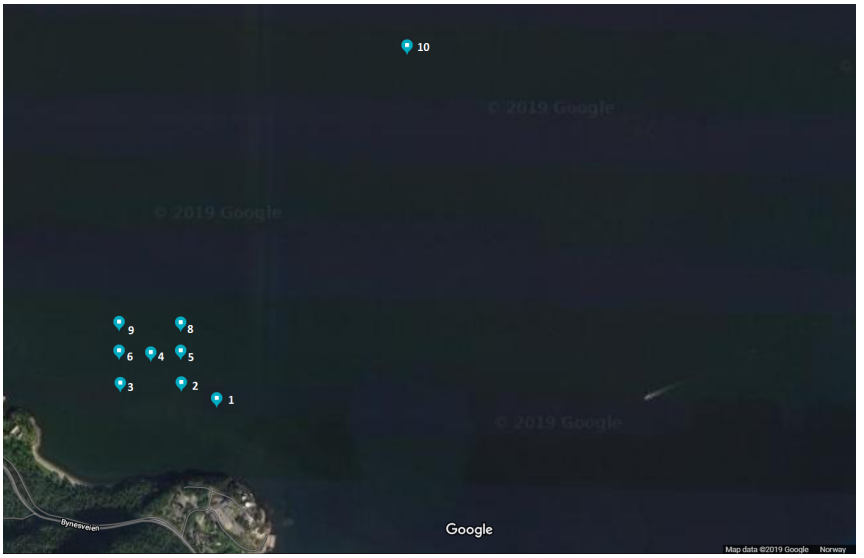


Figure 3.1: Overview over commercial boat lines in the sampling area. The map is taken from FFI.

The sampling of sediments were done as part of a bigger project to gain knowledge about the levels of different organic contaminants and elements in sediments from Trondheimsfjorden. Sediment samples were taken from the Høvringen area, and the location of the different sampling sites is given in Figure 3.2. Sediment samples (n=64) from Trondheimsfjorden were sampled on the 31st of May 2018 from the research vessel R/V Gunnerus using a sediment box corer. The sediment samples were brought up as blocks to a platform on the deck that had been washed with sea water to avoid contamination of the samples. The sediment blocks were cut with a stainless steel knife and stored in aluminum boxes for BTHs, BTRs, and PAHs analysis. 64 samples were also collected in CC cups for determination of elements. The sediment samples were sampled from three sides,

for sampling triplicates of the top and bottom sediments. For sediment samples that were of a larger size, the blocks could be divided into three groups, top, middle and bottom parts, that could be sampled individually. In cases where only smaller, residual sediments were collected (no block-like structure), sampling was done in a bulk. The sampling method was done in this way to make sure that replicates were collected for each sample. Further information about the individual samples and sample locations is given in Appendix A. All the sediment samples were freeze-dried using a Christ Alpha 1-4 LD plus laboratory freeze dryer and stored in a dark freezer at -22°C until analysis.

(a) Overview of the sampling locations



(b) Sampling locations for sediments

Figure 3.2: Sampling locations for the sediment samples taken in Høvringen in May 2018.

3.2 Chemicals and Materials

The sediments were analyzed for nine BTHs [BTH, 2-Me-BTH, 2-OH-BTH, 2-Cl-BTH, 2-NH_2-BTH, 2-Me-S-BTH, 2-S-BTH, 2-SCNMeS-BTH and 2-M-BTH], as well as seven BTRs [1-H-BTR, 1-OH-BTR, XTR, TTR (a mixture of 4-Me-1-H-BTR and 5-Me-1-H-BTR), 5-NH_2-BTR, 5-Cl-1-H-BTR, and BTR-COOH]. The sediments were also analyzed for 16 PAHs [NAP, ACY, ACE, FLU, PHE, PYR, BaA, CHR, BbF, BkF, FLT, ANT, BaP, DBA, BgP, and IND].

Standards of BTR (99%), XTR (≥99%), TTR (≥90%), 1-OH-BTR (≥97% (T)), BTR-COOH (99%), 5-NH_2-BTR (CPR), 5-Cl-BTR (99%), BTH (≥97%), 2-OH-BTH (98%), 2-Me-BTH (99%), 2-NH_2-BTH (97%), 2-M-BTH (CPR), 2-S-BTH (97%), 2-Me-S-BTH (97%) and BTR-d4 (≥98%, 10 $\mu g\,mL^{-1}$ in acetone) were purchased from Sigma-Aldrich (Steinheim, Germany). 2-Cl-BTH (99%) was obtained from Acros Organics (New Jersey, United States), 2-SCNMeS-BTH (98%) was from Advanced ChemBlocks (California, United States), BTH-d4 (98%) was from Toronto Research Chemicals (Toronto, ON, Canada), while 5-Me-BTR-d6 (96%) was obtained from Chiron (Trondheim, Norway). A stock solution containing a mix of the 16 EPA priority PAHs at a concentration of 100 $\mu g\,mL^{-1}$ in toluene was purchased from Chiron (Trondheim, Norway). In addition a stock solution containing a mixture of five fluorinated PAH internal standards in toluene [F-NAP, F-BIP, F-PHE, F-PYR, and F-CHR] at 200 $\mu g\,mL^{-1}$ and F-BkF (100 $\mu g\,mL^{-1}$ in toulene) were purchased from Chiron (Trondheim, Norway).

Acetonitrile (ACN, gradient grade for LC), dichloromethane (DCM, capillary grade for GC), acetone (HPLC isocratic grade), and hydrochloric acid (HCl, 37%) were purchased from VWR (Radnor, PA, United States), while methanol (MeOH, hypergrade for LC-MS) and ammonium hydroxide (ACS grade, 28-30 %) were obtained from Merck (Billerica, MA,

United States). Formic acid (98-100 % for LC-MS LiChropur®) were also obtained from Merck (Billerica, MA, United States). Milli-Q water was delivered by Millipore Water Purification system (Billerica, MA, United States).

Standard reference material (1941b Organics in Marine Sediment) for the PAH analysis was obtained from National Institute of Standards & Technology (NIST, Gaithersburg, United States). Concentrations of the different PAHs in the reference material from NIST is given in Appendix C (Table C.2). A total of 9 PAHs were present in the standard reference material.

SPE-cartridges, Strata™-X Polymeric Reversed Phase 3 cm^3/200 mg were obtained from Phenomenex (Germany). The particle specifications were as follows: 815 m^2/g specific surface area, 81 Å average pore size diameter, 28 µm mean particle diameter, and a pore volume of 1.17 mL/g. A 24-fold Visiprep DL SPE vacuum manifold system were obtained from Supelco (Bellefonte, PA, United States). Disposable liners, made of PTFE, were obtained from Sigma-Aldrich (Steinheim, Germany), and were used to eliminate the possibility of cross contamination during the extraction procedure.

3.3 Preparation of Standard Solutions

3.3.1 Standard Solutions for BTRs and BTHs

Standard stock solutions (1000 $\mu g\,mL^{-1}$) of all target analytes were prepared by weighing out about 0.100-0.200 g of standard to a 10 mL volumetric flask. All the standard solutions were prepared in MeOH (LC-MS grade), except BTR-COOH which was prepared in MeOH:MilliQ-water:Ammonium hydroxide (1:1:0.1, v/v). The standard stock solution

of 2-M-BTH was already prepared at a concentration of 2500 $\mu g\,mL^{-1}$. From the standard stock solutions, 10 ppm and 1 ppm working solutions were prepared. A mix of all the 16 standards were also prepared at different concentration levels. All the prepared stock solutions were stored in a freezer at -20°C for up to 1 month. The calibration standards for the organic target chemicals were prepared from this stock solution through serial dilutions with MeOH:ACN:Milli-Q water (1:1:2 v/v). A mix of the three internal standard (BTH-d4, BTR-d4, and 5-Me-BTR-d6) used for spiking the samples was also prepared at a concentration of 1 $mg\,L^{-1}$ (1 ppm).

3.3.2 Standard Mix for PAHs

From the standard mix of PAHs, a working stock solution was prepared in ACN. The calibration standards for the target analytes were prepared from this stock solution through serial dilutions with ACN. An internal standard mix was also prepared at a concentration of 1 $\mu g\,mL^{-1}$ in acetone to be added to all samples before the extraction. The standards were stored in the freezer at -20°C for up to 8 months.

3.4 Analysis of BTRs and BTHs

3.4.1 Sample Preparation

An aliquot of 0.5000 g ($\pm$0.0300 g) freeze dried sediment was transferred to a 15 mL Eppendorf® tube. The samples were spiked with the internal standard mix (20 µL of 1 ppm solution) prior to extraction. 5 mL of acidified MeOH/Milli-Q water (1:1, v/v, adjusted with 1M HCl to pH <3)

was then added to the samples. Liquid-solid extraction (LSE) was performed by vortex-mixing the samples for 1 minute, before they were ultrasonicated for 45 minutes at 40 °C. After centrifugation at 4000 rpm for 5 minutes, the supernatant was collected and diluted to 50 mL with acidified Milli-Q water (adjusted with 1M HCl solution to pH <3). Extraction and isolation of the target analytes was performed by Strata™-X RP cartridges (Phenomenex, Germany). The cartridges were conditioned by passage of 10 mL MeOH and equilibrated by passage of 10 mL acidified Milli-Q water (adjusted with 1M HCl to pH <3). Prior to loading onto the cartridges, the samples were vortex mixed for 1 minute. The acidified samples were passed through the cartridges, washed with 10 mL of acidified Milli-Q water (pH <3) and dried under vacuum for 15 minutes to remove excess water from the cartridges. The analytes were eluted with 10 mL of a mixture of MeOH/ACN (1:1, v/v) and collected in 15 mL Eppendorf® tubes. The samples were put on a heated tray (40°C) and concentrated to near-dryness (250 µL) under a gentle stream in nitrogen (N_2 (g)) using a TurboVap LV Biotage automated evaporation system. After evaporation, the samples were transferred to LC-vials and diluted to 1 mL with a final in-vial composition of MeOH/ACN/Milli-Q water (1:1:2, v/v) for LC-MS/MS analysis.

For the quality control step, sample matrix was spiked with internal standard and target analytes prior to extraction, and compared with sample matrix spiked with IS and target analytes post extraction (matrix matches). This was done to investigate how much the matrix would affect the signal for the individual compounds. Sample 64 was chosen for the quality control, and was spiked with 10 ppb, 25 ppb and 50 ppb target analytes, and for each concentration two replicates of the sample was extracted without adding any target analytes. A method blank was also extracted for each concentration level to account for possible contamination occurring during the sample preparation.

3.4.2 Instrumental Analysis

The chromatographic separation was carried out using an Acquity UHPLC Thermo system equipped with a column manager, a flow through needle, a sample manager, and a binary solvent manager (Waters, Milford, USA). The mass spectrometric system was a Xevo TQS triple quadrupole mass analyzer, equipped with a ZSpray Electrospray ionization source (Waters, Milford, USA). The LC column used was a Kinetix C18 column (30 mm $\times$ 2.1 mm, with 1.3 µm particle size) serially connected to a Phenomenex C18 guard column (4.0 mm $\times$ 2.0 mm, with 5.0 µm particle size). Determination of the precursor and product ions of the target analytes and internal standards were done by direct infusion of standards prepared in MeOH into the mass spectrometric ion source. The collision energies and cone voltages were also determined in this way. These paramaters are given for all target analytes and internal standards in Table 3.1. All the BTH and BTR derivatives presented satisfying fragmentation in positive mode, which is used to analyze the target analytes. The software IntelliStart (Waters, Milford, USA) was used to select two characteristic product ions for each target analytes; one quantification ion and one confirmation ion.

The chromatographic separation was performed by a Kinetex C18 column, where the column temperature was set to 40°C. The chromatographic analysis was carried out using a gradient elution program with ACN and Milli-Q water (both acidified with 0.1% v/v formic acid) as a binary mobile phase mixture at a flow rate of 0.4 µL min^{-1}. The gradient was set up as follows: 0 min 5% ACN, 3 min 100% ACN, 4.8 min 100% ACN, 5 min 5% ACN, and 6 min 5% ACN, for a total run time of 6.0 min. All gradients were linearly ramped. The electrospray ionization voltage was applied at +3.0 kV. The cone gas (N_2) flow rate was set at 150 L/Hr, and the desolvation gas (N_2) flow rate was set to 800 L/Hr. The source temperature was set to 150°C, while the desolvation temperature was 800°C. The cone voltage was set to 25 V, with a source offset of 50 V. The pressure for the

nebulizer gas was set to 6 bar. The final in vial composition of all samples and standard solutions were MeOH/ACN/Milli-Q water (1:1:2 v/v), and all samples were injected on column with partial-loop injection (5 µL). Data was obtained using the MassLynx and TargetLynx 4.1 software package (Waters, Milford, USA). Data treatment was performed with Excel (Microsoft Office, 2016).

Table 3.1: Parameters for the LC-MS/MS analysis of the target compounds

Component	Quantification transition (CE[b] [eV])	Confirmation transition (CE [eV])	CV[a] (V)
BTH	136>109 (28)	136>65 (22)	44
2-Cl-BTH	170.01>134.78 (24)	170.01>109.07 (22)	26
2-OH-BTH	152>124 (16)	152>80 (22)	2
2-S-BTH	168>135 (20)	168>92 (20)	2
2-Me-S-BTH	182>167 (22)	182>109 (32)	26
2-SCNMeS-BTH	239>180 (14)	239>136 (26)	8
2-Me-BTH	150.06>109.08 (20)	150.06>65.11 (32)	35
2-NH_2-BTH	151>124 (18)	151>109 (20)	28
2-M-BTH	221>177 (18)	221>109 (30)	2
BTR	120.02>92 (14)	120.02>65 (16)	28
TTR	134>79 (18)	134>77 (16)	18
XTR	148>93 (16)	148>77 (24)	16
BTR-COOH	164>108 (18)	164>80 (18)	2
5-Cl-BTR	154>99 (22)	154>73 (24)	24
5-NH_2-BTR	135>107 (16)	135>80 (16)	42
1-OH-BTR	136>119 (14)	136>91 (18)	24
BTH-d4 (IS)	140>113 (26)	140>69 (26)	40
BTR-d4 (IS)	124>96 (16)	124>69 (18)	38
5-Me-BTR-d6 (IS)	140.13>84.78 (20)	140.13>81.19 (22)	24

[a]: Collision energy, [b]: Cone voltage

3.5 Analysis of PAHs

3.5.1 Extraction of PAHs

Freeze dried sediment (5.00 g $\pm$ 0.10 g) was transferred to a clean glass beaker and mixed with about 2 mL activated silica. 200 µL F-PAH mix (IS) (1000 ng mL^{-1} F-PAH solution in acetone) were added to the sample, and air dried for a couple of minutes while it was simultaneously mixed with the sample. A cellulose filter was added to the extraction cell. Above the filter activated copper (1 g) and activated alumina (2 g) were added. The samples were added to the extraction cell and placed in the extraction chamber of the Accelerated Solvent Extractor (ASE 150, Thermo Scientific). The conditions for the ASE are given in Table C.1 in Appendix C. When the extraction process was finished, the extracts were concentrated to about 1 mL under a gentle stream of nitrogen at 40°C using a Biotage TurbVap LV Concentration Evaporator Workstation. To perform solvent exchange, 10 mL ACN were added to the concentrated extracts, before they were filtrated through disposable 0.45 µm PTFE syringe filters in order to remove particulates that could be present in the extracts. The extracts were then re-concentrated to about 1 mL at 45°C under a gentle stream of nitrogen. The final extracts were transferred to LC-vials and filled up with ACN to a final volume of 1.5 mL. The samples were stored in a freezer at -20°C until analysis. On the day of analysis the samples were filtered a second time using 0.45 µm PTFE syringe filters since precipitation were observed in the samples after they stored in the freezer.

3.5.2 Quantitative PAH Analysis by HPLC-FLD

The HPLC analysis of PAHs was carried out using an Agilent 1260 Innity II system (California, United States). The chromatographic separation was carried out on a Supelcosil LC-PAH HPLC column (25 cm $\times$ 4.6 mm, 5 µm particle size) serially connected to a Supelcosil LC-18 Supelguard Cartridge guard column (2.0 cm $\times$ 4.0 mm). The injection volume for the samples was 3 µL. A gradient of ACN and Milli-Q water were used as mobile phase, with a flow rate of 1.5 mL/min. The gradient used for the separation is given in Table 3.2, where the total run time for the method was 60 minutes.

Table 3.2: HPLC mobile phase gradient

Time [min]	**Acetonitrile (%)**	**Water (%)**
0-5	40	60
5-30	100	0
30-45	100	0

The PAHs were detected by two serially connected detectors; the diode-array detector (DAD) and the fluorescence detector (FLD). The UV detection (DAD) was performed at 254 nm for a general detection of the analytes, while the emission and excitation conditions for the fluorescence detection is given in Table 3.3. Target analytes were quantified using the Agilent OpenLab Chromatography Data System ChemStation software.

Table 3.3: Fluorescence excitation and emission wavelengths for detection of PAHs during the HPLC analysis

Time [min]	Excitation [nm]	Emission [nm]
0-19.5	270	333
19.5-21.0	245	350
21.0-26.5	260	420
26.5-29.5	265	380
29.5-37.0	290	430
37.0-45.0	300	500

Chapter 4

Results

4.1 Quality Assurance and Method Validation

4.1.1 Benzothiazoles and Benzotriazoles

The precision of the LC-MS/MS method was evaluated by investigation different parameters from the analysis, which are presented in Table 4.1. The parameters are reported as an average of the values obtained for the standard solutions used in the calibration curves at nine concentration levels (N=9). For the ion ratios (IR%) the number of concentration levels used for the estimation is based on the region where the IR% was stable, since the confirmation ion for several target analytes was not sensitive enough for the lowest concentration levels. This was especially the case for compounds with an elevated LOD (see Table 4.3). All the ion ratios, except for 2-Cl-BTH, were found to meet the performance criteria given by the European Commission (Decision EC No 657/2002) [135]. 2-Cl-BTH was found to yield low MS response, which was especially true for the confirmation

ion, resulting in fluctuations in the ion ratio. Chromatograms obtained for the individual compounds are given in Appendix F, where chromatograms are given for a sample, a spiked sample and a standard solution.

Table 4.1: Ion ratios (IR%), retention times (RT) and relative retention times (RRT) for BTHs and BTRs (RSD%, N=9 unless specified)

Compound	***IR%***	***RT***	***RRT***
BTH	38.0 (24.8)[a]	2.76 (0.18)	1.72 (0.32)
2-Cl-BT	87.6 (40.4)[b]	3.09 (0.31)	1.24 (0.31)
2-OH-BTH	106 (16.3)[c]	2.70 (0.46)	1.08 (0.46)
2-S-BTH	50.8 (7.58)[d]	2.84 (0.12)	1.77 (0.30)
2-Me-S-BTH	9.84 (7.11)[d]	3.12 (0.00)	1.25 (0.00)
2-SCNMeS-BTH	39.5 (3.21)	3.15 (0.00)	1.27 (0.00)
2-Me-BTH	14.7 (5.41)[b]	2.91 (0.00)	1.17 (0.00)
2-NH_2-BTH	108 (2.07)[c]	0.93 (3.90)	0.58 (3.81)
2-M-BTH	73.7 (2.36)	2.86 (0.00)	1.15 (0.00)
BTR	43.2 (4.42)[d]	1.64 (0.69)	1.02 (0.61)
TTR	43.3 (2.22)	2.50 (0.00)	1.00 (0.00)
XTR	89.0 (4.76)	2.72 (0.00)	1.09 (0.00)
BTR-COOH	77.0 (8.84)[c]	1.37 (1.11)	0.55 (1.11)
5-Cl-BTR	15.7 (6.68)[c]	2.65 (0.00)	1.65 (0.33)
5-NH_2-BTR	31.8 (8.20)[c]	0.25 (0.00)	0.15 (5.75)
1-OH-BTR	18.5 (7.31)[c]	0.78 (0.43)	0.49 (0.45)

[a] N=4
[b] N=5
[c] N=6
[d] N=7

The recovery of target analytes from the sediment matrix was examined in three replicate analyses by fortification of the sediment matrix with standards prior to extraction. A fortification level of 10, 25 and 50 ng for each

analyte was used for this purpose. BTH-d4, BTR-d4 and 5-Me-BTR-d6 were spiked at 20 ng. The absolute and relative recoveries for the BTHs and BTRs and the relative standard deviations (RSD%, N=3) are given in Table 4.2. The absolute recoveries for 9 of 16 target analytes were found to be >50%.

