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Preliminary Non-Targeted Screening of Organic Compounds linked with Elemental Analysis of Marine Sediments near Salmon Farms

Marco Skibnes Venzi

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Supervisor: Øyvind Mikkelsen, IKJ
Co-supervisor: Shazia N Aslam, IKJ
Rudolf Schmid, IKJ

Norwegian University of Science and Technology
Department of Chemistry

PREFACE

This thesis is a part of a master's degree in the Environmental Chemistry and Toxicology (ENVITOX) program from autumn semester 2016 until the spring semester 2018. The project was performed independently from any external interests.

The non-targeted approach presented has not been attempted before on marine sediments at this department. It is therefore to be considered a preliminary study, that aspires to create an experimental basis for future projects within this field. A wide spectrum of analytical techniques was utilized, making the interactions between the different categories of data especially interesting in our eyes.

Focusing on the environmental aspects of aquaculture has been a major motivation, given the dimension of this industry in the Norwegian context, the importance for the present and future economy worldwide as a potential source for healthy food and omega-3 fatty acids, and the environmental concerns that follows.

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Marco Skibnes Venzi

CONFLICT OF INTEREST STATEMENT

The author of this thesis has no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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ABSTRACT

Sediment samples from the vicinity of two active salmon farms in central west Norway (Smøla, Møre og Romsdal) have been analyzed for elements, organic compounds and physio-chemical parameters. Elements have been analyzed by ICP-MS, while organic compounds were characterized through non-targeted screening by GC-MS. Physio-chemical parameters included grain size distribution, total organic matter (TOM), pH and conductivity. Totally, the study included 52 samples for organic analysis and 37 samples for elemental analyses from 24 locations, approximately 100 m to 1 km from the perimeter edge of the installations.

Concentrations of trace elements suggested background (Class I) or good (Class II) conditions according to Norwegian marine sediment quality classification. However, by considering total organic carbon (TOC) content, most samples classified as very bad (Class V) near installation A (Hestøya) and bad (Class IV) near installation B (Nørholmen).

Lead (Pb) contents were significantly higher near installation B, while cadmium (Cd), arsenic (As) and antimony (Sb) were significantly higher near installation A ($p < 0.05$). Further, Cd, aluminum (Al), tin (Sn), chromium (Cr), iron (Fe) and manganese (Mn), showed elevated concentration closer than 500 m from the installations compared with distances above 500 m. However, negative correlations with distances from the installations were only confirmed for Fe, Mn and Al by Spearman correlations and Principal Component Analysis (PCA). On the other hand, the highest concentrations were found at intermediate distances (200-500 m) for Cd, Cr, Sn, TOM and pelite (grain size < 0.06 mm) contents, although this tendency was not statistically significant for the latter two parameters ($p > 0.05$). This could indicate an accumulation of the particles from the effluents at intermediate distances. However, assuming the elevated TOM/pelite levels were naturally occurring, elevated levels at intermediate distances could be explained by adsorption to clay particles and complexation with humic acids.

Presence of benzaldehyde and 3-bromophenol were successfully confirmed by analytical standards. Additionally, a list of 59 suggested organic compounds, including xylene and anthracene, was produced. Fifteen suggestions gave a match value above 850 according to the NIST library. Copper (Cu), Cd, As and/or molybdenum (Mo) showed a very strong positive correlations with five detected organic compounds ($r_s > 0.8$). However, correlation analyses and PCA showed insignificant relationships between abundance and frequency of organic compounds with distances from the installations. PCA showed a slight tendency for most organic compounds to accumulate at intermediate distances, as for trace elements. Further, PCA indicated a relationship between pH/TOM/pelite and trace elements of environmental concern and most organic compounds.

SAMMENDRAG (NO)

Sedimentprøver tatt i nærheten av to aktive lakseoppdrettsanlegg sør for Smøla (Møre og Romsdal), har blitt analysert for elementer, organiske forbindelser og fysiokjemiske egenskaper. Elementer ble analysert med ICP-MS, mens organiske forbindelser ble karakterisert gjennom «non-targeted screening» med GC-MS. Fysiokjemiske egenskaper inkluderte kornstørrelse, total organisk materiale (TOM), pH og ledningsevne. Studiet omfattet totalt 52 prøver til organisk analyse og 37 prøver til elementanalyse fra 24 lokaliteter ca. 100 m og 1 km fra anleggsområdene.

Konsentrasjoner av sporelementer viste til bakgrunnsforhold (Klasse I) eller gode forhold (Klasse II), i henhold til norske retningslinjer for klassifisering av marine sedimenter. Med grunnlag i innhold av totalt organisk karbon (TOC), klassifiserte sedimentprøvene imidlertid som veldig dårlig (Klasse V) for flertallet av prøvene nær anlegg A (Hestøya), eller dårlig (Klasse IV) nær anlegg B (Nørholmen).

Innhold av bly (Pb) var signifikant høyere nær anlegg B, mens kadmium (Cd), arsen (As) and antimon (Ab) var signifikant høyere nær anlegg A ($p < 0.05$). Cd, aluminium (Al), tinn (Sn), krom (Cr), jern (Fe) og mangan (Mn), hadde forhøyde verdier ved avstander under 500 m fra anleggene sammenlignet med avstander over 500 m. Likevel ble negative korrelasjoner med avstanden fra anleggene kun bekreftet for Fe, Mn og Al gjennom Spearman-korrelasjoner og «Principal Component Analysis» (PCA). På den andre siden, viste analysene de høyeste konsentrasjonene ved intermediære avstander (200-500 m) for Cd, Cr, Sn, TOM og pelitt (kornstørrelser > 0,06 mm). Tendensen var imidlertid ikke statistisk signifikant for de to sistnevnte parameterne ($p > 0.05$). Dette kan være en indikasjon på at partikler i effluenten akkumulerer ved intermediære avstander. På en annen side, antatt at TOM/pelitt-nivåene forekom naturlig, så kan de forhøyde verdiene ved intermediære avstander forklares gjennom adsorpsjon til leirpartikler og kompleksdannelse med humusstoffer.

Forekomst av benzaldehyd og 3-bromofenol ble bekreftet gjennom analytiske standarder. I tillegg ble det fremstillet en liste over 59 foreslåtte forbindelser, inkludert xylen og antracen. Av disse hadde 15 en match-verdi over 850 i henhold til NIST biblioteket. Kobber (Cu), Cd, As og/eller molybden (Mo) hadde svært sterke positive korrelasjoner med fem av de detekterte organiske forbindelsene ($r_s > 0.8$). Korrelasjonsanalyser og PCA viste imidlertid ingen sammenheng mellom mengden og frekvensen av organiske forbindelser med avstand fra anleggene. PCA viste at organiske forbindelser hadde en tendens til å akkumulere ved intermediære avstander, slik som for sporelementene. Videre indikerte PCA en sammenheng mellom pH/TOM/pelitt og flesteparten av sporelementene og de organiske forbindelsene.

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ACRONYMS

ASE: Accelerated Solvent Extraction

DCM: Dichloromethane

DE: Diatomaceous Earth

DI: Deionized

EPA: Environmental Protection Agency

GC-MS: Gas Chromatography - Mass Spectrometry

HPLC: High Performance Liquid Chromatography

ICP-MS: Inductively Coupled Plasma - Mass Spectrometry

IQR: Interquartile Range

KMO: Kaiser-Mayer-Olkin test for sampling adequacy

LC-MS/MS: Liquid Chromatography - Tandem Mass Spectrometry

LOD: Limit of Detection

LOQ: Limit of Quantification

MS: Mass Spectrometry

NGO: Non-Governmental Organization

NIST: National Institute of Standards and Technology

NTNU: Norges Teknisk-Naturvitenskapelige Universitet (Norwegian University of Science and Technology)

PBDE: Polybrominated Diphenyl Ethers

PC: Principal Component

PCA: Principal Component Analysis

PCB: Polychlorinated Biphenyl

PET: Polyethylene Tetraphthalate

PFA: Perfluoroalkoxy Alkane

PLE: Pressurized Liquid Extraction

PNEC: Predicted No Effect Concentration

POP: Persistent Organic Pollutant

PP: Polypropylene

PS: Polystyrene

PTFE: Polytetrafluoroethylene

RT: Retention Time

SIM: Single Ion Mode

TIC: Total Ion Chromatogram

TOC: Total Organic Carbon

TOM: Total Organic Matter

XIC: Extracted Ion Chromatogram

CONTENTS

1 INTRODUCTION

1.1 THE NORWEGIAN AQUACULTURE INDUSTRY

1.1.1 Economic and social aspects

Norwegian farming of Atlantic salmon (*Salmo salar*) ranks uncontested at the top, with the world's highest production volume during the last decades. As for today, more than 50 % of the global production of this species is cultivated along the coast and inside the fjords of the nation, with a total of 1.3 million tonnes in 2017 (Statistics Norway, 2018). In the last 20-years the industry has experienced a 10-fold increase in production volume for this species, and it is projected to sustain an exponential increase in the coming years. The next in line among species farmed in Norway is rainbow trout (*Oncorhynchus mykiss*), which constitutes 7.1 % of the total production volume of Atlantic salmon. The first hand value of the salmon and trout industry was 53.6 billion NOK in 2016, employing 7,270 persons (Statistics Norway, 2018). An overview of the geographical extent of the national aquaculture industry is shown in Figure 1.1.

Figure 1.1 Extent of aquaculture installations in Norway. Red dots represent aquaculture sites. In 2017 there was an average of 986 sites at sea, constituting 3604 net-pens containing Atlantic salmon and rainbow trout (Statistics Norway, 2018) (Map: The Norwegian Coastal Administration).

Environmental concerns regarding the externalities posed by the fish farming industry are continuously expressed, with an increasing severity and frequency especially the last decade, coming from government bodies, environmentalist NGOs, wild-fishing industries and the public in general. These concerns span from genetic introgression into the wild stocks from fish farm escapees, to the toxicity towards non-targeted organisms posed by dispersion of drugs into the marine ecosystem, and eradication of favorable spawning sites for other wild fish species of special economic interest. How well grounded these concerns are, is undoubtfully a matter of great controversy at all levels of society.

Generally, institutional economists describe the risk of "The Tragedy of the Commons", when individuals are administrating common-pool resources without a necessary degree of governmental regulation. This concept applies to environmental externalities as well as for resource management (Ostrom, 1990). The extent to which the Norwegian aquaculture is related to this phenomenon is certainly debatable. Different governance regimes have been implemented since the beginning of the 1970s, with varying degrees of regulatory power. In the mid-1990s the re-regulation slowed down the liberalization process from the institutional collapse in the 1980s. Generally, it can be concluded that in the re-institutionalization era from 2001 and beyond, the power structures have changed and representatives from expert institutions have achieved a more prominent position (Aarset and Jakobsen, 2009). Regarding attitudes, the industry has been historically characterized as one of "know-how" more than "know-why". An example was the low attendance by administrators on scientific seminars on new aquaculture technology, even though such attendance has been mandatory since 2006 (Christiansen, 2013).

On the other hand, it can be argued that there may exist an oversensitivity regarding these environmental issues, probably caused by Norway's long tradition for sustainable wild-fishing, and the competing interests between the wild-fish industry and the aquaculture industry. A general conservationist attitude internalized in people's mentality may also result in negative sentiments towards the industry, as the coastal landscape and private fishing conditions have been reported to change during the last decades.

1.1.2 Chemical pollution of the environment

The Institute of Marine Research releases an annual risk assessment concerning the entire Norwegian aquaculture industry ordered by the Ministry of Trade, Industry and Fisheries. This risk assessment examines many environmental aspects. Regarding chemical pollutants, which is the focus of this study, the sources from open net-pen fish farming are mainly discharge

by well-boats of water used for bath treatment with anti-parasite drugs (e.g. deltamethrin, cypermethrin, azamethiphos) and hydrogen peroxide (H_2O_2), excess feed and fish feces, and to some smaller extent antifouling agents in nets (Grefsrud et al., 2018). Figure 1.2 gives a schematic summary of the major sources of chemical pollution from open net-pen aquaculture, and dispersion mechanisms in the water column and sediments.

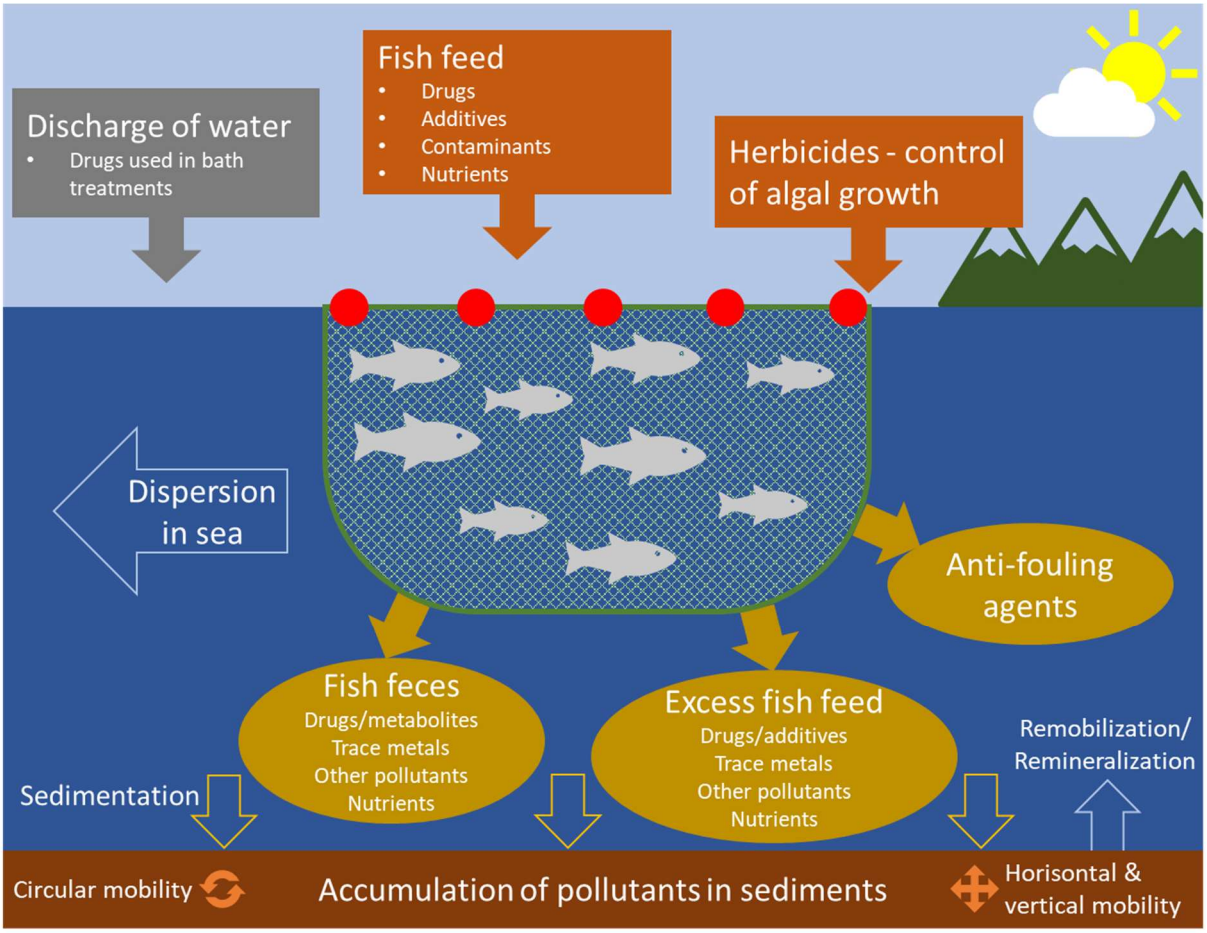

Figure 1.2 Schematics of potential chemical pollution from open net-pen aquaculture, showing the major sources for environmental contamination as well as processes in the environment. Based on information from the annual risk assessment for the industry (Grefsrud et al., 2018)

Fish feed used by the industry contains trace metals added as nutrient supplement for the benefit of the cultivated fish, as well as unwanted trace elements regarded as contamination. Veterinary drugs, especially for the abatement of sea-lice infestations (e.g. benzoylureas and emamectin benzoate), are also added to batches of fish feed, and administered during treatments. Consequently, fish feces and urine will most probably contain residues of drugs not taken up by the fish, as well as drug metabolites (Grefsrud et al., 2018). Given that 34 to 40 % of the input feed supplied to the fish farm systems is released either as excess feed or feces, it

would give a substantial flux of 592,000 to 696,000 tonnes annually to the recipient (Grefsrud et al., 2018).

Input of primary inorganic nutrients (nitrogen and phosphorus) to the environment is another consequence of excess feed and feces effluents from fish farms. This may lead to alteration of the ecosystem by increasing algal growth and favoring of opportunistic species (eutrophication) (Wang et al., 2012). Aquaculture is today the largest anthropogenic source of primary nutrients to the Norwegian fjords. A study estimated the annual discharge of nitrogen and phosphorus to be 50,600 and 9,400 tonnes respectively in 2009 (Wang et al., 2012). However, the risk of eutrophication beyond the immediate production area of the farm is considered low (Taranger et al., 2015).

1.1.3 Mandatory environmental monitoring

According to Norwegian regulations, all aquaculture installations aimed at food production must perform surveys of the environmental conditions on the site after Norwegian standard NS 9410, or an equivalent international standard (Regulations concerning operation of aquaculture installations, 2008, §35). NS 9410 gives the framework for two levels of surveys: B-surveys and C-surveys, previously referred to as MOM-B and MOM-C. Based on the measurements contained in the surveys, an environmental score from 1-4 is assigned, where level 4 is considered overloading of the surrounding sediments (Standards Norway, 2016).

B-surveys monitors benthic conditions underneath and in the immediate vicinity of the installation, including macrofauna characterization, sensory (e.g. odor, color and consistency) and chemical conditions. Chemical conditions are measured trough pH and redox potential (E_h). However, B-surveys does not include qualitative or quantitative analyses of chemical substances. The frequency depends on the result of the previous survey and implies on average annual surveys.

C-surveys are more extensive and include the transition zone, up to 500 m from the installation. Chemical parameters are extended and include total organic matter (TOM), total organic carbon (TOC), total nitrogen (TN) and copper (Cu). For source allocation of organic load, C-surveys may also include total phosphorus (TP) and zinc (Zn), as well as tracers like fatty acids and isotopes. C-surveys are imposed upon the operator by the Directorate of Fisheries when there is substantial reason for concern regarding the environmental conditions. In some cases, C-surveys are even a condition in the discharge permit. The frequency is usually every second or third year, depending on previous results.

The quality of these surveys can be considered reliable since the executive organ has to be accredited and not affiliated to the operator. Additionally, the quality of the surveys is assured through the standard NS 9410. However, the mandatory surveys do not demand analyses of trace organic pollutants like drug residues and their metabolites, nor trace elements apart from those mentioned above. For data on such analyses, one must turn to scientific publications.

1.2 PREVIOUS STUDIES ON CHEMICAL POLLUTION FROM FISH FARMS

1.2.1 Trace elements associated with aquaculture

In an international context, elevated concentrations of trace elements in sediments related to open net-pen aquaculture have been shown. Especially copper (Cu) and zinc (Zn) concentrations seems to be significantly higher near aquaculture sites (Burridge et al., 2010, Chou et al., 2002). Copper-based anti-fouling paints are usually applied to prevent algal growth on the net-pens. Copper leaks in the first 6 months (approximately) after the antifouling agent it is applied, but then seems to reach an equilibrium with the surrounding waters. However, accumulation in the surrounding sediments due to this leakage is not always an issue (Kalantzi et al., 2016). Other cases confirm elevated concentrations of Cu in sediments close to aquaculture installations, without detecting significant changes in composition or abundance of the benthic macrofauna (Vera et al., 2015).

Zn is used in the aquaculture industry as a supplement in salmon feeds (Burridge et al., 2010), and is shown to accumulate in the surrounding sediments (Farmaki et al., 2014). However, the benthic fauna seems to be unaffected as for Cu (Vera et al., 2015, Russell et al., 2011).

Accumulation of cadmium (Cd) is also related to aquaculture installations for some cases, as well as primary nutrient related elements, i.e. nitrogen (N) and phosphorus (P) (Farmaki et al., 2014). Lead (Pb) is also shown at significantly elevated levels in sediments related to aquaculture (Mendiguchía et al., 2006).

Cd, Zn and Pb may be considered the most toxic trace metals in coastal waters in that given order, considering the ranking of comparative toxicity potentials (CTP), with Cu following close behind (Dong et al., 2016). Elevated levels of these trace metals should be monitored closely, as they may cause substantial harm to marine ecosystems, and as well potentially bioaccumulate or biomagnify in species higher in the food web to cause harm or reduce the quality of food resources of great importance for human consumption and health. However, summing up the conclusions from the publications mentioned above related to trace

elements in sediments near aquaculture installation, levels seem to be below official limit values and not causing significant changes on benthic macrofauna.

1.2.2 Accumulation of drugs and their metabolites in sediments and biota

Several studies exist on sediment content of drugs commonly used in the industry. Earlier publications have focused on the accumulation and ecological fate of the antibiotic oxytetracycline (Samuelsen, 1989, Samuelsen, 1992, Coynea et al., 1994), but this drug is supposedly not used by the industry anymore. Of most environmental concern today in the Norwegian context, seems to be drugs used for abatement of sea-lice infestations: cypermethrin, deltamethrin (pyrethroid insecticides) and azamethiphos (organothiophosphate insecticide) are administered through therapeutic baths and emitted to the recipient by dumping of drug containing water from well-boats; teflubenzuron, diflubenzuron (benzoylurea insecticides) and emamectin benzoate (avermectin) are administered through fish feed, and enter the recipient through excess feed, or trough feces as unmetabolized parent compounds or as metabolized products (Grefsrud et al., 2018).

Use of the benzoylurea pesticides teflubenzuron and diflubenzuron, is considered a major risk concerning adversary effects on non-targeted crustaceans, as they are designed to inhibit the synthesis of chitin, which is the major constituent in the exoskeleton of all arthropods. Complete cessation of naupliar molting is shown at environmentally relevant concentrations for these drugs (Macken et al., 2015). Teflubenzuron seems also to bioaccumulate in rockpool shrimps, with significant effects on molting, exoskeletal development, stress and apoptosis (Olsvik et al., 2017).

Moreover, teflubenzuron and diflubenzuron have shown high stability and persistence in sediments in the field (Samuelsen et al., 2015) and in laboratory tests, where no significant reduction of concentrations in sediments was observed after 24 weeks (Samuelsen, 2016). About 30 % of the initial amount of benzoylureas have been previously demonstrated to transform within 12 days by bacterial fermentation in soil. The products of fermentation were 2,6-difluorobenzamide, 2,6-difluorobenzoic acid, 2,4-difluoro-3,5-dichloro-aniline and 1,2-bis(2,4-difluoro-3,5-dichlorophenyl)urea (Finkelstein et al., 2001).

Toxicity of cypermethrin and emamectin benzoate was demonstrated for an amphipod species with sediments containing concentrations down to μg kg^{-1} levels (Tucca et al., 2014). Emamectin benzoate is known to have various metabolites such as the 8,9-Z isomer, N-demethylated, N-formylated and N-methylformylated emamectins (Yoshii et al., 2004). It is suggested that emamectin benzoate has adverse effects on non-targeted crustaceans upon short

term exposure (Veldhoen et al., 2012). However, emamectin benzoate and its metabolites have failed to show toxic effects on organisms near fish farms after treatment. The drug has been quantified in blue mussels 10 m from the net-pens 12 months after treatment, although only after one week for distances up to 100 m (Telfer et al., 2006).

Regarding azamethiphos in combination with deltamethrin, there are indications that there may be an increased toxicity ("cocktail effect") when these agents are used in combination (Olsvik et al., 2014). Dumping of bath treatment water containing a combination of these drugs, is subject to new regulations introduced in 2017 (Grefsrud et al., 2018).

The sea-lice abatement drugs diflubenzuron, teflubenzuron, and emamectin benzoate can be detected at levels above environmental quality standards in sediments near several salmon farms in Norway, while deltamethrin and cypermethrin were not detected in the same study (Langford et al., 2014). However, in Chilean fjords, deltamethrin was found in sediments at several locations (Placencia et al., 2018).

Summed up, there are evidently reasons for concern regarding the use of anti-parasitic drugs in the aquaculture industry. Studies from many countries show especially adverse effects on non-targeted crustaceans and persistence in marine sediments for some of the drugs. Effects on crustaceans are also a major concern for resource management of commercial resources of e.g. shrimps, crabs and lobsters aimed at human consumption.

1.2.3 Other organic pollutants and xenobiotics

Formaldehyde is frequently used as a disinfectant (Grefsrud et al., 2018). However, a risk assessment aimed at Canadian aquaculture concerning the use of formaldehyde, concludes that even worst-case scenarios predicts that the environmental concentrations do not exceed estimated no-effect concentrations (Chénier, 2003).

Considering the xenobiotics polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDE), there are no evidence in a Norwegian context of contributions to marine sediments or biota related to aquaculture (Grefsrud et al., 2018). It is even shown that cod (*Gadus spp.*) feeding on excess feed from fish farms, have lower liver concentrations of PCB compared to the same species feeding on natural prey further inside the fjord system (Svåsand et al., 2016). This trend of background PCB levels or lower close to aquaculture installations, is backed up by a study on sediments close to Scottish freshwater fish farms (Russell et al., 2011). On the other hand, PCB levels have shown correlations with pollutants related to Faroese aquaculture in a pilot study, while PBDE did not show the same correlations (Gustavson et al., 2009)

1.3 NON-TARGETED SCREENING

Working with non-targeted analysis is a relatively new approach, and despite its complexity, demands from the government and popularity among environmental scientists have increased the last years, fueled by the increasing focus on emerging contaminants. Non-targeted screening can be defined as identification of environmental pollutants, their degradation products and metabolites, without a preceding selection of compounds of interest. Traditional targeted approaches give reliable identification and quantification for the selected compounds. The drawback is that compounds which are not selected as analytes are also not identified or determined. There is limited knowledge about residues and decomposition products from medical drugs used in e.g. fish farming, or other non-legacy trace organic pollutants from e.g. paints and surface treatments. The chemical industry synthesizes continuously new useful substances for a broad range of applications. Non-targeted analyses attempt to fill the knowledge gap concerning presence and accumulation of emerging pollutants in a qualitative and semi-quantitative matter (Norwegian Environment Agency, 2013).

The main analytical challenge lies in the very nature of the technique. Without a preceding selection of analytes, the analytical system cannot be optimized for the specific analytes and matrices. This makes quantification nearly impossible, while extraction and detection techniques will not work optimal for the specific groups of potential analytes. Fortunately, modern advanced mass spectrometry techniques give a possibility to screen many compounds over a short time (Vincent et al., 2016), and suggested identifications can be performed by matching mass spectra with a mass spectral database. Suggested identification based on mass spectra can produce a list of suspected compounds. In an iterative process, suspected compounds can be analyzed in a targeted fashion until confirmation by standard materials. The remaining peaks forms the basis for a new suspected compounds list.

Interlaboratory tests of non-targeted analysis of river water have been performed. The conclusion was that the procedures seem to be harmonized between the different laboratories, but data processing remains highly time-consuming due to lack of integration and connection of desired features in software packages, lack of exchange of suspected compounds lists, and lack of mass spectra in open databases (Schymanski et al., 2015). For future non-targeted screenings, a fully automated identification workflow will probably be available, facilitating this work radically.

1.4 PURPOSE OF THE STUDY

With the use of powerful techniques for chemical analysis like ICP-MS and GC-MS, in addition to descriptive and multivariate statistical analyses, this study intended a description of the chemical composition of the sediments surrounding two different marine aquaculture installations. Both inorganic parameters (trace elements) and results from organic non-targeted screening techniques, as well as basic physio-chemical characterizations, were used for this purpose. As mentioned, most previous studies focus on the abundance and presence of pollutants in fish feed, fish tissue, surrounding fauna and flora, and effluents from the installations during medical treatments, but few considers accumulation of pollutants, their metabolites and degradation products, in the surrounding sediments. The organic analyses are not mainly intended for identification and quantification, more as descriptions of the response by the applied chromatographic methods. Nevertheless, the analyses could give a foundation for future analytical studies based on the information provided.

The issues investigated in this study can be summed up in three main categories with their corresponding subcategories:

- Development of a methodology for non-targeted screening of organic pollutants in marine sediments related to aquaculture
    - Identification of emerging pollutants accumulating in sediments near fish farms
    - Identification of metabolites and degradation products
- Source allocation of eventual pollutants and relationship with nearby fish farms
    - Study of the changes in the frequency and concentrations with increased distances from installations
    - Comparison of sediment conditions near different installations
    - Sediment classification by content of trace elements of environmental concern
- Links between elements, physio-chemical characterizations, and organic compounds
    - Relationship between elements and organic compounds
    - Correlations between physio-chemical properties and chemical content

2 THEORETICAL BACKGROUND

2.1 FATE OF ENVIRONMENTAL POLLUTANTS

2.1.1 Origins of pollutants

The human race has probably synthesized hundreds of thousands of chemicals not previously present in the environment, with an exponential increase since the advent of synthetic chemistry in the early 19th century. An evaluation by the US Government Accountability Office (GAO) in 2013 estimated that up to 84,000 synthetic chemicals could be in use commercially at that point of time (US GAO, 2013). Increasingly more knowledge and sophisticated technology makes it possible to produce highly specialized chemicals for every aspect of human life, from home appliances, personal care products and medical drugs, to products aimed at industries for increasing efficiency and quality while reducing cost of production, to a wide range of new inventions adding to humanity's needs for commodities. There are reasons to believe that synthetic chemistry and its implications within engineering and medicine, may be one of the main factors responsible for the increased human wellbeing and life span in the last century (Nicolaou, 2016). However, this has a potential cost for the health of human beings and ecosystems, which is yet difficult to assess completely due to the vast complexity of the matter and relative lack of knowledge. The introduction of newly invented chemicals to the environment could be especially costly. Even if short-term effects could be well studied before introduction, there are substantial reasons for concern regarding their yet poorly known long-term effects, or their fate when subject to the numerous and multidimensional interactions within the complexity of the ecosystems (Bernhardt et al., 2017).

Another class of pollution is minerals and fossilized organic material extracted from deep under earth's surface and transferred to the biosphere to be used in service of humanity. By themselves, these chemicals are not strangers to the environment, but the mass balance is altered due to this resource extraction. The altered concentrations of e.g. trace elements or atmospheric CO_2 due to human activity, have probably already changed the planet (Notz and Stroeve, 2016).

A related form of pollution is the acceleration of geochemical cycles, causing an increased output of nutrients. An example is the large-scale production of fertilizer for agriculture by nitrogen fixation (Haber process), a process accomplished naturally only by bacteria (diazotrophs) and to a smaller extent by lightning (Hill et al., 1980). Increased primary nutrient input may alter the ecosystems and lead to eutrophication (Lessin et al., 2014).

2.1.2 General processes

The four top level ecosystems can be described with their main compartments as: atmosphere (air); hydrosphere (oceans, lakes, rivers); lithosphere (soil, sediments, exposed rock, bedrock); biosphere (plants, animals, fungi, microorganisms). The movement of pollutants between these ecosystems affects profoundly their bioavailability, defined as the ability of pollutants to be taken up by biota from abiotic compartments, and eventually into the food web. In soil and sediments, pollutants will remain relatively restrained to the local geographical area. However, once a pollutant enters a mobile compartment like air or water, it will disperse rapidly (Mohamed and Antia, 1998).

The fate of pollutants can be separated into three distinct processes: transfer/translocation, metabolism and decomposition. Transfer/translocation refers to the unmodified pollutants mobility from one place to another within the same compartment, or between different compartments. Metabolism is a transformation of the parent pollutant to a metabolite and happens usually within living organisms. Transformation may also happen abiotically through e.g. photolysis. As the compound is transformed, it will acquire new chemical properties, which in some cases neutralizes their damaging effects, but in other cases may increase the toxicity (e.g. microbial methylation of mercury forms methylmercury which is more toxic than its inorganic precursor). Finally, decomposition refers to the breakdown of a pollutant or a metabolite to its constituent parts by abiotic chemical processes like redox reactions. Degradation of an organic compound all the way to its inorganic constituents is referred to as mineralization (Mohamed and Antia, 1998).

In between and during these processes, chemical speciation will occur. Changes in physio-chemical properties of the natural vector, like acidity, oxygen content and particulate matter, may alter the form of the chemicals during their journey within or between environmental compartments. Processes like complexation, redox reactions and acid/base reactions, may alter the form of any given chemical to another chemical species, and consequently alter their pathways. Moreover, speciation alters the bioavailability of a chemical, e.g. a chemical bound to particulate matter may be inhibited from uptake by microorganisms and further up the food web (Menegario et al., 2017).

2.1.3 Bioaccumulation and biomagnification

Once a pollutant is taken up by an organism, lipophile pollutants could potentially be stored in the fatty tissues of the organism, unless metabolized into a product which is easier to excrete, usually a more hydrophilic compound. If the rate of uptake is larger than excretion,

accumulation within the organism will occur. This accumulation of pollutants within an organism is referred to as bioaccumulation. The potential of a chemical to bioaccumulate is related to its octanol/water partitioning coefficient (K_{ow}) as a measure for lipophilicity (Diepens et al., 2015).

Biomagnification refers to an increase of the concentration of a pollutant in animal tissue with increased trophic level, such that animals high in the food chain, e.g. top-level predators, could potentially have high concentrations of pollutants with biomagnification properties. Polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) are a classic examples of pollutants that show significantly higher concentrations among top-level predators than at lower trophic levels (Paolo et al., 2010). Biomagnification is one of the properties defining a persistent organic pollutant (POP) (Stockholm Convention, 2018).