Table 4.2: Absolute recoveries and relative recoveries for the individual BTHs and BTRs. The values are given as percentages (RSD%, N=3; [10 ng mL^{-1}])

Compound	Absolute recovery	Relative recovery
BTH	48.7 (11.7)	82.0 (19.2)
2-Cl-BTH[a]	19.1 (49.4)	26.7 (42.6)
2-OH-BTH	102 (19.9)	325 (19.4)
2-S-BTH[a]	19.9 (12.1)	29.1 (8.75)
2-Me-S-BTH	52.4 (11.6)	73.4 (4.88)
2-SCNMeS-BTH	55.1 (12.3)	77.7 (5.33)
2-Me-BTH	62.4 (9.54)	94.0 (3.77)
2-NH_2-BTH	41.3 (5.99)	60.8 (1.26)
2-M-BTH	30.6 (30.4)	42.2 (28.8)
BTR	69.9 (9.00)	108 (4.09)
TTR	76.6 (7.33)	107 (1.31)
XTR	62.3 (7.59)	86.6 (2.26)
BTR-COOH	71.3 (9.46)	106 (3.14)
5-Cl-BTR	67.0 (6.28)	97.9 (5.92)
5-NH_2-BTR	0.08 (51.5)	2.07 (24.8)
1-OH-BTR	18.9 (17.2)	31.2 (22.4)
BTH-d4	70.4 (9.65)	-
BTR-d4	68.6 (4.63)	-
5-Me-BTR-d6	72.2 (6.60)	-

[a]Due to high endogenous concentrations, the concentrations present in the samples could not be subtracted

The lower limit of quantification (LLOQ) and limit of detection (LOD) for the BTHs and BTRs are given in Table 4.3. The instrumental LLOQ was found from the calibration curve as the lowest calibration point that was identifiable and discrete. From this value the LOD could be calculated based on Equation 2.8. The method LLOQ and LOD were calculated to account for the fact that the concentrations of analytes are given in $\mathrm{ng\,g^{-1}}$ dry weight. Concentrations that were below the limit of detection were removed from the data set for calculations.

Table 4.3: Lower limit of quantification and limit of detection. The instrumental LLOQ and LOD are given in $\mathrm{ng\,mL^{-1}}$, while the method LLOQ and LOD are given in $\mathrm{ng\,g^{-1}}$

Compound	**Instrumental**		**Method**	
	LLOQ	LOD	LLOQ	LOD
BTH	5.00	1.67	10	3.33
2-Cl-BTH	0.50	0.17	1.00	0.33
2-OH-BTH	0.50	0.17	1.00	0.33
2-S-BTH	0.50	0.17	1.00	0.33
2-Me-S-BTH	0.10	0.033	0.20	0.067
2-SCNMeS-BTH	0.10	0.033	0.20	0.067
2-Me-BTH	0.10	0.033	0.20	0.067
2-NH_2-BTH	0.20	0.067	0.40	0.13
2-M-BTH	0.10	0.033	0.20	0.067
BTR	0.20	0.067	0.40	0.13
TTR	0.10	0.033	0.20	0.067
XTR	0.10	0.033	0.20	0.067
BTR-COOH	0.10	0.033	0.20	0.067
5-Cl-BTR	0.10	0.033	0.20	0.067
5-NH_2-BTR	0.10	0.033	0.20	0.067
1-OH-BTR	0.10	0.033	0.20	0.067

Table 4.4: Matrix factors and matrix effects for the different BTHs and BTRs. Negative values indicate ion suppression, while positive values indicate ion enhancement.

Compound	*MF*	*ME%*
BTH	1.22	22.3
2-Cl-BTH	0.50	-49.7
2-OH-BTH	0.60	-40.4
2-S-BTH	0.43	-56.5
2-Me-S-BTH	0.80	-19.9
2-SCNMeS-BTH	0.61	-38.8
2-Me-BTH	0.89	-11.4
2-NH_2-BTH	0.86	-14.2
2-M-BTH	0.63	-37.0
BTR	0.87	-12.6
TTR	0.73	-26.7
XTR	0.71	-28.7
BTR-COOH	0.72	-28.1
5-Cl-BTR	0.76	-24.3
5-NH_2-BTR	0.74	-25.9
1-OH-BTR	0.77	-22.9

The matrix effect and matrix factor for the different BTHs and BTRs analyzed by LC-MS/MS are given in Table 4.4. To the best of the authors knowledge, in previous studies done on these compounds in sediments, matrix effects have not been reported. From this study it was found that the analytes in general suffered from slightly negative matrix effects, except for BTH which experienced a positive matrix effect. The matrix effect for the individual BTH and BTR derivatives is also illustrated in Figure 4.1, where the compounds are arranged based in the degree of matrix effect experienced.

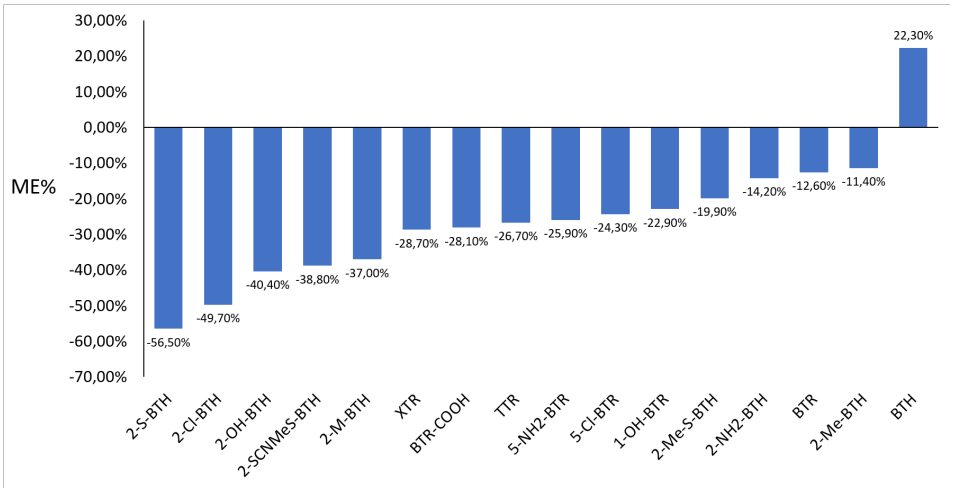


Figure 4.1: Matrix effect (ME%) for all BTH and BTR target analytes in sediments analyzed with ESI-LC-MS/MS.

4.1.2 Analysis of PAHs by HPLC-FLD

The precision of the HPLC-FLD method was evaluated through the stability of the retention times and relative retention times for the target analytes, which are given in Table 4.5. Since retention time is the only parameter that can be used to identify individual compounds in HPLC, it will be crucial that this parameter is stable between consecutive runs. The relative retention times reported are relative to F-PHE, since this compound was used as internal standard for the quantification of the PAHs in the sediment samples. In general it was found that the retention times and relative retention times were stable throughout the analysis. Chromatograms from the analysis of PAHs by HPLC-FLD are given in Appendix F.

Table 4.5: Retention times (RT) and relative retention times (RRT) for PAHs (RSD%, N=6).

Compound	*RT*	*RRT*
NAP	14.76 (0.09)	0.718 (0.04)
ACE	18.18 (0.08)	0.884 (0.04)
FLU	18.74 (0.13)	0.911 (0.05)
PHE	20.11 (0.11)	0.978 (0.004)
ANT	21.44 (0.16)	1.042 (0.07)
FLT	22.81 (0.12)	1.109 (0.03)
PYR	23.82 (0.11)	1.158 (0.03)
BaA	27.17 (0.11)	1.321 (0.05)
CHR	28.02 (0.10)	1.362 (0.05)
BbF	30.51 (0.11)	1.484 (0.05)
BkF	30.80 (0.10)	1.547 (0.06)
BaP	32.91 (0.10)	1.600 (0.06)
DBA	34.69 (0.18)	1.687 (0.11)
BgP	36.01 (0.19)	1.751 (0.13)
IND	37.23 (0.23)	1.810 (0.16)

To determine the accuracy of the extraction method for PAHs, target analytes were spiked into the sediment matrix at a concentration level of 300 ng. The recoveries of the PAHs are given in Table 4.6, which gives information about the efficiency of the extraction method. For the target analytes, all absolute recoveries were found to be >70%, ranging from 70.6% for naphthalene to 116% for fluoranthene. The only compound yielding a lower recovery was the internal standard F-NAP, with an absolute recovery of 69.4%.

Table 4.6: Absolute recoveries and relative recoveries for the PAHs. The values are given as percentages (RSD%, N=3)

Compound	Absolute recovery	Relative recovery
NAP	70.6 (4.58)	91.6 (2.05)
ACE	75.2 (4.27)	92.3 (0.84)
FLU	76.9 (5.32)	94.7 (0.62)
PHE	85.7 (13.6)	108 (5.55)
ANT	72.7 (19,2)	92.6 (14.2)
PYR	109 (21.9)	127 (7.85)
FLT	116 (27.2)	132 (12.5)
CHR	92.6 (17.7)	117 (6.09)
BaA	87.8 (14.0)	111 (4.67)
BkF	90.6 (13.9)	114 (5.35)
BbF	95.8 (27.3)	119 (10.5)
BaP	103 (52.1)	123 (12.3)
DBA	92.4 (8.54)	114 (3.77)
F-NAP	69.4 (3.85)	-
F-BIP	78.7 (2.40)	-
F-PHE	81.6 (4.16)	-
F-PYR	94.2 (4.97)	-
F-CHR	84.0 (4.24)	-

The LLOQ and LOD for the PAHs are presented in Table 4.7. These parameters were estimated in the same way as for BTHs and BTRs, which was by identifying the lowest point on the calibration curve that was identifiable and discrete. For all the compounds this corresponded to the lowest calibration standard which had a concentration of $50\,\mathrm{ng\,mL^{-1}}$. These parameters are only reported for the target analytes that could be identified from the chromatograms.

Table 4.7: Lower limit of quantification and limit of detection. The instrumental LLOQ and LOD are given in $\mathrm{ng\,mL^{-1}}$, while the method LLOQ and LOD are given in $\mathrm{ng\,g^{-1}}$

Compound	**Instrumental**		**Method**	
	LLOQ	LOD	LLOQ	LOD
NAP	50	16.7	15	5.0
ACE	50	16.7	15	5.0
FLU	50	16.7	15	5.0
PHE	50	16.7	15	5.0
ANT	50	16.7	15	5.0
PYR	50	16.7	15	5.0
FLT	50	16.7	15	5.0
CHR	50	16.7	15	5.0
BaA	50	16.7	15	5.0
BkF	50	16.7	15	5.0
BbF	50	16.7	15	5.0
BaP	50	16.7	15	5.0
DBA	50	16.7	15	5.0

4.2 Occurrence of BTHs, BTRs, and PAHs in Sediment Samples from Trondheimsfjorden

4.2.1 Benzothiazoles and Benzotriazoles

Benzothiazoles and benzotriazoles were detected in all 64 sediment samples. The total concentration of BTHs and BTRs in the sediments from Trondheimsfjorden are presented in Table 4.8. All target analytes were detected in at least one sediment sample, but several of the analytes were found to be present in very few samples, and were generally found below the limit of quantification. The total sum of BTHs and BTRs detected is also reported, and from this it is evident that benzothiazoles were found in higher concentrations in the sediment samples. The majority of the target analytes were found to be present in over 50% of the samples, indicating that these compounds are ubiquitous in aquatic environments.

The concentrations of target analytes in the sediments were estimated from their calibration curves, which all yielded R^2 values >0.99. The calibration curves for the BTHs and BTRs are given in Appendix E. The internal standard used for the different target analytes are given in Table B.2. The structure of the internal standards are given in Appendix D. The internal standards chosen for the different target analytes were generally based on the similarities between the analyte and internal standard, which was established based on their retention times.

Table 4.8: Total concentrations ($ng\,g^{-1}$) of BTHs and BTRs in sediment samples from Trondheimsfjorden.

Compound	Average (ng/g)	Median (ng/g)	Range	Detection Rate
BTH	27.2	20.0	<LOD-114	48/64
2-Cl-BTH	11.7	3.4	<LOD-134	30/64
2-OH-BTH	18.1	15.6	<LOD-140	55/64
2-S-BTH	4.95	2.5	<LOD-28.3	45/64
2-Me-S-BTH	0.60	0.53	<LOD-1.56	63/64
2-SCNMeS-BTH	0.57	0.13	<LOD-6.26	16/64
2-Me-BTH	29.9	19.1	<LOD-97.0	54/64
2-NH_2-BTH	0.25	0.21	<LOD-0.69	12/64
2-M-BTH	0.13	0.11	<LOD-0.23	17/64
$\sum$(9)BTHs	70.8	62.3	9.32-152	64/64
BTR	1.69	1.42	0.52-5.25	64/64
TTR	0.37	0.31	<LOD-0.96	64/64
XTR	0.17	0.15	<LOD-0.42	32/64
BTR-COOH	4.12	3.73	0.31-11.2	64/64
5-Cl-BTR	0.19	0.13	<LOD-0.52	35/64
5-NH_2-BTR	1.82	0.60	<LOD-6.99	59/64
1-OH-BTR	6.99	2.81	<LOD-58.3	21/64
$\sum$(7)BTRs	10.3	7.73	3.48-67.9	64/64

The contribution from the individual BTH and BTR derivatives on the total amount of BTHs and BTRs was also investigated. From the distribution of BTHs in the sediment samples that is given in Figure 4.2, it is evident that 2-Me-BTH, BTH, and 2-OH-BTH are the major components detected in the samples, and they also account for about 85% of the BTHs detected in the sediment samples. From the distribution of the different BTR derivatives (Figure 4.3) it is apparent that BTR-COOH, 1-OH-BTR, BTR, and 5-NH_2-BTR are the major components detected in the samples. BTR-COOH was found to account for almost 40% of the total concentrations of BTRs detected in the sediments from Trondheimsfjorden.

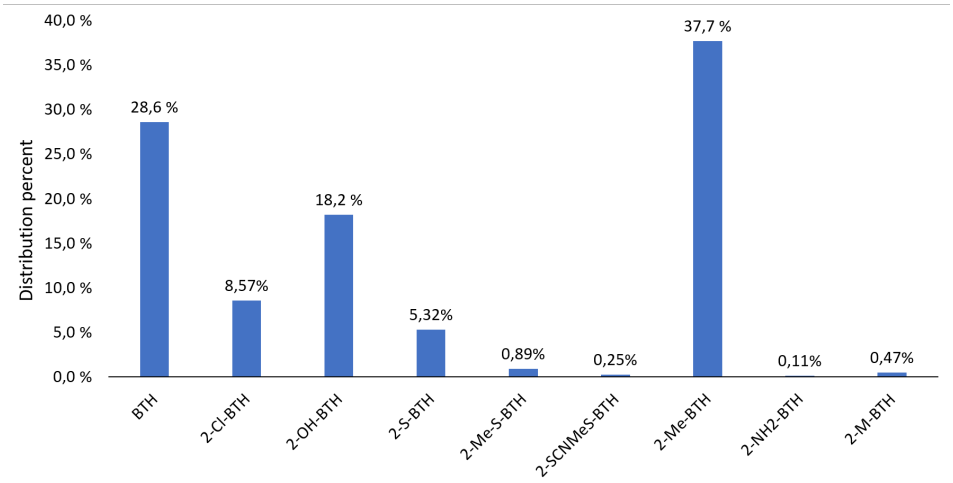


Figure 4.2: Distribution of benzothiazole derivatives in sediment, compared to ∑BTHs.

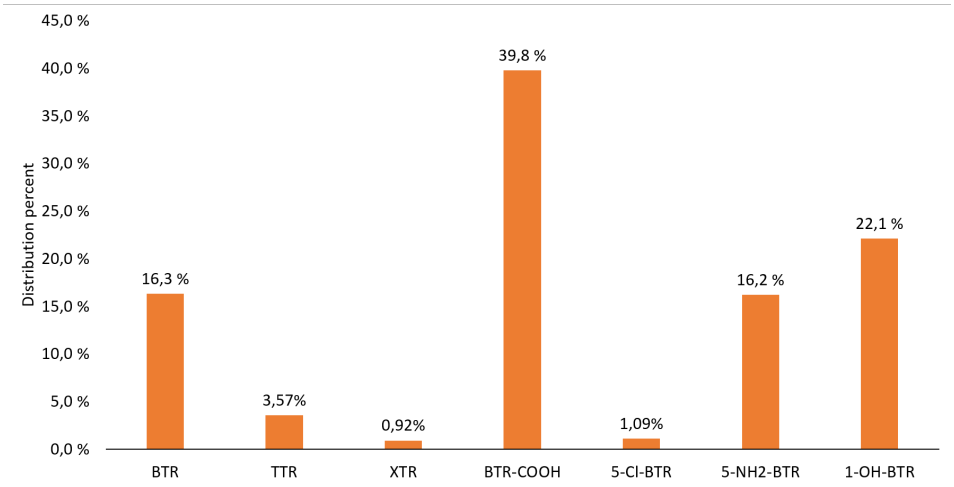


Figure 4.3: Distribution of benzotriazole derivatives in sediment, compared to ∑BTRs.

Sediment samples were taken from nine different locations in Trondheimsfjorden, eight of them located close together and one site further out in the fjord, where samples were taken to determine background levels. An overview of the sampling sites is given in Figure 3.2. The levels of contaminants detected at the different sampling sites were also considered, to investigate if there could be any apparent differences between the individual sampling sites. The distribution of the total levels of BTHs is given in Figure 4.4. The levels are in the same range at the different sampling sites, with highest concentrations observed at site 3 and 5. The distribution of the total levels of BTRs in the sediments at the different sites is given in Figure 4.5. BTRs were found in much lower concentrations than BTHs in the sediments, and there were also some minor differences in the distribution pattern between the two groups. The highest concentrations of BTRs were detected at site 1 and 10, where the high concentration observed at site 10 is attributed by high BTR concentrations at this location.

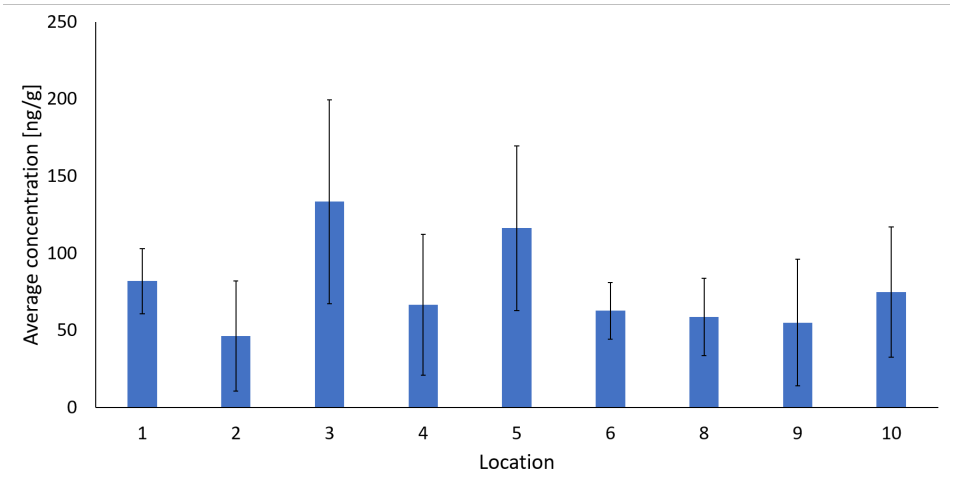


Figure 4.4: Total concentration of BTHs at the different sampling sites. The error bars represents the variation in concentrations between individual samples taken from the same location.

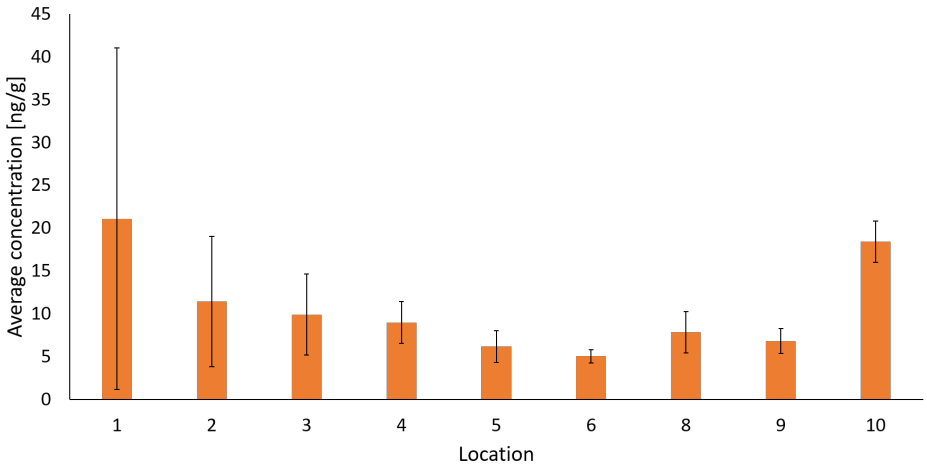


Figure 4.5: Total concentration of BTRs at the different sampling sites. The error bars represents the variability in concentrations of target analytes in the individual samples taken from the same location.

4.2.2 Polycyclic Aromatic Hydrocarbons

The total concentrations of the different PAHs in the sediment samples are presented in Table 4.9. Most of the PAHs were found in the samples, but some of the compounds could not be quantified due to low concentrations. BgP and IND was removed from this study since they were not found when looking at the recoveries with spikes and matrix matches. ACY is also not reported in this study since it was only visible under diode-array detection, in addition to not being present in any of the samples. The compounds are quantified by using F-PHE as internal standard, which for all target PAHs yielded calibration curves with R^2 values >0.99. The structures of the individual internal standards present in the internal standard mix are given in Appendix D.

Table 4.9: Total concentration (ng/g) of PAHs in sediment samples from Trondheimsfjorden.

Analyte	Average	Median	Range	Detection Rate
NAP	9.86	9.13	<LOD - 46.1	57/64
ACE	12.5	12.5	<LOD	1/64
FLU	9.99	9.99	<LOD	2/64
PHE	21.2	20.1	<LOD - 58.8	62/64
ANT	7.80	7.70	<LOD - 27.2	52/64
FLT	58.0	59.0	<LOD - 110	64/64
PYR	56.0	57.2	<LOD - 111	64/64
BaA	32.1	32.1	<LOD - 56.0	62/64
CHR	37.2	39.3	<LOD - 56.2	63/64
BbF	45.5	46.9	<LOD - 71.4	64/64
BkF	22.0	22.6	<LOD - 31.7	62/64
BaP	41.4	41.8	<LOD - 59.3	62/64
DBA	8.80	8.48	<LOD	54/64
∑(13)PAHs	336	351	29.1 - 548	64/64

The relative distribution between the 13 detected PAHs in the sediment samples is given in Figure 4.6. The relative distribution illustrates that fluoranthene and pyrene accounts for the majority of the PAHs detected in total, while acenaphthene and fluorene are responsible for only trace levels of the detected PAHs. This distribution profile seems to be in accordance with previous studies on PAHs in sediments from fjords in Norway [79].

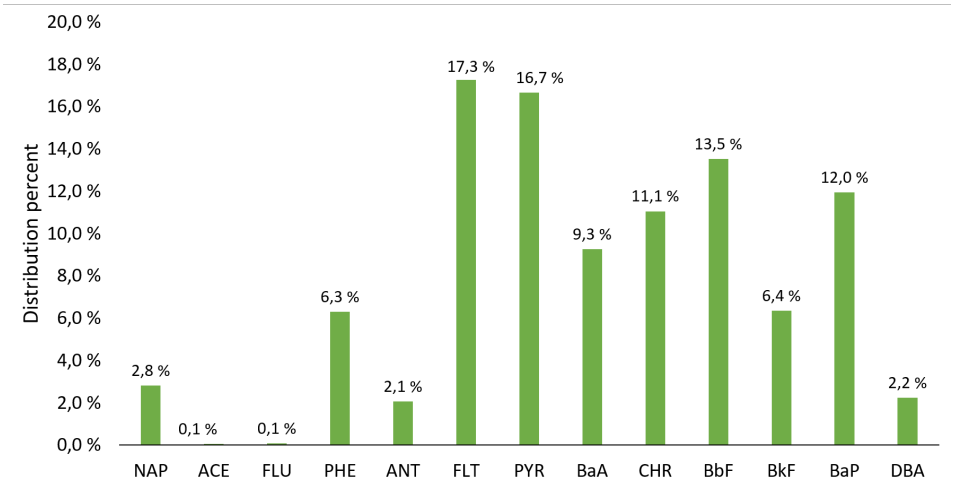


Figure 4.6: Distribution of the different PAHs in sediments, compared to ∑PAHs.

The distribution profile of the ∑PAHs at the nine sampling locations is given in Figure 4.7. The PAHs were found to be present in higher concentrations than BTHs and BTRs, and show a similar distribution pattern as the BTHs. From this distribution it is evident that the concentrations of PAHs are lowest for the sampling points furthest away from shore, while the highest concentrations were found at site 3, 5 and 6.

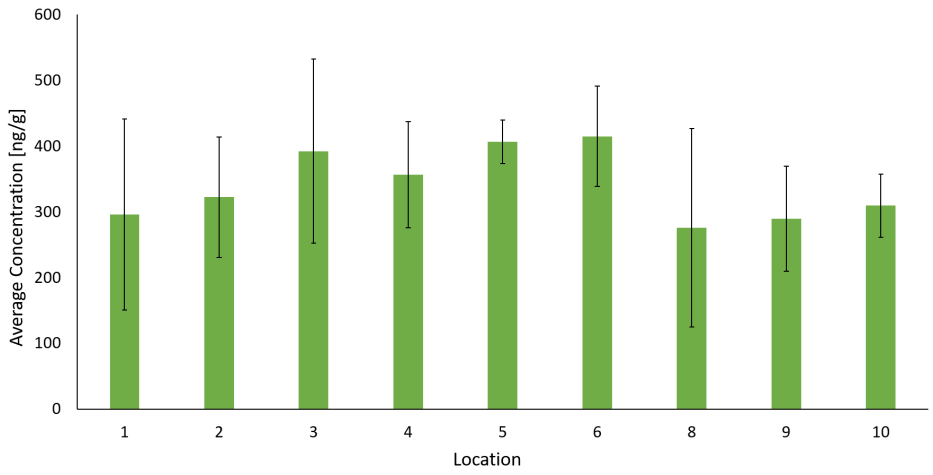


Figure 4.7: Total concentration of PAHs at the different sampling sites.

4.3 Associations Between BTHs, BTRs, PAHs, and Trace Elements in Sediments

The sediments were also analyzed for trace elements by inductively coupled plasma mass spectrometry (ICP-MS), in addition to determination of percentage organic matter by loss on ignition (LOI). This work was performed by another master student, Olav Leiros Bakkerud, and the results from those analyzes will be used to investigate possible associations between the organic compounds and trace elements present in the sediments.

4.3.1 PCA

Principal component analysis (PCA) was done to group and separate the compounds and samples, based on the variation in the samples. All the PCA biplots are given in Appendix G, where the organics and trace elements are separated into separate biplots. In these biplots the first principal component (Dim 1) accounts for 37.1% of the observed variations, while the second principal component (Dim 2) describes 16.6% of the observed variations, thus the biplot explains 53.7% of the variations in the samples. In these biplots the score plots are either sampling location, seafloor depth, or sediment section, while the loading plots are divided into organics and trace elements. A brief discussion of these plots is given in the next chapter.

The samples are clustered based on based on their similarity, meaning that samples which are clustered together will be related to each other. The biplot for the association between selected organic compounds and location/sample station is given in Figure 4.8, since this is the biplot of most interest for this study.

Figure 4.8: PCA for BTHs, BTRs, and PAHs in sediment samples

Chapter 5

Discussion

5.1 Sediment Sampling

All the sediment samples for this study were taken at the same day during the late spring of 2018. Since it is known that several of the analyzed compounds (e.g. BTR and TTR) are use as anti-freeze agents, it could have been interesting to collect samples from both the winter and summer season to investigate if there would haven been any noticeable differences in concentration levels between the two seasons. Another study have also found that the levels of BTHs and BTRs differed in particulate matter between studded and non-studded tire seasons, indicating that these compounds are present to different degrees in tires [9]. This could again have an effect on the concentrations found in the top layer of the sediments. If there would be any apparent differences this would probably only be visible by studying the top layer of the sediments, since the top layer is accountable for the most recent pollution. On the other hand, the sedimentation rate in fjords is expected to be quite slow [136], so it would be

reasonable to assume that there would be little, if any, observable changes in the distribution profiles of the different BTHs and BTRs between the two seasons.

Another aspect with the sediment sampling is that the samples were taken with a sediment box corer. The samples were brought up to deck as blocks, where each block was divided into a top, middle, and bottom section. The samples were divided into these three distinct groups to identify if there could be any differences in contamination levels between the different layers. Based on the detected levels of contaminants in the different layers, there seemed to be no apparent association between sediment section and contamination level. However, this might be a result of the sampling method used for collecting the sediment samples. When the samples were taken with the box corer, the sampling of sediments had to be done rapidly to make sure that the sediment still held its block like structure, so that sampling of the different sections could be achieved, and this division might therefore be a bit inaccurate. Crossover between different sections might also have occurred since the sectioning had to be performed out in the field. It might have been easier to study temporal trends in the sediments if a sediment corer had been available when the sampling was performed. The sole purpose of such a sampler it to gain a core of the sediment, so that different sections can be studied. This would have made the division into three sections based on their depth easier, without the risk of mixing the different layers of the sediments. Another study that looked at BTRs in sediment cores found that there was a temporal trend in BTR contamination, where they found a trend between the age of the sediments and contamination levels [40]. However, in order to gain interesting information from such a study it would have been necessary to be able to date the sediments, which was outside the scope of this project.

The sampling took place near Høvringen, where eight of the sampling points were located close to each other, while one site (location 10) was chosen as a reference area. This point was therefore located further out

in the fjord (see Figure 3.2a). It was observed, both for BTHs and BTRs, that the concentrations at site 10 were in the same range, if not higher than what was observed for several of the other sampling locations. This tendency was also observed for the PAHs, if not as clear as for the BTHs and BTRs. This might rise questions about whether this location was ideal as a reference point for background concentrations of pollutants. Since this area is quite far from the other sampling sites, there might be differences in the properties of the sediments, which again could have possible effects on the fate of contaminants in this area. For this to be certain further work needs to be done on the adsorption of BTHs and BTRs to sediments, and the different factors that may influence these processes. There was also found that there are quite a bit of ship traffic going on in the area where sampling site 10 is located (see Figure 3.1), and this may have resulted in higher concentrations of certain compounds (especially some elements), depending on how the activities associated with the ships. Further discussion on this is given with the interpretation of the PCA plots.

5.2 Benzothiazoles and Benzotriazoles

5.2.1 Accuracy and Precision of the Analytical Method

The recoveries of target analytes and the relative standard deviations (RSD%, N=3) suggest that there are some issues with the recoveries of several of the target analytes. From the absolute recoveries reported in Table 4.2, it is evident that some compounds were more challenging to recover than others. For two of the compounds, 2-S-BTH and 2-Cl-BTH, the concentrations already present in the samples were not subtracted due to high endogenous concentrations. The reported recoveries for these two compounds were, as a result, quite low compared to most of the other analytes. The recovery for 5-NH_2-BTR was found to be very low, with high relative

standard deviation. The low recovery observed for 5-NH_2-BTR can be due to little retention by this compound through the Strata-X RP cartridge. This might also be the case for 1-OH-BTR, since both of these compounds are quite polar analytes, and they will therefore experience little retention in reversed phase. Most of these BTHs and BTRs were analyzed in a different matrix by Asheim et al. (2018), where the same extraction procedure were followed. The reported recoveries in this study is a bit lower than what was reported in that study, but the analytes were in general found to follow a similar trend when it can to the efficiency of the extraction method [9]. The IS BTR-d4 and 5-Me-BTR-d6 provided adequate compensation for variations in the extraction percentages for BTHs and BTRs, which is illustrated by the higher values for the relative recoveries. This is the first time many of these compounds have been analyzed in sediments, and this method has in general demonstrated acceptable recoveries, illustrating that this method can be used to determine BTHs and BTRs in sediments. However, improvements can still be done to increase the recoveries of some of the target analytes, and further method development have to be done on this front.

All three internal standards yielded absolute recoveries $\sim$ 70%, and for BTR-d4 and 5-Me-BTR-d6 the recoveries were similar to the recoveries of their non deuterated compounds. The recovery for BTH-d4 was higher than the recovery of BTH, which might be due to the low MS-response of this compound. In the end, BTH-d4 was not used to quantify any of the target analytes, due to unreliable quantification of this compound. In general it provided low sensitivity compared to the other two internal standards, resulting in a low response for this IS. Some of the same issues were observed with BTH, especially in low concentrations, which is illustrated by the elevated LOD for this compound. The choice of internal standard for the different target analytes was based on the similarity between the target analyte and the internal standard. This is in general represented by the retention time of the analytes, and the internal standard that eluted closest to

the target analyte was in general chosen for quantification.

If the target analyte are very different from the chosen internal standard, this might lead to overcompensation of the relative recovery. This was observed for 2-OH-BTH, where the relative recovery was estimated to be 325%. This indicates that the chosen internal standard and target analyte have very different chemical characteristics, and illustrates the relevance of finding internal standards that are chemically similar to the analytes of interest. In the case of 2-OH-BTH, the relative recovery was estimated relative to both BTR-d4 and 5-Me-BTR-d6, but since both cases lead to an overcompensated relative recovery, the IS that eluted closest to 2-OH-BTH was chosen. It could have been an idea to estimate the recovery of 2-OH-BTH relative to BTH-d4, but since it was challenging to quantify this internal standard in the samples, this was not done.

For the determination of accuracy, target analytes were spiked into the sediment matrix at a fortification level of 10 ng, 25 ng and 50 ng with three (N=3) replicates each time. The reported recoveries are given as an average for the the replicates spiked with 10 ng target analytes, since this concentration in general gave stable recoveries between the three replicates. This is also the concentration level that is closest to the concentrations that were generally found in the sediments. The data from the samples spiked with 50 ng could not be used for this purpose since there was no data from the matrix matches spiked with 50 ng target analytes. This is likely due to an error during the injection of these samples into the LC, since it was noted that several samples in a sequence was not giving any response in the MS. This concentration level was therefore not used for any purpose in this study. This should, however, not affect the quality of the work since quality control steps were successfully done at two different concentration levels. The other concentration levels will also be more relevant since they are closer to the realistic concentrations found in the samples, and since they are located more in the middle of the calibration curves.

5.2.2 Procedural Blanks

Contamination that might arise from the sample preparation was evaluated through the analysis of procedural blanks. For every 23 samples (for each run on the SPE), one procedural blank was analyzed simultaneously, to investigate contaminant levels that might arise from sources in the laboratory. All surfaces in the laboratory were covered in aluminum foil to reduce the contamination risk from surfaces, and to avoid dust entering the samples. All glassware was also washed with methanol to make sure it was clean. All target analytes analyzed by LC-MS/MS were detected in all the procedural blanks in various degrees, sometimes exceeding the levels found in the individual samples. Consequently, the levels found in the blanks were subtracted from the measured concentrations in sediments to compensate for the contamination that occurred during the sample preparation. It is believed that it was the evaporation step that introduced most of the (cross-) contamination observed, and this has also been suggested in other studies of trace organic pollutants that use an evaporator to concentrate the analytes [125]. Since the LC-MS/MS is a very sensitive method, it will be able to detect even small levels of contaminates present, which might be why contamination was found in the procedural blanks for BTHs and BTRs, but not for the procedural blanks for PAHs which where analyzed with HPLC. Another study that analyzed for BTRs and BTHs in tap water, also found that small amounts of BTH were present in Milli-Q water, which derived from tap water, and HPLC grade water was therefore used instead [26]. Since Milli-Q water was used in several steps in the extraction process of the target analytes, it might be that some contamination of BTH has been introduced in this way. However, this by itself can not explain the relatively high concentrations in the blank samples, and it is more likely that the levels observed in the procedural blanks occurred during the evaporation step.

5.2.3 Matrix Effect

Since it is known that the ESI interface is susceptible to suffer from relatively strong matrix effects, the influence of the sample matrix was investigated by comparing the response of a sample matrix spiked with target analytes, against a standard in a solvent solution. This is done to investigate if the target analytes will experience ion suppression or ion enhancement, which could lead to false negative or false positive identification [137]. It was in general found that these compounds did not suffer from too strong matrix effects, and it was found that all the target analytes suffered from ion suppression, except for BTH which experienced some ion enhancement. The matrix effects for the analyzed BTHs and BTRs are reported in Table 4.4. It is uncertain why only BTH suffered from a positive matrix effect, but it might be related to the sensitivity issues experienced for this compound during the analysis. It is common for analytes to suffer from negative matrix effects, and even though it is unclear why the compounds suffer from different matrix effects, it is in general related to co-elution of matrix compounds, which may suppress the signal of target analytes. It has previously been reported that BTRs in general will suffer from greater matrix effects than BTHs, since BTRs will elute first in reversed phase chromatography [9]. The matrix effect is expected to be higher for early eluting compounds since hydrophilic compounds are not well retained in reversed-phase columns [123]. However, in this study it was found that the BTHs in general suffered from the strongest matrix effects, while the BTRs only experienced moderately negative matrix effects. In previous studies that have looked into the presence of BTHs and BTRs in sediments, matrix effects for the individual compounds have not been reported, therefore these values can not be directly compared with values recorded from the same matrix. On the other hand, it can seem like the target analytes experienced less matrix effect than what have been reported for these analytes in road dust [9], and much lower matrix effects were recorded in this study

compared to reports on BTHs and BTRs in urine samples [22].

The matrix effect for BTHs and BTRs was estimated based on the response of sediment matrix spiked with 25 ng target analytes into the final extract (post-extraction matrix spike), relative to a standard solution. There was not prepared a standard solution with a concentration of $25\,\mathrm{ng\,mL^{-1}}$, but since standards were made for $20\,\mathrm{ng\,mL^{-1}}$ and $50\,\mathrm{ng\,mL^{-1}}$, this concentration level could still be used, by estimating the response from a standard solution of $25\,\mathrm{ng\,mL^{-1}}$. This was the preferable concentration level to determine the matrix effect, since it falls in the middle of the calibration curve, and this point will therefore be representative for the calibration. It was decided to not use the $10\,\mathrm{ng\,mL^{-1}}$ matrix matches for this purpose, since this fortification level falls close to the limit of detected for some of the analytes, thus yielding unreliable estimations.