2.1.4 Accumulation of pollutants in marine sediments

The sediment compartment acts as sinks for chemical pollutants and can in many ways be regarded as the final destination for pollutants from both marine and terrestrial origin. This is however not entirely true since there is also a constant exchange of matter from the sediments to the water phase, although the gradient is usually larger downwards (Mohamed and Antia, 1998). Further, diagenesis occurs, which is the chemical conversion from sediment to rock. The rock will resurface again over a geological timespan due to tectonic displacement, and thus drive the geochemical cycle.

The composition of sediments is more complex and non-homogenous than a single body of water, with different physio-chemical characteristics within short distances. This gives rise to a higher variety of biological and chemical reactions in sediments compared to e.g. sea water. For example, anoxic conditions may occur just a few cm below the surface of the sediment, and thus altering the reaction pathways completely (Mohamed and Antia, 1998).

Further, chemicals may be immobilized by adsorption to particles in the sediment. However, fluctuations in physiochemical conditions like pH or oxygen content, may shift the equilibrium to favor chemical species with higher affinity for the water phase, and consequently remobilizing the chemical.

High organic content in sediments are shown to increase the ability to accumulate pollutants. Humic substances in the organic fraction of the sediments will work as ligands and create complexes with organic and inorganic pollutants (Mayer, 1985). Also, clay particles in the sediments have a high affinity to pollution by adsorption (Thiebault et al., 2015). A high

clay content will therefore probably increase the sediments ability to accumulate pollutants, but in the same way reduce the bioavailability of the pollutant.

2.2 COMPOUND IDENTIFICATION BY MASS SPECTROMETRY

Mass spectrometry is a powerful technique for identification of chemical compounds. The basic principle is the ionization of analytes by a plasma ray into ionic fragments. The ionic fragments are then refracted with strong magnet rods, usually referred to as a quadrupole. The degree of refraction depends on the ratio between the mass and the charge of the ion (m/z). A detector placed after the quadrupole, detects the degree of refraction and the intensity of the signal, i.e. the abundance of the ionic fragment. In this fashion, each compound will produce a fragmentation pattern, which reflects the structure of the compound. Coupled with a separation technique like gas or liquid chromatography, the aim is that individual separated compounds reach the mass spectrometer at different times, depending on their affinity to the chromatography column (Norwegian Environment Agency, 2013).

Extensive databases, like the National Institute of Standards and Technology's Mass Spectral Library (NIST library), contain mass spectra for numerous compounds. When identifying a compound through a mass spectral database, the fragmentation pattern, or mass spectrum, of a compound works like a fingerprint specific to that compound. Through a matching algorithm, the database returns a list of probable matches based on a query. The match value (Match) gives the degree of match between the mass spectrum of the analyte and that of the proposed compound. The reversed match value (RMatch) gives the degree of match by removing peaks that are not present in the proposed compound. In this way, the ratio between Match and RMatch gives an indication of how successfully noise has been removed from the mass spectrum. Ideally the difference between Match and RMatch should be as small as possible (Stein, 1994).

2.3 ANALYTICAL ERRORS

2.3.1 General considerations

When chemical analysis is performed, it is crucial to bear in mind the different sources of analytical errors specific to the analytical system applied, to be able to minimize their influence on the accuracy and precision of the results, as well as being able to describe the variations in the measurements. Absolute error is defined as the difference between observed values and true values, and can be either positive, i.e. higher observed value than the true value, or negative, i.e. lower observed value than the true value.

Deviations from true values can be distinguished as random errors or systematic errors. Random errors vary in an unpredictable manner between measurements, while systematic errors displace the measurements to one side. Random errors can in principle not be avoided, only minimized and described with statistics. On the other hand, systematic errors must be avoided when the origin becomes known, as they produce false results that cannot be described statistically (Reichenbächer and Einax, 2011).

Variation within a series of measurements are usually described by standard deviation (*s*), or by different values derived from standard deviation, like: variance (square of *s*); relative standard deviation (*s* divided by mean given as percentage); standard error of the mean (*s* divided by the square root of the number of observations) (Reichenbächer and Einax, 2011).

Common for all analytical methods are errors by contamination, i.e. transfer of unwanted substances to the sample or loss of analyte to materials in contact with the sample by sorption, which can give positive or negative errors respectively. Usually, contamination is avoided by using only materials which are known not to absorb, adsorb or leak compounds similar to the analytes. E.g. contamination could be minimized by assuring that samples for organic analysis are only in contact with acetone rinsed metal containers, and that samples for inorganic analysis are only in contact with high quality plastics like polytetrafluoroethylene (PTFE) (CEN, 2004). However, contamination could result from e.g. the analytical instruments, or impurities in the extraction solvents.

Related to contamination is cross-contamination, which refers to the carryover of analytical material from one sample to the other, and usually results in positive errors. Glass rods and spoons, in contact with the samples must be cleaned thoroughly between contact with each sample.

Chemical compounds are usually present as specific species in the environment, and in many cases, analysis requires that the results presented reflects the actual speciation in nature. Some analyses, e.g. pH measurements and a variety of electrochemical analyses, can be done directly in the field in the natural matrix, but most analyses require samples to be collected in a container and transported to a laboratory, and usually with a substantial time interval between sampling and analysis. Challenges are factors like redox reactions, microbial alteration, matrix effects, photochemical and thermic degradation, and reactions between the chemicals within the same sample. To minimize these effects, samples like soil or sediments are usually frozen as fast as possible after sampling. Common practice for water samples is to conserve the sample with an acid to prevent microbiological alterations (CEN, 2004).

2.3.2 Accelerated solvent extraction (ASE)

The classical method for extraction with organic solvents is with a Soxhlet extractor, which also gives satisfying recovery rates for many cases (Sporring et al., 2005). The main drawback with Soxhlet extraction, and consequently the main advantage with accelerated solvent extraction (ASE), is the time used for each extraction. Soxhlet extractions will typically use 6-8 hours for each sample, up to 48 hours for some cases, while ASE accomplishes the same task within 15 minutes (Vandenburg et al., 1998). ASE will also consume much less extraction solvent than by Soxhlet extraction (15 mL pr. 10 g sample) (Jensen et al., 1996).

Three major sources of contamination are considered crucial for ASE: 1) Impurity of the extraction solvent: with sensitive methods like LC-MS/MS, it is important to use solvents with a high grade of purity, preferably HPLC grade or better. 2) Impurities in the pressurizing gas: e.g. hydrocarbon residues in low quality gas can be transferred to the sample. 3) Tubing, fittings, extraction cells, collection vessels and septa: the instrument must be rinsed between each sample to minimize contamination and cross-contamination. Septa should be changed to a new and sterile one between each sample. Collection vessels must be cleaned thoroughly with appropriate solvents (Thermo Scientific, 2011).

Another general concern for all extraction systems is their ability to extract as much as possible of the analytes of interest from the samples. To assess the extraction systems capability, tests of recovery rates for each analyte are performed. The amount of recovered analyte must be considered when evaluating the amounts of the analyte present in nature. Regarding the operation of the ASE, recovery rates depends on the programming of the instrument (cycles, static time, pressure etc.), the ability of the solvent or solvent mixture to dissolve the analytes of interest, the matrix penetration capability of the solvent(s), and finally the efficiency and appropriate use of dispersant and cleaning agent (Thermo Scientific, 2011).

2.3.3 ICP-MS

The advantages of inductively coupled plasma - mass spectrometry (ICP-MS), as a technique to determine elemental composition, is mainly its multi element capability, speed and sensitivity. Sensitivity can reach down to ppb levels for a 0.1 g sample, and time of analysis makes it possible for a lab to analyze easily 15-20 samples/day including sample preparation (Longerich et al., 1990).

The primary factors limiting accuracy and precision using inductively coupled plasma mass spectrometry (ICP-MS) are machine drift and consequently the variation of the instrument response as a function of mass (Cheatham et al., 1993). Another challenge is that the type of

reference material used must be as similar as possible to the matrix of the samples to be analyzed. Since environmental samples usually have different composition within the same batch of samples, the properties of the chosen reference materials will seldom be the same for every sample.

Sample preparation is also a limitation since the sample must be liquid. Acid digestion of a solid will not always dissolve all the available analytes in the sample (Longerich et al., 1990). Also, the introduction of acids can potentially contaminate the sample, and moreover make it impossible to analyze the elements contained in the applied acid.

The cost of investment and operation is also a challenge regarding ICP-MS compared to other analytical techniques for elemental determination. For example X-ray fluorescence (XRF) is estimated to have a fixed cost pr. sample of at least six times lower than ICP-MS (Longerich et al., 1990).

2.3.4 Chromatography

Generally, in chromatography, separation of a mixture of analytes is possible due to each individual compound's different affinity to the mobile phase vs. the stationary phase. The retention time, i.e. the time before the analyte escapes the column and reaches the detector, is a function of the different affinities to the two phases. In this way, compounds with a low affinity to the stationary phase, but a high affinity to the mobile phase will pass through the column faster than compounds with opposite affinity.

In gas chromatography (GC), the carrier gas is the mobile phase, usually an inert gas, while the stationary phase is a microscopic layer of liquid or polymer on an inert solid support (the column). Therefore, gas chromatography is more correctly referred to as gas liquid chromatography. To enter the gas phase, the analytes must evaporate at the point of injection. It is therefore crucial to have temperatures at the inlet that are high enough for the analytes of interest to evaporate. However, temperatures should not be so high that the analytes are subject to thermal degradation (Lee et al., 2017). GC is therefore limited to analytes with a certain volatility, which excludes larger molecules like e.g. biopolymers and most medical drugs. Moreover, the stationary phase (column) is made of polymers with varying degrees of polarity in the non-polar to semi-polar range. Usually, only non-polar or semi-polar compounds will have enough affinity to the column to be retained and separated properly. Summed up, GC analysis is limited to volatile or semi-volatile analytes, that are semi-polar or non-polar (Norwegian Environment Agency, 2013).

Another popular technique within chromatography is liquid chromatography (LC), or more correctly liquid solid chromatography, where the mobile phase is liquid, while the stationary phase is a column packed with solid adsorbents. A more refined version is high performance liquid chromatography (HPLC), where a pump passes a pressurized liquid solvent though the column. LC is capable of analyzing a wide range of analytes, as they do not need to evaporate. Usually a polar solvent is used as mobile phase, while non-polar adsorbents constitutes the stationary phase (normal phase chromatography). The opposite polarity configuration is referred to as reverse phase chromatography.

Modern LC and GC instruments are usually coupled to a mass spectrometer (MS) to detect the abundance of the separated compounds, and to identify the analytes by their fragmentation pattern (Section 2.2).

A common limitation for both the chromatographic techniques mentioned, may be their ability to separate analytes properly. In many cases similar compounds have the same retention times, and therefore fails to separate and appears as one peak in the detector. Matrix effects and interactions between the analytes may also disturb the separation. Two-dimensional chromatography, e.g. GC × GC, can be used to attempt a better resolution of such peaks. Otherwise optimization, or method development, is necessary to find the optimal operational conditions necessary to analyze the analytes of interest, by selecting an appropriate column and by adjusting instrument configuration, like e.g. oven temperature program for GC.

Further, it can be a challenge that different groups of compounds give different magnitude of response depending on their potential for ionization. This may make detection of certain compounds difficult as they will give low response. Moreover, this makes it impossible to conclude anything about the relative abundance of compounds just by looking at the instrument response (Norwegian Environment Agency, 2013). For quantification, calibration curves must be constructed from analytical standard material.

2.3.5 Total organic carbon (TOC) by loss on ignition

Determination of total organic content by loss on ignition is considered a semi-quantitative method. Loss of ignition estimates total organic matter (TOM), which is then converted by a factor corresponding to the assumed fraction containing organic carbon. Conventionally this factor has been 1.724 based on the assumption of that organic matter contains 58 % organic carbon. However, the actual factor varies from soil to soil. Conversion factors range from 1.724 to as high as 2.5 (Schumacher, 2002). A recommended factor of 2 is for almost all cases a more accurate factor than the conventional factor (Pribyl, 2010).

Additionally, some clay materials contain structural water, which may be released at high temperatures and consequently overestimating the organic matter content. An alternative method that would avoid the issue of structural water contained in clay, is by oxidation with hydrogen peroxide (H_2O_2). However, as oxidation is incomplete, and as the extent of oxidation varies with different sediment compositions, this method is considered semi-quantitative at its best (Schumacher, 2002).

There are numerous ways for quantitative determination of organic content. One way is by collecting CO_2 after sample destruction, followed by quantification of CO_2 by e.g. titration. Destruction of the sample is usually achieved by oxidation or combustion (Schumacher, 2002). However, semi-quantitative determination by loss on ignition is commonly used due to its easy operation and relatively cheap equipment without any need for chemicals.

2.4 STATISTICAL ANALYSIS

2.4.1 Descriptive statistics

The first step in describing a dataset is to test whether the hypothesis of normal distribution is rejected or not. This step is important to examine initially, because the most powerful statistical tests assumes normal distribution. Data transformation like log-transformation, can be attempted to achieve normality. However, when normal distribution is unachievable, non-parametric methods, usually based on ranking of the data, can be applied (Stuart et al., 2010). Checking for normal distribution is usually done by looking at the histograms, and additionally by examining the numerical values for deviation from the Bell curve: skewness and kurtosis. Normality is also tested by the Shapiro-Wilk test, where the hypothesis of normality is rejected when the p-value is less or equal to 0.05 (Shapiro and Wilk, 1965). Usually all the methods mentioned above are used to test for normal distribution, as the Shapiro-Wilk test alone can in some cases be misleading.

Comparison between two groups can be performed by the student's t-test to assess the statistical significance of the difference. For the variables that do not follow the assumption of normal distribution, the Mann-Whitney U-test can be used. Comparison between more than two groups can be performed by one-way ANOVA for normal distributed data, or Kruskal-Wallis H-test for non-normal data. For pairwise comparisons, Tukey HSD post hoc is applied if the data passed the Levene test for homogeneity of variances, and only if the group sizes are equal, else the Games-Howell post hoc test is usually applied. Further, Mann-Whitney U-test can be used for pairwise comparison for data analyzed with Kruskal-Wallis.

Relationships between variables are usually calculated by Pearson correlation, which gives a coefficient (Pearson's r) from -1 to 1, describing the strength of the correlation and whether the variables move in opposite directions (i.e. negative/inverse correlation) or whether they move in the same direction (i.e. positive correlation). However, Pearson correlation assumes normal distribution within the variables. Alternatively, Spearman correlation can be applied, which calculate the correlation coefficients (Spearman's rho) based on ranking of the data. Spearman correlation coefficients are not to be overinterpreted as there are cases where they give positive values, while Pearson's coefficients are negative for the same data (Hauke and Kossowski, 2011). Pearson is generally preferred as the analysis is based on a linear relationship between the variables. The Spearman rho-value (r_s) is considered to represent a very strong correlation when its absolute value $|r_s|$ is above 0.8, strong when $|r_s|$ is between 0.6 and 0.8, moderate when $|r_s|$ is between 0.4 and 0.6, and finally weak or none with a $|r_s|$ less than 0.4 (Prion and Haerling, 2014).

2.4.2 Multivariate analysis

Principal component analysis (PCA) can be used to reveal several trends within a dataset. The general concept is to perform a dimensional reduction by projecting the data variables onto principal components (PCs) that are orthogonal to each other. This makes it possible to visualize a dataset with more than three variables within the same plot. In PCA, the original data matrix is described as the product of the loadings and the scores matrices according to the following formula (Abdi and Williams, 2010):

$$X = TP^T + E \tag{2.1}$$

where

X is the original data matrix;

T is the scores matrix;

P^T is the transposed loadings matrix;

E is the residuals matrix of variance not described by TP^T.

PCA plots can be visualized by three PCs in three-dimensional plots, but usually two-dimensional plots with two PCs are easier to understand. The goal when presenting two-dimensional plots, is that the first two PCs describe as much as possible of the variation within the dataset, preferably over 70 %. For cases where less than 70 % are described, deriving

conclusions from the PCA becomes very unreliable. PCs can be understood as latent variables, and descriptions of the PCs are attempted to understand the underlying phenomena that describes the variation in the dataset, i.e. the semantics of the PCs.

Scores plots visualize the entire dataset projected onto a graphically viable two-dimensional space by using the first two PCs. Each point in the scores plot represents one sample. Grouping among the samples may be revealed since samples with similar properties will probably be plotted closer to each other than samples with different properties. Usually, looking for grouping in PCA scores plots is the first step for further factor or cluster analysis. However, PCA is not a cluster analysis by itself since the grouping is more a mathematical consequence of the PCA, rather than a tool developed for cluster analysis including an evaluation of the distances between clusters. Further, factor and cluster analyses normally use the Kaiser-Meyer-Olkin test for sample adequacy (KMO), which excludes variables in the dataset based on their factorability. Factorable data should have some degree of collinearity among the variables, but not to an extreme degree causing singularity.

The loadings plots show the weights for each variable in the linear combinations of the first two or three PCs as vectors spanning from the origin. The degree of collinearity among these vectors are used to evaluate the correlations between the variables. In this way the relationship between the variables in a large dataset can be visualized within one plot. PCA is based on Pearson correlations (Abdi and Williams, 2010).

By combining the scores and the loadings plots, it is possible to evaluate which variables influence specific samples or groups of samples by inspecting the direction of the loadings vector for a variable relative to the position of the samples. This combination of scores and loadings plots is referred to as a biplot.

Before PCA, data undergoes preprocessing. Centering of the data is always performed. An example of centering is mean-centering, where the mean for a variable is subtracted from its values. The data can also be scaled. This is necessary if e.g. the variables originate from different analytical techniques, and therefore varies greatly by magnitude. An example of scaling is auto-scaling, where the values within each variable is divided by the standard deviation.

3 SAMPLING

3.1 STUDY AREA

The island of Smøla is located in Møre og Romsdal county, approximately 25 km north of Kristiansund and 110 km west of Trondheim (Figure 3.1). The region is subject to a high density of fish farming activity, as is the case for most of the Norwegian coast.

Sampling of marine sediments was performed near two fish farm installations, named Nørholmen and Hestøya, on the south side of the island (Figure 3.1). These installations represent different categories of production volumes, sediment conditions and history. Nørholmen was established in 1979, has a maximal allowed standing biomass of 6,240 tonnes, and a mainly rocky seafloor. On the other hand, Hestøya is a small installation with a maximal allowed standing biomass of 780 tonnes, operating since 2015, and located in a partly landlocked area with soft seafloor. Summary of data from the Directorate of Fisheries for the installations is given in Table 3.1.

Table 3.1 Overview of fish farm installations selected for this study (Directorate of Fisheries).

	Nørholmen	***Hestøya***
Owner(s):	Marine Harvest Norway AS	Atlantos AS Nekton Havbruk AS
Established:	1979	2015
Coordinates:	63°20'20.10"N 08°16'18.48"E	63°18'34.20"N 08°06'56.04"E
Loc. No.:	33937	34677
Species:	Salmon, Rainbow Trout	Salmon, Rainbow Trout
Maximal standing biomass:	6 240 tonnes	780 tonnes
Type of license:	Commercial	Commercial, Exhibition

Ocean currents for Nørholmen at different depths were modelled with SINMOD (SINTEF) and presented as rose diagrams, showing the frequencies of orientation and speed (Figure 3.2). Data from Hestøya were provided by the operator and based on measurements. According to the model, calculations for Nørholmen showed that the dominant ocean current direction was oriented towards southwest at both 7 m and 20 m depth, turning slightly westwards at deeper waters. For Hestøya ocean directions varied more than for Nørholmen during the time of measurement. However, there was a tendency for the south east directions to dominate at 7 m depth, while turning towards north east for bottom waters (29 m depth).

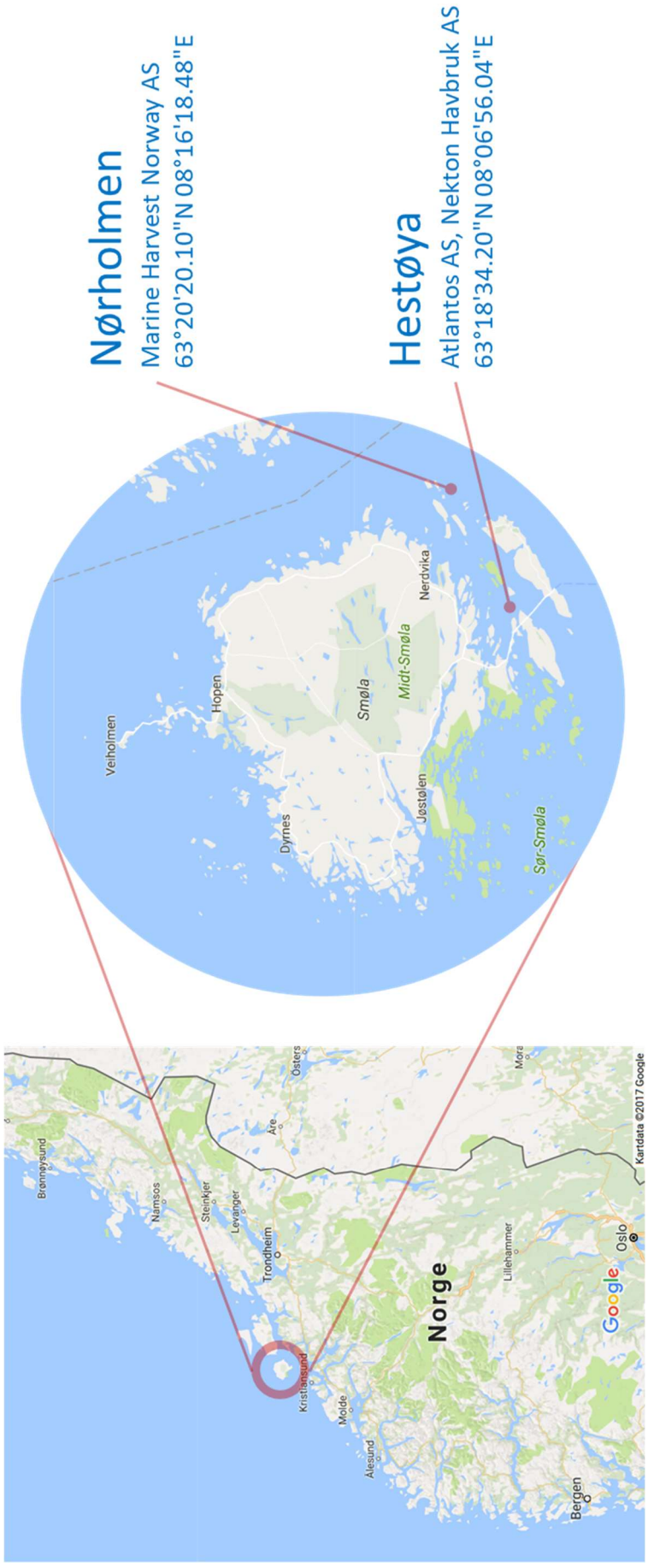

Figure 3.1 Locations of the island of Smøla and the fish farming installations 33937 Nørholmen and 34677 Hestøya (Map: Google).

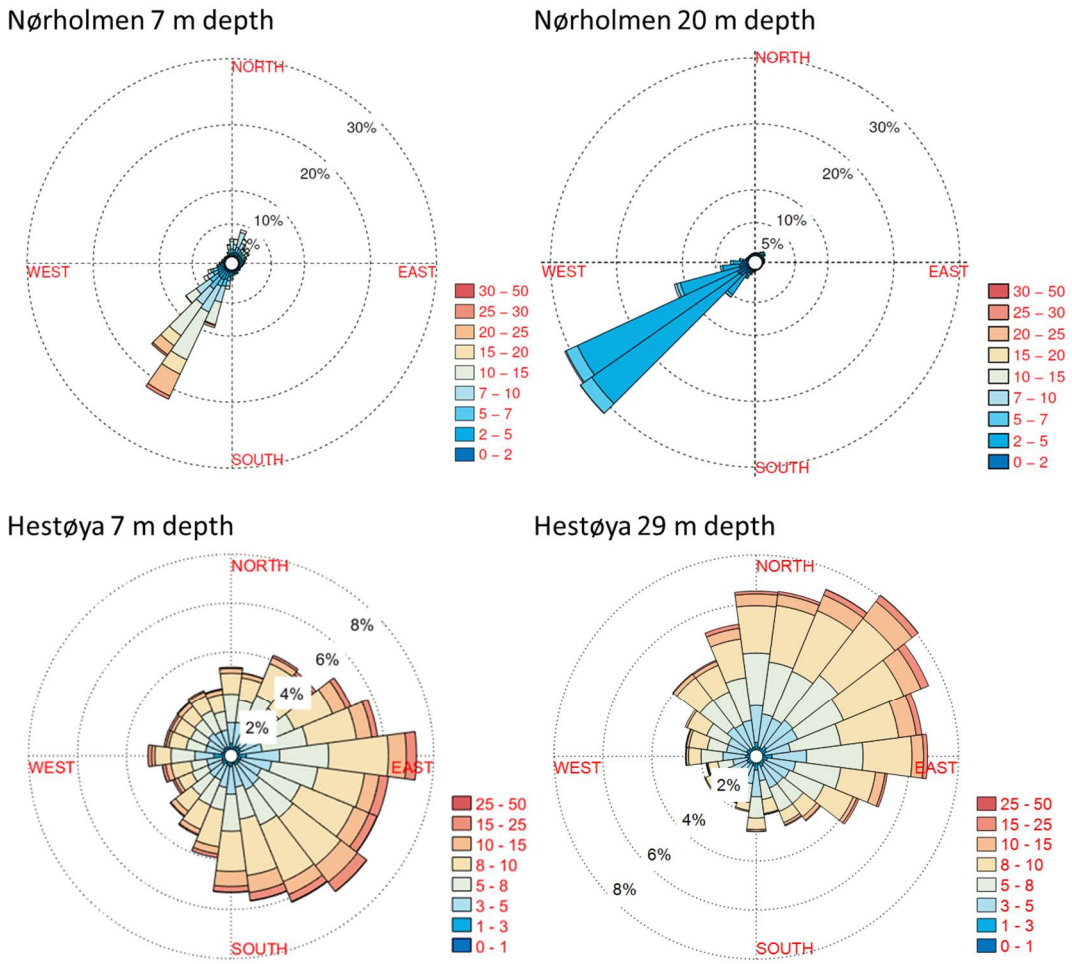

Figure 3.2 Ocean current roses for Nørholmen (top) and Hestøya (bottom) at two different depths. Data for Nørholmen is from SINMOD (Cred.: Sintef), while data from Hestøya is supplied by the operator (Cred.: Nekton Havbruk AS).

3.2 SAMPLING METHOD

All sampling was performed at daytime 28 March 2017 from NTNU's research vessel R/V Gunnerus. Samples were collected along gradients radiating out from the installations, spanning from approximately 100 m from the installation to approximately 1 km. Locations of sampling points along with the ocean floor topography are given in Figure 3.3 and Figure 3.4.

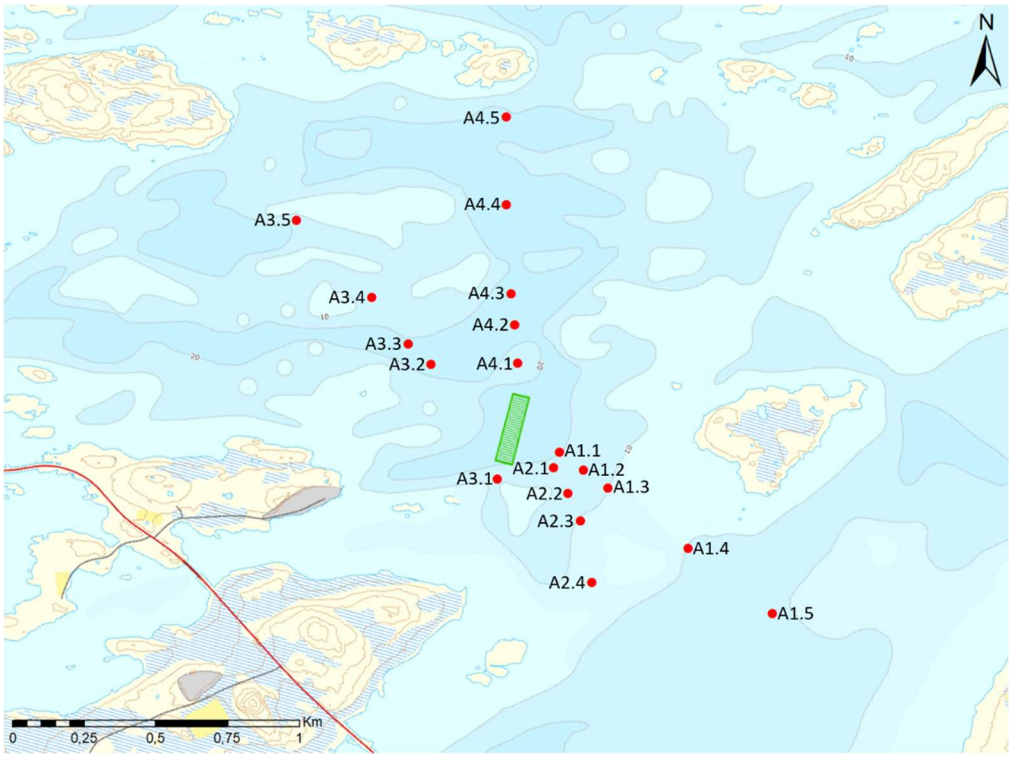

Figure 3.3 Sampling points for Hestøya fish farm (Installation A). The perimeter of the fish farm according to the official registry of aquaculture is marked in green. Depth is given as 10 m contours (Map: The Norwegian Mapping Authority). Coordinates for A3.5 are missing, however assumed to be within ±50 m of the appointed location.

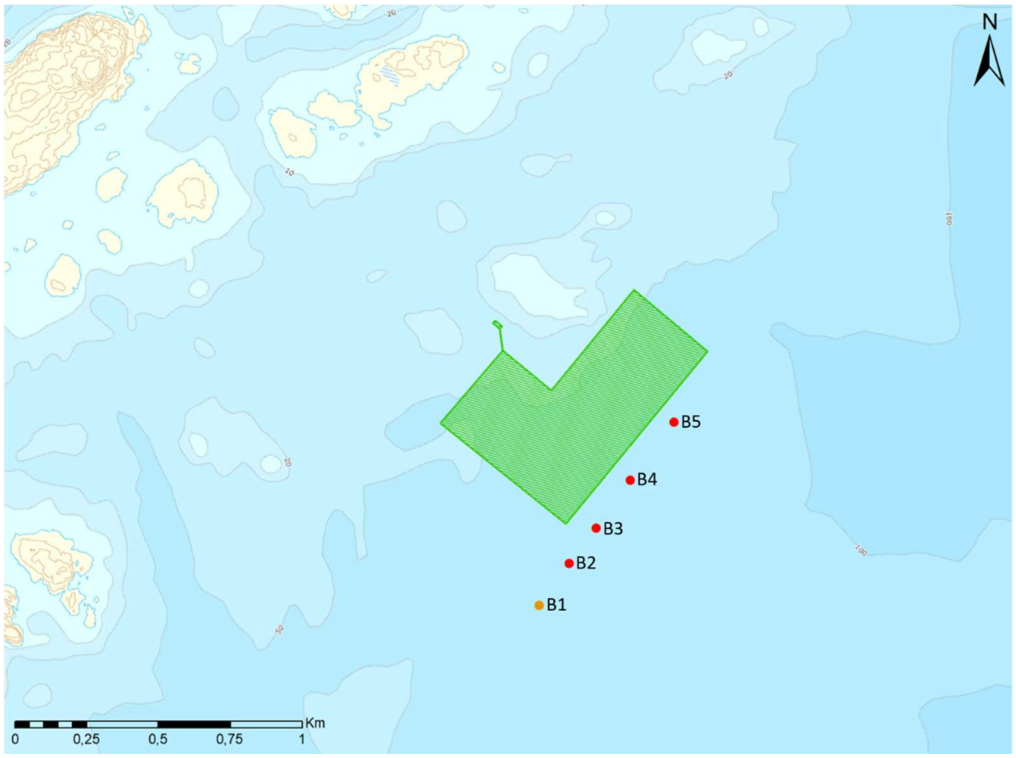

Figure 3.4 Sampling points for Nørholmen fish farm (Installation B). B1 was only analyzed for elements due to lack of material. The extent of the fish farm is shown according to the official registry of aquaculture is marked in green. Depth is given with 10, 20, 50 and 100 m contours (Map: The Norwegian Mapping Authority).

Sampling of marine sediments was performed in accordance to standard method EN ISO 5667-19 (CEN, 2004) with a box corer of dimensions 30 × 30 cm. The thickness of the sample varied from location to location, depending on the box corer's ability to penetrate the seafloor. For some cases with rocky seafloor, only scrapings from the top layer were possible to sample. Two representative sediment samples are shown in Figure 3.5, as well as the box corer. Overall, 24 sediment samples were collected from the locations shown in Figure 3.3 and Figure 3.4.

Figure 3.5 The top picture shows the box corer prepared for descent. The bottom left picture shows a sediment extract representative for soft sediments, while the bottom right shows a sediment extract from a rocky seafloor with a relatively thin sediment layer.