5.2.4 Limit of Detection and Lower Limit of Quantification

In general it was found that the target analytes were very sensitive, which is illustrated by their low limit of detection. Most of the target analytes were found to have a limit of detection $<0.2\,\mathrm{ng\,g^{-1}}$. This illustrates that even though these compounds were analyzed in a complex sample matrix, the chosen extraction and clean-up method eliminated most of the sample interferences, while efficiently retaining the target analytes. Some of the analytes, however, were found to have elevated LODs, and this was especially the case for some of the BTHs. This is most likely caused by low sensitivities for these compounds in the mass spectrometer. These analytes (BTH, 2-S-BTH, 2-OH-BTH, and 2-Cl-BTH) have also been reported to have elevated limits of detection in studies from different matrices [9, 26].

5.2.5 Sample Preparation

For the analysis of BTHs and BTRs approximately 0.5 grams of sediments were weighted out for the extraction of the target analytes. Since the concentrations of the different compounds were found to be quite low, the method experienced some difficulties in quantifying a few of the target analytes. This might have been improved if a larger quantity of sediment was used for the extraction, since larger amounts of target analytes would have been present in the extracts. However, this could have resulted in lower recoveries for the individual compounds since larger amounts would need to be extracted. It is also uncertain if this would have lead to larger amounts of co-extracted matrix compounds, which could have caused higher matrix effects due to a larger impact from interfering compounds. It might also be that a larger quantity of sediment would have lead to more particles in the extracts, which could have caused troubles during the solid-phase extrac, where large amounts of particles will clog the cartridges. In other studies on BTHs and BTRs in sediments it has generally been reported that 0.5-1 gram of sediment have been used for the extraction [32, 40], and it could have been interesting to see the effect of sample quantity, and investigate if this could have improved the detection rate for some of the analytes.

5.2.6 Distribution of BTHs and BTRs in Sediments from Trondheimsfjorden

From the concentrations of BTHs and BTRs that are reported in Table 4.8, it is evident that the concentrations of BTHs are much higher than the BTR concentrations. This distribution is in accordance with a study done on BTHs and BTRs in indoor dust samples, where they found much higher concentrations of BTHs compared to BTRs [25]. Higher concentrations of BTHs have also been reported to be found in wastewater from wastew-

ater treatment plants [4]. However, opposite distribution between these two groups of compounds have been observed in road dust, where benzotriazoles were responsible for the highest amount of contamination [9]. Since the production volumes of these compounds are unclear, it is difficult to determine if the observed profiles in the sediment samples is due to differences in production volume, or due to different behaviour in the environment. Based on the octanol to water partition coefficients for these compounds (Table B.3), it is expected that there are more BTHs present in the samples, since they in general have a higher affinity for the organic phase, compared with the BTRs which have a higher polarity.

The distribution of the different BTHs and BTRs in the sediments are illustrated in Figure 4.2 and Figure 4.3. For the BTHs it was found that 2-Me-BTH was accountable for a large percentage of the detected BTHs. In addition, there was found relatively high contributions from BTH and 2-OH-BTH. The analytes that are found in high concentrations will generally be end products from transformation processes, indicating that other derivatives can be biotransformed into these species. The high abundance of 2-Me-BTH in the sediments indicates that methylated species are end-products for BTHs in sediments. 2-Me-BTH is one of benzothiazoles with highest log K_{OW}, indicating that this derivative have higher affinity to particulates compared with many of the other derivatives, which also can explain the high abundance of this compound in sediments. Based on the octanol to water partition coefficients it should have been expected that 2-SCNMeS-BTH would be present in higher concentrations, but since this compound is very unstable, it is easily transformed into other derivatives [23]. For the BTRs a different distribution pattern was observed in the sediments. In general there was found that the oxidized species were accountable for the most of the detected BTRs, where BTR-COOH and 1-OH-BTR were responsible for 39.8% and 22.1% of the total BTRs detected. In addition, very little contribution came from the methylated species TTR and XTR, where the lowest contribution came from XTR (0.92%). TTR

is known to be used quite frequently in de-icing fluids, and high concentrations of this compound was found in a study of road dust in Trondheim [9]. This distribution indicates that the methylated species of BTR are transformed to different oxidation species in sediments, and that these oxidation species are relatively stable in sediments.

The detection rate of 2-S-BTH was quite high (70%), and in some of the samples the concentrations were found to be relatively high. The high abundance of this compound is a bit surprising since this compound is very unstable due to the free thio-group. The relatively low concentrations of 2-Me-S-BTH in the sediments is therefore unexpected, since several studies have reported that 2-S-BTH is easily transformed 2-Me-S-BTH [23]. Thus, one could expect an opposite profile to what was found in this study. The high detection of 2-S-BTH in the sediment samples can indicate that this compound was released to the sediments shortly before the sampling took place. It might also be that the free thio-group can be immobilized by organic matter in the sediments, and that these mechanisms keeps it from being transformed into other products.

To the best of the authors knowledge, this is one of the bigger studies done on benzothiazoles and benzotriazoles in sediments, where many of the derivatives have been investigated for the first time. This is also the first time these compounds have been reported in Trondheimsfjorden. Since BTHs and BTRs are not readily monitored in sediments,there is a lack of data on the occurrence of benzothiazoles in sediments. The levels of benzothiazoles and benzotriazoles found in the sediment samples from Trondheimsfjorden were in general in the same range as what have previously been reported. Since these compounds scarcely are studied in sediments, there are however quite large variations in the detected levels, and this is likely due to differences of the sampling locations, since the amounts found in sediment samples will be dependent on local contamination sources. Compared to other studies done on benzothiazoles and benzotriazoles in sediments, these levers were generally found to be in the lower end of the

scale. This was especially the case for benzotriazoles, where there have been reports on levels up to 630 $ng\,g^{-1}$ in sediments from Brazil [41], however these high concentrations were probably found due to the location of the river, which had 32 municipal WWTPs located close by. Compared to sediments from the United States and China the concentrations of BTR were found to be in the same range [32]. The levels of TTR detected in this study are lower than reported levels in sediments from a river in the United States (Detroit River) [32], which may be due to different usage of these compounds. The other benzotriazole compounds have not been reported in sediments, and it is therefore uncertain how the distribution profile in sediments from Trondheimsfjorden is compared to sediments from other areas.

The levels of 2-S-BTH seems to be similar to the levels observed in sediments from a river near Stockholm [46]. While 2-S-BTH was detected in sediment samples, it was not found in any of the surface water samples, suggesting that components present in the sediments can stabilize this compound, while it is transformed into different products in waters. The levels of 2-OH-BTH were found to be higher than the levels reported in sediments from the United States [47], while the levels of 2-M-BTH were a bit lower than reported in sediments from Japan [48]. Since these compounds in general are scarcely reported on in sediments, there is a lack of knowledge regarding their presence in this matrix. However, this study has indicated that even though these compounds are highly soluble in water, detectable amounts still finds their way to sediments where they may persist and constitute a heavy load on marine organisms. These effects may also be enhanced by the presence of other groups of contaminants in the sediments. This study highlights the need to do more research on these two groups of contaminants in sediments, since there is still many parameters that is unknown regarding their fate in this matrix.

5.3 Polycyclic Aromatic Hydrocarbons

5.3.1 Accuracy of the Analytical Method

To determine the accuracy of the analytical method, target analytes were spiked at a concentration of 300 ng (N=3) directly into the sample matrix. The absolute recoveries of the target analytes were in found to be satisfying, ranging from 70.6% (NAP) to 116% (FLT). When the recoveries were adjusted with the recoveries for the internal standard, it was found that the relative recoveries were between 91.6% and 132%, which illustrates that the internal standard provided efficient compensation for variations in the extraction recoveries. These high recoveries demonstrates that the target analytes were efficiently extracted with the developed method. All the internal standards showed high absolute recoveries ranging from 69.4% for F-NAP to 94.2% for F-PYR. In general, it was observed that recoveries were a bit higher for the target PAHs compared to their fluorinated variants, which may be due to the lower polarity of the fluorinated compounds.

Fluorinated PAHs were used as internal standards for the determination of the various PAHs. They work well as internal standards for this purpose since they have similar chemical and physical properties as the natural PAHs, and they do not appear naturally in the environment [138]. The production of F-PAHs is also quite low, so these compounds are not expected to be found in the environment. To be able to use F-PAHs as internal standard in HPLC, it is important that they can be separated from their parent PAHs, which they were found to be in this study. F-PHE was chosen to be used as the internal standard for the quantification of all the PAHs, since this internal standard have intermediate hydrophobicity and volatility. This compound would therefore be convenient to use as internal standard, since it can be applied to all the target PAHs. This internal standard was also found to be stable under the analytical procedure and was easily identified

in the individual chromatographic runs. The structures of all the F-PAHs present in the internal standard mix are given in Figure D.2.

5.3.2 Procedural Blanks

For every five sample a procedural blank was made to investigate for possible contamination occurring during the extraction process. The blanks showed no sign of contamination, which indicates that there was no significant contamination arising from the extraction process or from sources within the laboratory. However, the blanks showed signs of contamination for the analytes analyzed by UHPLC-MS/MS. Even though different methods were used for the extraction, both methods used a TurboVap-system to concentrate the extracts, and this device suspected to contaminate the samples analyzed for BTHs and BTRs. Since PAHs are semivolatile compounds it is reasonable to assume that contamination could occur during this step, especially for the lighter ones, but since these compounds were analyzed by HPLC with FLD detection, they were not detected in the procedural blanks. This is also illustrated by the limits of detection of the two methods, where the LODs were much lower for BTHs and BTRs due to the higher sensitivities of the mass spectrometer.

5.3.3 Standard Reference Material

For the analysis of PAHs, a standard reference material was available, and this was therefore also analyzed to investigate the accuracy of the analytical method. The results from this test is given in Table C.3 (Appendix C). First of all only eight of the target analytes were present in the reference material, so it was necessary to perform additional quality control steps to investigate the extraction efficiency of the remaining compounds. In general the recoveries was lower when calculated based on the reference

material, and some compounds yielded extremely high recoveries, compared to the recoveries that were found when it was estimated based on a spiked matrix compared to a standard solution. The reason for the observed effects may be that the standard reference material used was quite old, and the concentrations of target analytes in the material might therefore be a bit lower than the reported concentrations. There might also be that contamination of the standard reference material have occurred, since it has been stored in a busy laboratory for some time. To investigate this further, new reference material could have been obtained, but this is quite expensive, and in this study it was sufficient to use spiked samples for the quality assurance.

5.3.4 The Chromatographic Method for PAH Analysis

The presence of PAHs in sediment samples from Trondheimsfjorden were determined by HPLC with fluorescence detection. The first parameter to note from the chromatographic method is that the method was very long, with a total run time of 60 minutes. This lead to difficulties in quantification of the two latest eluting compounds (BgP and IND), and these compounds were therefore removed from this study. The long run times were due to the chromatographic column available, and since this was a minor part of the project, resources were not used on a new column that could have improved the chromatographic separation of the analytes. Another aspect from the analysis is that there were some issues with the baseline for the later eluting compounds, which may be due to the rapid change of the excitation and emittance wavelengths in this area. This has been suggested by Denis et al., where they observed a staircase baseline when many excitation and emittance wavelengths were applied in a short period of time [139]. These were the main two factors that complicated the quantification of the target PAHs, but in general the method illustrated sufficient separation of the target analytes, and a total of 13 PAHs could be determied

in the marine sediments from Trondheimsfjorden.

5.3.5 Distribution of PAHs

In general, the concentrations of PAHs were found to be quite low, where several compounds were found to be under the limit of quantification. BgP and IND are not reported in this study since they was not found when studying the recoveries with spikes and matrix matches. These were also the last eluting compounds, and they were due to the long analysis time also quite unreliably quantified. ACY is also not reported since this compound only was visible in the DAD, in addition this compound was not present in any od the samples. The highest contributions came from FLT and PYR, which accounted for 17.3% and 16.7% of the detected PAHs respectively. There is also quite high contributions from BbF and BaP to the total concentration of PAHs in the sediments. The distribution profile found in this study is quite similar to what was found in sediments from Trondheimsfjorden in 2003, and also to what was found during the project Renere havn [89]. The observed pattern of PAHs in the sediments is common for this group of compounds in sediments from Norway [79], where there is little contribution from the low molecular weight compounds. The low molecular weight PAHs will in general be found in low concentrations in sediments due to their higher water solubility and volatility. They will therefore be expected to be found in higher concentrations in the water column and in the atmosphere. This is also illustrated by their lower K_{OW} partition coefficients (Table C.5), which indicates that they have a higher affinity to the water phase.

Based on the classification system for PAHs in marine sediments it was found that the levels of PAHs in sediments from Trondheimsfjorden are between class I and class II, when the ∑PAH were looked at. This is approximately the same results that were found in sediments from Trondheimsfjor-

den after the project Renere Havn was finished in 2016. [89]. It is worth nothing that the ∑PAHs in this study is given for 13 compounds, and not 16 which is usually reported, but it is not expected that possible presence of the three remaining compounds will change the status for contamination of the sediments, since these compounds in general are less abundant in sediments [79]. The levels of the individual PAH compounds were also found to be categorized as class I and II under the new guidelines given by the Norwegian Environmental Agency (see Table C.6), where only ANT were close to be categorized under class III in a few samples. In general it can seem like the effects from the project Renere havn are lasting, where the levels of PAHs have remained low since the project finished up.

5.4 Principal Component Analysis

From the biplot given in Figure 4.8 some of the sampling locations could be grouped together, based on their placements in the plot. All the individual samples taken from location 10 were grouped together, which is sensible since these samples were taken from another area than the remaining samples. This site was located furthest away from the shoreline, and was chosen as a point where background concentrations in the sediments could be measured. From the plot samples from location 8 and 9 could also be grouped together, and a group for samples from location 1 and 2 could also be made. The rest of the samples were clustered together in the plot, and no further groups could be made up for the remaining sampling sites. The clear separation of location 10 from the other sampling locations can indicate that the samples from this location have different properties compared to the other sampling areas. This might lead to differences in compounds attracted to this area, which will result in different contaminant patterns at this site compared to the other sampling sites. It was also discovered that there are several commercial ship lines going over

this sampling site, which might introduce different contaminants than those found at the locations closer to land, since these sites will be more affected by contamination sources from land. It could therefore have been relevant to choose another location to do background checks, where there is less influence of contaminants from external sources.

When studying the different compounds included in the biplot, most of them are located in the top left section of the plot. In general the PAH compounds were found to be closely related, and there can also seem to be some relationship between the PAHs and two benzotriazole derivatives, namely TTR and XTR. There can also seem like there is a grouping within the PAHs, where the lighter PAHs are found in one group while the heavier ones are found in another group. TTR and XTR are mostly related to the lighter PAHs, which can be explained by their similarity in molecular weight, even though the partition coefficients for the benzotriazoles are a bit lower. The two other benzotriazoles included in the biplot, BTR and BTR-COOH, are found on the opposite side of the plot, and together with sampling the sampling site they explain most of the variance observed in dim 1. PAHs were in general found to be associated with the locations closer to shore, which indicates that there are some local contamination sources on land that distribute these compounds to the fjord. BTR was found to be located close to the samples taken from location 10, which may be an indication that BTR is associated with this location. This might indicate that BTR is influenced by the ship traffic in this area, but it remains uncertain weather products containing benzotriazole is commonly used on ships in Norway. It might also be that the higher abundance of BTR in sediments from location 10 is linked with different properties of the sediments from this area. To gain more knowledge about this, additional locations could have been chosen further from shore, to get an broader indication about the distribution of this compound. To investigate if the distribution of contaminants could be linked to different properties of the sediments, further characterization of the sediments from the different locations could

have been done. In the PCA plot, BTR-COOH is found in between the samples from location 10 and location 8 and 9, which may indicate that this compound is slightly associated with these three locations. From the PCA it can seem like BTR and BTR-COOH suffer from different fates in the sediments, compared with the other organic contaminants. There can also seem like there is some slight association between 2-Me-S-BTH, 2-S-BTH, and 2-Me-BTH, which is not surprising since there are several reports on the transformation of 2-S-BTH to 2-Me-S-BTH [23]. There can also be a slight association between 2-Me-BTH and BbF, but it is uncertain where this association arise from, since they have different physicochemical properties, and there are few reports on the specific usage of these two compounds.

PCA biplots were also made to investigate if there could be any connections between the different contaminants and the depth of the seafloor, and to investigate if there were any differences between the sediment sections. The PCA biplots for this is given in Appendix G. These plots gave no new information, and there seems like there is no association between sediment section and contaminants, which might be a result of the sampling method as mentioned earlier. There might also be that no pattern is found due to a limited number of replicates from the different sampling sites, which makes it difficult to see any groupings. From the PCA biplot where the seafloor depth is added as the PCA, there can seem like most of the PAHs are found in areas where there is not too deep waters, while BTR and to a certain degree BTR-COOH are found in areas where the seafloor is deeper. It is, however, difficult to know whether the observed differences are due to the seafloor depth or if this pattern is related to different characteristics of the sediments from the individual locations.

5.4.1 Associations Between the Organic Contaminants and Selected Trace Elements

Another goal of this thesis was to see if there could be any associations between the three groups of organic contaminants and selected trace elements in the sediments. The bioplots for the elements are given in Appendix G. The organics and a selection of the most interesting trace elements were separated into two groups for the PCA, but since they use the same axis, they can be compared across the plots, as long as the principal components are the same. In general it was found that most of the elements appeared on the opposite side of the biplot when compared to the organic contaminants. It can also seem like there is an association between sampling site 10 an several elements, like Zn, Pb, Cu, S, Se, Cd, and P. Several of these elements might also be associated with benzotriazole, since they are located near each other in the bipot. The association between BTR and several metals is expected, since this compound is used as a corrosion inhibitor due to its metal complexing characteristics. The general association of trace elements with the sites furthest away from shore might be linked to boat traffic further out in the fjord. The association of phosphorous (P) with sampling site 10 might be due to re-suspension of bottom sediments as a result of the ongoing boat traffic [140], which results in higher available phosphorous concentrations in the sediments collected. From the PCA it was found that several metals were closely connected to sulphur (S), and this might be due to the formation of precipitates between sulphur and several of the trace metals in the sediments. This is especially believed to be the case for cadmium (Cd) where Cd rapidly will displace iron in FeS to form cadmium sulfide precipitate [141]. There might also be a slight association between 2-Me-BTH, BbF and the metals tin (Sn) and mercury (Hg), but it is uncertain where this correlation might arise from, since there are few mentions on the usage of 2-Me-BTH.

5.5 Correlations

A correlation plot was also done in order to see if there was any correlation between the different organic contaminants. The correlation plot is given in Figure G.6 in Appendix G. From this it can seem like there is a positive correlation between BTR and percentage organic matter, which may indicate that BTR binds to organic matter, and will be found in higher abundance in sediments with a higher percentage of organic matter. This is supported by reports on the sorption of BTRs to soil, which indicated that this adsorption varied as a function of organic matter in the soil [66]. There can also seem like there is a slight positive correlation between the different benzothiazoles, which is expected since these compounds are known to be biotransformed to each other, and in addition they are generally used for the same purposes. Some of the benzotriazole derivatives, 5-Cl-BTR, 5-NH_2-BTR, and 1-OH-BTR, were found to be negatively correlated with most of the PAHs, which illustrates the physicochemical differences between these compounds. In the correlation plot it was also evident that there was a positive correlation between the different PAHs, which was also illustrated in the PCA plots. The heaviest PAHs were also found to have a positive correlation with the percentage of organic matter, which is as expected since these are the most lipophilic compounds. Of the benzothiazoles only 2-Me-S-BTH were found to have a positive correlation with percentage organic matter, indicating that this compound deposits in the same fashion as the PAHs. The negative correlation between BTR-COOH and TTR (+XTR) suggests that the latter compounds are transformed to BTR-COOH in sediments, which was also indicated by the distribution of the different BTRs in the sediments.

Chapter 6

Conclusion

The extensive use of benzothiazoles and benzotriazoles has lead to their release to a variety of environmental matrices. In this study a total of 16 target BTHs and BTRs, as well as 13 PAHs were detected in sediments from Trondheimsfjorden, thus indicating their omnipresence in the environment. The concentrations of ∑(9)BTHs were found to range from 9.32-152 $ng\,g^{-1}$, while the concentrations ranged from 3.48-67.9 $ng\,g^{-1}$ for ∑(7)BTRs. The concentrations of PAHs in the sediments ranged from 29.1-548 $ng\,g^{-1}$. This study illustrates that despite the hydrophilic properties of BTHs and BTRs, adsorption to sediments occur through different mechanisms that still remain unknown. The concentrations of BTHs and BTRs were in general found to be relatively low in the sediments, where 2-Me-BTH and 2-OH-BTH were responsible for most of BTHs detected, while BTR-COOH was accountable for most of the BTRs. It is proposed that the methylated species of benzothiazoels are end products for BTHs in sediments due to the high detection of 2-Me-BTH, while oxidation species are the end products of BTRs. The low concentrations of TTR and to a certain degree BTR, and the consequently high presence of BTR-COOH

and 1-OH-BTR, indicates this scenario.

It was in general found that the levels of PAHs were low in the sediments, falling under class I and II in the Norwegian classification system for contaminated sediments. These low values illustrates that the effects from the clean-up of the harbor in Trondheim have been effective, and that they are still in effect. Despite this, it should be noted that PAHs still are ubiquitous contaminants in sediments from Trondheimsfjorden, and further actions should be made in order to reduce their levels even further.

There was found few associations between the three groups of organic contaminants included in this study. This is probably due to their different physicochemical properties, and illustrates that their fate in the environment may be different due to these differences. From the PCA there was an indication that the lighter PAHs and TTR were associated, which may be explained by their somewhat similar characteristics. The PCA also indicated that BTR were associated with several different trace metals, which may be due to the usage of BTR as an corrosion inhibitor. Since these compounds where found to be associated with the reference sampling site, while the rest of the organic compounds were found closer to shore, there may be that these compounds suffer from different fates in sediments, highlighting the importance of establishing data on contamination patterns for trace organic pollutants.

Chapter 7

Further work

This study illustrated that several benzothiazoles and benzotriazoles are found in sediments, but knowledge about the behaviour of these contaminants in sediments remain unknown. In the future work should be done on the interactions between BTHs, BTRs, and sediments, to gain more knowledge about factors influencing the distribution of these compounds in sediments. In this study only a fraction of samples were taken, and it would be interesting to take more samples from several areas of the fjord to gain more knowledge about the distribution of BTHs and BTRs in Trondheimsfjorden. For a future project samples could be taken near the treatment plant at Ladehammeren, to see if there are any differences in pollution level next to the two main treatment plants in Trondheim.

In future studies on BTHs and BTRs in sediments there could be an idea to take water samples as well, since the concentrations of BTHs and BTRs are expected to be higher in the water phase. This could give a more comprehensive picture of the contamination status of the area, and it could also give an insight into the rate to which BTHs and BTRs deposit onto sediments.

The recoveries for some of the analytes in this study were in the mid-range, and in the future further work should be done to improve the recoveries of some of these analytes. Sensitivity issues were also a challenge for some of the analytes, and work should be done to try and improve the peak sensitivity for the most challenging analytes (BTH, 2-S-BTH, 2-Cl-BTH). Further work should also focus on improving the chromatographic separation of the PAH analytes, since the method proposed is very time consuming and therefore it also uses large volumes of solvents.

In this study it was also found that some benzotriazoles may correlated with PAHs, but there are no information about the combined toxic effects of these pollutants. Further work should therefore look into the combined effects of these contaminants on organisms, to see if they could have synergistic effects. The same should be done for trace elements that are known to be toxic and benzotriazole, since these were found to be co-present near sampling site 10. Further studies on the toxicity of these chemicals should also investigate environmental relevant levels, to see if the concentrations found in the environment can pose a risk for aquatic organisms.

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Appendices

Appendix A

Sampling of Sediments from Trondheimsfjorden

Table A.1: Coordinates, dimensions, seafloor depth and sediment block section depths for the samples collected from R/V Gunnerus on the 31st of May 2018.

Sample no.	Position no.	Time	Latitude	Longitude	Seafloor depth	No. of replicates	Depth of sediment section	Sediment section size	Position of sediment section	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, 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 sample 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	Sample 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				

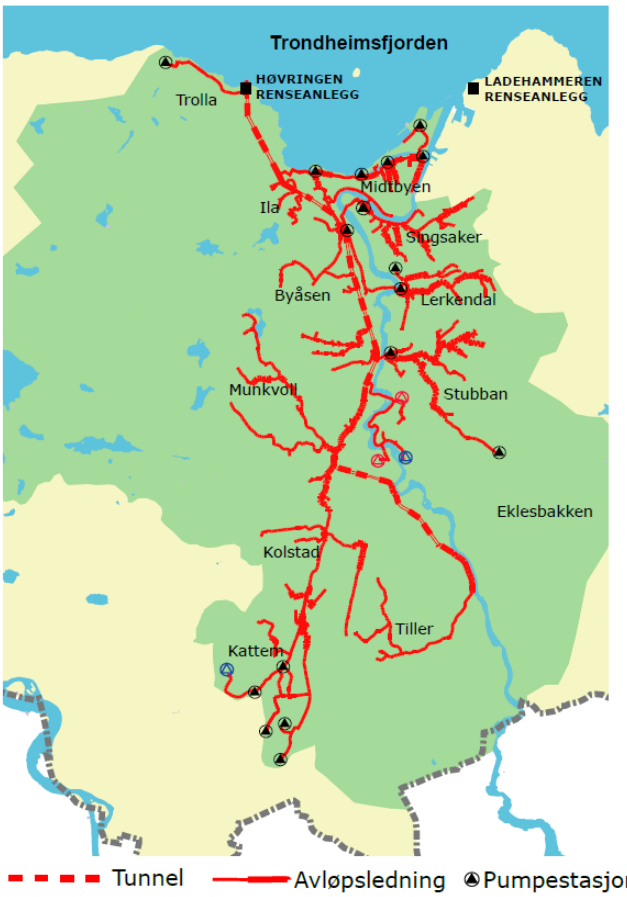


Figure A.1: Høvringen wastewater treatment plant. The map illustrates where the discharge pipes are located. The picture is taken from Trondheim muicipality.

Appendix B

Additional Data for BTHs and BTRs

Table B.1: Weight of BTHs and BTRs used for the preparation of standard stock solutions and the concentration of these stock solutions

Compound	Weight [g]	Concentration [ppm]
BTH	0.0100	1000
2-Cl-BTH	0.0174	1740
2-OH-BTH	0.0108	1080
2-S-BTH	0.0102	1020
2-Me-S-BTH	0.0120	1200
2-SCNMeS-BTH	0.0103	1030
2-Me-BTH	0.0118	1180
2-NH_2-BTH	0.0106	1060
2-M-BTH	-	2500
BTR	0,0090	900
TTR	0,0095	950
XTR	0,0110	1100
BTR-COOH	0,0095	950
5-Cl-BTR	0,0104	1040
5-NH_2-BTR	0,0097	970
1-OH-BTR	0,0121	1210

Table B.2: Internal standard used for quantification of the different BTHs and BTRs

Compound	Internal standard
BTH	BTR-d4
2-Cl-BTH	5-Me-BTR-d6
2-OH-BTH	5-Me-BTR-d6
2-S-BTH	BTR-d4
2-Me-S-BTH	5-Me-BTR-d6
2-SCNMeS-BTH	5-Me-BTR-d6
2-Me-BTH	5-Me-BTR-d6
2-NH_2-BTH	BTR-d4
2-M-BTH	5-Me-BTR-d6
BTR	BTR-d4
TTR	5-Me-BTR-d6
XTR	5-Me-BTR-d6
BTR-COOH	5-Me-BTR-d6
5-Cl-BTR	BTR-d4
5-NH_2-BTR	BTR-d4
1-OH-BTR	BTR-d4

Table B.3: Some physicochemical properties of benzothiazoles and benzotriazoles

Compound	Molecular weight [g/mol]	log K_{OW} [a]
BTH	135	2.17
2-Cl-BTH	170	2.81
2-OH-BTH	151	2.35
2-S-BTH	167	1.83
2-Me-S-BTH	181	1.63
2-SCNMeS-BTH	238	3.12
2-Me-BTH	149	2.72
2-NH_2-BTH	150	2.00
2-M-BTH	220	2.62
BTR	119	1.17
TTR	133	1.71
XTR	147	2.26
BTR-COOH	163	1.05
5-Cl-BTR	154	1.81
5-NH_2-BTR	134	0.25
1-OH-BTR	135	0.11

[a]Predicted data cited from chemspider.com, which are generated using the U.S. Environmental Protection Agency's EPISuite.

Appendix C

Additional Data for PAHs

Table C.1: Conditions for the extraction of PAHs using an Accelerated Solvent Extractor (ASE 150) from Thermo Scientific (Waltham, MA, United States)

System information	Value
System pressure [psi]	1500
Oven temperature [°C]	100
Cell size [mL]	10
Sample size [g]	5
Static extraction time [min]	5
Static cycles	2
Rinse volume [mL]	6 (60% of PLE cell)
Nitrogen purge [s]	90
Extraction time [min]	19

Table C.2: Concentrations of PAHs in the standard reference material

PAH	Concentration [$\mu g\,kg^{-1}$ dw]
PHE	406±44
ANT	184±18
PYR	581±39
CHR	291±39
BbF	453±21
BkF	225±18
BaP	358±17
DBA	36.7±5.2
BGP	307±45

Table C.3: Absolute recovery of target analytes based on the standard reference material

Analyte	Absolute recovery %
NAP	-
ACE	-
FLU	-
PHE	53.9
ANT	74.8
FLT	-
PYR	44.2
BaA	-
CHR	77.7
BbF	68.4
BkF	61.3
BaP	49.1
DBA	358

Table C.4: Retention times and elution order for the different PAHs and F-PAHs

Order of elution	Retention time [min]	PAH
External standards		
1	12.148	NAP
3	15.874	ACY
5	18.309	ACE
6	18.877	FLU
7	20.261	PHE
9	21,582	ANT
10	22.965	FLT
11	23.985	PYR
13	27.355	BaA
14	28.208	CHR
16	30.691	BbF
17	31.986	BkF
18	32.886	F-BkF
19	33.122	BaP
20	34.926	DBA
21	36.303	BGP
22	37.564	IND
Internal standards (F-PAHs)		
2	14.892	F-NAP
4	17.795	F-BIP
8	20.702	F-PHE
12	25.797	F-PYR
15	28.496	F-CHR

Table C.5: Some physicochemical properties of the detected PAHs

Analyte	Molecular weight [g/mol]	log K_{OW}
NAP	128	3.34
ACE	154	4.00
FLU	166	4.22
PHE	178	4.57
ANT	178	4.68
FLT	202	5.20
PYR	202	4.98
BaA	228	5.91
CHR	228	5.81
BbF	252	6.12
BkF	252	6.11
BaP	252	6.13
DBA	278	6.50

Table C.6: Classification of PAHs in sediment, based on the Norwegian Environmental Agency supervision M-608 (2016) [86]. The values for ∑PAH-16 are taken from the old guidelines, since no values were given for this in the new ones [85]

PAH	Class I Background [µg kg^{-1}]	Class II Good [µg kg^{-1}]	Class III Moderate [µg kg^{-1}]	Class IV Bad [µg kg^{-1}]	Class V Very bad [µg kg^{-1}]
NAP	2.0	27	1754	8769	>8769
ACE	2.4	96	195	19500	>19500
ACY	1.6	33	85	8500	>8500
FLU	6.8	150	694	34700	>34700
PHE	6.8	780	2500	25000	>25000
ANT	1.2	4.6	30	295	>295
FLT	8.0	400	400	2000	>2000
PYR	2.2	84	840	8400	>8400
BaA	3.6	60	501	50100	>50100
CHR	4.4	280	280	2800	>2800
BbF	90	140	140	10600	>10600
BkF	90	135	135	7400	>7400
BaP	6.0	183	230	13100	>13100
DBA	12	27	273	2730	>2730
BgP	18	84	84	1400	>1400
IND	20	63	63	2300	>2300
∑PAH-16	<300	300-2000	2000-6000	6000-20000	>20000

Appendix D

Internal Standards

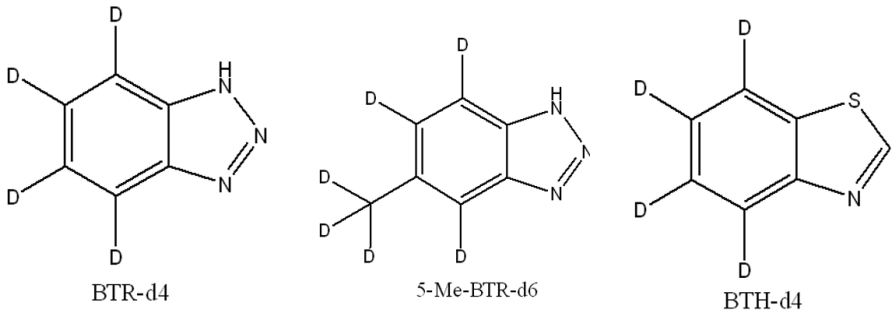


Figure D.1: Internal standards used for quantification of BTHs and BTRs.

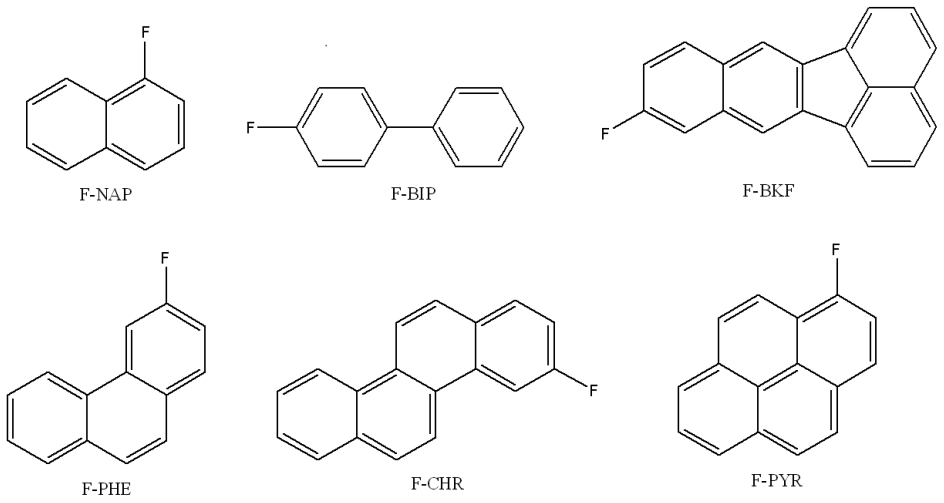
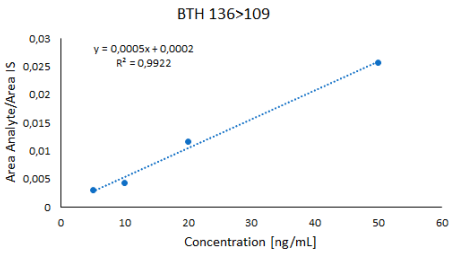
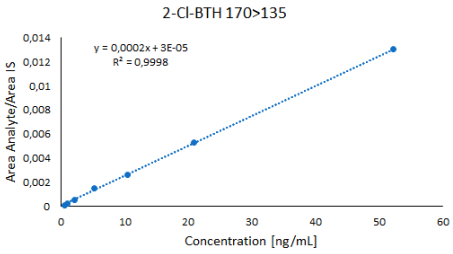


Figure D.2: Internal standards used for the determination of PAHs. F-PHE was used for the quantification of al the target PAHs.

Appendix E

Calibration Curves for BTHs, BTRs and PAHs





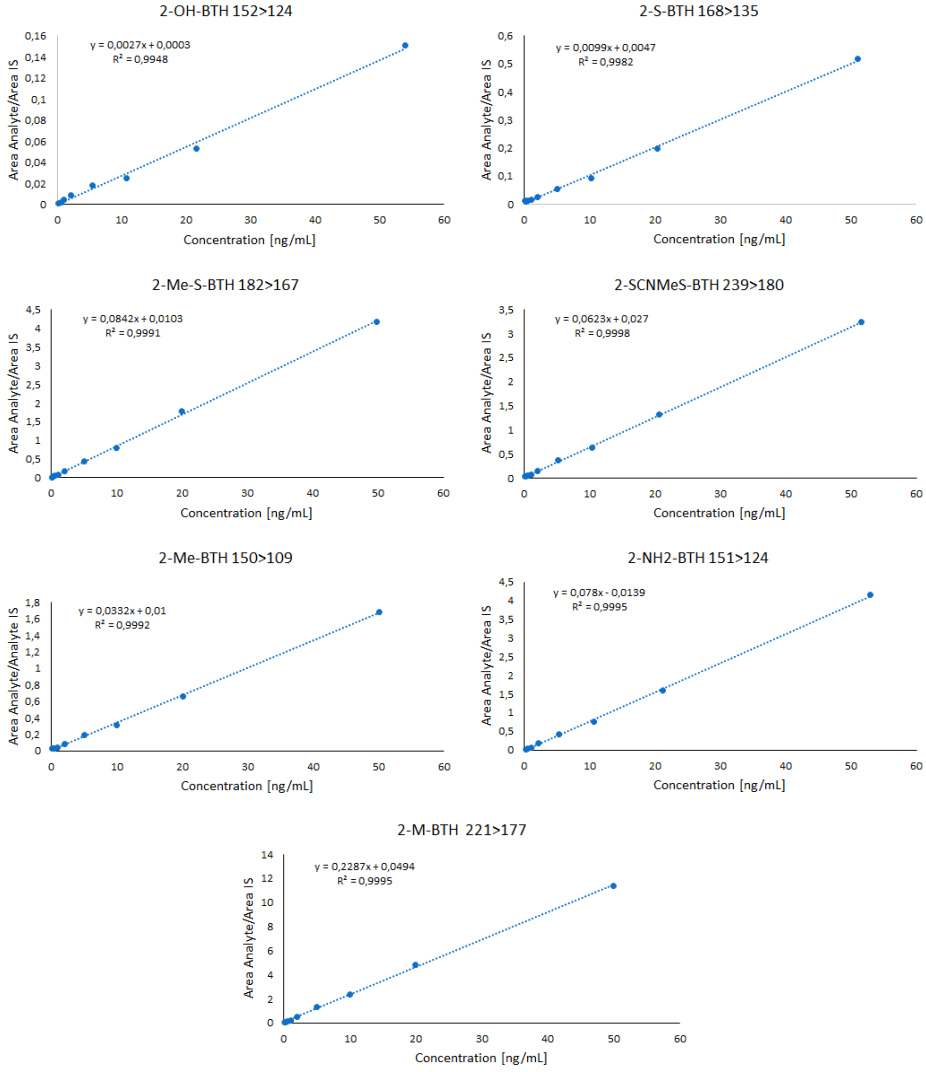
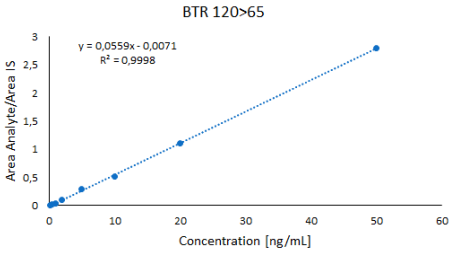
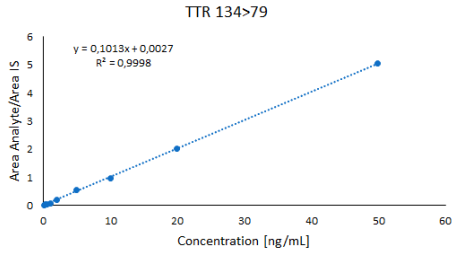
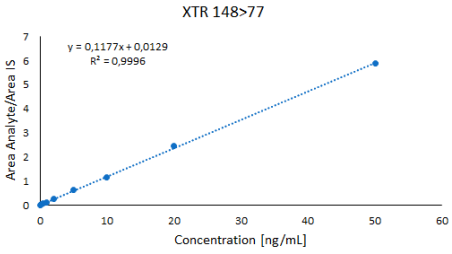
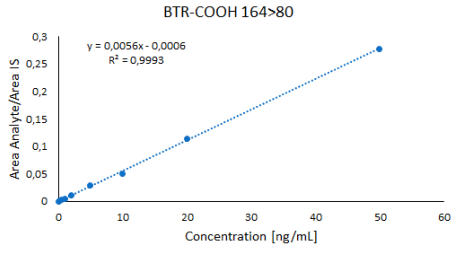


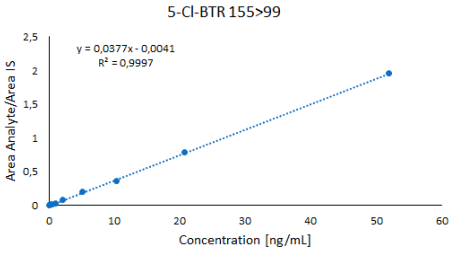
Figure E.1: Calibration curves for the BTHs included in this study.