For organic analyses, the top 2 cm was scraped off with a clean polytetrafluoroethylene (PTFE) scrape and transferred to a 500 mL aluminum container with aluminum coated lid. Whenever possible, two or three replicates (subsamples) were collected from the same sediment sample. If the thickness of the sediment was more than 5 cm, the bottom 2 cm was also collected. The containers were then wrapped with aluminum foil. Table 3.2 gives a complete overview of the sampling data. For inorganic analysis, subsamples were collected simply by scrapping the sediment surface with a 25 mL polystyrene (PS) cup, and these cups were then capped with a polyethylene terephthalate (PET) lid.

In total, sediment sampling yielded 53 subsamples for organic analysis and 51 subsamples for inorganic analysis collected from 24 box core extracts. All samples were then stored immediately at -4°C and were maintained at the same temperature until arrival at the laboratory within 24 hours of sampling, where they were stored at -20°C until analysis was performed.

The term *sample* refers technically to the sediment samples extracted by the box corer, while *subsamples* refer to replicates collected from box core samples. In the following chapters the terms *sample* and *subsample* are used interchangeably. However, *sample* refers to the replicate unless specified otherwise.

Table 3.2 Overview of sample data.

Sample location ID	*Coordinates*	*Distance[1] (m)*	*Depth (m)*	*Sample ID organic*	*Sample ID inorganic*	*Layer depth*
A1.1	63°18'31.80"N 08°07'07.68"E	178	24	01A	1	0-2 cm
				01B	2	0-2 cm
A1.2	63°18'29.82"N 08°07'13.56"E	278	18	02A	3	0-2 cm
				02B	4	0-2 cm
A1.3	63°18'27.84"N 08°07'19.74"E	383	12	03A	5	0-2 cm
				03B	6	0-2 cm
				03C	7	8-10 cm
A1.4	63°18'21.12"N 08°07'39.72"E	728	10	04A	8	0-2 cm
				04B	9	0-2 cm
A1.5	63°18'13.92"N 08°08'00.78"E	1 095	12	05A	10	0-2 cm
A2.1	63°18'30.06"N 08°07'06.18"E	190	21	09A	18	0-2 cm
				09B	19	0-2 cm
				09C	20	5-7 cm
A2.2	63°18'27.18"N 08°07'09.78"E	289	15	08A	14	0-2 cm
				08B	15	0-2 cm
				08C	16	0-2 cm
				08D	17	8-10 cm
A2.3	63°18'24.12"N 08°07'12.96"E	390	10	07A	13	0-2 cm
A2.4	63°18'17.22"N 08°07'15.90"E	593	9	06A	11	0-2 cm
				06B	12	0-2 cm
A3.1	63°18'28.74"N 08°06'52.32"E	176	20	10A	21	0-2 cm
				10B	22	0-2 cm
				10C	23	8-10 cm
A3.2	63°18'41.52"N 08°06'35.58"E	363	29	11A	24	0-2 cm
				11B	25	0-2 cm
				11C	26	14-16 cm
A3.3	63°18'43.74"N 08°06'29.88"E	468	26	12A	27	0-2 cm
				12B	28	0-2 cm
A3.4	63°18'48.90"N 08°06'20.76"E	668	15	13A	29	0-2 cm
				13B	30	0-2 cm
A3.5[2]	63°18'57.60"N 08°06'01.80"E	1 043	15	14A	31	0-2 cm
				14B	32	0-2 cm
A4.1	63°18'41.70"N 08°06'57.06"E	232	13	19A	43	0-2 cm
				19B	44	0-2 cm
				19C	45	12-14 cm
A4.2	63°18'45.96"N 08°06'56.28"E	363	15	18A	40	0-2 cm
				18B	41	0-2 cm
				18C	42	12-14 cm
A4.3	63°18'49.44"N 08°06'55.26"E	471	30	17A	38	0-2 cm
				17B	39	0-2 cm
A4.4	63°18'59.40"N 08°06'54.00"E	779	22	16A	36	0-2 cm
				16B	37	0-2 cm
A4.5	63°19'09.24"N 08°06'53.82"E	1 083	22	15A	33	0-2 cm
				15B	34	0-2 cm
				15C	35	6-8 cm
B1	63°19'55.98"N 08°16'09.66"E	755	78	-	46	0-1 cm
B2	63°20'00.72"N 08°16'17.04"E	599	79	20A	47	0-2 cm
				20B	48	0-2 cm
B3	63°20'04.62"N 08°16'23.76"E	484	63	21A	49	0-2 cm
				21B	-	0-2 cm
B4	63°20'10.02"N 08°16'32.10"E	364	72	22A	50	0-2 cm
				22B	-	0-2 cm
B5	63°20'16.50"N 08°16'42.96"E	357	82	23A	51	0-2 cm
				23B	-	0-2 cm
Total number of subsamples from 24 locations				*53*	*51*	

[1]Distances are measured from the center of the fish farm perimeter according to the official aquaculture registry.
[2]Coordinates for this sampling point are missing but assumed to be within ± 50 m from the stated coordinates.

4 MATERIALS AND METHODS

4.1 NOTES ON METHOD DEVELOPMENT

The strategy for this particular study was to screen the sediment samples for inorganic and organic compounds with advanced instrumentation available at the Department of Chemistry at NTNU. Adding basic sediment characterization (sedimentology) together with geodata (e.g. distance from fish farm installation), and in the end combine all data for statistical computing such as descriptive statistics and principal component analysis (PCA) to reveal potential trends and correlations within the dataset. A summary of the methodology is given in Figure 4.1.

The elemental analysis was highly quantitative, and the measurements were used further for sediment condition classification based on trace metal concentrations, as the analysis complies to EPA Method 6020 (U.S. EPA, 2014b). For the organic part, the aim was not primarily to quantify eventual pollutants/organic compounds, but rather to perform qualitative analyses and characterizations of chromatograms from the analyses of sediment extracts. This study used semi-quantitative methods for the organic analyses, such as total ion chromatogram (TIC) peak area, to generate data for statistical computation, which was similar to the approach described in EPA Method 8260 (U.S. EPA, 1996). TIC peaks were identified by mass spectrum query via the NIST Mass Spectral Library (NIST Standard Reference Database 1A v17). Additionally, two TIC peaks were confirmed and quantified with reference materials from Sigma-Aldrich. Recovery rates were also calculated with the use of these reference materials.

One sample (08A) was chosen for testing and method development. Different instrumentation and solvents were tested to find the best possible way to screen for organic compounds. The initial choice of extraction solvents was based on an analysis of the solubilities of potential contaminants related to aquaculture industry. Dichloromethane (DCM) was chosen for further extraction of all sediment samples after the method development phase, based on its ability to produce interesting results with GC-MS. Column chromatography and LC-MS/MS was only conducted during method development, as this method failed to detect any compounds. The decision was not to use any cleaning agent to maximize the diversity of extracted compounds.

Tests with both cleaning agent (activated copper) and filtration showed reduced performance. High care was therefore implemented to not overload the mass spectrometers, such as dilution before injection and split ratio for GC-MS.

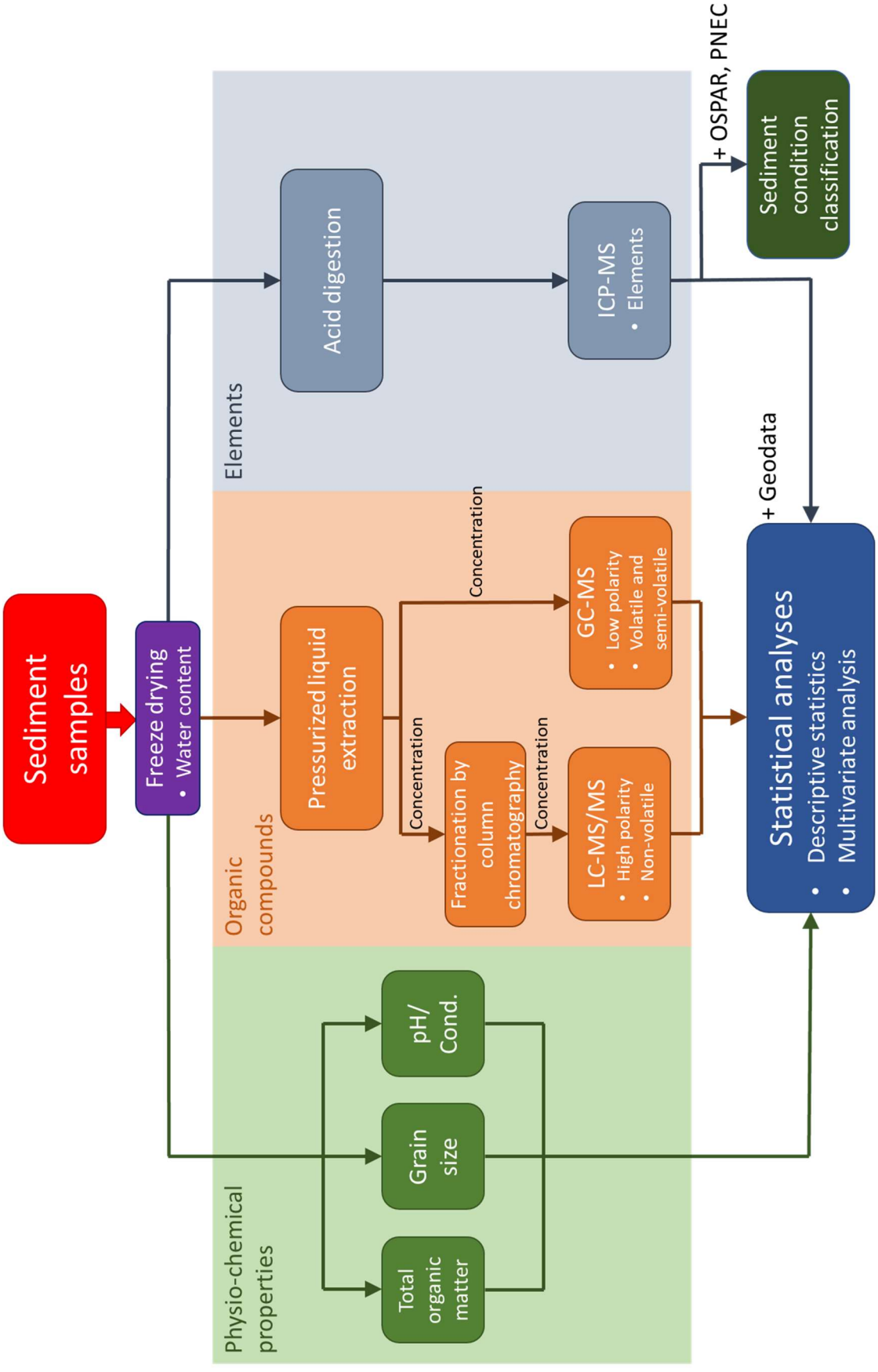

Figure 4.1 Flowchart for non-targeted organic screening of sediments samples linked with analysis of elements and physio-chemical characterizations.

4.2 PHYSIO-CHEMICAL ANALYSIS

4.2.1 Water content

Calculation of water content in the sediment samples is performed in accordance to EN ISO 16720 (CEN, 2007b). With this method, the weight difference before and after lyophilization indicates the amount of water retained in the samples after box core extraction. The lyophilization procedure is given in Section 4.3.1.

4.2.2 Total organic matter (TOM) and total organic carbon (TOC)

The fraction of total organic matter (TOM) in the sediment samples was determined by loss on ignition, in accordance with standard method EN 15169 (CEN, 2007a). Three grams of each of 37 freeze dried samples (see Section 4.3.1) were transferred to a pre-weighed porcelain crucible, first dried at 45°C for 2 hours and then incinerated in a furnace (Carbolite) at 550°C for 3 hours. The samples were weighted with a four-decimal analytical scale (Precisa 240A) after drying and after incineration.

Loss on ignition was calculated according to the following formula:

$$W\% = \frac{m(c) - m(b)}{m(c) - m(a)} \times 100 \tag{4.1}$$

where

$W\%$ is the loss on ignition in percent of the dry sample;

$m(a)$ is the mass of the empty crucible;

$m(b)$ is the mass of the crucible containing the ignition residue;

$m(c)$ is the mass of the crucible containing the dried sample.

To convert between loss on ignition and total organic carbon (TOC), a recommended conversion factor of 2 was used, assuming an organic carbon content of 50 % of total organic matter (TOM) (Pribyl, 2010).

4.2.3 Grain size distribution

Sediment texture was determined by dry sieving in accordance to ISO 16665 (ISO, 2014). Two mesh sizes were used, 2 mm and 0.06 mm, to produce three fractions: gravel (>2 mm), sand (0.06 – 2 mm) and pelite (<0.06 mm). Twenty grams of freeze dried sediment was first sieved with a 2 mm mesh sieve by horizontal hand shaking until no more product was visible. The sieved fraction was weighted, then added to the 0.06 mm sieve, and treated the same way as for the 2 mm sieve.

4.2.4 Conductivity and pH

Conductivity and pH of 52 sediment samples were determined as described by standard method ISO 10390 (ISO, 2005). Five grams (±1%) of each sample was weighted and transferred to a 50 mL polypropylene centrifuge tube (Fischer Scientific). To this 25 mL of DI water (18.2 MΩ, MilliQ) was added and vortexed at 40 Hz for 2 minutes (Heidolph REAX top). After vortexing, the samples were allowed to stand for 1 hour before measurements were taken.

While stirring the samples, conductivity was measured with a conductometer (model IONcheck 30, Radiometer analytical), while pH was measured with a pH meter (Model 3510, JENWAY). Calibration was carried out just prior to both the conductivity and pH measurements, using 0.01 M KCl solution and buffer solutions of pH 4, 7, and 10 (VWR) respectively. Three blanks were also measured according the same procedure, containing only DI water.

4.3 ELEMENTAL ANALYSIS

4.3.1 Sample preparation

All samples were stored in 25 mL polystyrene (PS) cups with lids, and frozen at -4°C immediately after sampling while onboard R/V Gunnerus. Arriving at the laboratory less than 24 hours after sampling, the samples were stored at -20°C. Approximately 1 month after sampling, samples were freeze dried with an Alpha 1-2 LDplus (Martin Christ), at a pressure of 0.94 mbar and temperature of -25°C for 24 hours in correspondence with EN ISO 16720 (CEN, 2007b), before acid digestion by autoclave.

Digestion was performed by weighing 200-300 g freeze dried sediment into perfluoroalkoxy (PFA) vessels (18 mL volume). Then 9 mL of 50 % concentrated HNO_3 (Ultra-Pure grade, distilled by Milestone SubPur unit) was added to the samples and digested with the use of a high-pressure digestion unit UltraCLAVE (Milestone).

4.3.2 ICP-MS conditions

After digestion by nitric acid as described above, the samples were analyzed for the presence of 47 elements by ICP-MS under conditions shown in Table 4.1. The instrument was calibrated using 0.6 M HNO_3 solutions of matrix-matched multi-element standards (Elemental Scientific) run after every 10 samples. The calibration curve consists of five different concentrations made from multi-element standards. Method detection limits (MDL) were based either on three times the standard deviation of the blanks, or on the instrument detection limits (IDL). Detection limits are given Table A.1 (Appendix A). The IDL results from the

concentration yielding to 25 % of relative standard deviation at three scans, which were calculated for the used sample amount. To assess possible contamination during sample preparation, blank samples of HNO_3 and ultrapure water (18.2 MΩ, MilliQ, ELGA DV25 reverse osmosis water purifier) were prepared using the same procedure as for the samples. Results were corrected for reagent blank values. A certified reference material (Soil GBW 07408) with water content set to 5 %, was used to validate the reference. Accuracy was determined running this reference material in duplicates.

Table 4.1 Specification for ICP-MS instrumentation.

Instrument	ICP-HR-MS Element 2 (Thermo Scientific)
Sample introduction system	Auto-sampler – SC2 DX
Gas flow	Splitting of sample gas, 10 % methane in argon
Analysis resolution	Low (400)
	Medium (5 500)
	High (10 000)

4.4 ORGANIC ANALYSIS

4.4.1 Accelerated solvent extraction (ASE) and concentration

Sediment samples were defrosted at 4°C for 6 hours, and consequently divided into 4 parts. Each part was then transferred to a 25 mL aluminum cup, wrapped in aluminum, and refrozen at -20°C. One part was lyophilized using 1-2 LDplus (Martin Christ) as described on last page, and were stored again at -20°C until extraction. All materials in contact with the samples were either aluminum or stainless steel, and pre-rinsed with acetone before drying.

Extraction was performed by accelerated solvent extraction (ASE) using Dionex ASE 150 (Thermo Fisher), in accordance to EPA Method 3545 (U.S. EPA, 1998). Lyophilized samples were weighted to 10 g, added 3 g diatomaceous earth (DE) and mixed with a glass rod. Finally, the mixture was transferred to a 34 mL extraction cell. All equipment in contact with the samples were rinsed thoroughly with deionized water (18.2 MΩ, MilliQ, ELGA DV25 reverse osmosis water purifier) and acetone (Technical grade, VWR), and dried before use. The DE was incinerated at 550°C for 3 hours in a furnace (Carbolite) before use. Table 4.2 sums up the conditions for accelerated solvent extraction performed on the sediment samples.

Note that the terms *accelerated solvent extraction* (ASE) and *pressurized liquid extraction* (PLE) are used interchangeably but refers to the same method in this study. The

reason is that ASE is a Dionex trademark, while PLE is a generic term. The method can also be referred to as *pressurized fluid extraction* (PFE) in the literature.

Table 4.2 Conditions for accelerated solvent extraction.

Instrument	Dionex ASE 150 (Thermo Fischer)
System pressure	1.4 MPa
Oven heat up time	5 min
Static time	5 min
Oven temperature	100°C
Sample size	10 g
Cell size	34 mL
Filter	Cellulose (Thermo Fischer)
Collection vial	60 mL amber with Ultra Clean septum (Thermo Fischer)
Dispersion agent	Diatomaceous earth, 3 g (Flux-calcined, Sigma-Aldrich)
Cleanup agent	None
Flushing volume	60 %
Purge time	80 seconds
No. of cycles	2
Nitrogen purge	1 MPa
Solvent	Dichloromethane (Pestinorm GC, VWR)
	n-Hexane (Chromanorm HPLC, VWR)
	n-Hexane:Acetone 1:1 (v/v) (Chromanorm HPLC, VWR)
	Ethyl acetate (Chromanorm HPLC, VWR)
	Methanol (Chromanorm HLPC, VWR)

After extraction each sample was immediately concentrated from 74 mL to approximately 1 mL by rotary evaporation (BÜCHI R-200) in a 35°C water bath (BÜCHI B-490) at 600 mbar, 225 mbar, 140 mbar or 125 mbar, for dichloromethane, n-hexane, ethyl acetate and methanol respectively. After concentration, each sample was transferred to a 1.5 mL amber vial (VWR) with a glass Pasteur pipette, and concentrated further to 200 µL by atmospheric evaporation in the ventilation hood for approximately 3 hours, before capping with a polypropylene (PP) screw cap with natural rubber septum (VWR).

Some samples formed colloids after concentration by rotary evaporator, which were omitted from being transferred to the vials. By visual inspection, colloids never exceeded 10 % of the total volume of the concentrate.

After concentration, volumes were adjusted to 200 µL ± 10 % either by further evaporation, or by adding more solvent. Finally, the 200 µL extracts were transferred to vials with 0.1 mL micro-inserts (VWR).

4.4.2 Column chromatography

Column chromatography of the ASE extracts was performed in the initial method development phase with the purpose of fractionating the analytes according to their properties (polarities in this case). The details of extraction and fractionation is shown schematically in Figure 4.2. A glass column with sintered glass filter and a bottom valve was packed with silica gel (high purity, pore size 60 Å, mesh 200-400, Sigma-Aldrich) by making a slurry in in n-hexane. Once the column was packed, approximately 1 mL extract after rotary evaporation was added carefully and given time to settle. Fractionation of the extract was started with non-polar solvent and the polarity was increased stepwise afterward (Figure 4.2). Only the n-hexane extract was eluted without further fractionation, thus ending up with 13 different fractions for each sample.

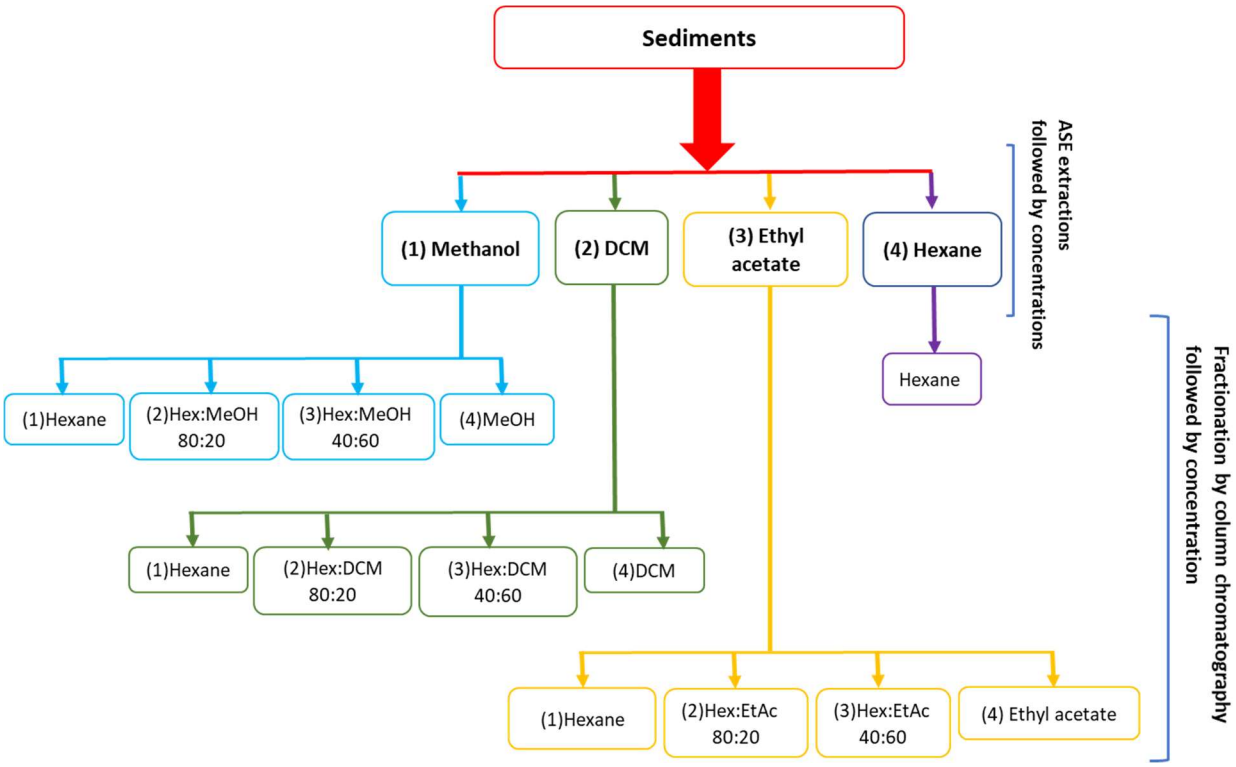

Figure 4.2 Fractionation of sediment extracts by column chromatography (Cred.: Dr. Shazia N. Aslam).

After elution, the fractions were concentrated by rotary evaporator to approximately 1 mL. To prepare for LC-MS/MS analysis, 1 mL methanol was added to the concentrated fractions, and the original solvent evaporated under N_2 flow to transfer the analytes to the methanol phase. The final sample was then pipetted to a 1.5 mL vial and stored at -20°C until analysis. Method blanks were also produced at this stage, where solvents underwent the same procedures as the samples.

4.4.3 LC-MS/MS

During method development, the fractions separated with column chromatography were analyzed by LC-MS/MS according to the specifications in Table 4.3. Non-targeted analysis was performed on an UPLC system (ACQUITY UPLC I-Class, Waters) coupled to a Synapt G2-S (Waters) electrospray Q-TOF instrument in positive mode. Analytical columns, BEH C18 (100 mm × 2.1 mm, 1.7 µm) and HSST3 (100 mm × 2.1 mm, 1.7 µm), were tested for chromatographic separation. The mobile phase comprised of water (Chromanorm HPLC, VWR) with 0.1 % formic acid (HPLC, Sigma-Aldrich) and acetonitrile (Chromanorm HPLC, VWR) that contained 0.1 % formic acid introduced at a flow rate of 300 µL/min. Acetonitrile was first held at 5 % for 0.5 min, increased from 5 % to 95 % within 18 min, increased again to 100% within 2 min and reverted to 5 % within 2 min. Data processing was done by Waters TM Software Masslynx V4.1 SCN871.

Table 4.3 Specification for LC-MS/MS instrumentation.

LC Instrument	ACQUITY UPLC I-Class (Waters)
MS Instrument	Synapt G2-S (Waters)
Column(s)	BEH C18 (100 mm × 2.1 mm, 1.7 µm) HSST3 (100 mm × 2.1 mm, 1.7 µm)
Mobile phases	A: Water with 0.1 % formic acid
	B: Acetonitrile with 0.1 % formic acid
Flow rate	300 µL/min
Mobile phase gradient	Hold 0.5 min: A at 95 %, B at 5 % 0.5 - 18 min: A to 5 %, B to 95 % 18 - 20 min: A to 0 %, B to 100 % 20 - 22 min: A to 95 %, B to 5 %
MS type	Q-TOF
Ionization	Electrospray (ESI), positive mode

4.4.4 GC-MS

After testing different temperature profiles during method development, 52 sediment samples were analyzed with a GC system (7890A, Agilent Technologies) coupled to an inert mass selective detector (5975, Agilent Technologies) with ionization by electron impact according to the specifications given in Table 4.4. A capillary intermediate polar column (Equity-1701, Sigma-Aldrich) was used with an injection split ratio of 5:1 and helium gas flow of 10.327 L/min at constant pressure of 10 psi. Oven program was set to a temperature span from 50 – 280°C at a rate of 4°C/min, with 4 min hold at 50°C and 4 min hold at 280°C. In addition to untreated solvent blanks before each run, mine method blanks were included in the analysis. The response from the method blanks were taken into consideration when evaluating

the chromatograms for the samples. The computer software MSD ChemStation E.01.01.335 (Agilent Technologies) and OpenChrom Community Edition (Alder) was used for analysis and data treatment.

Table 4.4 Specification for GC-MS instrumentation.

GC Instrument	GC-system 7890A (Agilent Technologies)
MS Instrument	Inert Mass Selective Detector 5975 (Agilent Technologies)
Column	Equity-1701 (Sigma-Aldrich)
	Capillary intermediate polar
	L × I.D. 30 m × 0.25 mm, d_f 0.25 µm
Carrying gas	Helium
Split ratio	5:1
Total flow	10.327 mL/min
Pressure	10 psi
Oven program	Temperature span 50 - 280°C
	Rate 4°C/min
	Hold 4 min at 50°C and at 280°C
Injection volume	1 µl
Injector heater	275°C
Interface heater	300°C
Start MS	5 min
Scan mode	Total ion
Ionization	Electron impact (EI)

Calibration curves for quantification

Two calibration curves were constructed for the estimation of concentrations of benzaldehyde and 3-bromophenol, after identification from the TIC diagrams using the NIST library. Specifications for the standard materials are given in Table 4.5. The method of quantification was based on EPA Method 8260 (U.S. EPA, 1996).

Table 4.5 Standard materials used for peak identification, quantification and calculation of recovery rate.

	Benzaldehyde	***3-Bromophenol***
CAS Number	100-52-7	591-20-8
Grade	Analytical standard (≥ 99.5 %)	98 %[1]
Distributor	Sigma-Aldrich	Sigma-Aldrich
M	106.12 g/mol	173.01 g/mol
Bp	178-179°C	236°C
Mp	-26°C	28-32°C

[1]The 3-bromophenol standard is strictly speaking not an analytical standard, but considered adequate for the scope of this study

The calibration formulas were established by linear regression through seven points, each representing the average of measured triplicates. Each data point was annotated with standard deviation, and R^2 values were calculated for both regressions. Calibration curves and regression formulas are presented in Appendix A (figures A.2 and A.3).

Limits of detection and quantification

Limit of detection (LOD) for the pseudo-quantification, was estimated by looking at the response depending on a series of dilution of the standard materials for benzaldehyde and 3-bromophenol. The concentration where the peak could be distinguished from the baseline, was considered the LOD for these compounds. For the peak characterizations of the unidentified compounds, LOD was set to three times the noise baseline for each peak.

Limits of quantification (LOQ) for the pseudo-quantification of benzaldehyde and 3-bromophenol, were decided by evaluating the linearity for the calibration curve at the lowest concentrations. LOQ was set at the concentration where the response began to show a linear trend. The peak characterizations of the unidentified compounds did not have a LOQ as quantification was not performed.

Identification of selected peaks

Peaks detected from the sample TICs and not present in the method blanks were identified through the NIST Mass Spectral Library (NIST Standard Reference Database 1A v17). Two of the peaks were selected based on their response and frequency of appearance in the samples. For confirmation of identity, three samples (01A, 09B, 14A) were spiked with 10 µg benzaldehyde, and three samples (05A, 10B, 17A) with 100 µg 3-bromophenol solved in in dichloromethane, by adding standards directly into the extract vials (standard materials are shown in Table 4.5). The spiked samples were then run under the same conditions as shown in Table 4.4.

Characterization of unidentified peaks

For the purpose of this study, unidentified peaks include those not confirmed by a standard material even if they scored a high match value against the NIST library. The methodology for characterization of these peaks was developed by trial and error.

Initially, peak maxima were identified by the first derivative, and abundance was decided by reading the peak maxima. Secondly, all peaks with an abundance (peak intensity) lower than three times the noise baseline for the area of the peak, were removed as they were considered below the limit of detection (LOD). The remaining peaks were then lined up so that their retention time (± 0.05 minutes) corresponded between the different samples and method

blanks. To confirm that a given retention time corresponded to a specific compound within the different samples and method blanks, the MS spectrum for every peak was checked for similarities. As this was done manually, it would be too time consuming within the time limit of this project to check each peak for all the samples. However, the MS spectra for the nine method blanks were thoroughly compared. Further, eight of the 52 samples were analyzed the same way. The remaining samples were assumed to be in correspondence given the similarity analysis by the first eight samples. Finally, all peaks found at least in one of the nine method blanks were considered contamination from the analytical system, and the remaining peaks were assumed to be extracted from the sediment samples

Estimation of recovery rate

One lyophilized sediment sample (06A) was spiked with both 1 µg benzaldehyde and 10 µg 3-bromophenol before extraction by ASE. Triplicates from the same sample were prepared the same way. Specifications for the reference materials are shown in Table 4.5. The spiked replicates were then extracted and analyzed according to the procedures described in Sections 4.4.1 and 4.4.4.

Concentrations were estimated according to the calibration curves, and recovery rate was calculated separately for each standard material by the following formula based on EPA Method 8000 (U.S. EPA, 2014a):

$$R\% = \frac{c(a) - c(b)}{c(c)} \times 100 \tag{4.2}$$

where

$R\%$ is the recovery rate in percent;

$c(a)$ is the estimated concentration of the spiked sample;

$c(b)$ is the estimated concentration of the un-spiked sample;

$c(c)$ is the concentration expected if the recovery rate was 100 %.

The average of the recovery rate of the three replicates was then calculated for both compounds and annotated with standard deviation.

Stability of the analytical system

Four sediment samples (01B, 03A, 08B, 21B) were selected for evaluation of the stability of the extraction and instrumentation. The selection criterium was to include samples that represented low, medium and high levels of organic content. All four samples mentioned above were extracted and analyzed in triplicates, each according to the same procedures as described in Sections 4.4.1 and 4.4.4.

4.5 STATISTICAL METHODS

4.5.1 Data description

Descriptive statistics was performed with SPSS® version 25 (IBM). Data was first checked for normal distribution by looking at the histograms, skewness and kurtosis. Additionally, normality was examined by the Shapiro-Wilk test. If the hypothesis of normality was rejected, log-transformation was performed in an attempt to achieve normal distribution.

Comparison between two groups was performed by student's t-test for normally distributed data, and by Mann-Whitney U-test for non-normal data. Comparison between more than two groups was performed by one-way ANOVA for normal distributed data, or Kruskal-Wallis H-test for non-normal data. For pairwise comparisons, Tukey HSD post hoc were applied if the data passed the Levene test for homogeneity of variances, and only if the group sizes were equal, else the Games-Howell post hoc test was applied. Mann-Whitney U-test was used for pairwise comparison for data analyzed with Kruskal-Wallis.

Interaction between the variables were calculated by Spearman correlations, since normal distribution was not possible to obtain even by log-transformation, as required by Pearson correlation.

Regarding measurements described throughout the text, the averages are expressed as averages (means) with standard deviation (SD) in the format (AVG ± SD), unless specified otherwise. The boxplots in Section 5 excludes values as outliers when 1.5 × interquartile range (IQR) is exceeded.

4.5.2 Principal component analysis

Principal component analysis (PCA) was applied to the combined dataset, and subgroups of the dataset, with three purposes: to identify which variables followed each other, to discover tendencies of grouping among the samples, and to examine which variables influenced a specific sample or group of samples. As the combined dataset in this study had

126 variables, it would be practically impossible to visualize them graphically within one plot. This was solved with PCA by dimensional reduction. PCA was performed with R version 3.4.3.

The dataset underwent preprocessing by mean-centering and by autoscaling since the variables originated from different analytical techniques, and therefore varied greatly by magnitude. In the final stage, PCA was performed after selecting variables confirming to the Kaiser-Meyer-Olkin test for sample adequacy (KMO). The latter was done to improve the performance of the PCA.

PCA requires that there are no blank values in the dataset. Usually samples/rows that have at least one blank should be removed entirely. However, according to this criterium every sample disqualifies since each sample lacks at least one organic peak. This has been solved by giving non-detected peaks the value zero. Regarding samples for inorganic analysis, the amount (37 in total) were fewer than for the organic analyses (52 in total). This inconsistency has been solved by assuming that whenever replicates are missing for inorganic analyses, replicates are assumed to have the same values. This approach is justified by looking at actual replicates when they are present, where levels do not differ much for the majority of the samples.

Results are shown in separate scores and loadings plots. The choice was to not present biplots, as too many variables produced unnecessary visual clutter. However, the plots are placed side by side to facilitate the visualization of the relationship between loadings and scores.

5 RESULTS

5.1 PHYSIO-CHEMICAL ANALYSIS OF SEDIMENTS

5.1.1 Sediment composition and TOM

Overall, the marine sediments were rich in sand (0.06 - 2 mm), with an average contribution of 79 ± 13 %. After sand, pelite (< 0.06 mm) was the second major fraction (15 ± 11 %), while gravel (>2 mm) only contributed 7.0 ± 8.6 % (Kruskal-Wallis H-test, $H_{(2)} = 112.541$ at $p = 0.001$) (Overview of grain size distributions is shown in Figure A.1, Appendix A). Comparing two different installations (Figure 5.1), the content of gravel was significantly higher ($p < 0.05$) near installation A (Hestøya) (8.2 ± 8.8 %) than near installation B (Nørholmen) (0.22 ± 0.12 %) (Mann-Whitney U-test, $U = 5.000$ at $p = 0.001$). However, sand and pelite contents were not significantly different. All raw data mentioned in the results can be made available upon request.

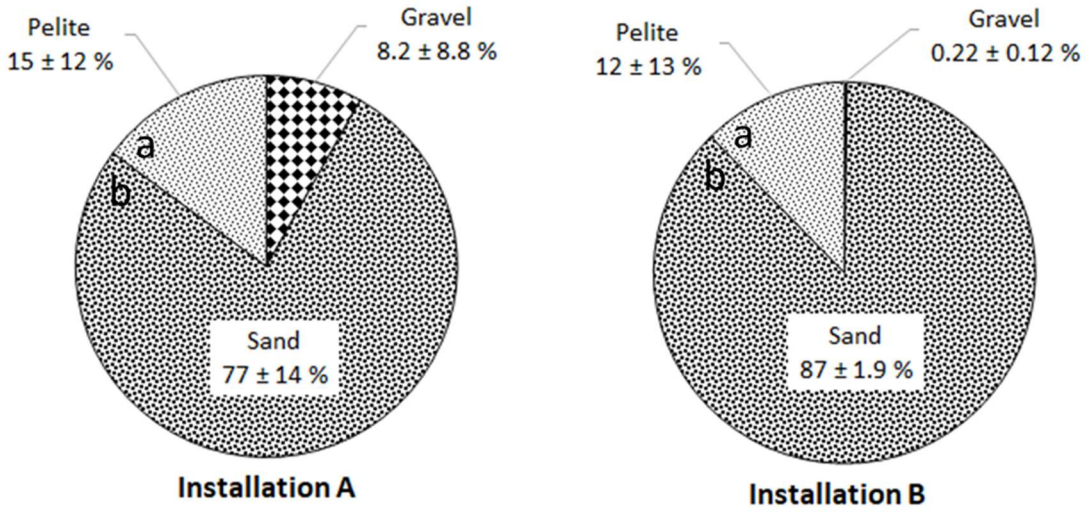

Figure 5.1 Comparison of average grain size distribution between installation A (Hestøya) and B (Nørholmen). Standard deviations are shown with the averages. Equal letters indicate no significant difference ($p > 0.05$). $N = 44$ for installation A, and $N = 8$ for installation B.

Distances from the installations did not seem to have any impact on the sediment grain size distribution represented by the pelite content (Figure 5.2). In contrast, sampling directions showed a significant impact on the sediment composition, as pelite levels were significantly different between different sampling directions (Kruskal-Wallis H-test, $H_{(4)} = 27.475$ at $p = 0.001$). Pairwise comparison by post hoc tests, revealed significantly lower ($p < 0.05$) pelite

contribution in sediments at directions A1 and A2 compared with A3. Direction A2 was also significantly lower than A4 ($p < 0.05$).

Average total organic matter (TOM) content was 7.3 ± 4.9 %, with 7.9 ± 5.1 % and 4.0 ± 0.5 % for installations A and B respectively. TOM levels varied significantly (Kruskal-Wallis H-test, $H_{(4)} = 30.535$ at $p = 0.001$) for different sampling directions, with significantly higher ($p < 0.05$) levels for the northbound directions (A3 and A4) compared to the southbound directions (A1 and A2) around installation A, and compared to the sediments near installation B (B1). In a similar fashion to pelite, TOM content did not vary significantly between the two installations considering the averages of all directions, nor did TOM vary significantly as a function of distance away from the installations (Figure 5.2).

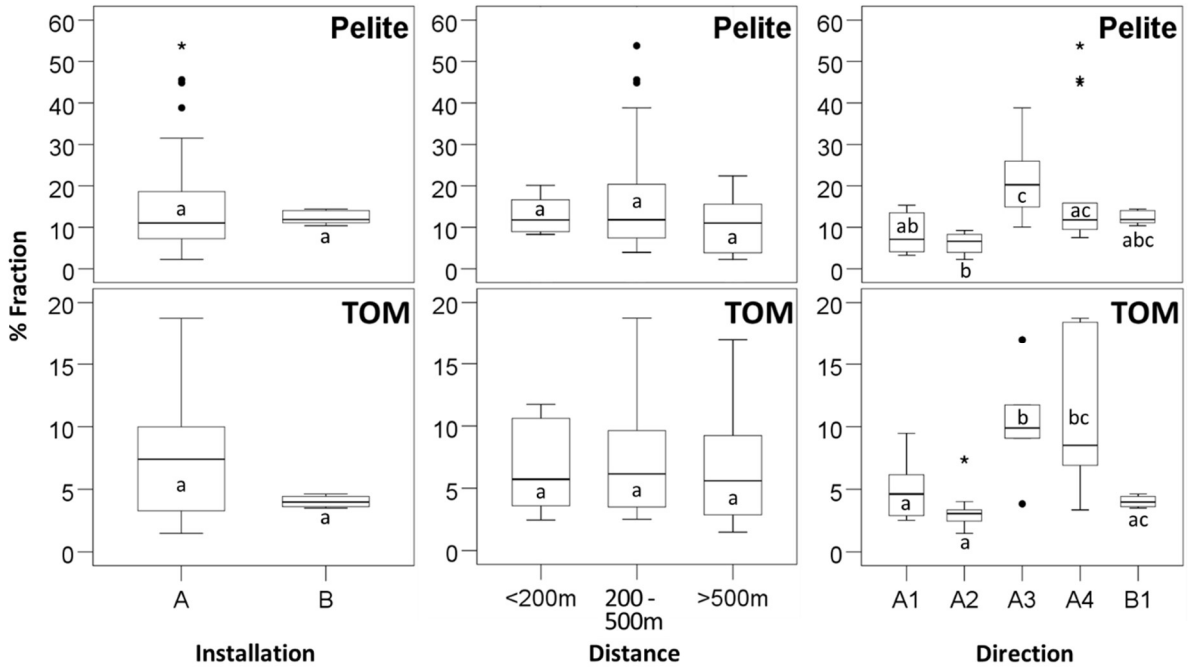

Figure 5.2 Sediment content of pelite and TOM, comparing: installation A (Hestøya) and B (Nørholmen); distances (<200 m, 200-500 m, >500 m); directions of sampling from the installations (A1: southeast; A2: south; A3: northwest; A4: north; B1: installation B has only one axis). Boxes: median and 25/75 percentiles; bars: minima and maxima; dot: outlier (1.5×IQR); starred dot: outlier (3×IQR). Significant differences ($p < 0.05$) between groups are shown by letter codes (same letter means no significant difference). Installations: $N = 44$ for A, $N = 8$ for B; distances: $N = 8$ for <200 m, $N = 29$ for 200-500 m, $N = 16$ for >500 m; directions: $N = 10$ for A1, $N = 9$ for A2, $N = 12$ for A3, $N = 13$ for A4, $N = 8$ for B1.

5.1.2 Conductivity and pH

The pH values were significantly higher ($p < 0.05$) for installation B (Nørholmen) (8.46 ± 0.16), than for installation A (Hestøya) (8.09 ± 0.32) (Mann-Whitney U-test, $U = 65.500$

at $p = 0.003$) (Figure 5.3). Conductivity values were also higher for installation A (13.6 ± 7.3 mS cm^{-1}) than for installation B (8.97 ± 0.63 mS cm^{-1}), however these differences were not significant (Figure 5.3).

Considering the different sampling directions, there were significant differences ($p < 0.05$) for pH (Kruskal-Wallis H-test, $H_{(4)} = 23.361$ at $p = 0.001$) and for conductivity (Kruskal-Wallis H-test, $H_{(4)} = 30.860$ at $p = 0.001$). Pairwise comparison by post hoc tests for pH showed significantly lower ($p < 0.05$) values at directions A3 and A4 compared with B1. Direction A3 was also significantly lower ($p < 0.05$) than A2. Regarding conductivity, directions A3 and A4 were significantly higher ($p < 0.05$) than in A2. Direction A3 was also significantly higher ($p < 0.05$) than A1.

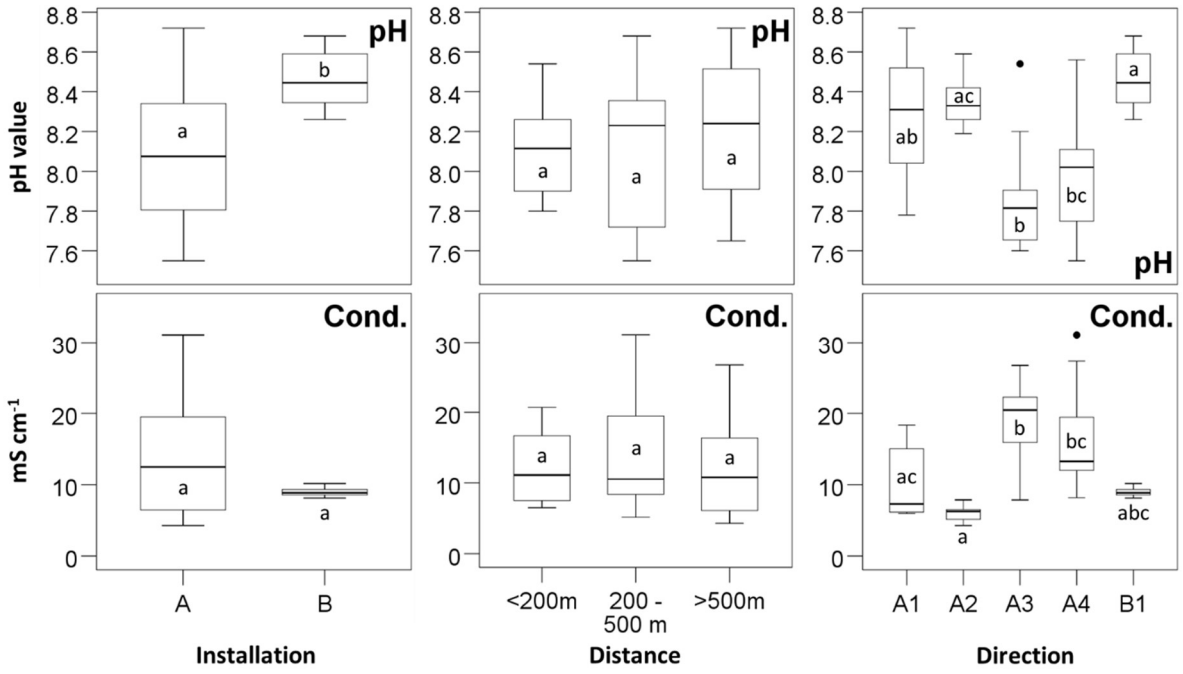

Figure 5.3 Sediment pH and conductivity, comparing: installation A (Hestøya) and B (Nørholmen); distances (<200 m, 200-500 m and >500 m); directions from the installations (A1: southeast; A2: south; A3: northwest; A4: north; B1: Installation B has only one axis). Boxes: median and 25/75 percentiles; bars: minima and maxima; dot: outlier (1.5×IQR). Significant differences ($p < 0.05$) between groups are shown by letter codes (same letter means no significant difference). Installations: $N = 44$ for A, $N = 8$ for B; distances: $N = 8$ for <200 m, $N = 29$ for 200-500, $N = 16$ for >500 m; directions: $N = 10$ for A1, $N = 9$ for A2, $N = 12$ for A3, $N = 13$ for A4, $N = 8$ for B1.

5.2 ELEMENTAL COMPOSITION OF SEDIMENTS

In total 47 elements were analyzed in 37 sediment samples. However, only 16 elements were chosen for further analysis based on an evaluation of their significance in the context of pollution from aquaculture installations: lead (Pb), mercury (Hg), cadmium (Cd), arsenic (As),

Copper (Cu), Nickel (Ni), aluminum (Al), tin (Sn), Chromium (Cr), zinc (Zn), iron (Fe), manganese (Mn), barium (Ba), antimony (Sb), sulfur (S) and phosphorus (P).

Average values (± standard deviation) for the selected elements are presented in Table 5.1. The abundance of these elements was in the descending order, as following: Fe > Al > S > P > Mn > Ba > Zn > Cr > Ni > Cu > Pb > As > Sn > Cd > Sb > Hg. Regarding Zn, one outlier was removed from the dataset (Sample 09A: 4360 µg g^{-1} dw, 325 × IQR).

Table 5.1 Average concentrations (± standard deviation) near two fish farm installations of elements selected by their potential environmental concern. $N = 37$ except for Zn where $N = 36$.

	Avg. conc. *µg g^{-1} dw*		***Avg. conc.*** *µg g^{-1} dw*		***Avg. conc.*** *µg g^{-1} dw*		***Avg. conc.*** *µg g^{-1} dw*
Fe	8590 ± 2250	Mn	180 ± 56	Ni	9.24 ± 3.30	Sn	0.401 ± 0.150
Al	7820 ± 200	Ba	30.2 ± 4.6	Cu	6.91 ± 3.95	Cd	0.362 ± 0.033
S	3410 ± 2190	Zn	24.6 ± 9.1	Pb	6.28 ± 3.29	Sb	0.078 ± 0.075
P	554 ± 176	Cr	19.6 ± 5.2	As	3.18 ± 1.82	Hg	0.031 ± 0.016

Measurements for Cr, Sn, Ba and Zn were normally distributed according to the Shapiro-Wilk test for normality ($p > 0.05$). For the remaining elements, the null-hypothesis that the population is normally distributed was rejected ($p < 0.05$).

5.2.1 Sediment classification

The environmental state of the sediments were assessed based on current Norwegian legislation (Norwegian Environment Agency, 2016) and measurements of element concentration in sediment samples by ICP-MS. Table 5.3 gives an overview of measured concentrations for the metals and metalloids of most concern, as well as total organic carbon (TOC), measured through loss on ignition by assuming that 50 % of the total organic matter (TOM) contained carbon. A legend describing the color codes is given in Table 5.2. Limit values for classification of elements of special concern and TOC are presented in Table A.2 (Appendix A).

Table 5.2 Description of color coding for sediment classification. Background values are given by the Oslo/Paris convention (OSPAR) (Norwegian Environment Agency, 2016). PNEC is an acronym for Predicted No Effect Concentration.

	Class I	< OSPAR	"Background"
	Class II	< $PNEC_{Chronic}$	"Good"
	Class III	< $PNEC_{Intermittent}$	"Moderate"
	Class IV	< $PNEC_{Intermittent}$ × 2-5	"Bad"
	Class V	> $PNEC_{Intermittent}$ × 2-5	"Very Bad"

This assessment was based on 24 sediment samples near installation A (Hestøya) and 6 samples near installation B (Nørhomen). Only samples from the top layer (0-2 cm) were included. Regarding the selected metals and the metalloid As, all the measured concentrations suggested either Class I "Background" or Class II "Good". Cd concentrations were mostly above background levels around installation A. Some Hg concentation were also above background levels.

Table 5.3 Sediment classification according to Norwegian legislation (Norwegian Environment Agency, 2016) near two fish farm installations. TOC concentrations are normalized by pelite content. All concentrations are for dry weights (dw) of sediments.

Sample ID	***Inst.***	***Distance*** *m*	***Hg*** *µg g^{-1}*	***Cr*** *µg g^{-1}*	***Ni*** *µg g^{-1}*	***Cu*** *µg g^{-1}*	***Zn*** *µg g^{-1}*	***Cd*** *µg g^{-1}*	***Pb*** *µg g^{-1}*	***As*** *µg g^{-1}*	***nTOC*** *mg g^{-1}*
01A	A	178	0.047	25	13	9.9	31	0.48	6.7	3.8	63
01B	A	178	0.034	23	11	7.8	25	0.35	5.9	3.2	53
02A	A	278	0.026	18	7.1	4.6	17	0.24	3.8	2.2	46
02B	A	278	0.013	21	8.3	5.2	21	0.21	4.4	2.6	46
03A	A	383	0.025	20	7.7	4.7	20	0.18	3.8	2.1	33
04A	A	728	0.012	13	5.1	2.0	11	0.055	2.0	1.2	30
05A	A	1095	0.013	9.1	4.8	1.6	19	0.056	2.6	1.2	34
06A	A	593	0.016	15	5.5	1.7	13	0.033	2.0	1.2	25
07A	A	390	0.036	22	11	8.6	39	0.46	6.1	3.7	54
08B	A	289	0.030	19	7.2	2.9	16	0.10	2.8	1.6	32
09A	A	190	0.011	17	6.4	3.4	*	0.21	4.6	1.4	29
09B	A	190	0.019	21	8.3	4.1	21	0.17	3.9	2.2	36
10A	A	176	0.049	21	12	11	31	0.55	7.3	5.0	73
11A	A	363	0.055	30	17	12	38	0.89	10	6.8	61
12A	A	468	0.051	28	14	12	32	0.81	8.0	5.8	61
13A	A	668	0.038	21	10	8.8	23	0.58	5.8	4.2	65
14A	A	1043	0.055	23	14	14	36	0.69	8.2	6.4	100
15A	A	1083	0.027	10	5.4	5.3	13	0.21	3.4	2.5	56
15B	A	1083	0.025	16	8.3	7.5	19	0.37	5.3	3.6	57
16A	A	779	0.024	17	6.6	5.6	16	0.27	4.5	2.6	51
17A	A	471	0.087	34	20	20	50	1.6	14	9.4	100
18A	A	363	0.057	25	14	13	40	1.0	10	4.8	110
19A	A	232	0.031	14	7.8	7.2	21	0.40	5.5	3.8	59
19B	A	232	0.042	16	9.2	9.4	28	0.55	7.4	4.1	65
19.5A	B	778	0.044	21	9.3	10	35	0.087	12	1.9	*
20A	B	599	0.035	20	9.4	6.3	26	0.086	12	2.3	34
20B	B	599	0.030	20	9.2	6.0	26	0.10	12	2.0	35
21A	B	484	0.017	18	7.6	5.1	25	0.091	9.1	2.0	34
22A	B	364	0.034	21	9.4	8.6	32	0.12	11	2.1	37
23A	B	357	0.025	22	8.1	6.6	27	0.22	9.9	2.0	39

*Blank cells are either outliers (Zn) or non-measured values (nTOC)

On the other hand, normalized total organic carbon (nTOC) had an average level of a factor 2.8 and 1.8 higher than stipulated background levels for installation A and B respectively. The majority of the samples near installation A classified therefore as Class V "Very bad", only one sample (06A) classified as Class II "Good". Regarding installation B, samples classified as Class IV "Bad" except one sample, which classified as Class III "Moderate".

5.2.2 Comparison of elemental concentration between two installations

Concentrations at installation A (Hestøya) and B (Nørholmen) for 16 selected elements related to potential pollution from human activities, are presented as boxplots in Figure 5.4. Concentration of Pb was significantly higher ($t_{(28)}$ = -4.200 at p = 0.001) by a factor of almost two at installation B (10.9 ± 1.2 µg g^{-1} dw) than A (5.77 ± 2.89 µg g^{-1} dw). However, the average concentrations of Cd (0.438 ± 0.369 µg g^{-1} dw and 0.116 ± 0.052 µg g^{-1} dw for installation A and B respectively); As (3.55 ± 2.05 µg g^{-1} dw and 2.04 ± 0.14 µg g^{-1} dw for installation A and B respectively); Sb (0.0719 ± 0.0397 µg g^{-1} dw and 0.0324 ± 0.0119 µg g^{-1} dw for installation A and B respectively) were significantly higher (pairwise comparison by Mann Whitney U-test at $p < 0.05$) near installation A compared to B. For the remaining elements, there were no significant differences.

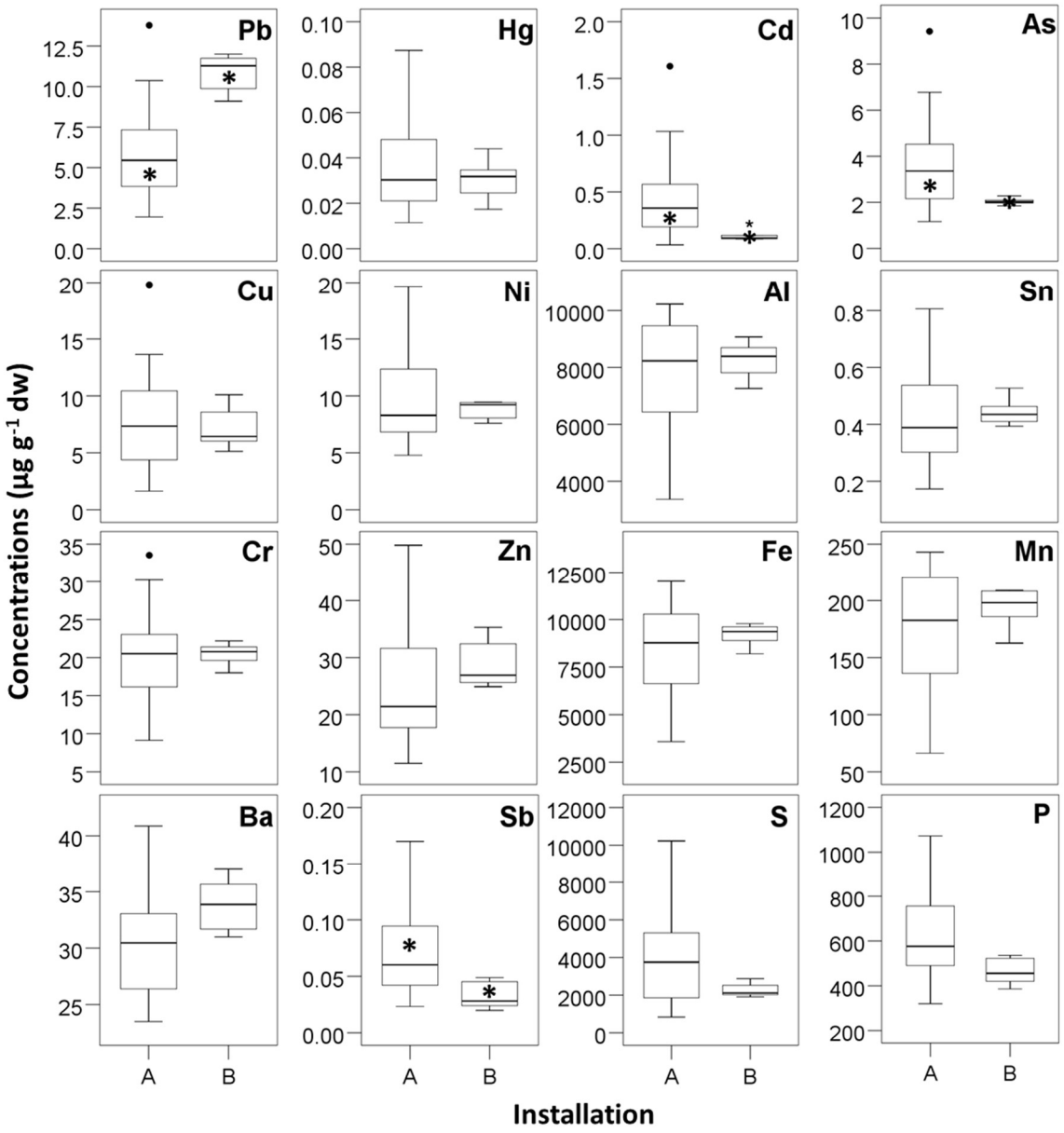

Figure 5.4 Concentrations (µg g^{-1} dw) of 16 elements for installation A (Hestøya) and B (Nørholmen). Boxes: median and 25/75 percentiles; bars: minima and maxima; dot: outlier (1.5×IQR); starred dot: outlier (3×IQR). Only samples from the top layer (0-2) cm are included. Asterisks (*) represents significant difference ($p < 0.05$) between the installations. $N = 24$ for installation A, except for Zn where $N = 23$. $N = 6$ for installation B.

5.2.3 Elemental concentrations on different distances from the installations

Concentrations at distances closer than 500 m from the installations and above 500 m for 16 selected elements related to potential anthropogenic sources, are presented as boxplots in Figure 5.5. A radius of 500 m from marine fish farms is roughly considered the zone of influence regarding the sediments (*transition zone*) according to Norwegian Standard NS 9410:2016 (Standards Norway, 2016).

The following elements were significantly higher at distances below 500 m compared with distances above 500 m from the installations: Cd (Mann-Whitney U-test, $U = 57.0$ at $p = 0.042$); Al (Mann-Whitney U-test, $U = 37.0$ at $p = 0.003$); Sn (t-test, $t_{(28)} = 2.916$ at $p = 0.007$); Cr (t-test, $t_{(28)} = 2.777$ at $p = 0.010$); Fe (t-test, $t_{(28)} = 3.231$ at $p = 0.003$) ; Mn (t-test, $t_{(28)} = 2.542$ at $p = 0.017$). In contrast, none the 16 selected elements show significantly higher concentrations at distances above 500 m than for distances under 500 m.

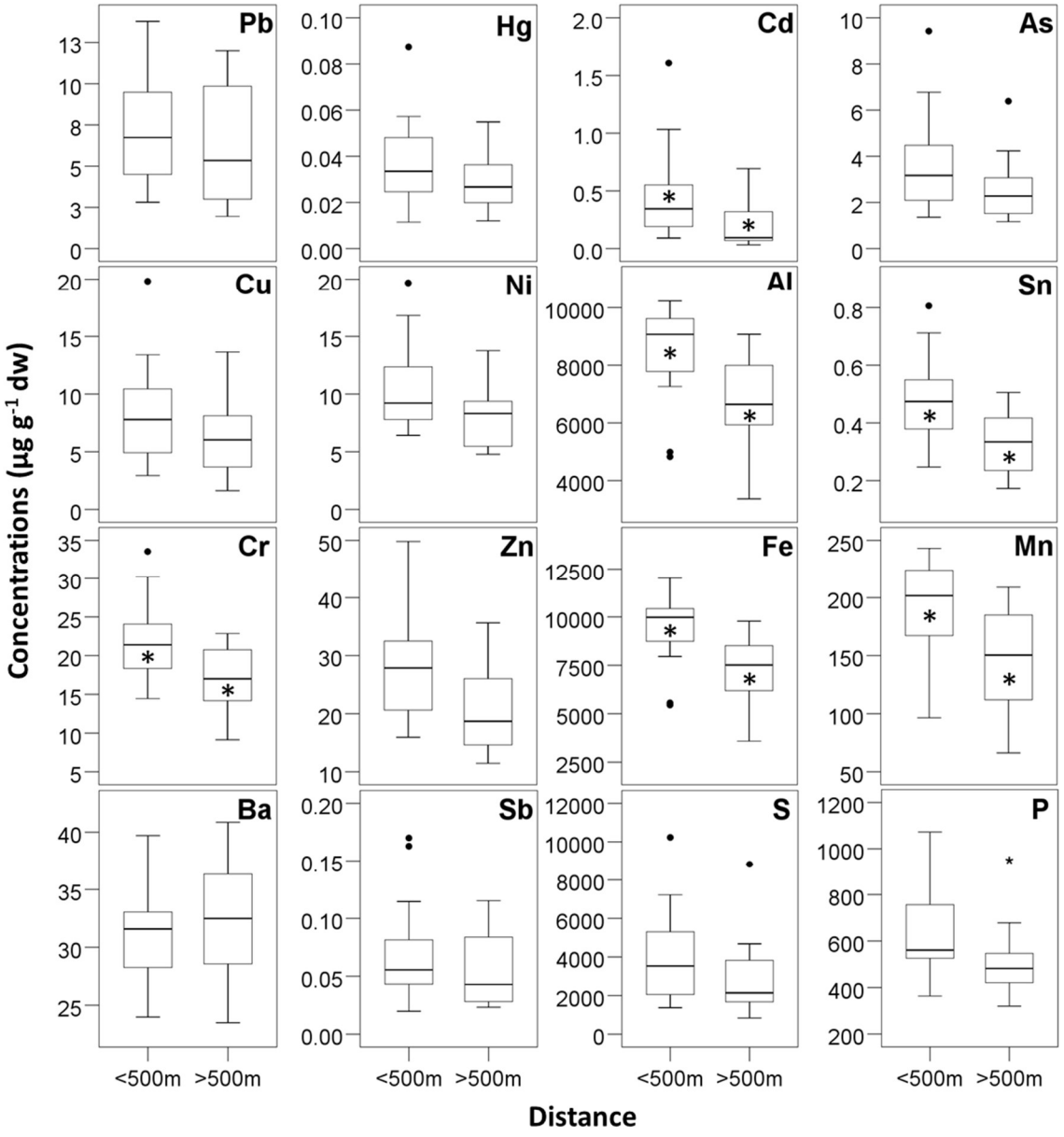

Figure 5.5 Concentrations (µg g^{-1} dw) of 16 elements for distances <500 m and >500 m. Boxes: median and 25/75 percentiles; bars: minima and maxima; dot: outlier (1.5 × IQR). Only samples from the top layer (0-2 cm) were included. Asterisks (*) represent a significant difference ($p < 0.05$) between the installations. $N = 19$ for distances <500 m except for Zn where $N = 18$. $N = 11$ for distances >500 m.

The elements that had a significantly higher ($p < 0.05$) concentration closer to the installations as shown in Figure 5.5, were analyzed further by dividing distances into three groups: <200 m, 200–500 m and >500 m. Only samples from installation A were considered, because the number of samples from installation B was not statistically sufficient ($N < 3$) at this resolution. Overall, the concentrations of Al (Kruskal-Wallis H-test, $H_{(2)} = 9.495$ at $p = 0.009$), Cr (ANOVA, $F_{(2,21)} = 4.329$ at $p = 0.027$), Mn (ANOVA, $F_{(2,21)} = 5.474$ at $p = 0.012$), Sn (ANOVA, $F_{(2,21)} = 4.072$ at $p = 0.0329$) and Fe (Kruskal-Wallis H-test, $H_{(2)} = 10.403$ at $p = 0.006$) were significantly higher close to the installations (< 200 m and 200-500 m) and decreased significantly at distances greater than 500 m away from the installations (Figure 5.6). The concentrations of Cd did not show any significant difference between the distance groups at this resolution, which differed from the conclusion derived from Figure 5.5. This is probably due to the use of only data from installation A for the data in Figure 5.6.

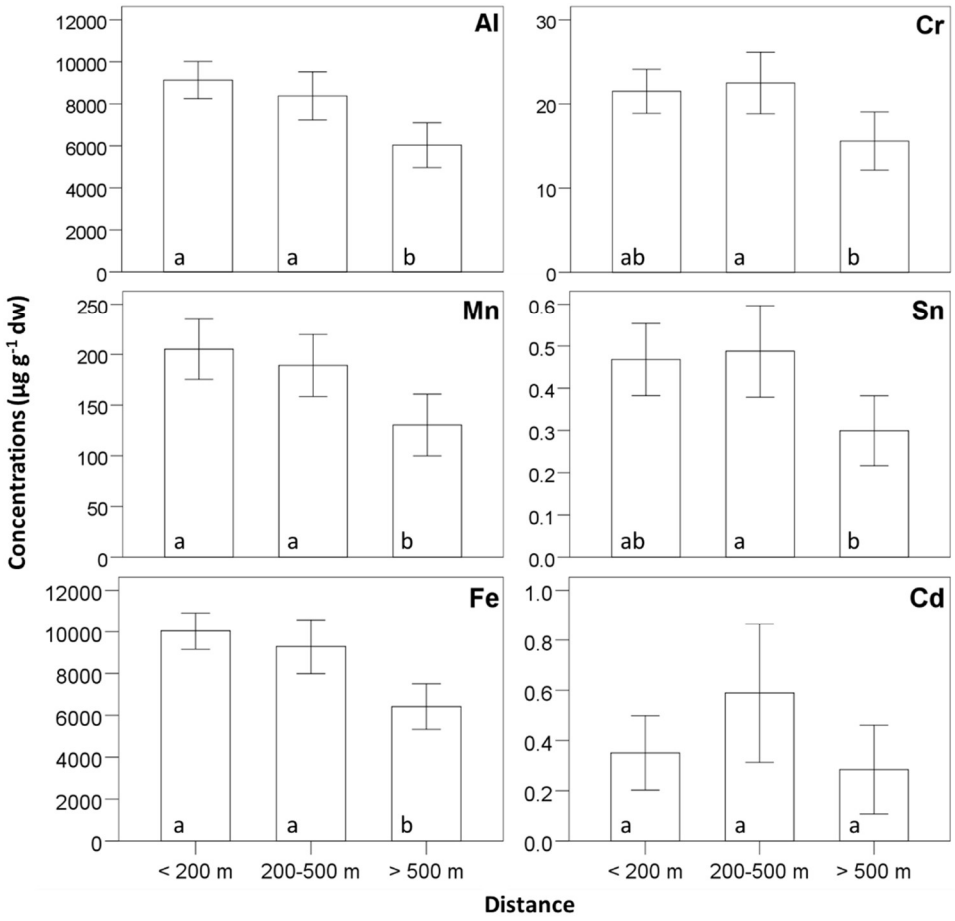

Figure 5.6 Concentrations (µg g^{-1} dw) of six selected elements at distances <200 m, 200-500 m and >500 m for installation A (Hestøya). Bars shows average concentrations with error bars 2×standard error. Equal letters inside the bars marks distance groups that show no significant difference ($p > 0.05$). $N = 5$ for distances <200 m, $N = 11$ for distances 200-500 m and $N = 8$ for distances >500 m.