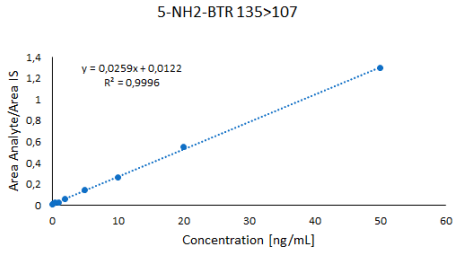

BTR 120>65
y = 0,0559x - 0,0071
R² = 0,9998
Area Analyte/Area IS
Concentration [ng/mL]

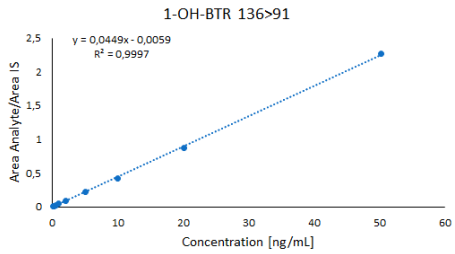

TTR 134>79
y = 0,1013x + 0,0027
R² = 0,9998
Area Analyte/Area IS
Concentration [ng/mL]


XTR 148>77
y = 0,1177x + 0,0129
R² = 0,9996
Area Analyte/Area IS
Concentration [ng/mL]


BTR-COOH 164>80
y = 0,0056x - 0,0006
R² = 0,9993
Area Analyte/Area IS
Concentration [ng/mL]


5-Cl-BTR 155>99
y = 0,0377x - 0,0041
R² = 0,9997
Area Analyte/Area IS
Concentration [ng/mL]


5-NH2-BTR 135>107
y = 0,0259x + 0,0122
R² = 0,9996
Area Analyte/Area IS
Concentration [ng/mL]


1-OH-BTR 136>91
y = 0,0449x - 0,0059
R² = 0,9997
Area Analyte/Area IS
Concentration [ng/mL]

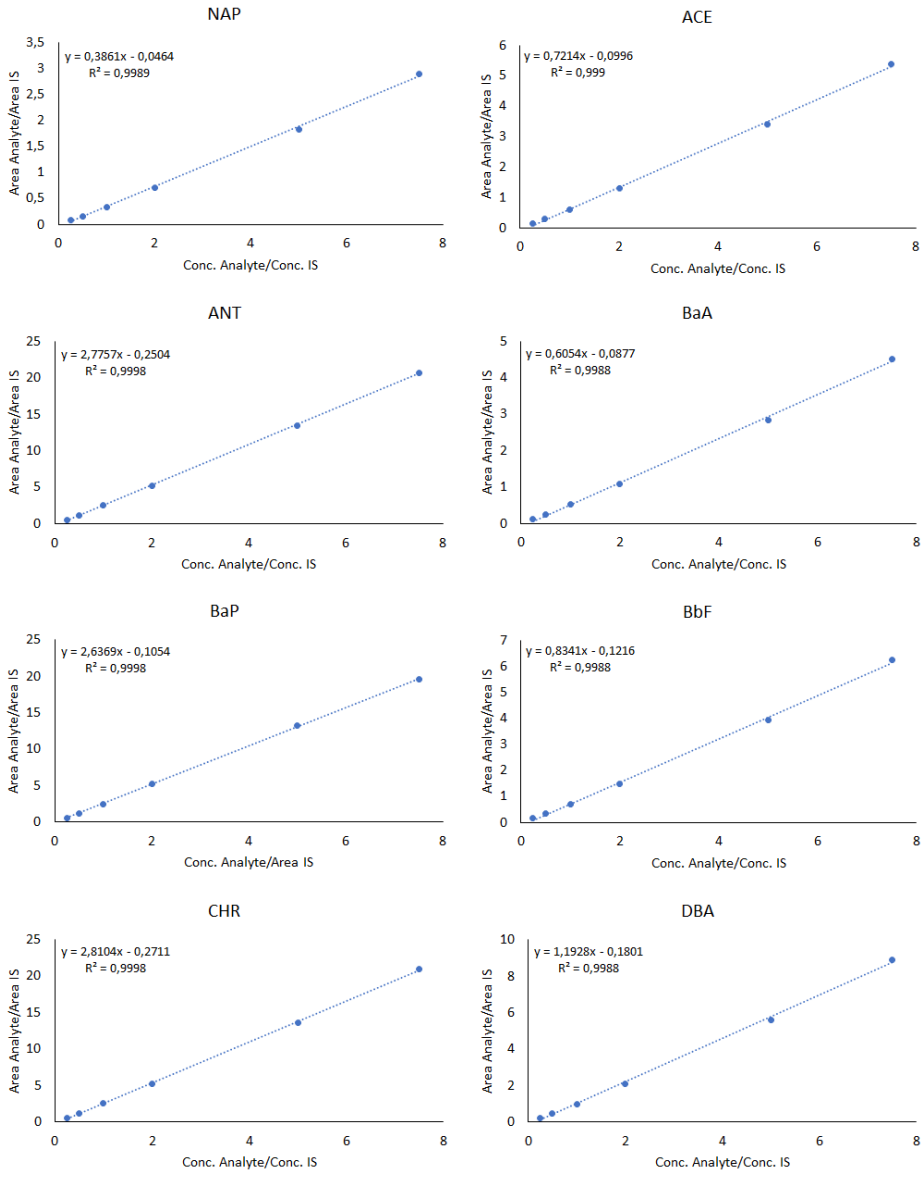


Figure E.3: Calibration curves for BTRs

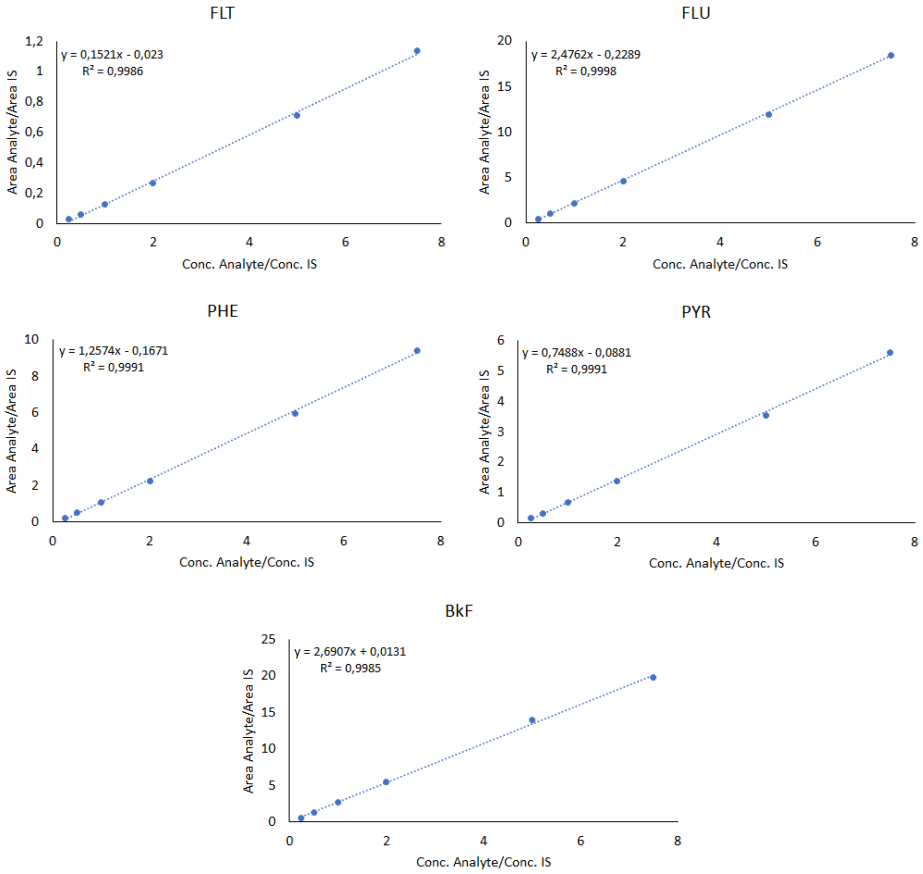


Figure E.3: Calibration curves for PAHs.

Appendix F

Chromatograms

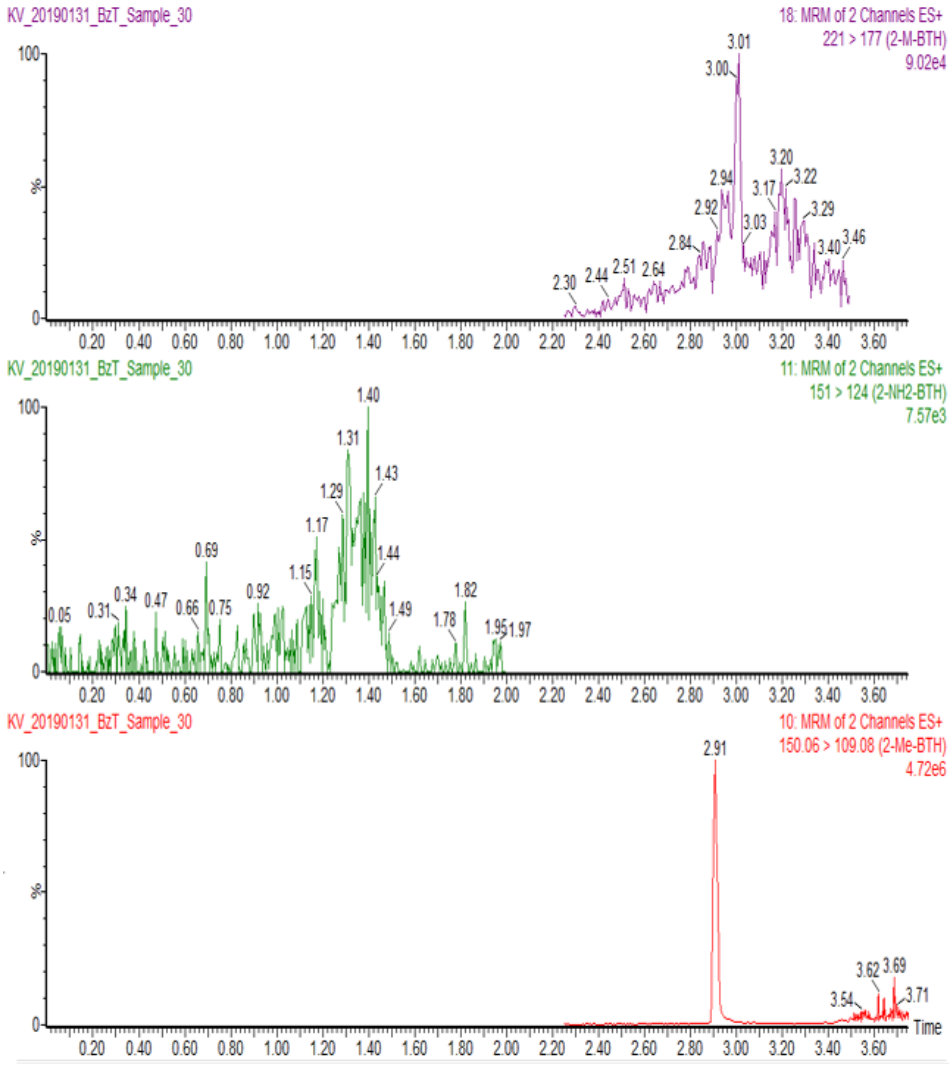


Figure F.1: MRM chromatograms for benzothiazoles in a real sample. The remaining chromatograms are on the next page