Pairwise comparison by post hoc tests revealed that distances less than 200 m did not differ significantly ($p > 0.05$) from distances 200-500 m for all selected metals. However, concentrations at distances under 500 m (<200 m and 200-500 m) were significantly higher than distances above 500 m for Al, Mn and Fe. In contrast, Cr and Sn were significantly higher at 200-500 m and above 500 m. Surprisingly, Cr and Sn concentrations did not differ significantly between samples collected at below 500 m and above 500 m from the installation (Figure 5.6).

5.3 NON-TARGETED SCREENING OF ORGANIC COMPOUNDS

5.3.1 Total ion chromatograms

Total ion chromatograms (TICs) for 52 sediment samples extracted with dichloromethane by pressurized liquid extraction (PLE) and analyzed with GC-MS, were characterized. Two samples (08B and 12A) are presented as examples in Figure 5.7 as they produced relatively low and high responses (abundances) respectively. The distinct peak around 42 minutes seemed to be mainly elemental sulfur according to the mass spectra based on database queries (NIST library), hereby named the "Sulfur peak". This peak was present at varying degrees in all analyzed sediment samples. Peaks present after the "Sulfur peak" were considered unreliable in this study. Therefore, the focus was only on retention times between 5 to 40 minutes for all chromatograms.

Since the response (abundance) was low for most peaks (lower than 10^5), measures were taken to distinguish contamination of the analytical system from analytes extracted from the sediment samples. Nine method blanks were included for this purpose. One example of a method blank is shown in Figure 5.8, as well as the TIC of an analysis of the extraction solvent concentrated by the same method as the sample extract, showing what were probably impurities in the solvent and what were contamination from the extraction system.

As running bake-out between each sample would be too time consuming to perform, the cross contamination between each run as shown in the bottom right TIC in Figure 5.8, must be taken into consideration. Noise seemed to accumulate after approximately 45 minutes for each run. However, retention times below 40 minutes seemed to be unaffected. This was also a reason why this study only considered retention times from 5 to 40 minutes.

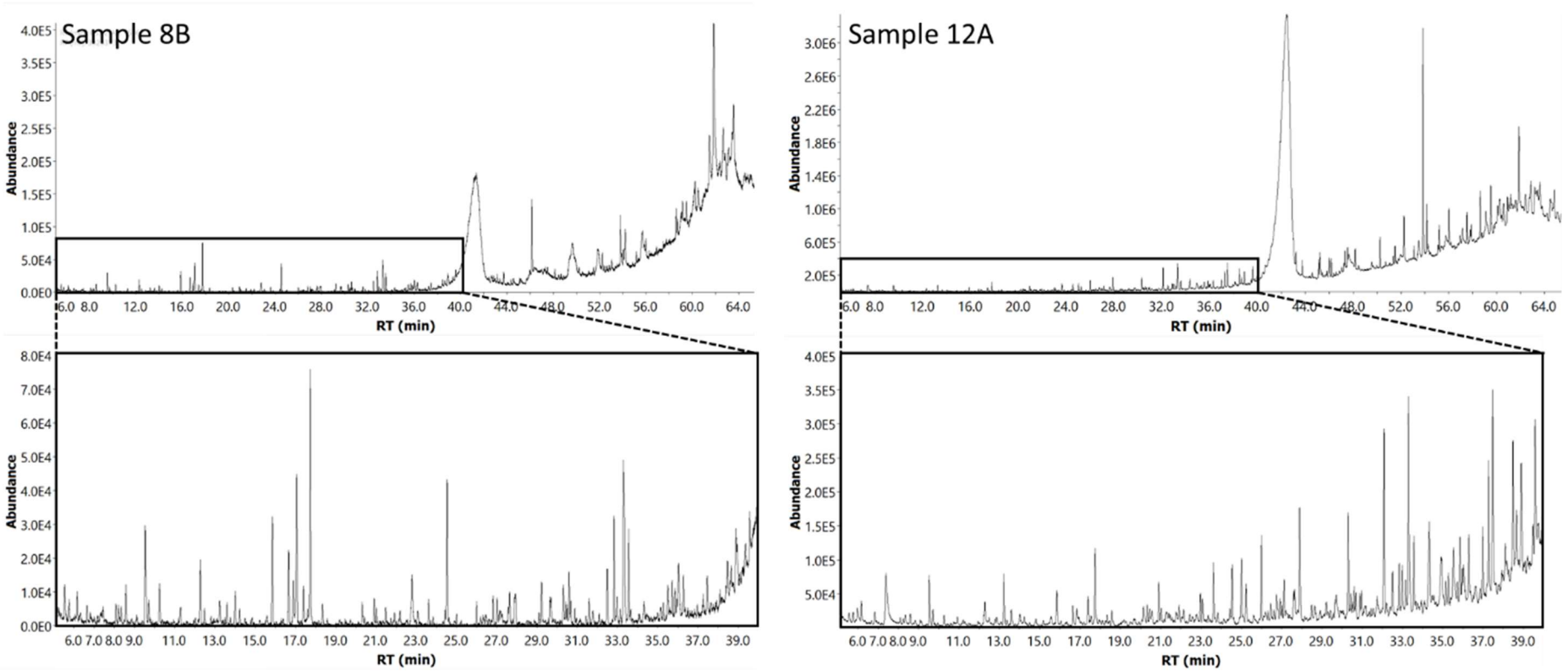

Figure 5.7 Examples of GC-MS TICs analyzed from untreated dichloromethane ASE extracts. The bottom row zooms into retention times 5-40 minutes.

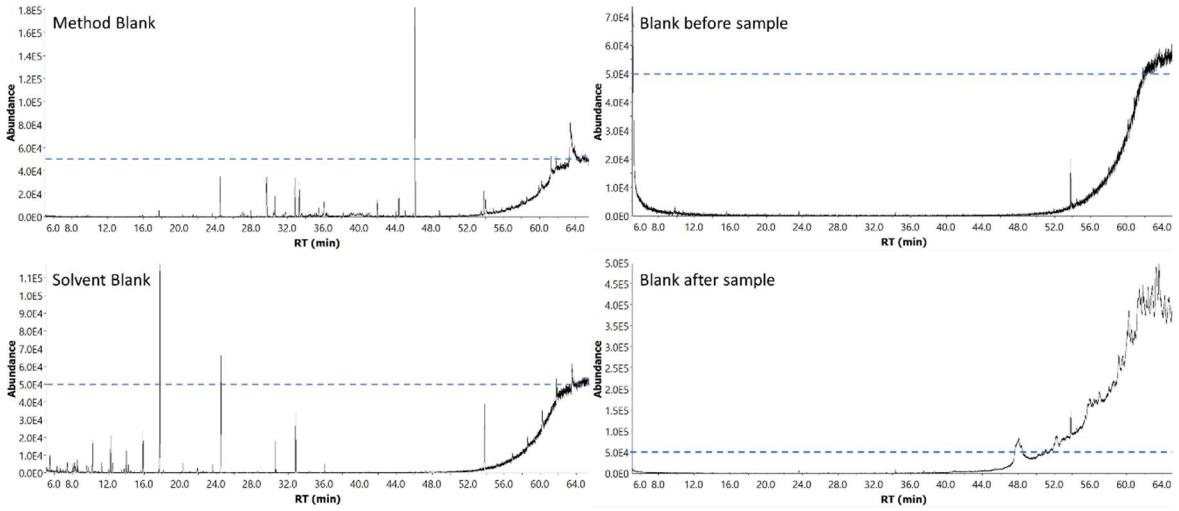

Figure 5.8 Examples of TICs from method blanks, concentrated solvent blanks, and blanks run before and after the samples. Abundance of 50,000 is marked by a dotted line for easy comparison. The blanks shown to the right side are untreated solvent injected directly into the GC-MS. The top right TIC shows the blank before samples are run, i.e. right after bake-out, and the bottom right TIC shows the blank after samples are run.

5.3.2 Peak identification

Mass spectra for the peaks present in the TICs for both samples and method blanks were compared with mass spectra in the National Institute of Standards and Technology's Mass Spectral Library (NIST Standard Reference Database 1A v17) supplied by the software package for the GC-MS used in this study (Agilent ChemStation). Three examples of matched mass spectra are shown in Figure 5.9, all with a high match value above 900.

Identification by matching mass spectra against the NIST library did not provide any final confirmation. For this it was necessary to confirm the peak by adding standard material as internal standard. Figure 5.10 shows an example of positive confirmation of benzaldehyde and 3-bromophenol at retention times 13.2 and 30.3 minutes respectively.

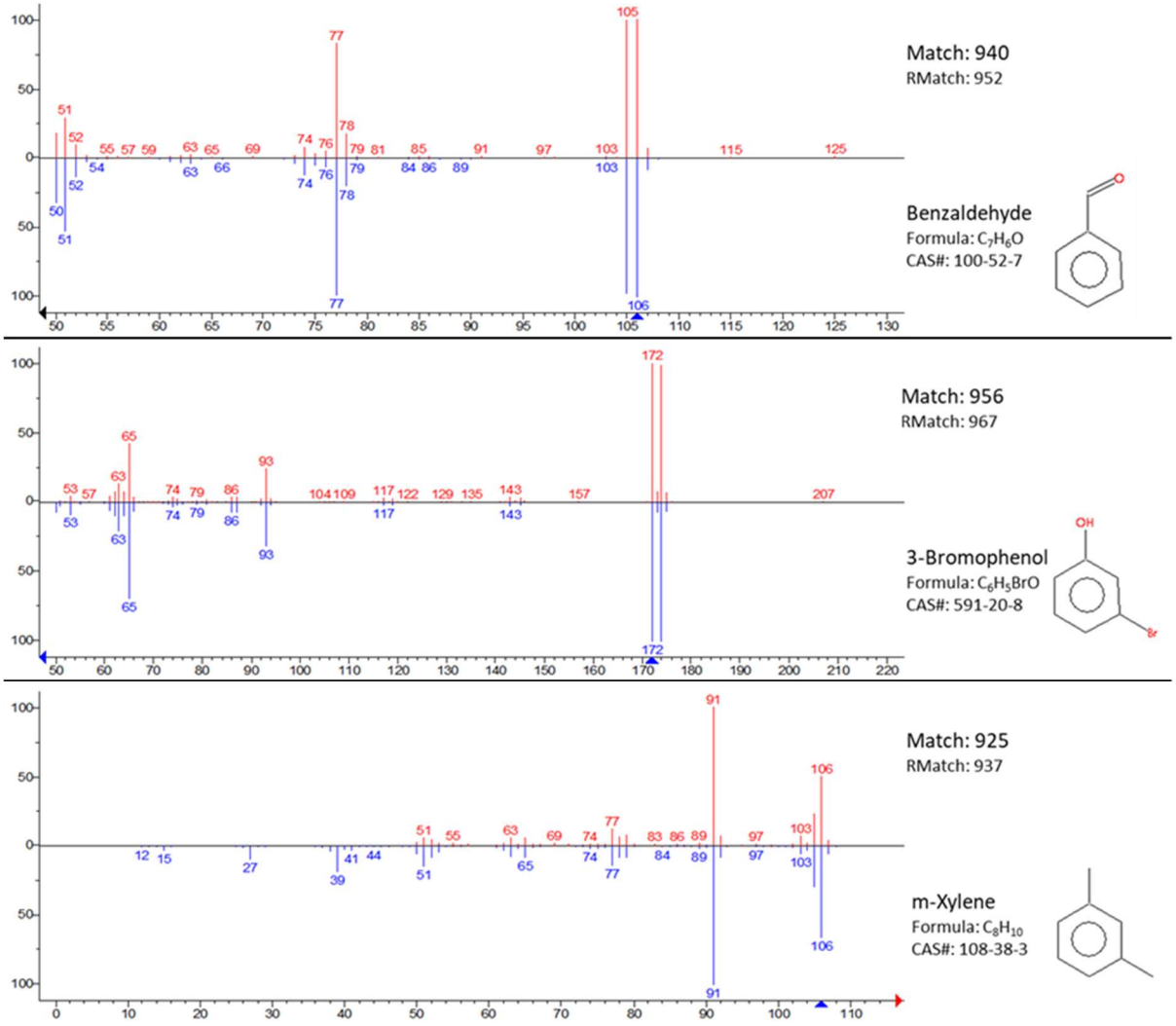

Figure 5.9 Three examples of mass spectra from analyzed sediment samples (red) matched with mass spectra in the NIST Mass Spectral Library (blue). The most probable compound is shown to the right.

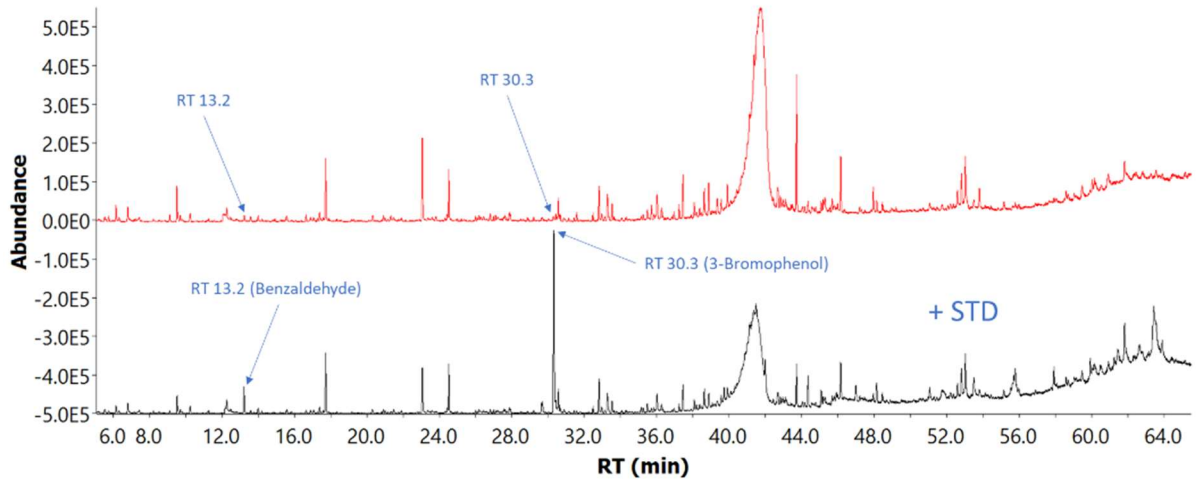

Figure 5.10. Peak confirmation by adding reference material. Top TIC is before addition, bottom TIC is after addition of benzaldehyde and 3-bromophenol standard materials acquired from Sigma-Aldrich.

5.3.3 Suggested compounds

After excluding the peaks present in the method blanks, among other exclusion criteria described in Section 4.4.4, a list of 61 suggested compounds related to each TIC peak was produced (Table A.3, Appendix A). The mass spectra of 15 of these 61 compounds gave a high match value above 850 against the NIST library, which are shown in Table 5.4. Lower match values could be attributed to poor noise removal, but nonetheless gave a suggestion of possible compound. The average peak maxima and relative frequencies of the 61 detected peaks presumably from the sediment samples, is represented graphically in Figure 5.11. Raw data can be made available upon request.

Table 5.4 Compounds possibly identified in 52 sediment samples with NIST library match values above 850. Also shown is the frequency (FRQ) of appearance, average abundance by peak maxima (AVG) with standard deviation (STDEV), and the best match/reversed match achieved together with the id of the corresponding sample (MATCH@ID).

RT Min	*CAS#*	*NAME (non-IUPAC italicized)*	*FRQ*	*AVG*	*STDEV*	*MATCH@ID*
RT7.4	106-42-3	p-Xylene	0.60	32961	21901	911/934@12A
RT9.7	111-71-7	Heptanal	0.90	15804	7068	894/896@14A
RT13.2	100-52-7	Benzaldehyde	0.92	30731	24974	940/952@14A
RT16.7	2548-87-0	2-Octenal	0.73	16777	8128	882/888@14A
RT16.9	111-87-5	1-Octanol	0.25	25782	29670	946/962@22A
RT20.4	5205-34-5	5-Decanol	0.40	90590	199798	932/932@22A
RT20.6	2471-84-3	*1-Methylideneindene*	0.23	17576	5558	884/923@23A
RT20.9	1125-21-9	*4-Oxoisophorone*	0.90	26429	19143	919/948@11C
RT25.0	475-03-6	*α-Ionene*	0.77	35852	29891	911/932@18A
RT25.2	30364-38-6	1,1,6-Trimethyl-1,2-dihydronaphthalene	0.58	26084	17488	905/952@11C
RT26.3	23950-04-1	*α-Nicotine*	0.23	11241	4869	858/915@01A
RT27.9	575-37-1	1,7-Dimethylnaphthalene	0.94	54975	48433	946/966@11C
RT28.9	615-58-7	2,4-Dibromophenol	0.10	22456	30594	920/924@22B
RT30.3	591-20-8	3-Bromophenol	1.00	78815	91665	956/967@21B
RT37.5	102608-53-7	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.98	93993	97116	860/936@18B
RT39.7	120-12-7	Anthracene[1]	0.75	25261	22601	824/918@23A

[1]Anthracene has been included because of its environmental relevance, even though the match is below 850.

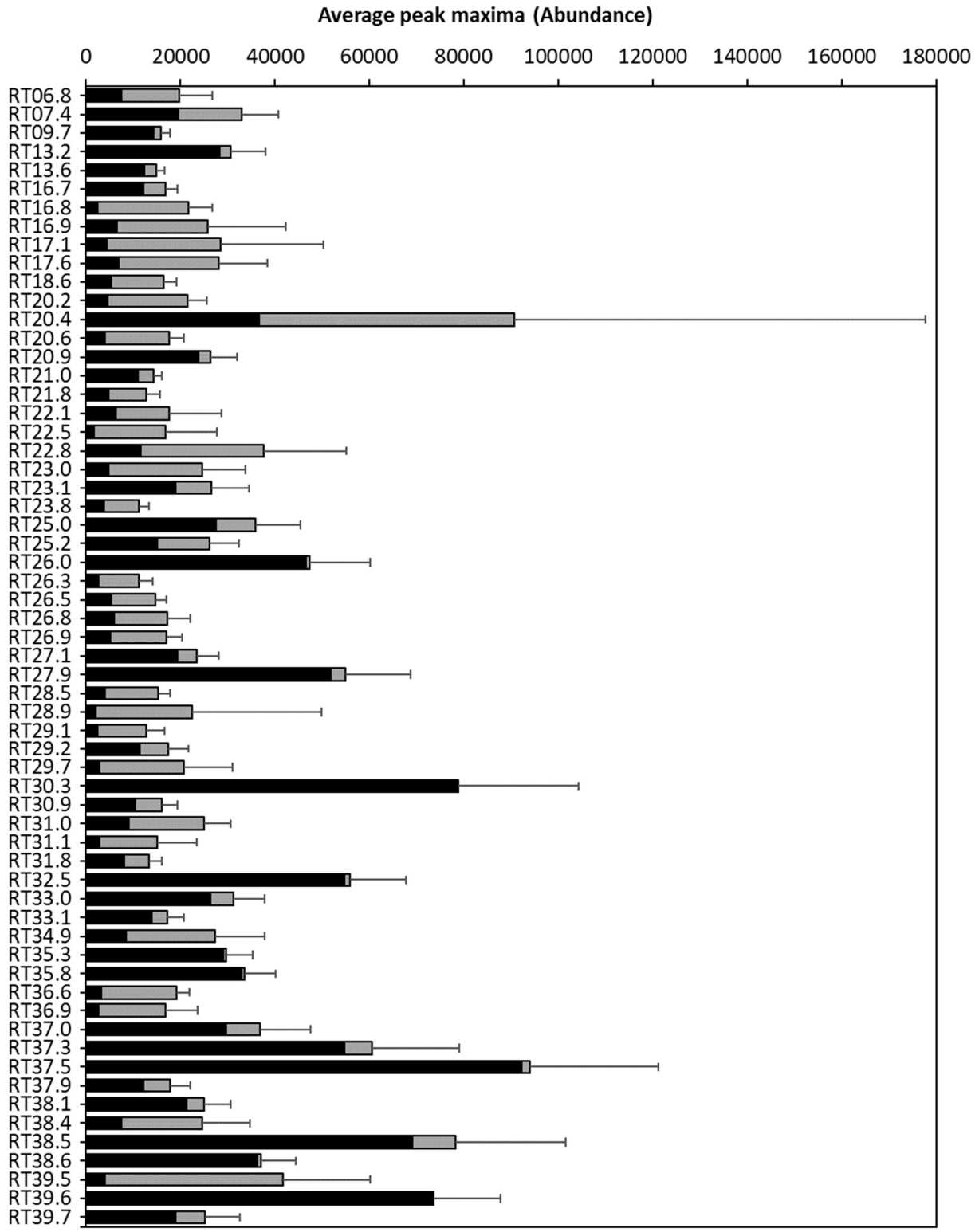

Figure 5.11 Graphical presentation of average peak maxima of the peaks found in the sediment samples. The black area of the bar shows the frequency of samples with positive detection of that specific peak relative to the average peak maxima (avg. peak max. × frequency), while the sum of black and grey areas shows the average peak maxima. Error bars represent 2 × standard error.

5.3.4 Stability of analytical system

The stability of the analytical system, i.e. extraction, concentration and GC-MS analysis, was inspected based on analyses of three replicas of four different samples: 01B, 03A, 08B and 21B, representing different levels of overall peak abundances. Overlaid TICs are shown in Figure 5.12. Visual evaluation suggested a close match between the replicas within a sample, which can be confirmed by comparing with the relatively large difference between the different samples.

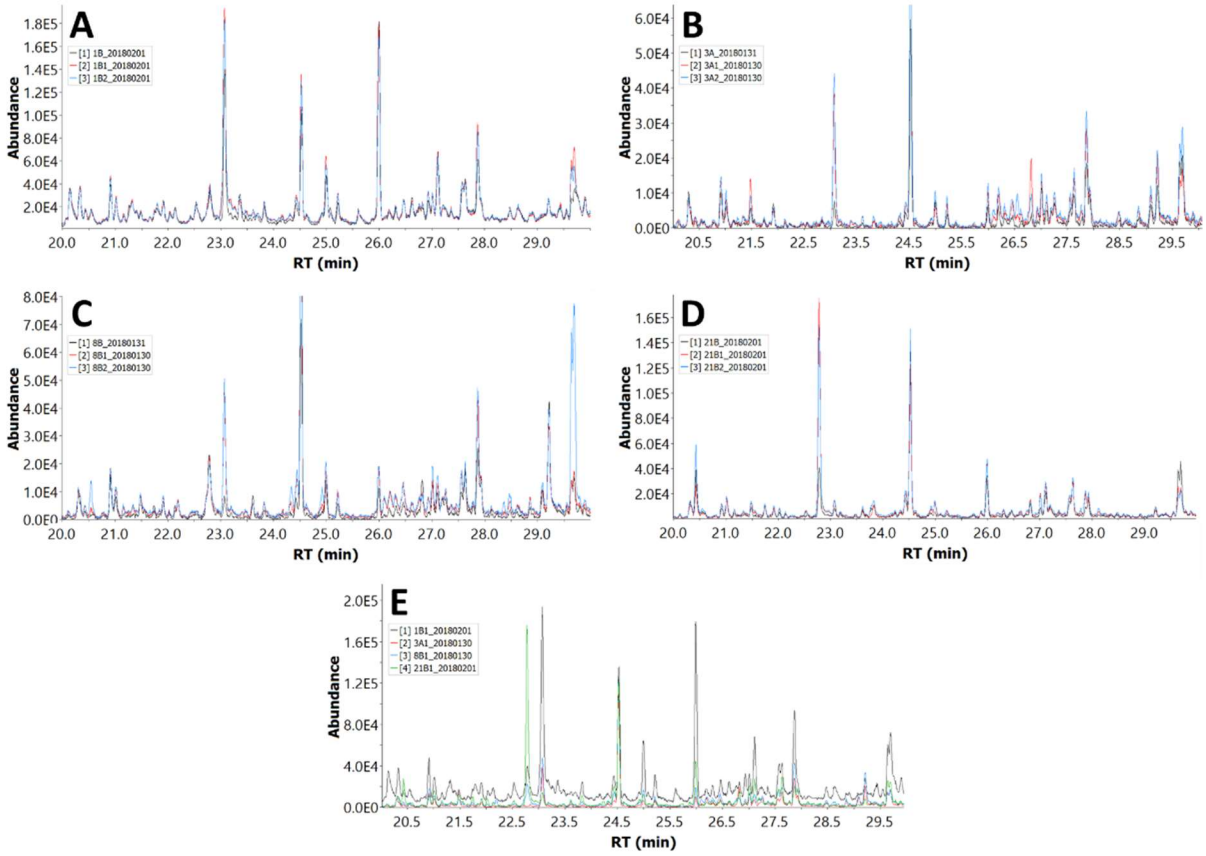

Figure 5.12 Overlaid TICs for visual evaluation of the stability of the analytical system. Retention times between 20 and 30 mins are shown Three replicas of: A: Sample 01B; B: Sample 03A; C: Sample 08B; D: Sample 21B. E: One replica of each of the mentioned samples.

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5.3.5 Pseudo-quantification of selected compounds

Benzaldehyde and 3-bromophenol were quantified in 52 sediment samples with the use of standard materials as described in Section 4.4.4. Quantification was not performed in a strict analytical sense, as the MS scan was not conducted in single ion mode (SIM), and as the analytical system as a whole was not optimized for detection of the specific compounds, thereby the term pseudo-quantification. However, this serves the purpose of relating the peaks to

estimated concentrations in the sediments. Concentrations are calculated based on calibration curves constructed for benzaldehyde (R^2 = 0.999, N = 6) and 3-bromophenol (R^2 = 0.997, N = 3), and presented graphically in Figure 5.13. Calibration curves are given in the figures A.2 and A.3 (Appendix A).

Average recovery rate was calculated by spiking three replicas of one sample with known amounts. Sample 06A was selected for this purpose based on its undetectable levels of the compounds of interest. The limits of detection (LOD) and limits of quantification (LOQ) estimated according to the method described in Section 4.4.4, as well as the rates of recovery, are given in Table 5.5.

Table 5.5 Analytical information: Limit of detection (LOD), limit of quantification (LOQ) and average rate of recovery given with standard deviation.

	Benzaldehyde	***3-Bromophenol***
LOD (µg g^{-1} dw)	0.001	0.02
LOQ (µg g^{-1} dw)	0.001	0.1
Avg. Recovery ± SD (%)[1]	63 ± 5	158 ± 8

[1] N = 3 for both standards

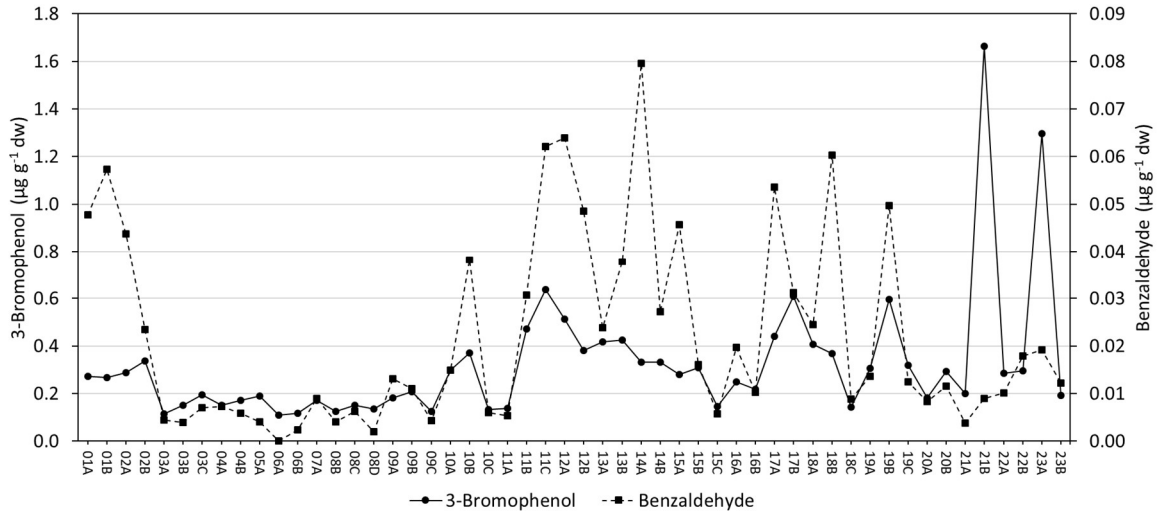

Figure 5.13 Graphical representation of pseudo-quantification of benzaldehyde and 3-bromophenol. Values are not corrected for recovery rate. N = 1.

Stability of the analytical system was evaluated as shown in Section 5.3.4. Using the same samples (01B, 03A, 08B, 21B), concentrations of benzaldehyde and 3-bromophenol were calculated with standard deviations (Table 5.6). Relative standard deviations (RSD) ranged from 3.9 % to 42 % for benzaldehyde, and from 2.4 % to 13 % for 3-Bromophenol.

Table 5.6 Sediment concentrations with standard deviation based on three replicates ($N = 3$), presented with relative standard deviation (RSD). Values are not corrected for recovery rate.

Sample ID	***Benzaldehyde***		***3-Bromophenol***	
	µg g^{-1} dw	*RSD*	*µg g^{-1} dw*	*RSD*
01B	0.081 ± 0.003	3.6 %	0.43 ± 0.05	12 %
03A	0.011 ± 0.004	37 %	0.14 ± 0.01	6.5 %
08B	0.017 ± 0.007	42 %	0.16 ± 0.02	12 %
21B	0.020 ± 0.004	21 %	2.2 ± 0.1	2.4 %

5.3.6 Differences between two installations

The abundance of organic compounds, represented by the number of peaks detected by GC-MS (#Peaks), as well as concentrations of benzaldehyde and 3-bromophenol for installation A (Hestøya) and installation B (Nørholmen), are presented as boxplots in Figure 5.14. Although there were differences in the abundance of organic compounds, as well as concentrations of benzaldehyde and 3-bromophenol between the two installations, these differences were not significant in any case.

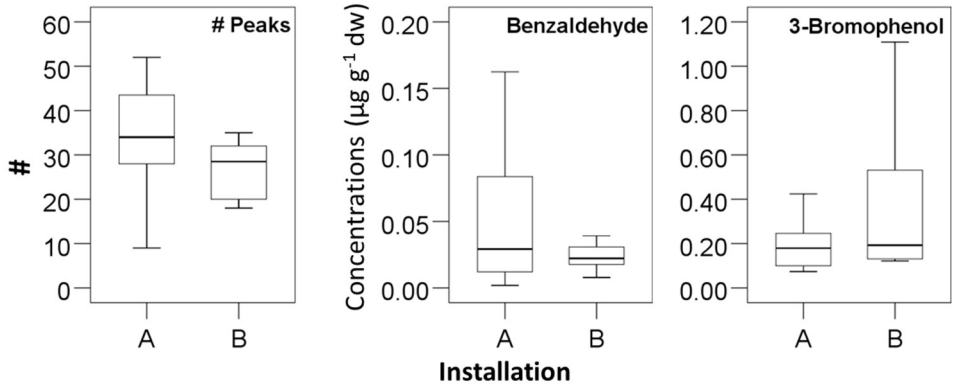

Figure 5.14 Number of detected peaks and concentrations (µg g^{-1} dw) of benzaldehyde and 3-bromophenol for installation A (Hestøya) and B (Nørholmen). Boxes: median and 25/75 percentiles; bars: minima and maxima. Lack of presence of asterisks (*) shows that there are no significant differences ($p > 0.05$) between installation A and B. $N = 44$ for installation A, $N = 8$ for installation B.

Five specific peaks, represented by their retention times, were selected for comparison in Figure 5.15. The criteria for selection was that these peaks gave a very strong correlation ($r_s > 0.8$ at $p < 0.05$) with either Cd or As or both (Figure 5.19), which could indicate a possibility of anthropogenic influence.

Although all selected peaks had higher average abundance near installations A compared to B, there was only a significant difference ($p < 0.05$) between the installations for

RT25.0 (Mann-Whitney U-test, $U = 58.5$ at $p = 0.003$), where installation A had higher levels than installation B. RT7.4 and RT25.2 was not detected at all near Installation B.

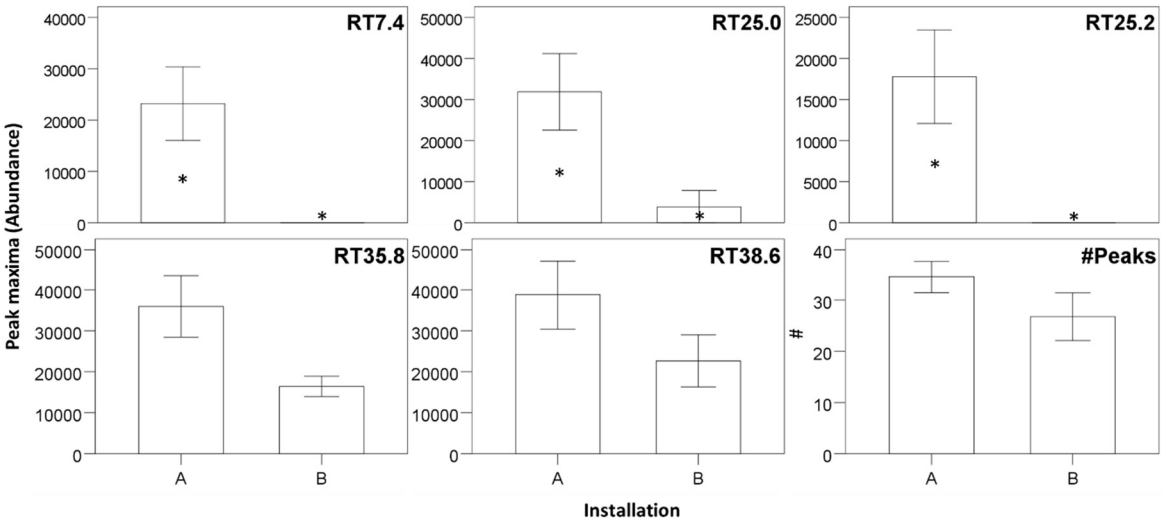

Figure 5.15 Abundance by peak maxima (abundance) for five selected peaks represented by their retention times, as well as number for peaks, for installation A Hestøya and B Nørholmen. Bars show average abundances with error bars 2×standard error. Asterisk (*) marks a significant difference ($p < 0.05$). $N = 44$ for installation A, and $N = 8$ for installation B.

5.3.7 Different distances from the installations

The number of peaks detected by GC-MS as well as concentrations of benzaldehyde and 3-bromophenol for distances less than 500 m and above 500 m from the installations, are presented as boxplots in Figure 5.16. Levels are generally higher near installation A compared to installation B, however there were no significant differences between the distance groups for all cases.

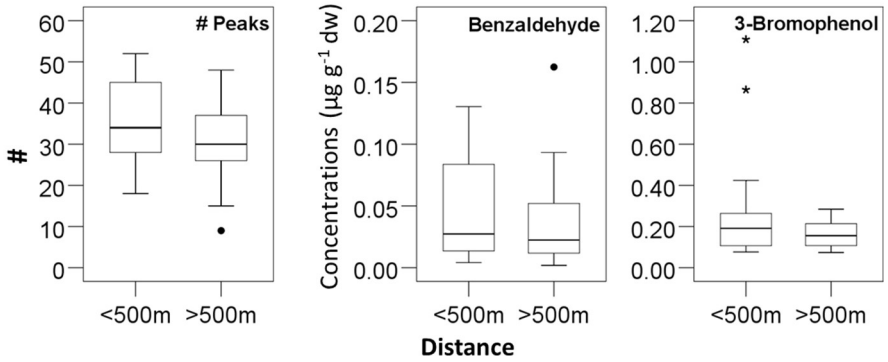

Figure 5.16 Number of detected peaks with concentrations (µg g^{-1} dw) of benzaldehyde and 3-bromophenol for distances <500 m and >500 m. Boxes: median and 25/75 percentiles; bars: minima and maxima; dot: outlier (1.5×IQR); starred dot: outlier (3×IQR). Lack of presence of asterisks (*) within the bars shows that there are no significant differences between the distance groups. $N = 36$ for distances <500 m, $N = 16$ for distances >500 m.

Five specific peaks represented by their retention times, based on their average peak maxima, as well as the number of peaks detected, were selected for comparison in Figure 5.17. The comparison considers the distances <200 m, 200-500 m and >500 m. The criterion for selection of peaks was the same as for comparison between the installations described above.

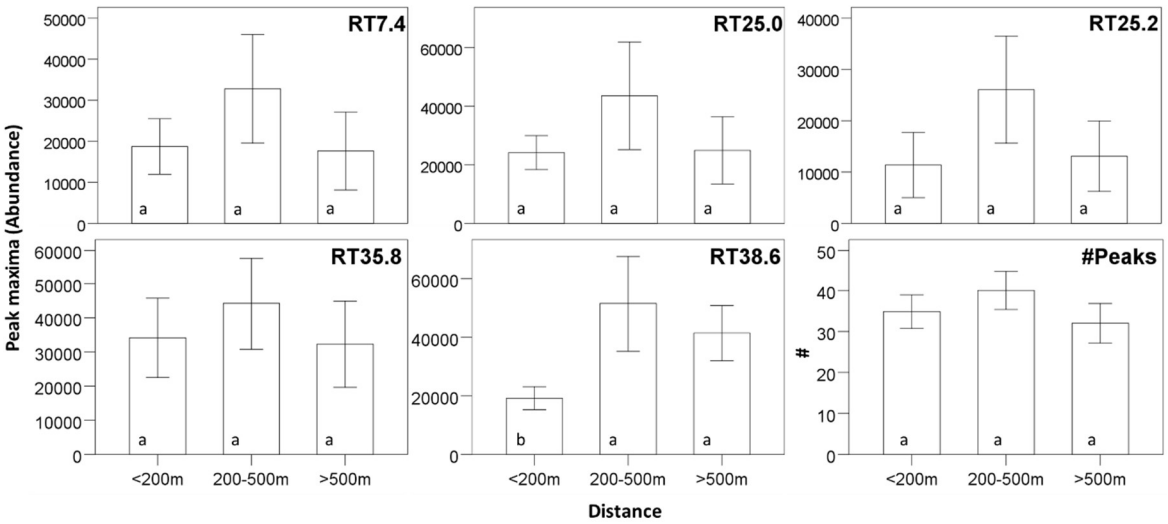

Figure 5.17 Abundance by peak maxima (abundance) for five selected peaks represented by their retention times, as well as number for peaks, at distances <200 m, 200-500 m and >500 m around installation A (Hestøya). Bars shows average concentrations with error bars 2×standard error. Equal letters marks distance groups that show no significant difference ($p > 0.05$). $N = 6$ for distances <200 m, $N = 17$ for distances 200-500 m, $N = 13$ for distances >500 m.

There was a significant difference ($p < 0.05$) between the distance groups only for RT38.6 (ANOVA, $F_{(2,33)} = 3.536$ at $p = 0.041$). Pairwise comparison by post hoc tests for RT38.6 showed a significantly lower ($p < 0.05$) level at distances less than 200 m than at distances 200-500 m and above 500 m. However, distances 200-500 m and above 500 m did not differ significantly from each other. Although levels were higher for distances 200-500 m for all cases, there were no significant differences between the groups other peaks than RT38.6, as well for the number of peaks.

5.4 BIVARIATE CORRELATIONS

Relationships between each variable in this study were examined by Spearman correlation analysis. The strength of the correlation is defined in Table 5.7 based on Spearman's rho (r_s). Since a graphical presentation of all 126 variables in this study combined would be literally unreadable, groups of variables are shown in different figures. By only selecting the variables that have a very strong correlation ($|r_s| > 0.8$) with at least one other variable, the

number of variables was reduced to 65, suggesting that at least 50 % of the variables conform to this criterion. The combined correlations for variables selected by this criterion is presented in Figure A.4 (Appendix A).

Table 5.7 Legend for interpretation of Spearman correlations.

	"Very strong"	$\|r_s\| > 0.8$
	"Strong"	$0.6 < \|r_s\| < 0.8$
	"Moderate"	$0.4 < \|r_s\| < 0.6$
	"Weak" or "None"	$\|r_s\| < 0.4$
-	Negative corr.	$r_s < 0$
	Positive corr.	$r_s > 0$

Correlations between a selection of elements are presented in Figure 5.18, while correlations between elements and organic analyses are presented in Figure 5.19. Distances of the samples from the installations are also included in these figures, as they were used to evaluate the potential sources. Elements included this correlation analysis, were those considered of primary environmental concern in Section 5.2. extended with molybdenum (Mo) and magnesium (Mg) (totally 18 elements).

Most of the elements were strongly correlated with each other in a positive manner ($r_s > 0.6$). Elements that especially followed each other seemed to be Cd, As, Cu and Ni, as all these had very strong correlation with each other ($r_s > 0.8$). However, only Al, Fe and Mn were negatively correlated with the distance from the installations, and only in a moderate fashion ($r_s < -0.4$).

Of special interest in an environmental context, regarding correlations between elements and organic parameters, were the very strong positive correlations ($r_s > 0.8$) between Cd and As with the TIC peaks RT7.4, RT25.0, RT25.2, RT35.8 and RT35.3. Also, Cu was correlated very strongly ($r_s > 0.8$) with RT35.8.

Correlations between organic parameters are shown in Figure A.5 (Appendix A), which may be used as a basis for discussions regarding degradation products of organic compounds. Other correlation trends will be discussed further under Section 6 (Discussion) as they are linked to an evaluation of potential anthropogenic contamination. All correlation coefficients can be made available upon request.

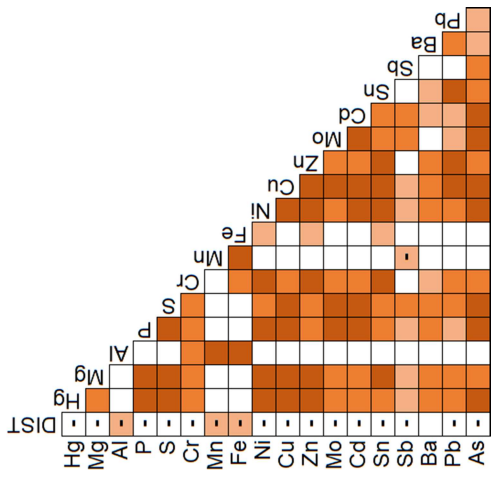

Figure 5.18 Spearman correlations for a selection of elements.
Distance from installations (DIST) is included. Legend for color codes are given in Table 5.7. $N = 52$.

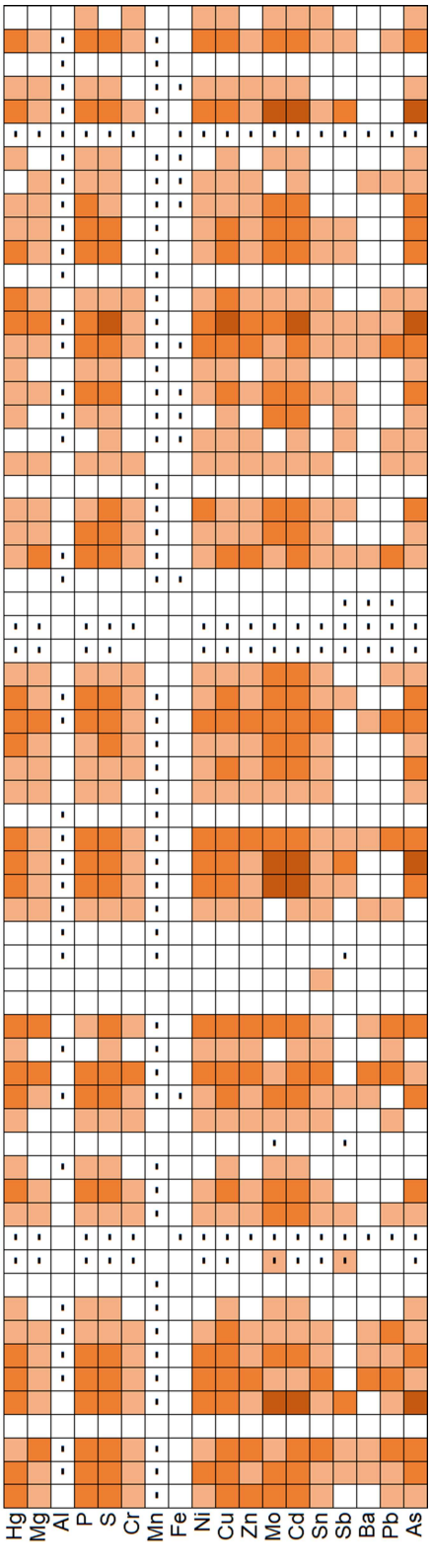

Figure 5.19 Spearman correlations between selected elements and organic analyses. Legend for color codes are given in Table 5.7. $N = 52$.

5.5 MULTIVARIATE ANALYSIS

5.5.1 Elemental data

In order to study the relationships between each element and physio-chemical properties, principal component analysis (PCA) was performed. The number of elements included was reduced to the same as for the correlation analysis (Section 5.4). Together with physiochemical properties and distances from the installation, the number of variables totaled to 26. With this dataset, the variance explained by the first two principal components reached 78.1 %.

According to the scores plots in Figure 5.20, samples from installation B (Nørholmen) showed a tendency to group together. The loadings plot may explain this by higher levels of Mn, sand and pH compared with installation A (Hestøya), since the loadings vectors for these variables points to the upper right quadrant where all the samples from installation B are located (Figure 5.20). This trend was coherent with the measurements presented in Section 5.1 and 5.2.

Moreover, the scores plot showed a slight tendency to group together the members of the same distance groups (< 200 m, 200-500 m or > 500 m). Samples close to the installations (< 200 m) gathered more around the origin, while intermediate distances (200-500 m) extend towards the top left quadrant, and distances above 500 m extend more towards the bottom right. Conferring with the loadings vectors (Figure 5.20), they indicated that most of the heavy metals were directed towards the left, with a major weight towards the top left quadrant. This was coherent with the measurements shown in Section 5.2, which showed a tendency of accumulation of some heavy metals at intermediate distances. Distances above 500 m were influenced to a lesser degree by the trace element contents. However, the loadings vector for distances from the installations (DIST), pointed in the same direction as the members of distances above 500 m, which is self-explanatory (redundant) and may give a false indication of separation between the members of distance groups closer vs. further away from the installations.

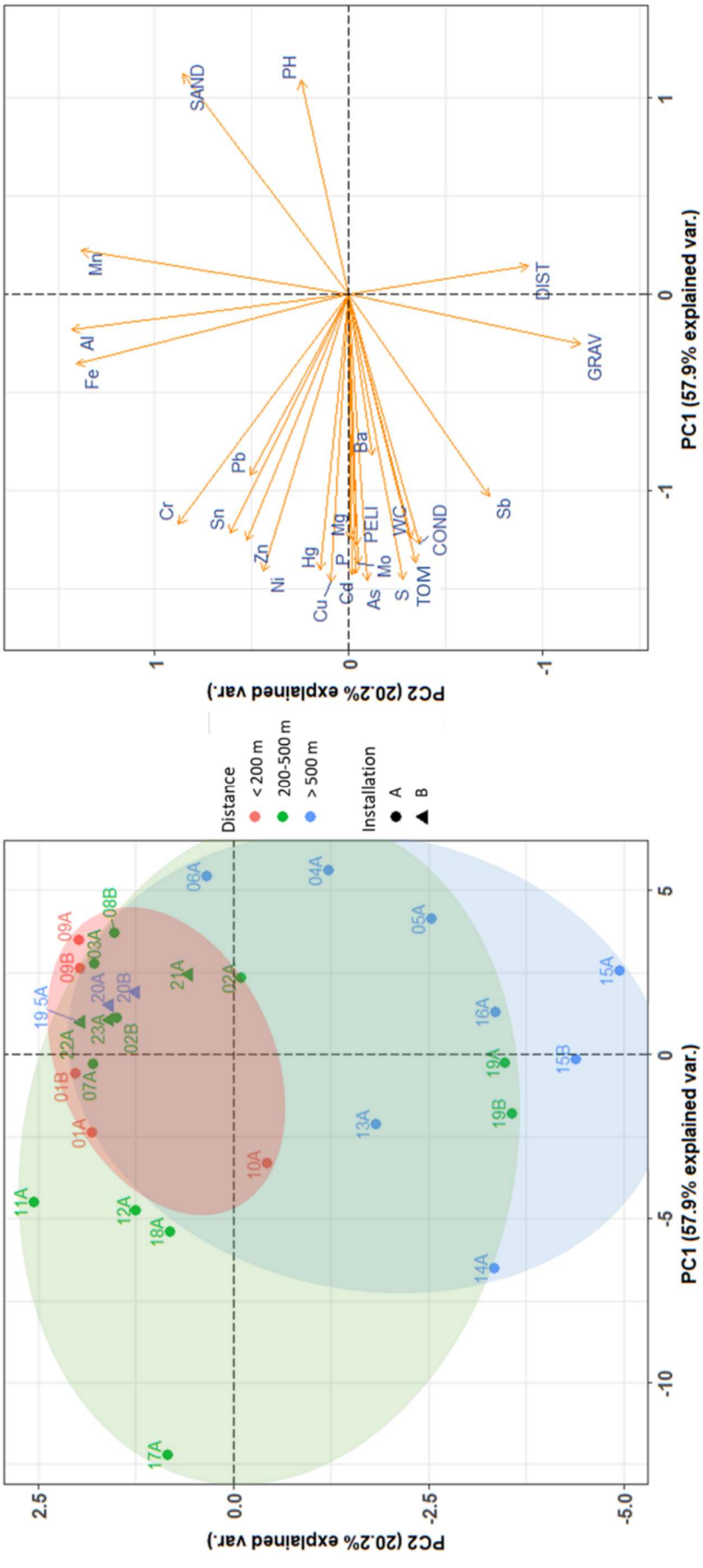

Figure 5.20 Scores and loadings plots for selected elements combined with physio-chemical characterizations (totally 26 variables). Color codes in the scores plot represents different distance groups, different shapes represent different installations, as shown in the legend. Grouping tendencies for different distance groups are illustrated. $N = 30$.

Sample 17A seemed to form a separate group, and according to the loadings seemed to be heavily influenced by elements. Comparison with element concentrations in Section 5.2 confirms that sample 17A had highest levels of Hg, Pb, Cd, Ni, Cu, Cr and Zn of all samples. In this way, PCA may have identified the most polluted sample in this study regarding trace elements of special concern. To a lesser degree, this was also true for Sample 18A, which pulled in the same direction as sample 17A, and together with the samples 11A and 12A, seemed to be highly influenced by the heavy metals Cr, Sn, Pb, Zn, Ni, Hg and Cu, while samples 01B and 07A seemed more influenced by Fe, Al and Mn.

Looking further at the loadings plot in Figure 5.20, Mn, Fe and Al were negatively correlated with respect to the distances from the installations (DIST). These metals were also near orthogonal to the other elements, pH and total organic matter content (TOM), and therefore probably not correlated. Additionally, the elements except Mn, Fe and Al were inversely correlated with pH, i.e. increases as the pH becomes more acidic. Other correlations shown by the loadings plot were the close relationship between pelite and TOM, and the inverse relationship between these two variables with sand content.

Summed up, PC1 as a latent variable seemed to represent the degree of content for the majority of trace elements, positively or negatively correlated with TOM/pelite and pH respectively. PC2 on the other hand represented the content of Mn, Fe and Al, negatively correlated with the distance of the samples from the installations.

The top 20 variables contributing to PC1 and PC2, as well as the scree plot showing the percentage variation explained by the ten first PCs, are shown in Figure A.8 (Appendix A). Al, Fe and Mn are most influential on PC2, which may explain its ability to separate distances closer than 500 m from those above 500 m, since these elements are negatively correlated to distance as mentioned above and in Section 5.2.

5.5.2 Organic data

Relationships between each organic variable and physiochemical property (totally 72 variables), were investigated by PCA (Figure 5.21). The scores plot did not show any particular grouping among the samples when considering the different distance groups, as seen in the previous PCA (Figure 5.20). The two first principal components for this case described just 56.0 % of the variance in the dataset, which made the interpretations of the scores and loading more unreliable than in the previous case.

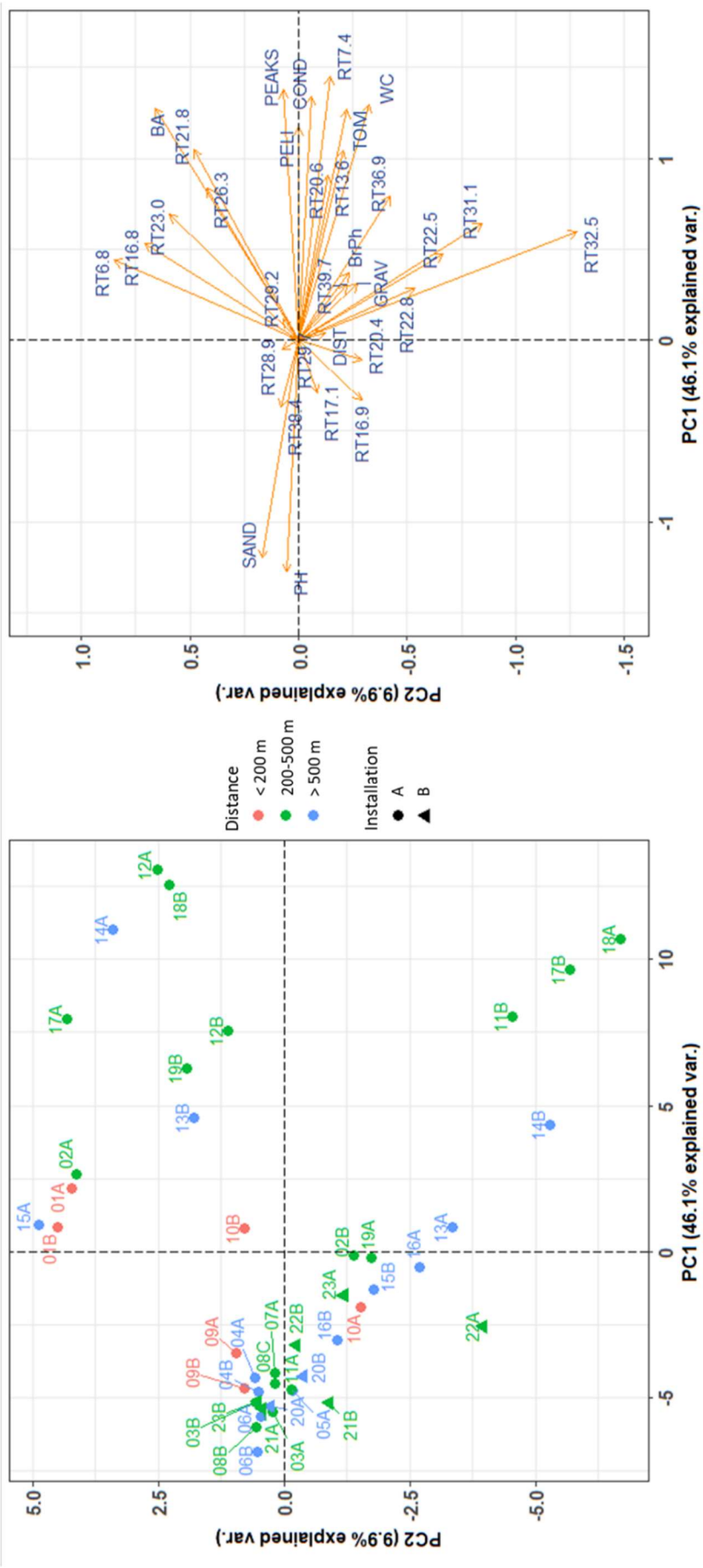

Figure 5.21 Scores and loadings plots for organic data combined with physio-chemical characterizations (totally 72 variables). Color codes in the scores plot represents different distance groups, different shapes represent different installations, as shown in the legend. Variables have been excluded in the visual presentation of the loadings to reduce clutter. $N = 44$.

To reduce visual clutter in the loadings plot in Figure 5.21, peaks with strong Spearman correlation ($r_s > 0.6$) were represented visually only by the one with lowest retention time. However, all peaks were included in the PCA calculations. Most organic peaks excluded in the visual presentation were pointing towards the right side of the plot roughly along the vectors for the number of detected peaks (PEAKS) and total organic matter (TOM). Loadings plot visualizing all variables is shown in Figure A.6 (Appendix A).

Most of the organic peaks gathered around the loadings vector for total organic matter (TOM), as was expected. The fraction of sand seemed to follow pH, and consequently a lower pH is observed with increasing pelite (PELI) content.

By conferring with the loadings vectors for the majority of the organic peaks, samples on the right side of the scores plot were more influenced by organic compounds (Figure 5.21). Especially the number of peaks detected, had a large influence on PC1 in that direction. Samples on the right side of the sores plot were slightly dominated by intermediate distances (200-500 m), while samples from installation B seemed less influenced by organic compounds.

Looking at collinearity of the loadings vectors, most organic compounds were inversely correlated with pH to varying degrees, and positively correlated with pelite and TOM. However, a small number of organic compounds, represented by RT16.9, RT17.1, RT28.9 and RT38.4 seemed to follow an increased pH. Benzaldehyde (BA) followed the loadings vector for RT21.8, which may indicate that this peak was a chemical precursor or product of the compound. Further, the inverse relationship between sand and pelite stands out clearly in this loadings plot.

Summed up, PC1 as a latent variable can be described as the amount and abundance of the majority of the organic compounds, which were also correlated with pH, TOM and ratio of pelite to sand. PC2 on the other represents some of the organic compounds, which were not correlated with physiochemical properties of the sediments, except the gravel content.

The scree plot and contribution of the variables to the first two PCs, are shown in Figure A.9 (Appendix A). RT32.5 distinguished itself by having a higher influence on PC2 compared with the other peaks.

5.5.3 All data combined

The Principal Component Analysis (PCA) scores and loadings plots for the combined dataset including organic, elemental and physio-chemical analyses are shown in Figure 5.22. Initially, there were 126 variables in the combined dataset, but for this analysis, the total has been reduced to 90 variables by omitting those elements that are believed not to be relevant for

the environmental context of this study. In this case only 55.6 % of the variance was explained by the first two principal components.

The scores in Figure 5.22 plot showed a similar pattern as that for only the organic analyses (Figure 5.21). However, as for the elemental analysis (Figure 5.20), a slightly higher tendency was observed for the samples from installation B (Nørholmen) to group somewhat together, while samples from installation A (Hestøya) were scattered around the area of the scores plot. There was also a slight tendency for distances closer than 200 m to gather around the origin, while distances 200-500 m extended towards the right-side quadrants, and distances above 500 m towards the top right. In other words, PC1 seemed to some extent to separate distances 200-500 m from distances above 500 m. It also seemed that PC1 described better the variations for distances 200-500 m, while PC2 described better the variations for distances above 500 m. Nevertheless, these tendencies are vague, and it is problematic for this analysis that most data points gather around one area with no distinct grouping and poor separation, and that the variance explained by the first two principal components were low.

The visual presentation in the loadings plot (Figure 5.22) were treated as for the organic dataset in the previous section, i.e. reducing visual clutter by omitting those organic peaks that were strongly correlated ($r_s > 0.6$), without removing them from the calculation. Likewise, most excluded peaks pointed roughly along the vectors for the number of detected peaks (PEAKS) and total organic matter (TOM). Loadings plot visualizing all variables is shown in Figure A.6 (Appendix A).

Interpretations of the placement of samples in the scores plot by conferring with loadings, gave no more information compared to the previous cases. However, by combining all data, collinearity between the different dataset could be discovered. Distance from the installations seemed to be negatively correlated with the peaks RT16.9 and RT20.4, and slightly negatively correlated with Mn and Al. There seemed also to be a relationship between the majority of organic peaks with the elements, except for Mn, Fe and Al. Nevertheless, Al and Fe followed RT22.5, RT22.8 and RT32.5, while Mn were more correlated with RT16.9 and RT20.4.

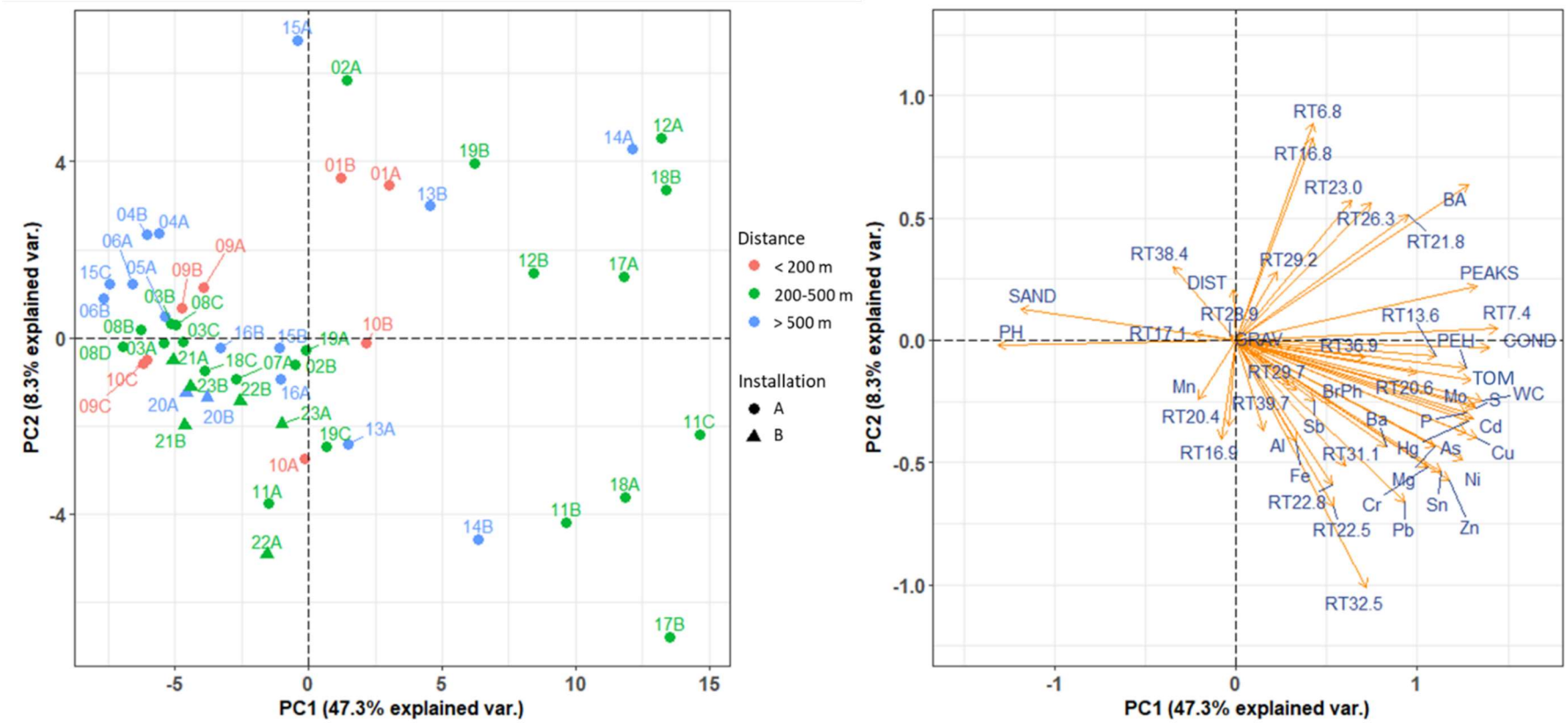

Figure 5.22 Scores and loadings plots for the combined dataset containing 90 variables. Color codes for the scores plot represents different distance groups, different shapes represent different installations, as shown in the legend. Variables have been excluded in the visual presentation of the loadings to reduce clutter $N = 52$.

This capability of the variation within pH, TOM and pelite to separate the samples by PC1, can also be explained by the observing how the loadings for these parameters are aligned along this principal component. Considering the semantic of PC1, it may indicate that this principal component can be described as representing the physio-chemical characteristics of the sediments. On other hand, PC2 can be described as representing those parameters not correlated with the physio-chemical characteristics.

Scree plot as well as plots describing variable contributions to PC1 and PC2, are shown in Figure A.7 (Appendix A). The highest contributions originate from the organic peaks including benzaldehyde, but also Pb and Cu seem to have some influence on the PCs.

5.5.4 Further optimization of PCA

Two different approaches for improving the PCA for the combined dataset are shown in Figure 5.23. Attempts to perform the PCA without autoscaling, or other types of scaling, are not included in the figure, as this approach seemed to give a deteriorated result even when outliers were removed, which can be explained by the wide array of different analytical techniques in the dataset. Looking at the plots, attempting to group the organic peaks according to retention time seemed to give a better result with an explained variance of 69.1 % by the first two principal components. The challenge with this approach was that a lot of information was lost, since organic compounds with similar retention times does not necessarily have similar chemical properties except their affinity to the chosen GC column, and may have widely different meaning for the context of this study.

Considering an eventual factor or cluster analysis based the dataset in this study, the variables should be selected such that the whole dataset confirms to the Kaiser-Mayer-Olkin test for sampling adequacy (KMO). The criterion is that the KMO index for the dataset should be > 0.6 or > 0.5, according to different publications (Tabachnick and Fidell, 2001). In this analysis, variables with individual low KMO index were excluded until the dataset combined gave an index > 0.6. The resulting scores plot shown in the bottom of Figure 5.23 gives grouping patterns equivalent to the untrimmed dataset in Figure 5.22, however in an inverted fashion. Variance explained by PC1 and PC2 reached 60.6 %, which was slightly better than the untrimmed dataset where 55.6 % was explained. Furthermore, the loadings plot showed similar correlation trends as the loadings for the untrimmed dataset.