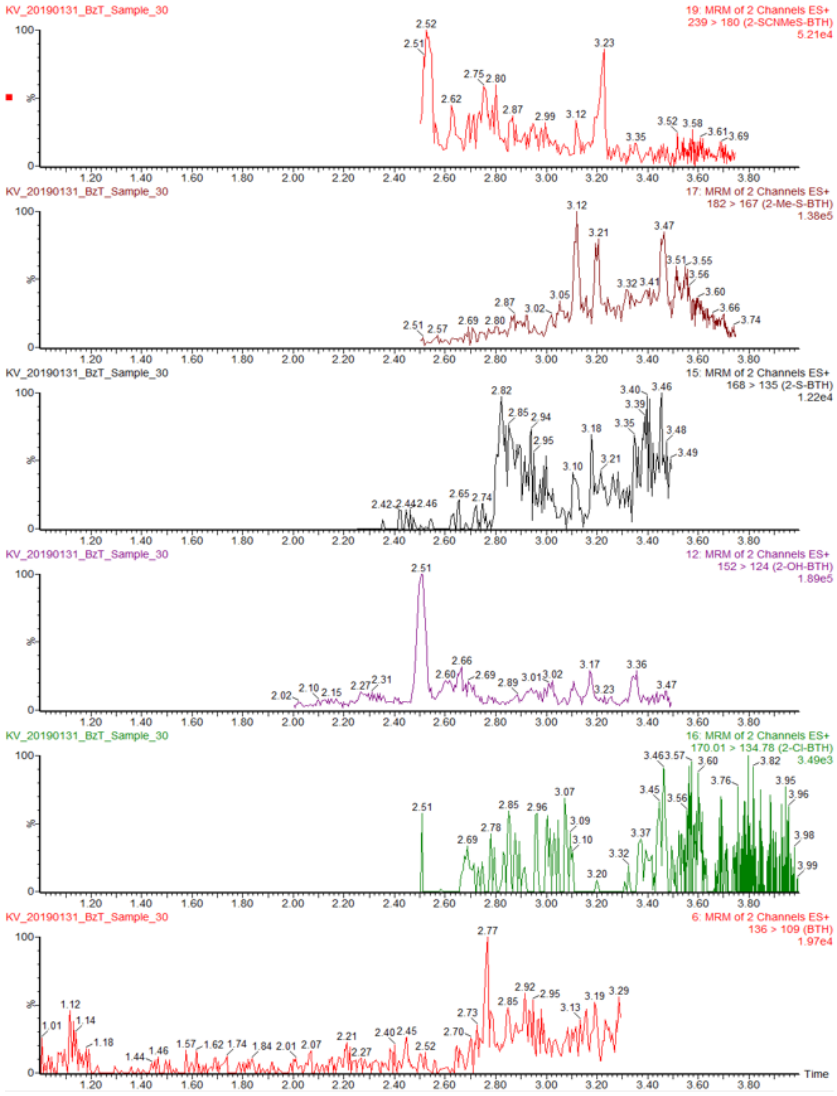


Figure F.1: MRM chromatograms for benzothiazoles in a real sample

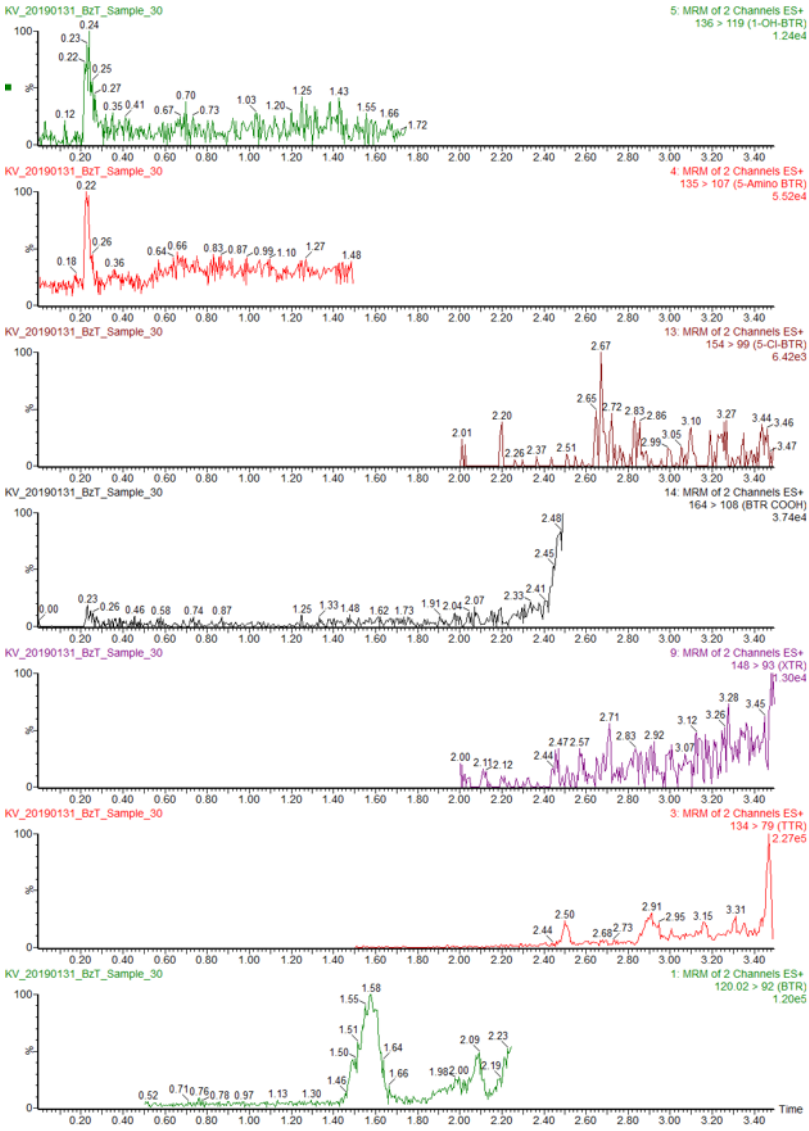


Figure F.2: MRM chromatograms for benzotriazoles in a real sample

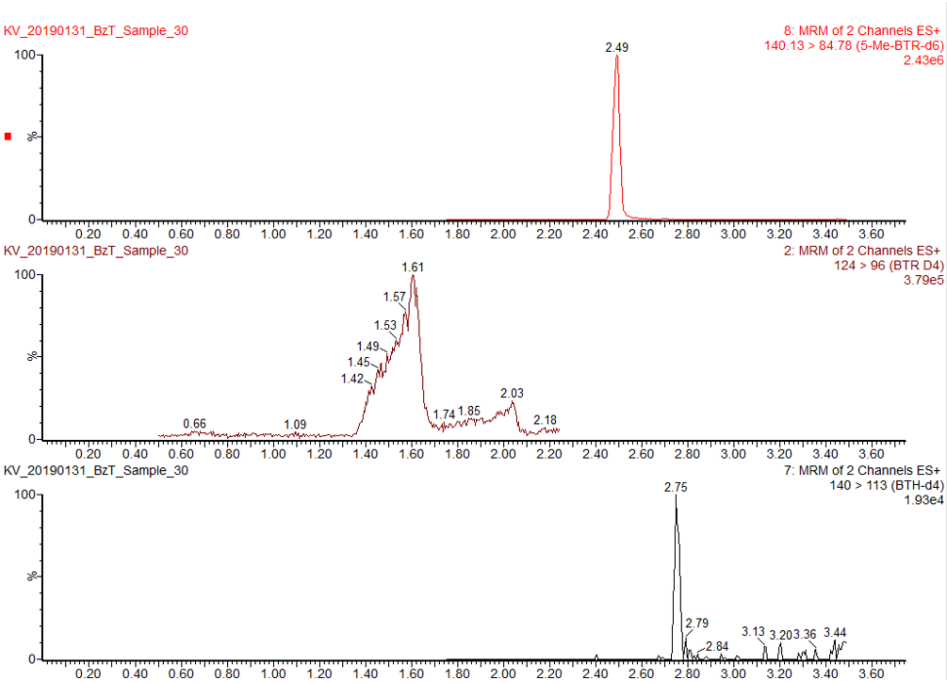


Figure F.3: MRM chromatograms for the internal standards in a real sample

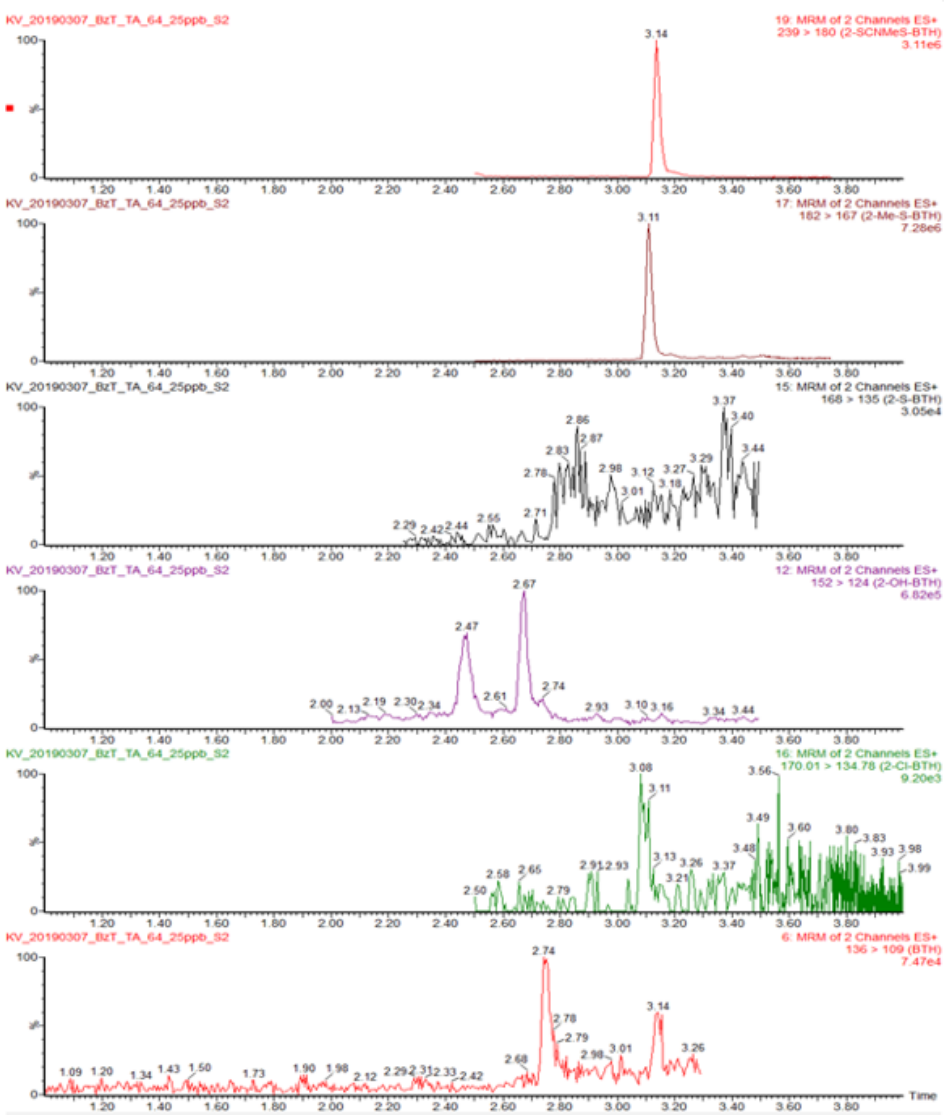


Figure F.4: MRM chromatograms for benzothiazoles of spiked sediment sample at a fortification level of 25 ng target analytes. Part 1

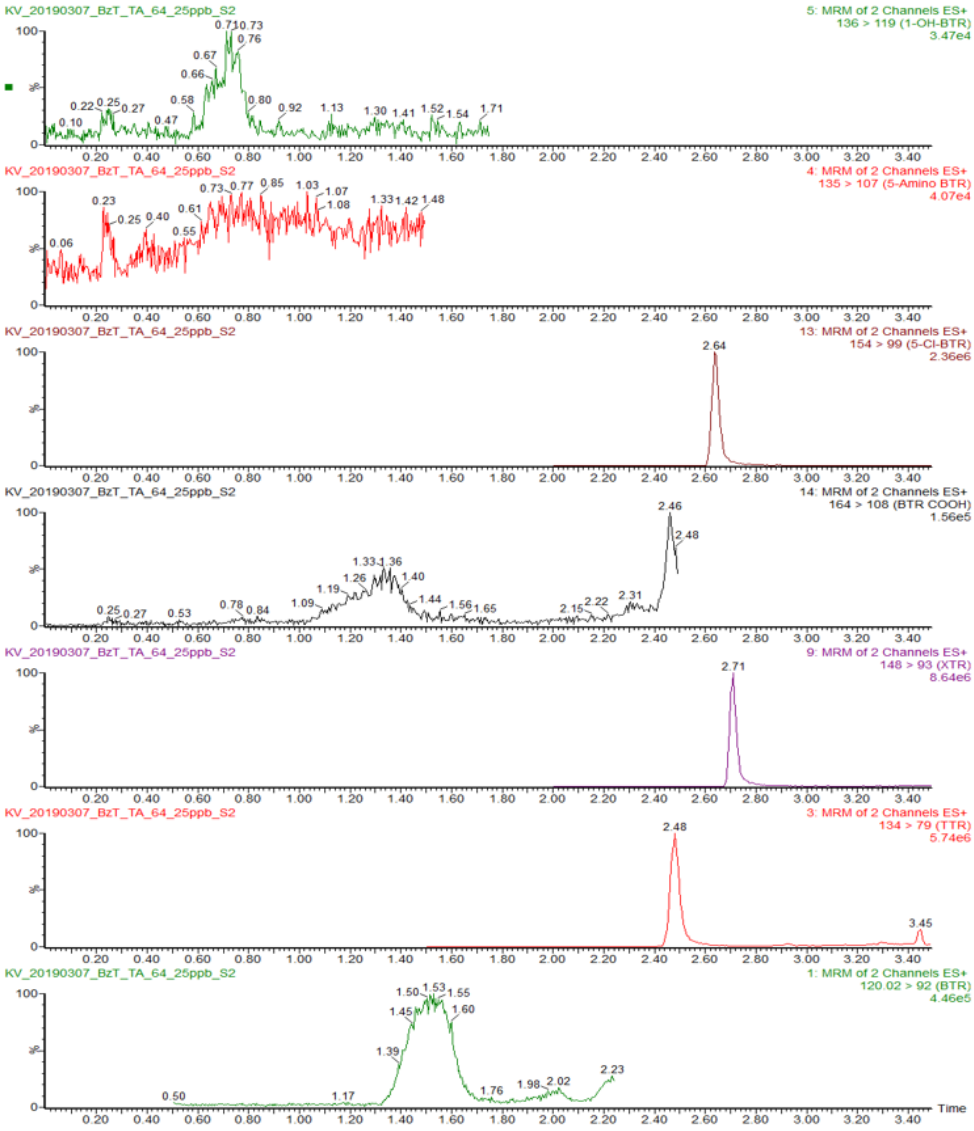


Figure F.5: MRM chromatograms for benzotriazoles of spiked sediment sample at a fortification level of 25 ng target analytes

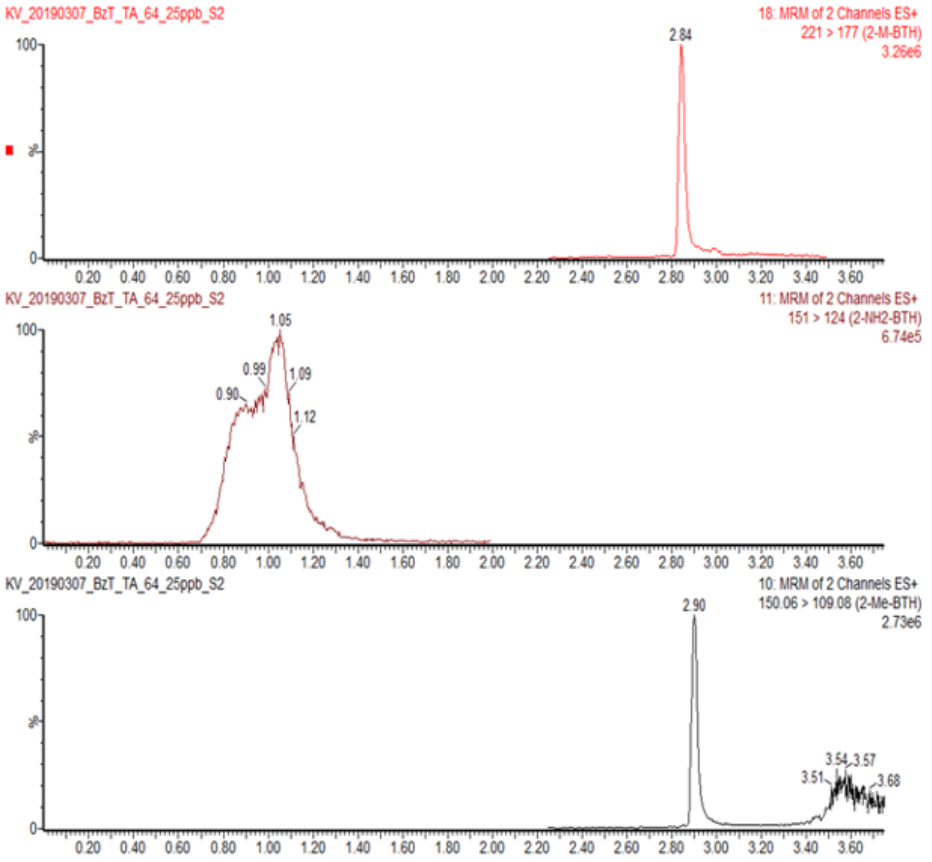


Figure F.4: MRM chromatograms for benzothiazoles of spiked sediment sample at a fortification level of 25 ng target analytes. Part 2

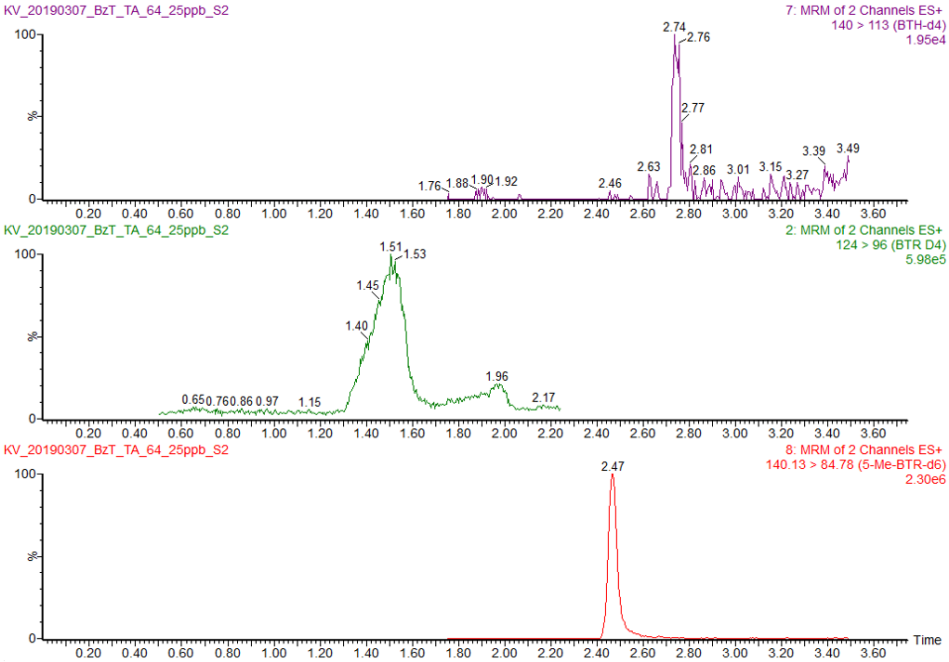


Figure F.6: MRM chromatograms for internal standards in sediment sample spiked with 25 ng target analytes

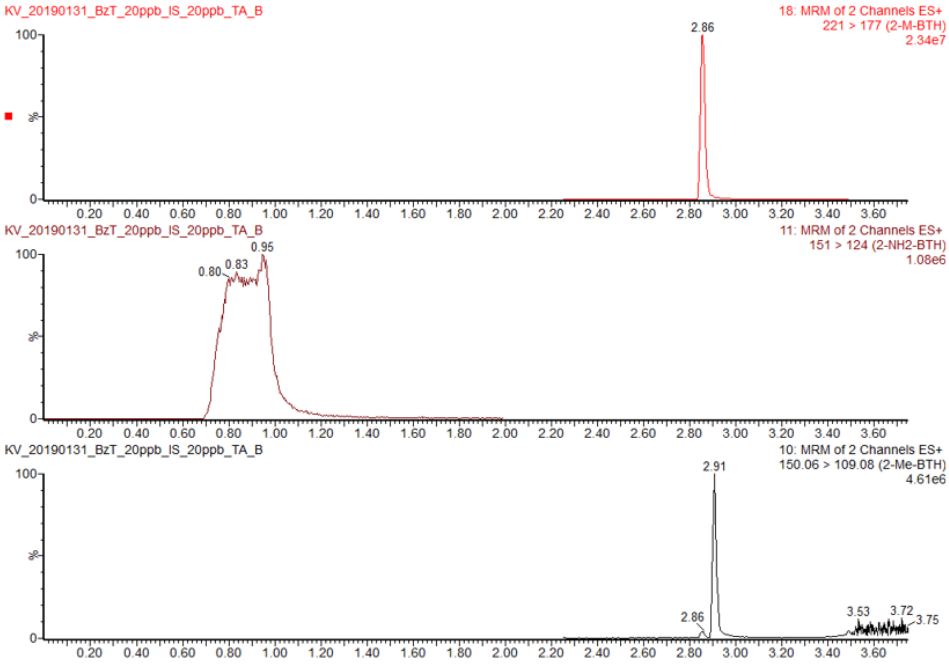


Figure F.7: MRM chromatograms of benzothiazole standards at a concentration of 20 ng mL^{-1}

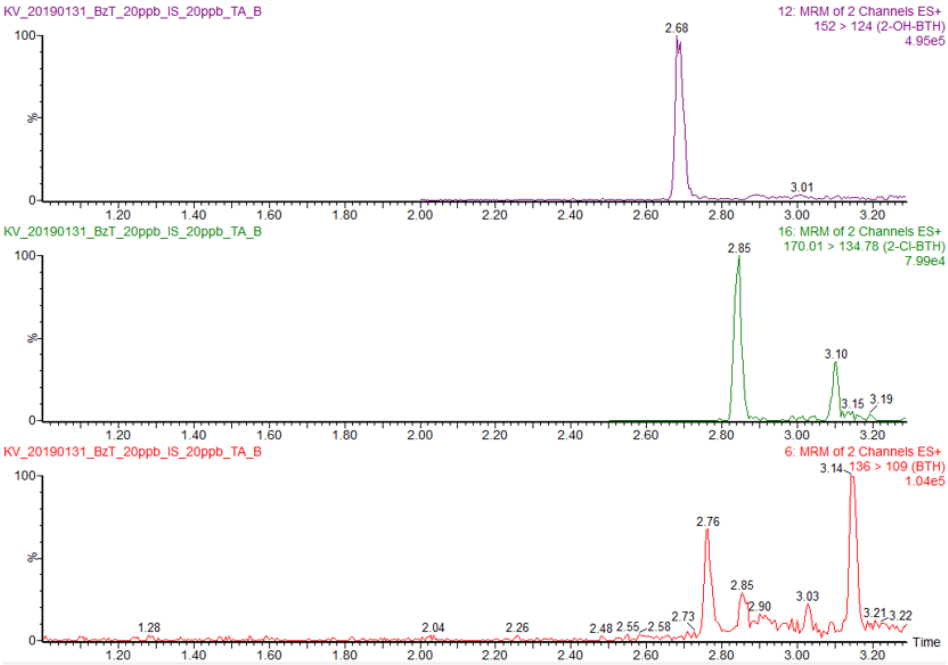


Figure F.7: MRM chromatograms of benzothiazole standards at a concentration of 20 ng mL^{-1}

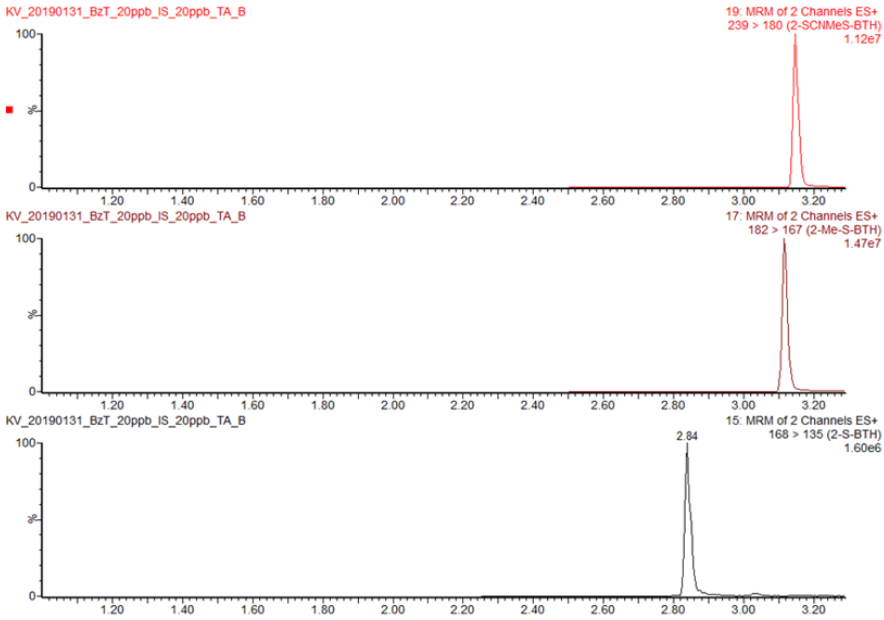


Figure F.7: MRM chromatograms of benzothiazole standards at a concentration of 20 ng mL^{-1}

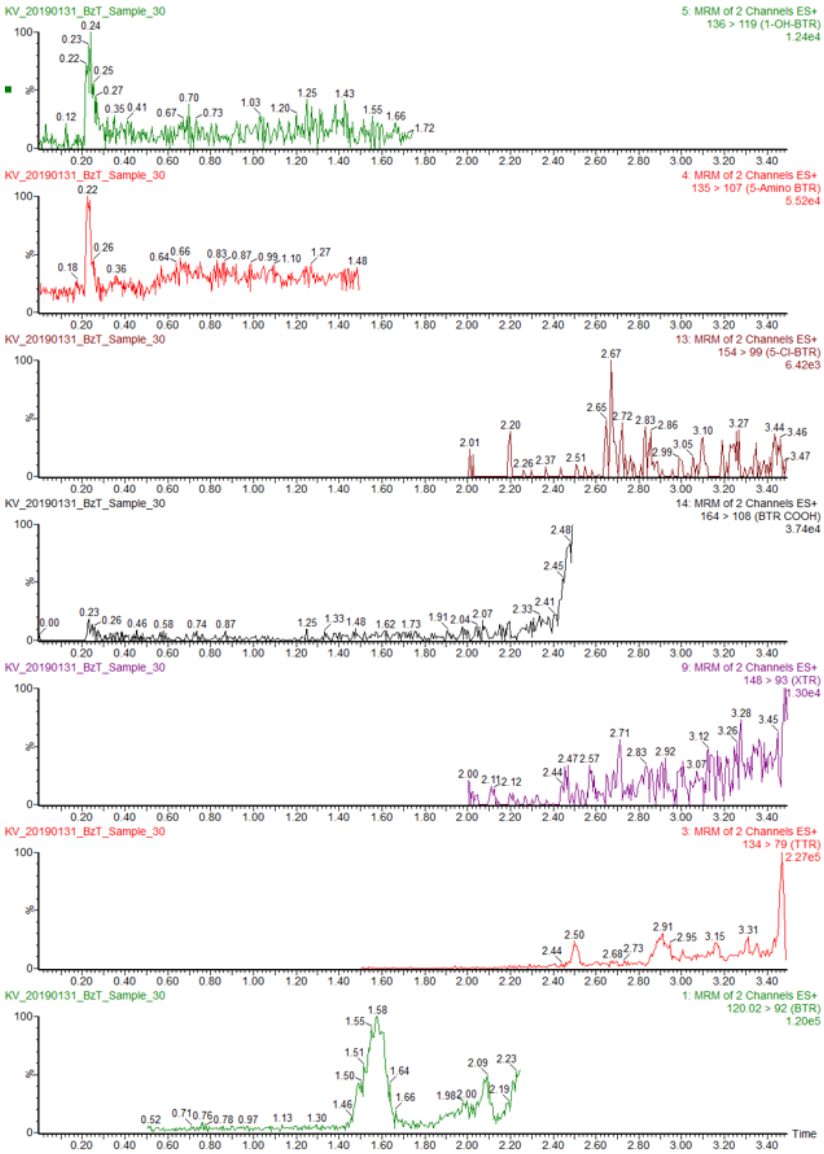


Figure F.8: MRM chromatograms of benzotriazole standards at a concentration of 20 ng mL^{-1}

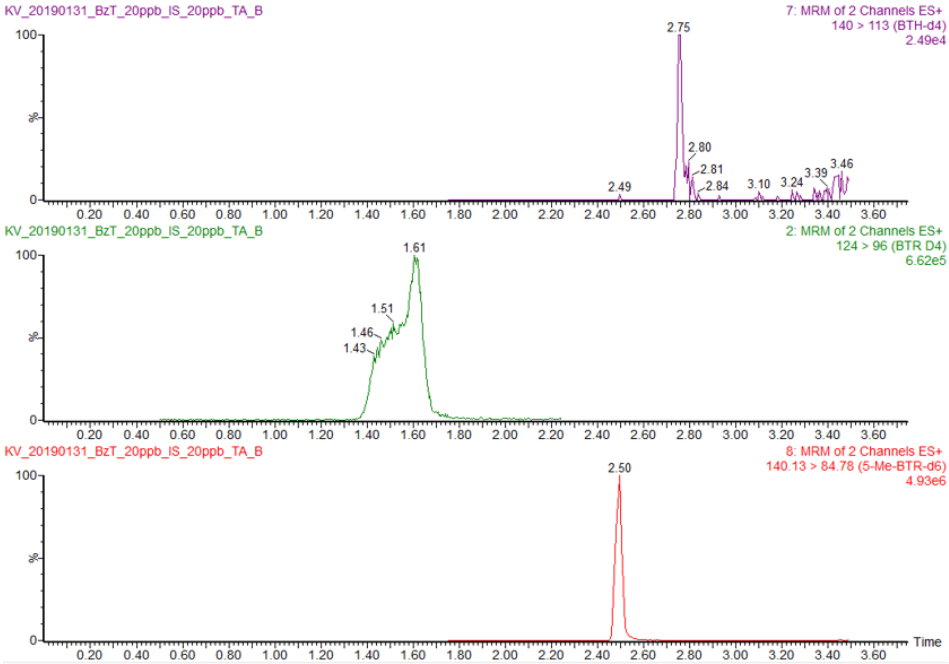


Figure F.9: MRM chromatograms of internal standards at a concentration of 20 ng mL^{-1}

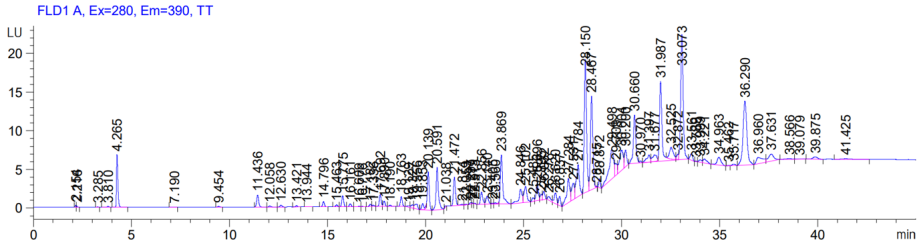


Figure F.10: Chromatogram of separation of PAHs in a real sample

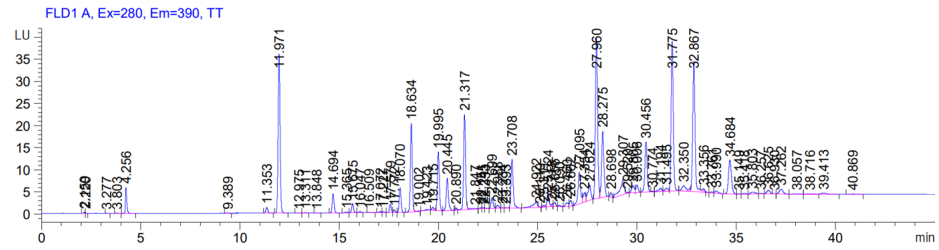


Figure F.11: Chromatogram of sediment matrix spiked with 300 ng target analytes

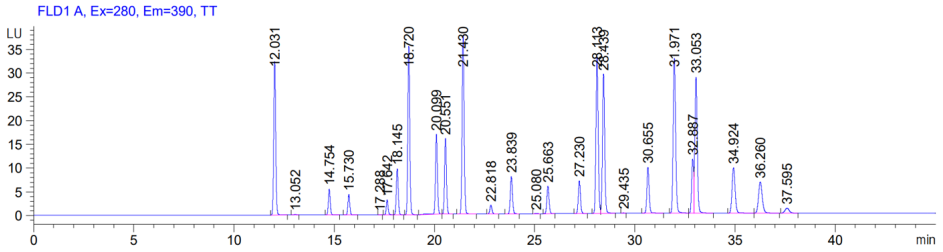


Figure F.12: Chromatogram of a PAH standard solution

Appendix G

PCA data

The different PCA biplots that were estimated are given below. These biplots are used to discuss various correlations between the samples and different analytes. The three biplots for a selection of elements are also presented here, since these were used to investigate if there was any connections between certain elements and the organic pollutants. Correlation of concentration (log) for the BTHs, BTRs, and PAHs studied is also given.

Figure G.1: PCA biplot for BTHs, BTRs, and PAHs at different seafloor depths.

Figure G.2: PCA biplot for BTHs, BTRs, and PAHs looking at the different sediment sections.

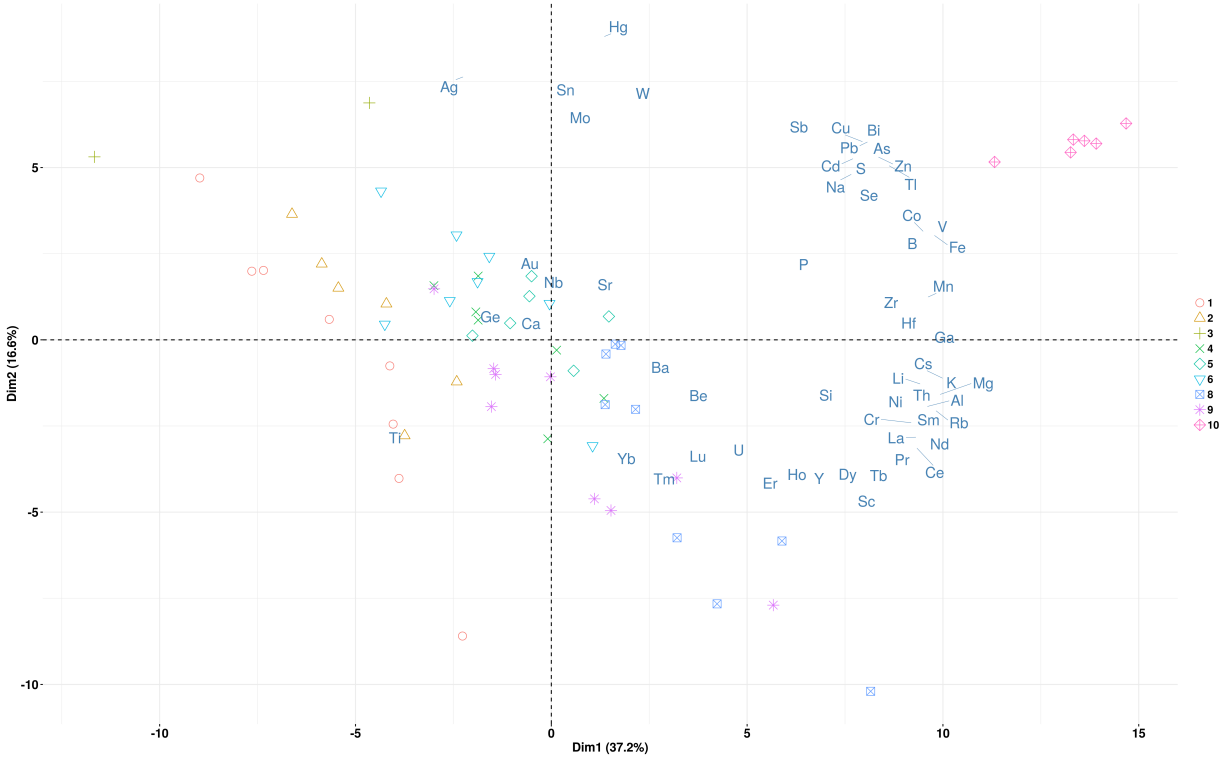


Figure G.3: PCA biplot for selected elements looking at the different sampling locations

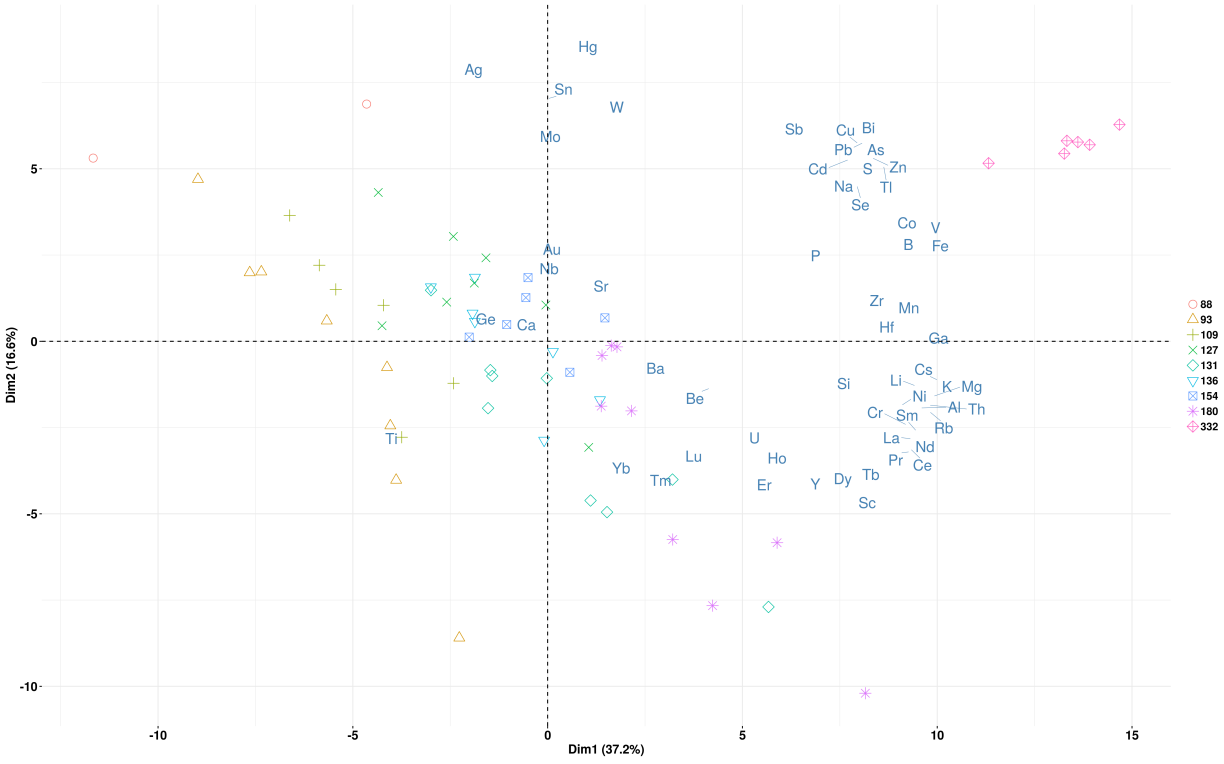


Figure G.4: PCA biplot for selected elements looking at the depth of the seafloor

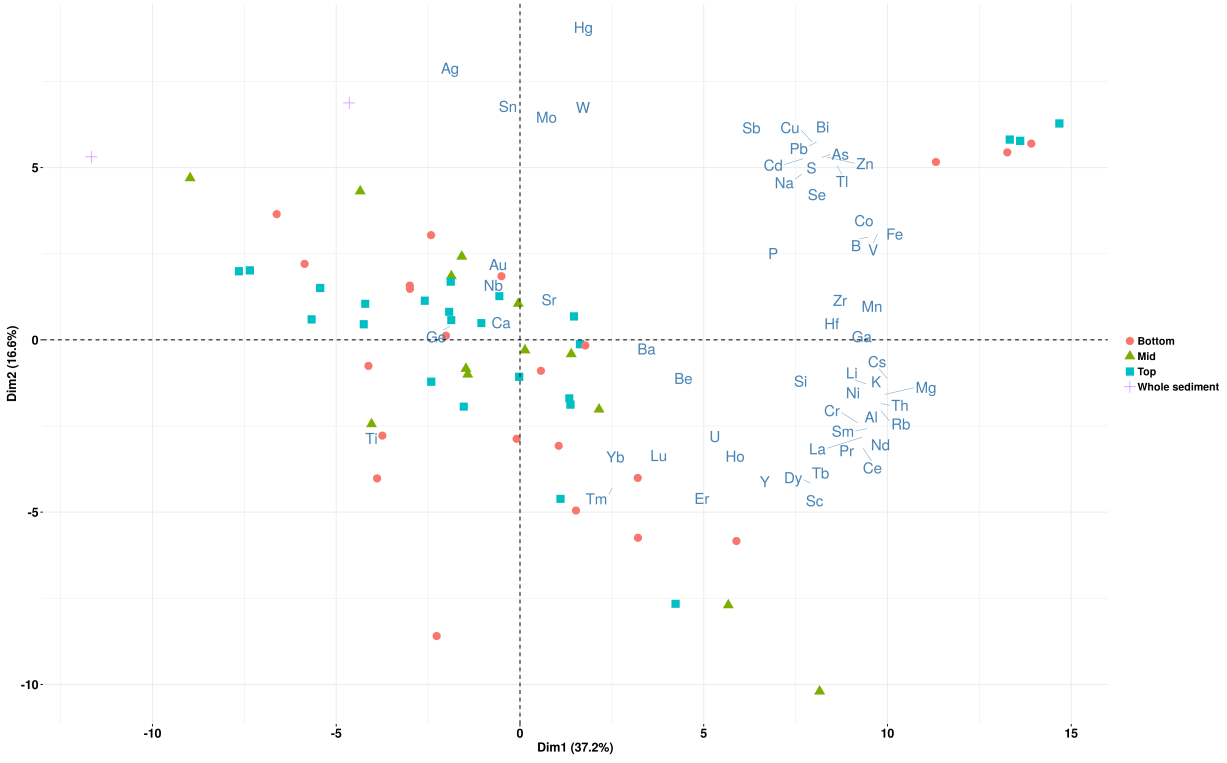


Figure G.5: PCA biplot for selected elements looking at the depth of the different sediment sections

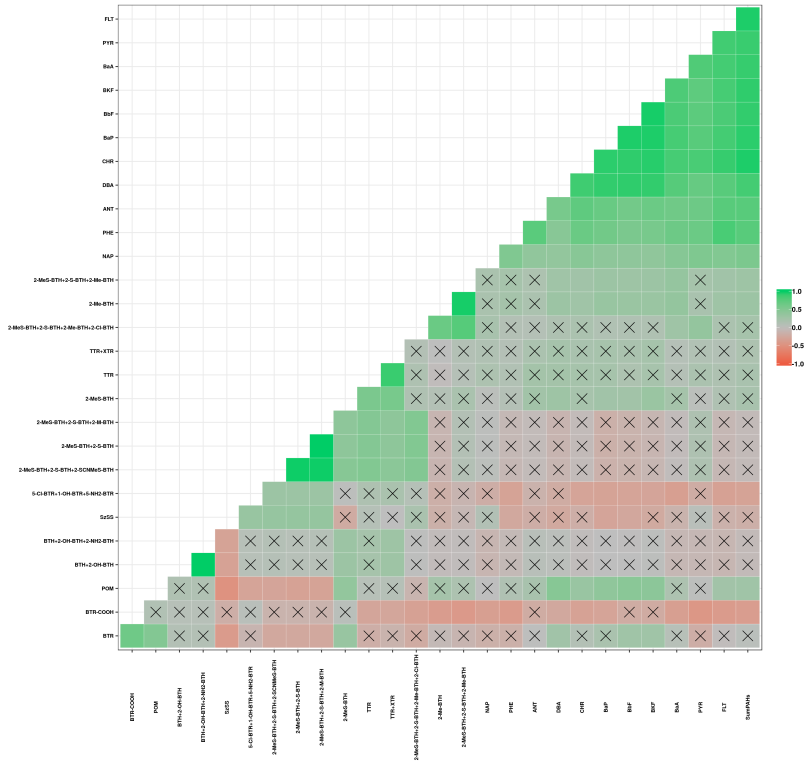


Figure G.6: Correlations of concentration of BTHs, BTRs, and PAHs used for PCA

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