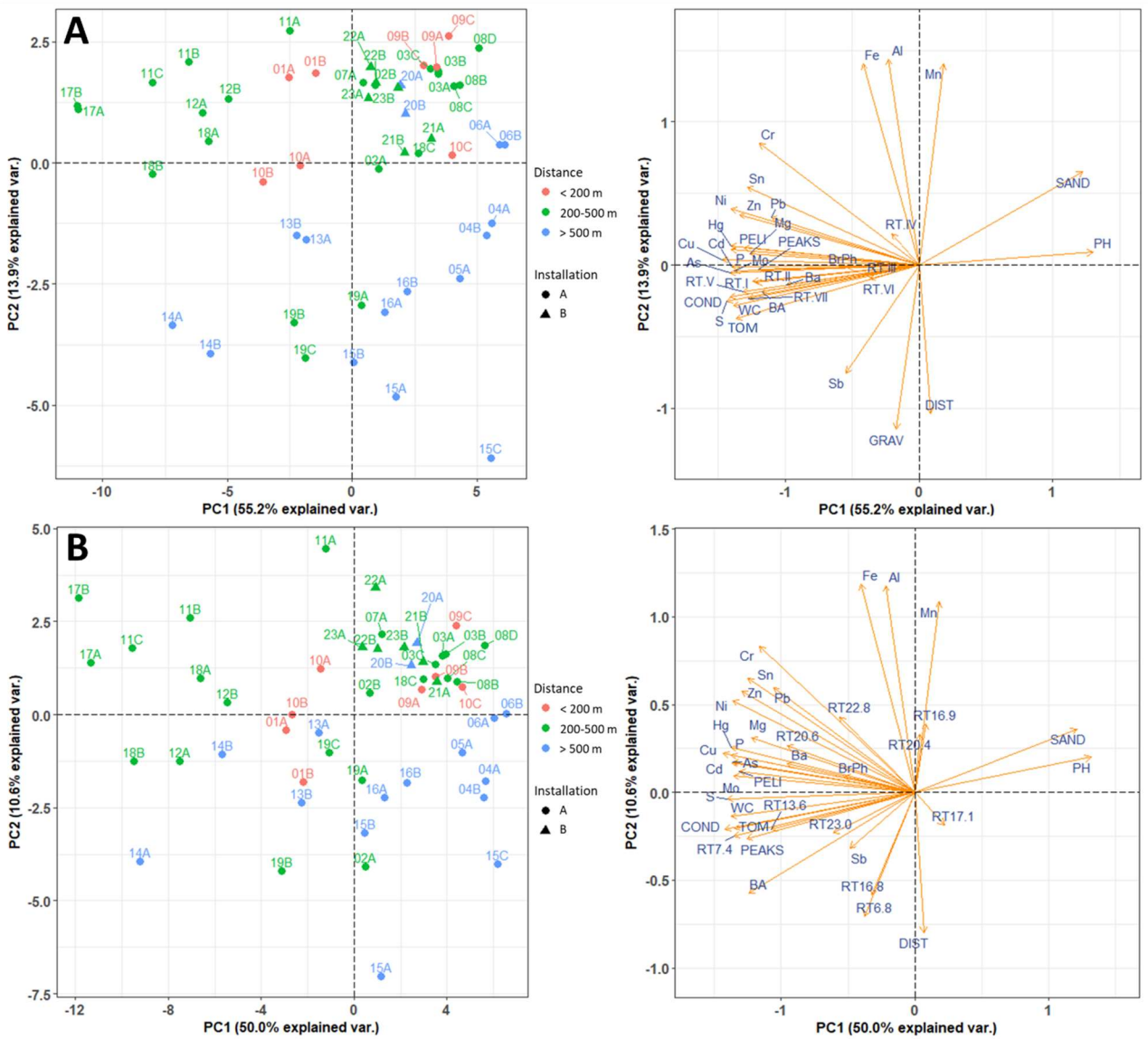

Figure 5.23 Scores plot for two different cases to the left with their corresponding loadings plot to the right. A: Combined dataset with organic data grouped by retention times with a resolution of 5 minutes (36 variables). $N = 52$. B: Combined dataset using Keiser-Meyer-Olkin test for sampling adequacy as a selection criterion (48 variables). Variables have been excluded in the visual presentation of the loadings plot to reduce clutter. $N = 52$.

6 DISCUSSION

6.1 COMMENTS ON METHODOLOGY AND METHOD DEVELOPMENT

6.1.1 Non-targeted screening

Usually, non-targeted screening studies incorporates advanced mass spectrometry like time-of-flight-MS (TOF-MS), to achieve higher sensitivity and better characterization of fragmentation patterns. Two-dimensional GC is also a commonly used technique to achieve better resolution of the chromatographic separation (Norwegian Environment Agency, 2013). However, this study was based on screening by GC-MS, with an electron impact mass spectrometer. GC-MS is a powerful tool for targeted analyses of volatile or semi-volatile compounds, but in the context of non-targeted screening, this instrument will limit the amount of successfully detected compounds compared to more state-of-the-art screening techniques.

Nevertheless, analysis was attempted with liquid chromatography (LC) coupled with quad time of flight (Q-TOF) mass spectrometer. This approach produced negative results, and possible explanations are discussed further in Section 6.1.3.

What seems to be coherent with previous non-targeted screening studies, were the suggested identifications by matching mass spectra with a database, and following confirmation by a standard material. However, it is recommended to use extracted ion chromatograms (XICs), yielding a more reliable quantification in the targeted analyses (Schymanski et al., 2015). All interpretation and quantification in this study were based on total ion chromatograms (TICs). Further, a list of suspected compounds was produced. Future studies of their potential relevance in effluents from the industry under scrutiny, could discover potential pollutants. Correlations with elements and within detected compounds is another approach that could reveal metabolites and degradation products.

The response of most peaks was in the same magnitude as for impurities in the extraction solvent, as well as contaminants presumably from the extraction system. An extensive use of method blanks was therefore applied to separate contamination from actual extracted compounds. Whether this approach was successful can be debated, since for a few cases, detected peaks appeared in just one of the nine method blanks. There is therefore no guarantee that some contamination was regarded as extracted analytes by mistake. However, it was assumed with a satisfying degree of confidence, that if a compound was present in more than ten of the 52 samples but in none of the nine method blanks, this compound probably originated from the sediment samples.

Different approaches for cleanup of the extracts were performed during method development. Micro-filtering of the extract resulted in a high degree of contamination and was therefore abandoned. Removal of sulfur compounds was also attempted by introducing a layer of active copper in the extraction cell. However, this approach resulted in loss of detected peaks and contamination, as was the case with micro-filtering. The conclusion was therefore not to perform any cleanup or filtering of the extracts to minimize contamination and loss of analytes, in accordance with the basic principle of non-targeted analysis.

6.1.2 Selected aquaculture installations and sampling strategy

The sediments around the two installations selected as objects of examination for this study, were considered in very good conditions according to previous surveys (Directorate of fisheries, 2018). Installation A (Hestøya) has even a special "exhibitory" license and has only been in operation since 2015, so exemplary operation with respect to environmental consideration is expected from this installation. It is therefore important to underline that these installations far from represented the worst cases from an environmental perspective, rather possibly the best cases, considering that only 18 out of 142 C-surveys nationally in 2015-16 could showed background levels in the surrounding sediments, according to normalized ecological quality ratios (nEQR) for benthic fauna (Directorate of fisheries, 2018).

Regarding installation A (Hestøya) the ocean current directions varied substantially according to the rose diagrams in Figure 3.2, and seemed to cover more or less all directions, but considering installation B (Nørholmen), the sampling axis was parallel to the installation and the dominant current direction. It is therefore possible that the effluents from this installation have not been included at all in the samples.

External factors also influenced the sampling strategy. Restrictions due to governmental hygiene regulations was interpreted to limit our distance from the installations to a minimum of 100 meters (Regulations concerning operation of aquaculture installations, 2008, §18). Other factors causing deviation from the original sampling plan were the anchor points for the net pens, buoys and fishnets from surrounding fishing activities, shallow waters, islets and rocky seafloor.

Considering these aspects regarding the sampling strategy and choice of installations to examine, it was not expected that this study revealed accumulation of organic pollution originating from the aquaculture activity, nor deteriorated sediment conditions. However, the methodology developed could be a framework for further studies aimed at screening pollutants

related to aquaculture installations. Method development remained therefore the main focus of this study, rather than a survey of pollution from the aquaculture industry in general.

6.1.3 Analytical errors

Sampling and sample handling

Even if great care was taken to avoid contamination and cross contamination, the samples would probably be altered slightly in one way or another before analysis. The box corer was made of stainless steel, and hypothetically iron and impurities may have been transferred to the sample. All equipment used under sampling were rinsed by tap water onboard the ship to avoid cross contamination between the samples. However, this water was not deionized or distilled, and could probably contribute to a small degree with contamination. Aluminum containers were used for samples aimed at organic analyses and polystyrene cups for inorganic analysis. This should avoid relevant contamination from the container itself, but since the containers were not rinsed prior to sampling, some residues from factory production of the containers could hypothetically contaminate the samples. Contamination from the box corer and the containers was minimized by avoiding utilizing sample material in direct contact with the container for further analysis.

Samples were frozen at -4°C immediately after collection onboard the ship, and at -20°C when arriving at the lab. However, the storage time before analysis could alter the analytes due to chemical reactions like oxidation or by sublimation for the most volatile compounds. For the inorganic analyses, all analyses were performed within 6 months after sampling, but for some of the organic samples about 8 months passed before the final analyses. Nevertheless, samples seemed to be preserved well since replicates of samples analyzed within 6 months and after showed similar patterns.

Freeze-drying under vacuum could have resulted in cross contamination. The lids of the containers used for lyophilization were perforated. As pressure changed, small particles could hypothetically contaminate the space within the freeze-drier. A measure taken to avoid this, was to quickly start the freeze-drying process when samples were removed from the freezer, such that as much as possible of the water was removed by sublimation and not by evaporation.

Samples aimed at ICP-MS analysis were not in contact by materials other than the container and acid solution used for digestion. Transfer of sample material was done exclusively by decantation. The advantage is that the risk of contamination was minimized. On the other hand, the samples were not homogenized, and due to the nature of environmental samples, a

large variation of chemical composition could exist within just a small batch contained in the container.

Considering the organic samples, a large variation was observed within the replicates from the same box core sample. These variations probably represent natural variations within the box core area of 30 × 30 cm. Homogenization of the samples was poorly performed by stirring with a glass rod, after defrosting and separation into smaller containers for freeze drying. Hypothetically, this could result in differences between analyses from the same sample. Homogenization by grinding with a mill could be a solution. However, contamination from the mill must be minimized by choosing appropriate mill materials, and additionally by thorough rinsing of the mill between the samples. The latter probably being a time-consuming procedure considering the number of samples and method blanks to be analyzed.

Comparison of grain size distribution between the replicates could indicate the degree of homogeneity, between samples and replicates. According to Figure A.1 (Appendix A), when considering the ratio of pelite to sand, 16 out of the 21 samples with at least two replicates from the top layer, showed some degree of homogeneity between replicates (percent difference < 25 %). However, by comparing 11A to 11B and 18A to 18B, the percent differences were up to 103 % and 144 % respectively. As a conclusion homogeneity between the replicates, and probably also within each replicate, cannot be assumed.

Extraction and concentration

The solvent used for extraction (DCM) of organic substances showed substantial presence of peaks at the same magnitude as the analytes, as was demonstrated in the concentrated solvent blanks. The solvent used for extraction was of GC-MS quality, but due to substantial concentration of the extracts, impurities were highly visible in the chromatograms. Due to the degree of concentration, and low levels of the compounds of interest, a higher quality solvent, like HPLC-grade, should have been used to reduce contamination in the method blanks. This issue was to some degree evaded by subtracting the peaks detected in the method blanks from the samples. Nevertheless, this approach prevented detection of compounds with retention times equivalent to that of the impurities.

The extraction system was also shown to add contamination to the samples, by comparing concentrated solvent blanks with full method blanks, even if this resulted in only a couple of distinct peaks in the chromatograms. Contamination from the ASE is thought to stem from fittings and/or worn septa, contaminants in the diatomaceous earth use as dispersant, or contaminants in the N_2 gas used for pressurizing. Attempts were made to pass the gas through

a coal filter, but this resulted in a delay during pressure changes required by the method, which halted the instrument. The extractions cells were maintained according to the protocol, including e.g. sonication of the metal filters within the cells, so the extraction cells by themselves were probably not the source of contamination. However, contamination from the extraction system, was dealt with by subtracting peaks detected in method blanks from the peaks detected in the samples (Section 6.1.1).

Some samples formed colloids during extraction and concentration, which were removed manually by a glass pipette. The colloids could hypothetically remove potential analytes by sorption. However, one colloid was analyzed by GC-MS after being dissolving in acetone, which did not show any detected peaks within the retention time window used in this study.

Glassware and vials for collection of extract, as well as the glassware used for rotary evaporation, were also potential sources for contamination and cross contamination. All glassware was either previously unused and GC-MS certified, or rinsed thoroughly by DI water and acetone before drying. Blank analyses of the acetone showed low levels of contamination, showing that residues from acetone after rinsing was probably not significant. To ensure minimal contamination, all glassware was also scoured by the appropriate solvent before use.

The recovery rate calculated for benzaldehyde was relatively low compared to general performance previously shown for the ASE (Zhang et al., 2011). This could be due to poor optimization of the extraction procedure, or due to properties specific for the analyte relative to the solvent used. However, as quantification was not the main focus of this study, poor extraction performance did not get much attention. More important was a certain repeatability, which was demonstrated by running three replicates of four samples through the whole analytical system including extraction. The stability testing showed satisfying stability between replicates, while the differences where substantial between different samples. This showed as well that homogenization after freeze-drying of the samples was satisfying.

However, storage time and lyophilization were not considered in the stability testing. Hypothetically both steps could induce alteration on the chemical content of the samples. Most critical was probably lyophilization, where the more volatile compounds could theoretically be lost.

GC-MS errors

The major issue related to the gas chromatography in this study seemed to be the carryover of analytes from one sample run to the next, by failure to evacuate all compounds out

of the column with the chosen GC program. Running blanks in between samples proved this to influence the peaks detected with retention times above approximately 45 minutes. Carryover was avoided by including a one-hour bake-out of the GC oven between samples. However, this would increase the time of analysis by more than the double, as it would require running bake-out manually between each sample. Analysis was therefore run in batches of 7 to 8 samples, with the most "dirty" samples at the end, and with a bake out step between each batch. However, the chromatograms still showed noise at retention times above 45 minutes. Therefore, only retention times below 40 minutes were considered in this study, as the blanks for this time window showed satisfying results.

Substantial matrix effects were expected with this methodology, especially considering that the extracts were not subject to cleanup steps before GC-MS analysis, as explained in Section 6.1.1. Even if the reason for not including cleanup was to include as many compounds as possible, this may on the other side have resulted in a loss of the number of compounds detected due to masking by matrix interactions.

Stability test of the analytical system showed satisfying repeatability for the mentioned retention time window. Other error sources mentioned in the theory chapter for this type of instrumentation seemed therefore to be insignificant for the scope of this study.

The so called "sulfur peak" masked a relatively large area of the chromatograms, and the compounds within have probably removed interesting analytes by various interactions (e.g. adsorption or complexation). Further analysis of the content of the "sulfur peak" could be performed with two-dimensional GC, to get a better resolution for this specific peak. Slowing down the rate of temperature increase around the appropriate retention time interval from 4°C/min to 1°C/min, was attempted with no improvement.

Quantification of benzaldehyde and 3-bromophenol was performed by the formulas derived from linear regression of the calibration curve for each compound. As this quantification was not based on any standardized protocol, and moreover not run in single ion mode (SIM), nor by extracted ion chromatograms (XIC), it was considered a pseudo-quantification, implying that the resulting sediment concentrations are only to be regarded as indications. To get a better identification, extracted ion chromatograms (XICs) should have been used for this purpose (Schymanski et al., 2015).

LC-MS/MS errors

Liquid chromatography coupled with a Q-TOF mass spectrometer could in theory give a more sensitive detection than GC-MS. Nevertheless, no peaks were detected using this

instrumentation in the method development phase. One explanation could be that this technique with the applied column, mainly detects polar and semi-polar compounds. Hypothetically, more polar substances could dissolve in the sea water, while the more non-polar stuck to the sediment. However, the pore water in the sediment should have contained these polar substances, which would precipitate to the sediment during lyophilization. Polar substances have been detected in sediments in numerous previous studies (Lubcke-von Varel et al., 2011, Benskin et al., 2016), so this explanation seems very unlikely.

A more likely explanation is that the column chromatography used for fractionation prior to LC-MS/MS, failed to elude any analytes, or reduced the concentration substantially. However, the eluate had different colors depending on the fraction, indicating that they contained something. The fractions were further evaporated after adding methanol, to transfer the analytes to a methanol phase. This process could as well have reduced the concentrations below detection limits. The final explanation seems to be that the fractions were diluted too much to be detected by the instrument, out of the precaution to avoid damaging the MS filament by overloading. It can therefore not be ruled out that further attempts on detection by LC-MS/MS could have succeeded. The reason this project turned to the use of GC-MS seems therefore to be more an issue of budget and instrument availability.

ICP-MS errors

Analyses by ICP-MS follow strict procedures established by the Department of Chemistry at NTNU. The samples were never in contact with other materials than their containers, the acid of ultrapure quality used for digestion, as well as ultrapure water for dilution. This procedure is tested for numerous samples, and considered to induce minimal or no contamination detectable by the instrument. As commented before, the main issue relating ICP-MS data to environmental concentrations seems to be due to the lack of homogenization of the samples.

However, for some samples, a red residue was seen in the bottom of the PTFE tubes after acid digestion by autoclave. By judging the color, this could implicate that some Fe was lost by adsorption to the container. On the other hand, repeatability tests show relatively stable analyses.

Another source of error was the use of soil reference material (Soil GBW-07408), which differs from the marine sediment matrix of the samples analyzed. The extent to which difference matrix effects of soil and marine sediments affected the analysis, was not evaluated.

6.2 SEDIMENT CLASSIFICATION BASED ON TRACE ELEMENTS AND TOC

Concentrations of trace elements of special environmental concern showed mostly background levels. However, levels of Cd were above background levels in 19 out of 30 sediment samples, which could imply an anthropogenic influence. Still, the concentrations were below PNEC and sediments could easily be classified as Class II ("good" conditions). Regulations states that cost-benefit analyses or remediation measures, generally are not necessary for classifications below Class III ("moderate" condition) (Norwegian Environment Agency, 2015). Background levels are given by the OSPAR agreement, and therefore does not necessarily reflect the background levels of this specific area (Norwegian Environment Agency, 2016), such that it cannot be concluded if the levels measured above Class I are to be considered as pollution.

Levels of Hg were above background levels in five out of 30 sediment samples, but none above PNEC. Same considerations concerning background levels and regulations applies for Hg, as mentioned above for Cd.

On the other hand, total organic carbon (TOC) contents were very high compared to the expected Norwegian background levels. For installation A, the majority classified as Class V ("very bad" condition), while for installation B all but one classified as Class IV ("moderate" condition). However, there are reasons to consider classification limits for TOC in particular as too strict. This notion is supported by the fact that the same sediments classified as "very bad" according to normalized TOC in several B- and C-surveys, often classifies as "good" or "very good" for benthic fauna (Directorate of fisheries, 2018). Whether the shown elevated levels of TOC are anthropogenic or naturally occurring in the area, was not possible to conclude on basis of these analysis. Use of tracers like fatty acids as mentioned in NS 9410, could be a way to assess the source (Standards Norway, 2016).

Previous C-surveys from 2016 for installation A (Hestøya) and from 2011 for installation B (Nørholmen), contained inter alia measurements of sediment contents of Zn, Cu, P and TOC (Directorate of fisheries, 2018), as shown in Table 6.1. Directions from the installations and exact location of sampling for these surveys were not evaluated. However the values were considered to be comparable as they represent sediments within similar radia from the installations. Cu and Zn resulted in the same sediment classification as for the measurements from 2017 presented in this study. For installation A, Cu was measured to an average of 7.6 ± 4.5 mg kg^{-1} in 2017 compared to 12 ± 3 in 2016, Zn to an average of 25 ± 10 mg kg^{-1} in 2017 compared to 34 ± 10 mg kg^{-1} in 2016, and P to an average of 610 ± 189 mg kg^{-1} compared to 773 ± 201 in 2016. Likewise, for installation B, Cu was measured to an average of 7.1 ± 1.8 mg

kg^{-1} in 2017 compared to 8.4 ± 1.5 mg kg^{-1} in 2012, Zn to an average of 29 ± 4 mg kg^{-1} in 2017 compared to 38 ± 18 mg kg^{-1} in 2012, and P to an average of 563 ± 62 mg kg^{-1} in 2017 compared to 403 ± 73 in 2012.

On the other hand, TOC levels were higher compared to the results from previous C-surveys, as the sediments classified as Class III or lower in the C-surveys, but mostly Class V for installation A and Class IV for installation B, with the measurements presented in this study. It seems unlikely that this reflects actual change from one year to another caused by the aquaculture activity, since such an accumulation should have been detected in 2012 considering the installation under operation since 1979. A more likely explanation could be seasonal variations in TOC content, as more terrestrial runoff with high organic content could be expected in the thawing season around time of sampling at the end of March. Another likely explanation could be potential differences in the analytical techniques and/or the normalization of TOC values. However, TOC was analyzed by loss of ignition for all cases. Moreover, the reported pelite contents were comparable. There could be a different use of conversion factors from loss on ignition to content of organic carbon. However, this study uses a more conservative factor of 2, which should probably give lower results compared to the conventional conversion factor of 1.724. Differences could also be explained by different sample locations, which makes the 2017 measurements more reliable since the sample population was higher ($N = 30$ compared with $N = 6$).

Table 6.1 Sediment condition according to previous C-surveys for installation A (Hestøya) and B (Nørholmen) (Directorate of fisheries, 2018). Color codes shows sediment classification according to Table 5.2. TOC was normalized by pelite content (nTOC). All concentrations are for dry weigh (dw) of sediments.

Installation	***Distance[1]***	***Zn*** *mg kg^{-1}*	***Cu*** *mg kg^{-1}*	***P*** *mg kg^{-1}*	***nTOC*** *mg g^{-1}*
A. Hestøya	Near (< 30 m)	42	14	830	21.6
	Intermediate (< 500 m)	23	9.1	550	20.3
	Recipient (> 500 m)	37	14	940	21.8
B. Nørholmen	Near (< 30 m)	23	7.5	410	16.1
	Intermediate (< 500 m)	58	10	470	30.2
	Recipient (> 500 m)	32	7.6	330	19.6

[1]Distances according to NS 9410:2016 (Standards Norway, 2016)

Speciation and bioavailability are generally not considered with this approach for environmental classification. Here, we considered only the total concentration of an element extracted with an extraction system designed to dissolve as much of the sample as possible.

Speciation is important for a more thorough environmental assessment, as many forms will be immobilized and not available for uptake into the ecosystem. The classification system applied here, assumes that the element is present in a bioavailable form when calculating PNEC levels. Also considering background levels, it is not known in what form the element is present, which consequently could mean that background levels in one case consists of highly bioavailable species, while for another case consist mainly of immobilized species, and therefore in reality of quite different environmental statuses.

Further, as mentioned earlier, the sediment classification in this study cannot be considered representative for the general conditions in Norwegian aquaculture. Considering the location of the sampling points relative to the dominant ocean current direction, it can be discussed if they are even representative as measurements of influence from installation B.

6.3 DETECTION AND IDENTIFICATION OF ORGANIC POLLUTANTS

6.3.1 Compounds associated with aquaculture industry

Diflubenzuron, teflubenzuron, and emamectin benzoate have been detected in the sediments close to Norwegian aquaculture installations, exceeding quality limits by up to 67 % compared to UK quality standards (Langford et al., 2014). Further, teflubenzuron has previously shown unchanged concentrations in sediments up to 204 days after medication (Selvik et al., 2002). However, for the last case, the drug was only above detection limit within 20 m from the edge of the cage.

In previous studies, LC-MS/MS instrumentation were applied to detect the benzoylureas (diflubenzuron and teflubenzuron) and emamectin benzoate, and GC-MS to detect deltamethrin and cypermethrin (Langford et al., 2014), which means the instrumentation used in this study could potentially have picked up the latter assuming they were extracted by the applied method. However, regarding the benzoylureas, none of the parent compounds would have been detected by GC-MS due to the limitations given by the instrumentation, but hypothetically more volatile metabolites and degradation products could be detected by the applied technique (Section 2.3.4). Metabolites of benzoylureas should contain fluorine (Finkelstein et al., 2001). However, no fluorine containing compounds were detected within the window of this analysis according to the Table A.3 (Appendix A).

Whether the list in Table A.3 (Appendix A) includes metabolites of decomposition products of other organic pollutants associated with the aquaculture industry needs to be examined further by structural analysis. Based on the analysis done in this study, no such conclusions can be drawn. Further, based on the arguments presented above regarding the

limitations of instrumentation, the most likely conclusion is that none of the drugs associated with the industry, nor their metabolites and degradation products, were detected within the analytical window of this study. Another reason could be the sampling distances from the installations, being more than 100 m from the edge of the cages. Future studies should sample from closer distances, as the regulation in fact only restricts fishing closer than 100 m, but non-fishing related activities can approach as close as 20 m (Regulations concerning operation of aquaculture installations, 2008, §18).

Apart from the analytical limitations, there may be reasons to believe that the use of veterinary drugs, among other pollutants, at installation A (Hestøya) is generally limited due to its small size and special license of operation. Regarding installation B (Nørholmen), the potential effects of the installation upon the sediments may not have been measured at all, as discussed in Section 6.1.2.

6.3.2 Compounds identified by Mass Spectrometry (MS)

The solvent used for extraction (DCM) and the analytical instrumentation for separation and detection (GC-MS), represents a limited window into all compounds potentially present in the sediment samples. Only compounds with a certain volatility and affinity to the GC column would be picked up by the analytical system. Therefore, the analysis excludes many potential compounds of interest in the context of the study. Further, only peaks detected between 5 – 40 minutes are considered. However, it would be far beyond the limit of the time and budget for this project to include all compounds present in the sediments. Nevertheless, the findings in this study can be valuable. Especially if complemented by eventual future studies that attempts other windows into the total picture, by using different extraction solvents and instrumentation.

Detection of benzaldehyde and 3-bromophenol was confirmed by standard material. For the remaining compounds listed in Table A.3 (Appendix A), detection was only performed by mass spectra matched against the NIST library. Therefore, the possible detected compounds are only to be considered as qualified suggestions. However, RT7.4 gave a good match (match value 911) with xylenes (o-, m- or p-xylene varies between different samples), which are members of the BTEX aromatics and considered toxic (Dawson et al., 2008). Xylenes are present in small amounts in gasoline and air fuel, and usually related to air pollution. The compounds are also components of crude oil and present naturally in plant material.

Another possibly identified trace organic pollutant was anthracene (RT39.7), although with a relatively low match (match value 824). Anthracene is one of 16 polycyclic aromatic

hydrocarbons (PAHs) registered as persistent organic pollutants (POPs) according to the Stockholm convention (Stockholm Convention, 2018).

Fifteen compounds had a match values above 850 against the NIST library. Whether their presence was a consequence of anthropogenic input, was not confirmed at this stage. The remaining 42 suggested compounds gave low match values. Better match could have been achieved by e.g. subtracting noise from the TICs or using extracted ion chromatograms (XICs) for specific ions of interest. Further work could also attempt targeted approaches to identify and quantify the suggested peaks found in these sediment samples.

6.4 SOURCE ALLOCATION OF SEDIMENT POLLUTION

6.4.1 Fluctuations of trace element content related to aquaculture

Elevated levels of trace metals in the vicinity of aquaculture installations have been demonstrated in previous scientific studies in an international context. The most significant trace metals related to aquaculture activity seems to be Cu, Pb, Zn and Cd (Russell et al., 2011, Vera et al., 2015, Mendiguchía et al., 2006, Farmaki et al., 2014, Burridge et al., 2010).

Comparing the elemental contents of the sediments between the two installations, there were significantly higher concentrations of Pb near installation B, which has been in operation much longer than installation A (since 1979 compared to 2015). It may seem like Pb has accumulated in the nearby sediments during the decades. Inferior regulations and knowledge of environmental impacts in the past, especially in the 1970s and 1980s, could be an explanation. However, the levels are below environmental concern according to Norwegian regulations (Norwegian Environment Agency, 2016).

Of unwanted trace elements in fish feed used by the industry, As, Cd, Hg and Pb have been previously detected (The National Institute of Nutrition and Seafood Research, 2015), and could therefore be considered potential contaminants from aquaculture activity. However, assuming the elevated Cd levels shown, according to environmental classification, were not naturally occurring, the source could as well be other anthropogenic activities in the vicinity (e.g. land-based agriculture and road traffic). By looking at distance gradients from the installations, there were significantly higher levels of Cd below 500 m than further away, which could indicate some influence from the fish farms. However, this conclusion was not backed up by the Spearman correlation between Cd and distance (r_s = -0.17), nor by the PCA which showed a near orthogonal relation between the loadings vectors.

Regarding Hg, there was no significant difference below 500 m than further away. Like for Cd, the Spearman correlation with distance was considered weak (r_s = -0.10) and the PCA

showed near orthogonal relation between the loadings vector. Therefore, the slightly elevated Hg levels according to the classifications, were probably even less related to the aquaculture activity than Cd. Further, neither As nor Pb showed significant differences below 500 m than above, although maximum levels were higher closer to the installations than further away. However, no statistical significant conclusions can be made upon maximum levels alone. PCA loadings showed near orthogonal relationships between these elements and distance from the installation, which is backed up by the weak Spearman correlation coefficients (r_s = -0.12 and r_s = -0.05 respectively). Summed up, trace elements previously reported present in fish feed (The National Institute of Nutrition and Seafood Research, 2015), seemed not to be related to the fish farms in this study in a statistically significant way.

On the other hand, Al, Mn and Fe had higher concentrations closer than 500 m from the installations than above 500 m. The trend of increasing concentrations of these metals closer to the installations was verified by Spearman correlations, with negative moderate correlations between the metal and distance (r_s = -0.47, r_s = -0.44 and r_s = -0.44 respectively), and by PCA where the loadings vector of these three metals roughly followed that of distance. Al in some of its forms is known to be a gill toxicant to freshwater fish, especially in acidic lakes (Rosseland et al., 1990). However, this toxicity is not reported for marine organisms.

Cr and Sb had also significantly higher levels closer to the installation, but both the Spearman correlation (r_s = -0.24 for both) and the PCA loadings, showed a less distinct relationship with distances.

Comparing distances closer than 200 m with those between 200-500 m, there was a tendency for Cd, Cr and Sb to have higher concentrations at the intermediate distances. However, this does not exclude that the source was the fish farms. Hypothetically, there could be a tendency for the smaller particles in the effluents to travel some distance before reaching the sediment, which could explain the observed pattern. This pattern is probably not previously documented, as accumulation of effluents seems to deposit primarily in the immediate vicinity of fish farms (Carroll et al., 2003). However, dispersion of effluent particles may vary significantly from case to case, depending on ocean currents, stratification and seafloor topography.

Regarding outliers, the utilized software (SPSS) considers automatically all values above 1.5 × interquartile range (IQR) as outliers. This is considered a rudimentary approach for outlier detection, with a high risk of labeling too many outliers. More sophisticated probabilistic methods have been proposed (Yuen and Mu, 2012). If some of the values regarded as outliers by SPSS were instead not regarded as outliers, there could have been detected other significant

differences between the compared groups. E.g. Hg, As, Cu, Ni and Sb had outliers in the high range for distances below 500 m, and therefore significantly higher levels at closer distances could have been established if outliers were determined in a different manner.

PCA has been used to a limited extent regarding source allocation in this analysis. Other studies have successfully used chemometric methods for this purpose, but usually PCA in combination with cluster analysis (Retnam et al., 2013), or by factor analysis (Christensen and Bzdusek, 2005). In this study, PCA proved a useful tool for visualizing the big picture based on the available dataset, to support findings done by group comparisons and Spearman correlations, and to reveal correlation trends within the dataset not seen by the other statistical approaches.

6.4.2 Altered physio-chemical characteristics related to aquaculture

Generally, soil containing high amounts of organic content is known to accumulate more pesticides (Bollag and Loll, 1983), and elevated TOC levels have been previously measured in sediments close to aquaculture (Mendiguchía et al., 2006).

Total organic matter (TOM) content was not significantly different by comparing the installations nor distances. However, it seemed like the directions of sampling from installation A (Hestøya) mattered significantly. The two northwards directions had a higher TOM than southwards. Relating this to the ocean currents, the dominant directions were mostly southeast for surface water and east for bottom water. This suggests that the high TOM content, and consequently high TOC content, did not originate from installation effluents, but rather from terrestrial runoff. Moreover, Installation A is located in a relatively land locked area, which may be a reason for elevated TOM/TOC levels. The latter argument can explain lower TOM/TOC levels near installation B, which is located in a relatively open and ventilated area.

Looking further into the physio-chemical characteristics of the sediments, Spearman correlations showed a very strong link between pelite content and TOM (r_s = 0.82), with a strong inverse correlation with pH (r_s = -0.79 for both). These trends were confirmed by the PCA loadings, and by patterns shown by comparisons of the averages for different distance groups. This is consistent with established theories of relations between TOM, pelite and pH. Organic materials will usually decompose mechanically into fine particles and therefore increase the pelite fraction. Moreover, organic material contains humic acids which may reduce the pH concomitantly (Perdue, 1985).

TOM was also strongly correlated with Cu, Cd and As (r_s = 0.91, r_s = 0.89 and r_s = 0.82 respectively). This may suggest that the elevated TOM originates from anthropogenic sources,

since Cu, Cd and As, among others, are typically associated with industrial and wastewater effluents (Everaert et al., 2017). TOM and pelite contents did not have significantly different levels as a function of distance. Nevertheless, they did have higher maximum values at intermediate distances (200-500 m), which may slightly strengthen the hypothesis that effluent particles sedimented at these distances. Considering the rudimentary determination of outliers by the software as discussed in the previous section, there could be established a significantly higher pelite content at intermediate distances (200-500 m) if the outliers were treated differently, as this parameter had particularly many outliers in the high range for this distance group.

Seen from another point of view, sediments with high organic content have a higher affinity for metals by complexation with humic substances, especially at estuarine salinity levels (Mayer, 1985). Assuming the levels of TOM were naturally occurring, complexation with humic substances may also explain the distinct correlation between TOM and some trace elements. Therefore, it cannot be excluded that TOM and the correlated pelite content is naturally occurring. This argument is strengthened by the fact that TOM and the correlated pelite had higher levels in the samples directed towards north of installation A, which is closer to land and terrestrial runoff. Moreover, this may also explain why installation A had significantly higher levels of Cd, As and Sb compared with installation B, even if the latter has been in operation much longer.

6.4.3 Trace organic pollutants related to aquaculture

Confirmed organic compounds

Comparing the results with a suggested predicted no effect concentration (PNEC) in marine sediments for benzaldehyde at 2.21 µg kg^{-1} dw (European Chemicals Agency, 2018a), just 2 out of 52 sediments samples showed concentrations below this limit. However, considering the limitations for this quantification discussed earlier, the weak reference for the PNEC value, and because one point in the figure represented only one value (N = 1), no conclusions should be based on this analysis. Benzaldehyde is used as a taste enhancer in the food industry ("synthetic almond oil") (Scott and Scott, 1920), but is not previously reported as an additive in fish feed, or to have any connection with aquaculture whatsoever. The compound is also present naturally in plant material.

Regarding 3-bromophenol, no PNEC or other limit values for marine sediments were found. Bromophenols are previously detected in marine algae. However, their ecological function is unknown. Bromophenols are also detected in industrial flame retardants like

polybrominated diphenyl ethers (PBDEs) (Hassenklover et al., 2006), which are legacy pollutants with several PBDEs registered as POPs by the Stockholm convention (Stockholm Convention, 2018).

Benzaldehyde and 3-bromophenol could as well be naturally occurring at the measured levels, which was supported by comparing average concentrations and different distances, as well as the PCA loadings vectors, where benzaldehyde was near orthogonal to distance and 3-bromophenol even positively correlated.

Organic compounds linked with metals and metalloids

Strong Spearman correlations between metals that are usually associated with anthropogenic effluents and detected organic peaks, may potentially indicate that the specific peak is of interest in an environmental context, and could therefore be subject to further examination in future studies. However, causality is not given by correlation studies, and the association many as well be due to other factors, e.g. sediment composition that favors accumulation of certain metals and organic compounds. Only very strongly correlated ($r_s > 0.8$) parameters are discussed in this section, referring to the figures in Section 5.4 and Appendix A for information on the weaker correlations.

Cu, Cd and As were very strongly correlated with RT35.8 ($r_s = 0.84$, $r_s = 0.83$ and $r_s = 0.80$ respectively), which matched with dihydroactinidolide (IUPAC name: (7aR)-4,4,7a-trimethyl-6,7-dihydro-5H-1-benzofuran-2-one; CAS#: 17092-92-1), although the match value was low (match value 783). This compound is a volatile terpene and has been prepared synthetically and used as a fragrance. It may as well be naturally occurring in the sediments as it is present in macroflora and even as a pheromone for a variety of insects (Yao et al., 1998).

There were also very strong positive correlations ($r_s > 0.8$) between Cd, Mo and As with RT7.4, RT25.2 and RT38.6, as well as for RT25.0 with Cd and Mo. RT7.4 matched with xylenes, which are discussed in the previous section. RT25.2 was matched with 1,1,6-trimethyl-1,2-dihydronaphthalene (CAS#: 30364-38-6) (match value: 901), which is responsible for certain aromas in Riesling wines (Sacks et al., 2012), strongly suggesting it is a naturally occurring substance. RT25.0 matches with α-ionene (IUPAC name: 4,4,7-trimethyl-2,3-dihydro-1H-naphthalene; CAS#: 475-03-6) (match value: 911) which belongs to the family of tetralins. This compound could be of potential interest in this context as it is used as flavor agent in the food industry and also listed as toxic to aquatic life with long lasting effects (European Chemicals Agency, 2018b). However, as concentrations were not

quantified, it was not possible to conclude whether the detected abundance was present naturally in the sediments, or whether it was anthropogenic. RT38.6 matched with nonadecane (CAS#: 629-92-5) (match value: 840) which is a paraffin hydrocarbon and is found naturally in vegetable oils (Hsouna et al., 2011).

The TIC peaks discussed above, RT7.4, RT25.0, RT25.2, RT35.8 and RT38.6 did not show significant differences among the distance groups, except RT38.6 which had significantly higher levels for distances above than 200 m. Also, the number of detected peaks (#Peaks) showed the same pattern. However, there was a non-significant tendency for higher abundances at intermediate distances (200–500 m), compared to distances less than 200 m or above 500 m. This was coherent with tendencies shown for Cd, Cr and Sn. Assuming the source of these seemingly elevated concentrations at intermediate distances stem from the fish farms, it could strengthen the hypothesis that fine particular matter in the fish farm effluents can travel a certain distance before reaching the sediments. On the other hand, if the compounds were naturally occurring, this observed pattern could be explained by the slightly higher pelite and TOM contents at the intermediate distances. Even if the latter trend was not statistically significant, treating outliers differently as previously discussed, could have shown significantly higher pelite values at intermediate distances.

7 CONCLUSIONS

Elemental analysis by ICP-MS showed significantly ($p < 0.05$) elevated concentrations closer to the fish farm installations (< 500 m) than further away (> 500 m) for the following elements: cadmium (Cd), aluminum (Al), tin (Sn), chromium (Cr), iron (Fe) and manganese (Mn). Among the 16 elements selected for further analysis, none showed significantly higher levels at distances above 500 m compared to closer distances. This could indicate that the elevated levels closer to the installations were caused by the nearby aquaculture activity. However, according to sediment classification based on Norwegian regulations (Norwegian Environment Agency, 2016), none of the elements exceeded "good" conditions (Class II) in all samples, and consequently they were within predicted no effect concentrations (PNEC).

Comparisons between installation A (Hestøya) and installation B (Nørholmen), showed that lead (Pb) was significantly higher near installation B. This could be a consequence of accumulation from aquaculture activities, since installation B has been in operation since 1979, while installation A only since 2015. However, cadmium (Cd), arsenic (As) and antimony (Sb) were higher near installation A.

Total organic carbon (TOC) content classified sediment conditions as "very bad" (Class V) for 17 out of 30 samples. Only one sample classified as "good" (Class II). However, it was not possible to conclude if the high TOC contents were connected to effluents from the fish farms. TOC did not show any significantly higher concentrations in samples closer to the installations compared with further away. Elevated TOC levels could therefore be a consequence of the relatively land locked location of installation A, consequently with a potentially large input of organic matter from terrestrial sources.

Non-targeted organic screening by LC-MS/MS failed to detect any compounds in the sediment samples. However, it was not clear whether this was caused by the applied analytical procedures, or whether it reflected the conditions in the sediments. On the other hand, GC-MS identified two compounds (benzaldehyde and 3-bromophenol), which were confirmed by analytical standards. Additionally, 59 peaks were detected with suggested corresponding compounds according to mass spectra comparisons with the NIST library. Fifteen peaks gave reliable match values above 850. Some of these compounds may be potential pollutants, metabolites or degradation products, which could be harmful to ecosystems also at trace concentrations. However, these compounds may also occur naturally in marine sediments. Further targeted studies could reveal more information through identification, quantification and structural analyses.

Some peaks were correlated very strongly with the elements cadmium (Cd), arsenic (As) and copper (Cu), which suggest that these organic compounds have an anthropogenic origin. However, whether these correlations were natural or related to human activities, was not confirmed by this study.

Principal component analysis (PCA) revealed several correlations between the measured variables. To some degree, the first two principal components were able to separate different samples on elemental content. Samples at intermediate distances (200-500 m) seemed to be influenced more by content of elements of environmental concern, than distances closer to the installation (< 200 m) and distances further away (> 500 m). Moreover, pelite (grain sizes < 0.06 mm) and total organic matter (TOM) content, had a strong correlation with both the frequency and abundance of organic compounds. Correlations with pelite/TOM were also shown for most trace elements, which again correlated negatively with pH. However, the elements that correlated with pelite/TOM content, did not correlate with distances from the installations. On the other hand, aluminum (Al), iron (Fe) and manganese (Mn) did not correlate with pelite/TOM, but correlated negatively with distances, which suggested influence from the installations. Principal component 1 (PC1) could be described as the magnitude of pelite/TOM, most organic compounds and trace elements, while principal component 2 (PC2) described distances from the installations and content of Al, Fe and Mn.

A general conclusion is that, even if there are interesting trends linking slightly elevated element concentrations to the fish farms, influence on the surrounding sediments was probably insignificant from these specific installations. However, the results from this study showed that it is possible to detect potential pollutants by a non-targeted approach in combination with descriptive and multivariate statistics. Moreover, linking the organic analyses with elemental and physiochemical analyses, gave information concerning the dispersion mechanisms and potential anthropogenic influence. Further studies are necessary to conclude whether specific organic compounds can be related to the aquaculture activity. The use of cluster analysis in combination with PCA has been effective for source allocation in previous studies (Retnam et al., 2013). Future studies into these matters should also be conducted on samples from fish farms with an already established influence on the surrounding sediments, and possibly by including microbiological approaches, like i.e. immunoassays or community DNA sequencing (metagenomics) (Handelsman et al., 1998). Introducing such biological dimensions could make it easier to arrive at conclusions on eventual anthropogenic ecological impacts on sediments near aquaculture installations.

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1 APPENDIX A: ADDITIONAL INFORMATION

Table A.1 Detection limit (DL) in sediments for 47 elements analyzed by ICP-MS. Resolution (Res.) is given as Lr, Mr, Hr = Low, Medium and High.

Sign	*Isotope*	*Element*	*Res.*	*DL* $\mu g\ g^{-1}$	*Sign*	*Isotope*	*Element*	*Res.*	*DL* $\mu g\ g^{-1}$
Al	27	Aluminium	Mr	0.50	**Ni**	60	Nikkel-60	Mr	0.038
As	75	Arsenic	Hr	0.063	**P**	31	Phosphor	Mr	1.00
Au	197	Gold	Lr	0.0005	**Pb**	208	Lead	Lr	0.0050
Ba	137	Barium	Mr	0.033	**Pr**	141	Praseodymium	Lr	0.0008
Ca	43	Calcium	Mr	25.0	**Rb**	85	Rubidium	Mr	0.030
Cd	114	Cadmium	Mr	0.0250	**S**	34	Sulphur	Mr	50
Ce	140	Cerium	Lr	0.0005	**Sb**	121	Antimony	Mr	0.0050
Co	59	Cobalt	Mr	0.0100	**Sc**	45	Scandium	Mr	0.0100
Cr	52	Chromium	Mr	0.0125	**Si**	28	Silisium	Mr	10.0
Cs	133	Cesium	Lr	0.0013	**Sm**	147	Samarium	Lr	0.0013
Cu	63	Cupper	Mr		**Sn**	118	Tin	Mr	0.025
Dy	163	Dysprosium	Mr	0.0050	**Sr**	88	Strontium	Mr	0.063
Er	166	Erbium	Lr	0.0008	**Tb**	159	Terbium	Lr	0.0005
Fe	56	Iron	Mr	0.050	**Th**	232	Thorium	Lr	0.0013
Hg	202	Mercury	Lr	0.0025	**Ti**	47	Titanium	Mr	0.050
Ho	165	Hollium	Lr	0.0005	**Tl**	205	Thallium	Lr	0.0006
K	39	Potassium	Hr	12.5	**Tm**	169	Thulium	Lr	0.0013
La	139	Lantan	Mr	0.0050	**U**	238	Uranium	Lr	0.0006
Lu	175	Lutetium	Lr	0.0005	**V**	51	Vanadium	Mr	0.0075
Mg	24	Magnesium	Mr	0.25	**W**	182	Wolfram	Lr	0.0025
Mn	55	Manganese	Mr	0.0150	**Y**	89	Yttrium	Lr	0.0010
Mo	98	Molybdenum	Mr	0.050	**Yb**	172	Ytterbium	Lr	0.0010
Nb	93	Niob	Hr	0.063	**Zn**	67	Zink-67	Mr	0.100
Nd	146	Neodymium	Lr	0.0005					

Table A.2 Limit values for classification of sediments based on trace element content and normalized total organic carbon (nTOC) content (Norwegian Environment Agency, 2016).

			Upper limit value for each class				
Element	***Sign***	***Unit***	I	II	III	IV	V
Cadmium	Cd	µg g^{-1} dw	0.2	2.5	16	157	>157
Lead	Pb	µg g^{-1} dw	25	150	1480	2000	>2000
Nickel	Ni	µg g^{-1} dw	30	42	271	533	>533
Mercury	Hg	µg g^{-1} dw	0.05	0.52	0.75	1.45	>1,45
Copper	Cu	µg g^{-1} dw	20	84	84	147	>147
Zink	Zn	µg g^{-1} dw	90	139	750	6690	>6690
Arsenic	As	µg g^{-1} dw	15	18	71	580	>580
Chromium	Cr	µg g^{-1} dw	60	660	6000	15500	>15500
Normalized Total Org. Carbon	nTOC	mg g^{-1} dw	20	27	34	41	>41

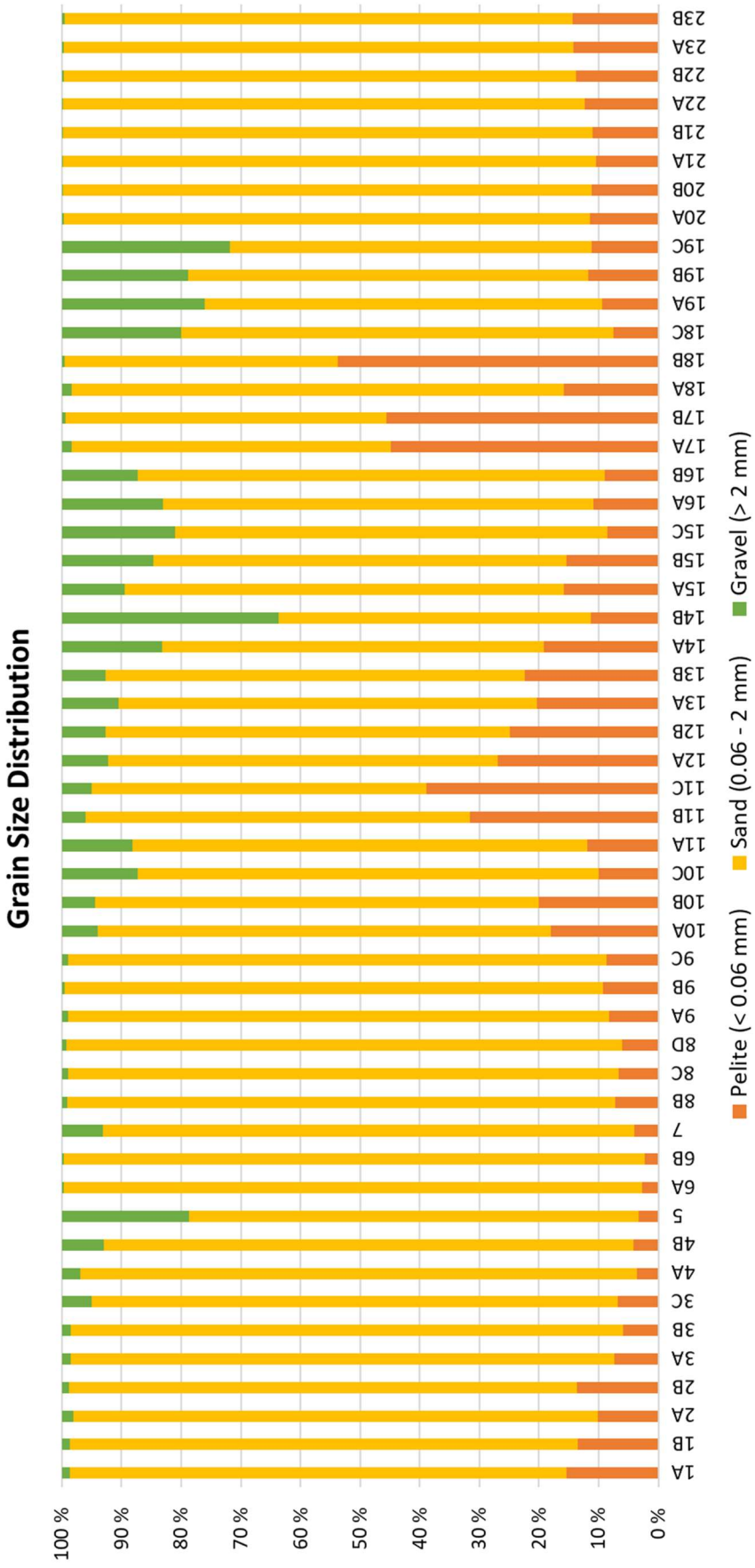

Figure A.1 Grain size distribution for sediment samples determined by dry sievage.

Table A.3 Possible compounds identified in the 52 analyzed sediment samples sorted by retention time (RT min). Also shown is the frequency (FRQ) of appearance among the samples, the average abundance by peak maxima (AVG), the variation among the samples by standard deviation (STDEV), and the best match/reversed match achieved together with the id of the sample that gave best match (MATCH@ID).

RT Min	***CAS#***	***NAME (non-IUPAC italicized)***	***FRQ***	***AVG***	***STDEV***	***MATCH@ID***
RT6.8	111-67-1	2-Octene	0.38	19757	15460	735/791@01B
RT7.4	106-42-3	p-Xylene	0.60	32961	21901	911/934@12A
RT9.7	111-71-7	Heptanal	0.90	15804	7068	894/896@14A
RT13.2	100-52-7	Benzaldehyde	0.92	30731	24974	940/952@14A
RT13.6	124-13-0	Octanal	0.83	14842	5778	824/926@11C
RT16.7	2548-87-0	2-Octenal	0.73	16777	8128	882/888@14A
RT16.8	122-78-1	Phenylacetaldehyde	0.12	21784	6126	749/943@19B
RT16.9	111-87-5	1-Octanol	0.25	25782	29670	946/962@22A
RT17.1	143-28-2	*Oleyl Alcohol*	0.15	28490	30910	798/809@15B
RT17.6	289-16-7	1,2,4-Trithiolane	0.25	28100	18693	833/899@18B
RT18.6	17429-02-6	4-Hydroxy-4-methylcyclohexanone	0.33	16487	5478	759/776@14A
RT20.2	118-71-8	*Maltol*	0.21	21574	6776	808/857@18B
RT20.4	5205-34-5	5-Decanol	0.40	90590	199798	932/932@22A
RT20.6	2471-84-3	*1-Methylideneindene*	0.23	17576	5558	884/923@23A
RT20.9	1125-21-9	*4-Oxoisophorone*	0.90	26429	19143	919/948@11C
RT21.0	112-31-2	Decanal	0.77	14304	5596	843/873@11B
RT21.8	58422-67-6	1-Cyan-1-(2-methylphenyl)ethyl ethaneperoxoate	0.38	12690	6861	620/703@17A
RT22.1	432-25-7	*β-Cyclocitral*	0.37	17596	23988	784/853@11C
RT22.5	6920-24-7	1,10-Hexadecanediol	0.10	16915	12049	710/761@17B
RT22.8	7320-37-8	2-Tetradecyloxirane	0.31	37540	34993	759/781@22A
RT23.0	10510-54-0	*Cresyl violet acetate*	0.19	24668	14301	545/578@17A
RT23.1	99858-37-4	(5-Iodopentyl)benzene	0.71	26616	23997	742/761@02A
RT23.8	294-62-2	Cyclododecane	0.35	11177	4426	787/796@11C
RT25.0	475-03-6	*α-Ionene*	0.77	35852	29891	911/932@18A
RT25.2	30364-38-6	1,1,6-Trimethyl-1,2-dihydronaphthalene	0.58	26084	17488	905/952@11C
RT26.0	20189-42-8	*Ethylmethylmaleimide*	0.98	47405	45232	765/877@14A
RT26.3	23950-04-1	*α-Nicotine*	0.23	11241	4869	858/915@01A
RT26.5	106-23-0	*β-Citronellal*	0.37	14703	5027	656/670@12A
RT26.8	50786-09-9	*2-Isopropylidenecyclohexanone oxide*	0.35	17302	9944	673/734@11C
RT26.9	92485-93-3	*2-Methyl-3-oxocyclohexanebutanal*	0.31	17053	6582	743/755@11C
RT27.1	21494-57-5	*Methylvinylmaleimide*	0.83	23376	15566	717/927@01B
RT27.9	575-37-1	1,7-Dimethylnaphthalene	0.94	54975	48433	946/966@11C
RT28.5	571-58-4	1,4-Dimethylnaphthalene	0.27	15218	4755	757/879@11C
RT28.9	615-58-7	2,4-Dibromophenol	0.10	22456	30594	920/924@22B
RT29.1	112-55-0	1-Dodecanethiol	0.19	12660	6434	709/748@09A
RT29.2	3489-28-9	1,9-Nonanedithiol	0.65	17442	12291	724/761@09B
RT29.7	126-86-3	*Surfynol 104*	0.13	20745	13614	752/752@11A
RT30.3	591-20-8	3-Bromophenol	1.00	78815	91665	956/967@21B
RT30.9	83005-01-0	Dodecyl dichloroacetate	0.65	16065	9417	587/718@14A
RT31.0	14901-07-6	*β-Ionone*	0.37	24920	12369	800/811@18B
RT31.1	23262-34-2	*Dendrolasine*	0.19	15051	13247	766/836@18A

RT Min	*CAS#*	*NAME (non-IUPAC italicized)*	*FRQ*	*AVG*	*STDEV*	*MATCH@ID*
RT31.8	23267-57-4	*β-Ionone 5,6-epoxide*	0.60	13434	7580	744/845@18B
RT32.5	638-66-4[b]	Stearaldehyde	0.98	55860	42508	829/854@18A
RT33.0	645-72-7	*Dihydrophytol*	0.85	31218	21885	716/741@13B
RT33.1	2136-70-1	*Myristyl monoethoxylate*	0.81	17321	11050	729/729@11C
RT34.9	2152-44-5	*Betamethasone 17-valerate*	0.31	27350	21022	518/544@17A
RT35.3	638-66-4[b]	Stearaldehyde	0.98	29747	19853	837/868@14B
RT35.8	17092-92-1	*Dihydroactinidolide*	0.98	33596	23703	783/837@12A
RT36.6	65646-68-6	*Fenretinide*	0.17	19075	4309	636/644@18B
RT36.9	53384-71-7	(E)-N-(3,4-Dimethylphenyl)-1-(4-methoxyphenyl)methanimine	0.15	16878	9511	568/618@19A
RT37.0	14237-73-1[a]	3,7,11,15-Tetramethyl-2-hexadecene	0.81	36754	35028	777/890@18A
RT37.3	14237-73-1[a]	3,7,11,15-Tetramethyl-2-hexadecene	0.90	60617	63010	847/914@18A
RT37.5	102608-53-7[c]	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.98	93993	97116	860/936@18B
RT37.9	638-66-4[b]	Stearaldehyde	0.69	17720	12909	841/872@22A
RT38.1	102608-53-7[c]	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.85	25077	18331	781/923@18A
RT38.4	3045-76-9	2-Methylenecyclododecanone	0.31	24625	20064	780/815@04B
RT38.5	629-92-5	Nonadecane	0.88	78215	78882	840/883@18A
RT38.6	102608-53-7[c]	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.98	37045	26272	807/929@18A
RT39.5	14016-29-6	*Averufin*	0.10	41695	20752	520/597@17A
RT39.6	502-69-2	*Hexahydrofarnesylacetone*	1.00	73587	51179	754/894@11C
RT39.7	120-12-7	Anthracene	0.75	25261	22601	824/918@23A

[a,b,c] Identical letters after CAS# indicates that the same compound is also identified at other retention times

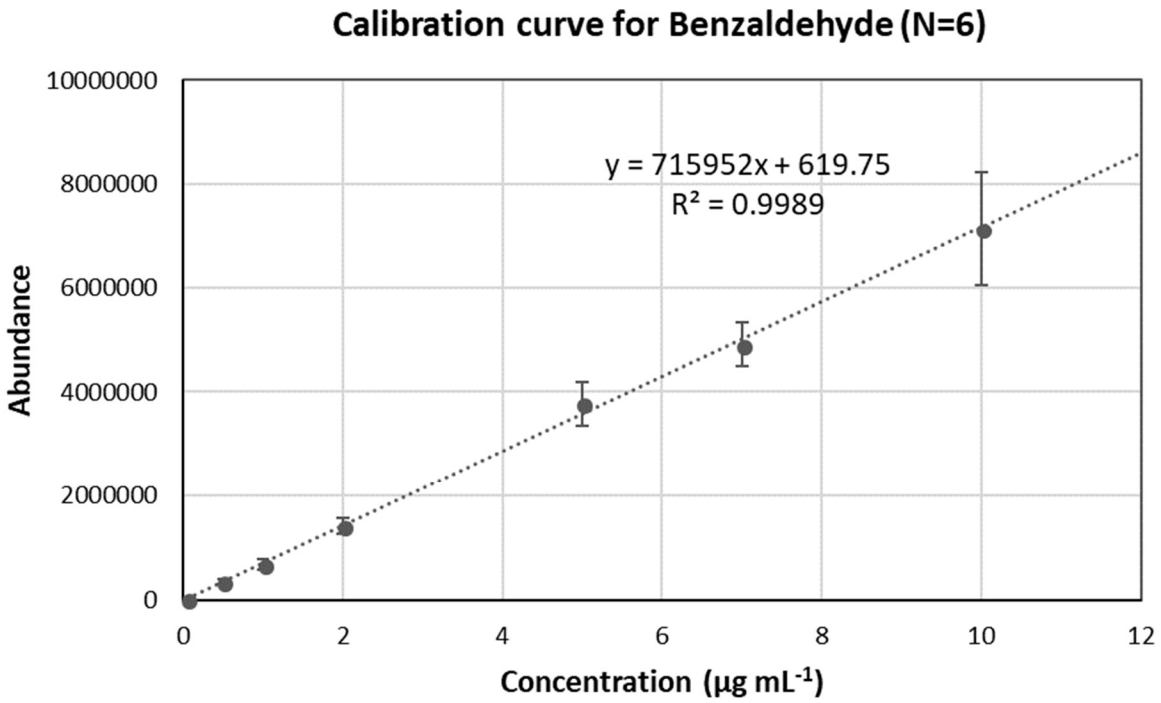

Figure A.2 Calibration curve for benzaldehyde for pseudo-quantification by GC-MS. Error bars represent standard deviation.

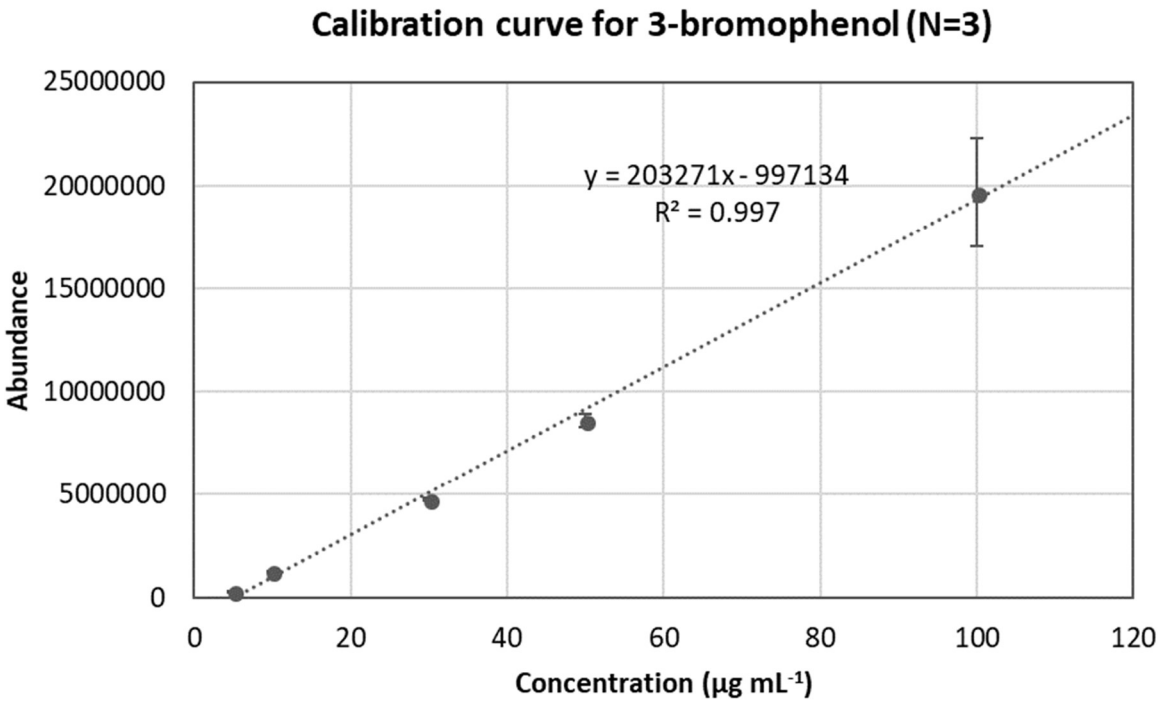

Figure A.3 Calibration curve for 3-bromophenol for pseudo-quantification by GC-MS. Error bars represent standard deviation.

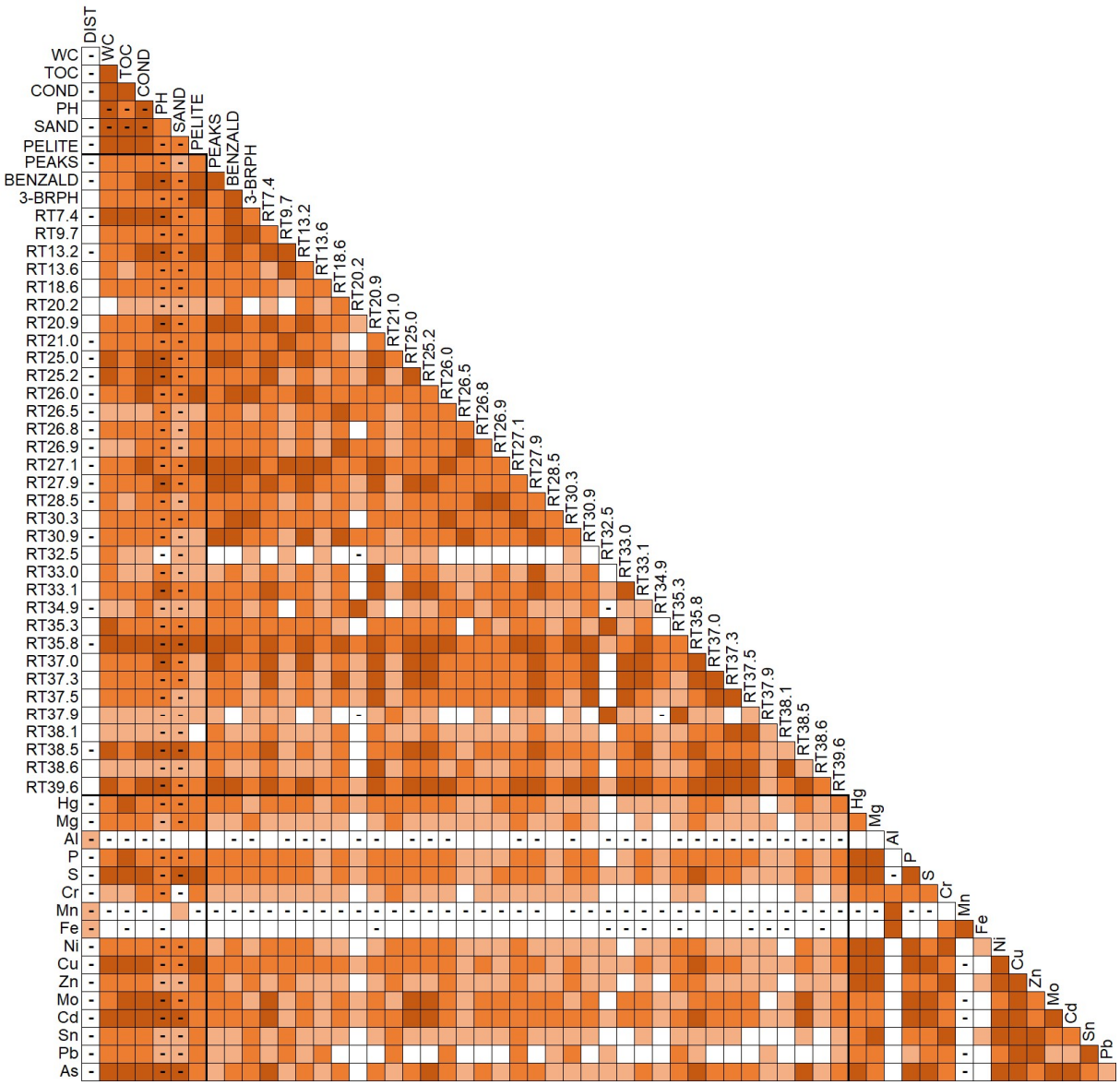

Figure A.4 Spearman correlations for all variables that was very strongly correlated ($|r_s| > 0.8$) with at least one other variable. Distance from installation (DIST) is included as well. Legend for color codes are given in Table 5.7. Thick cell border shows boundaries between groups of variables; sediment characterization, organic analyses, elemental analyses.

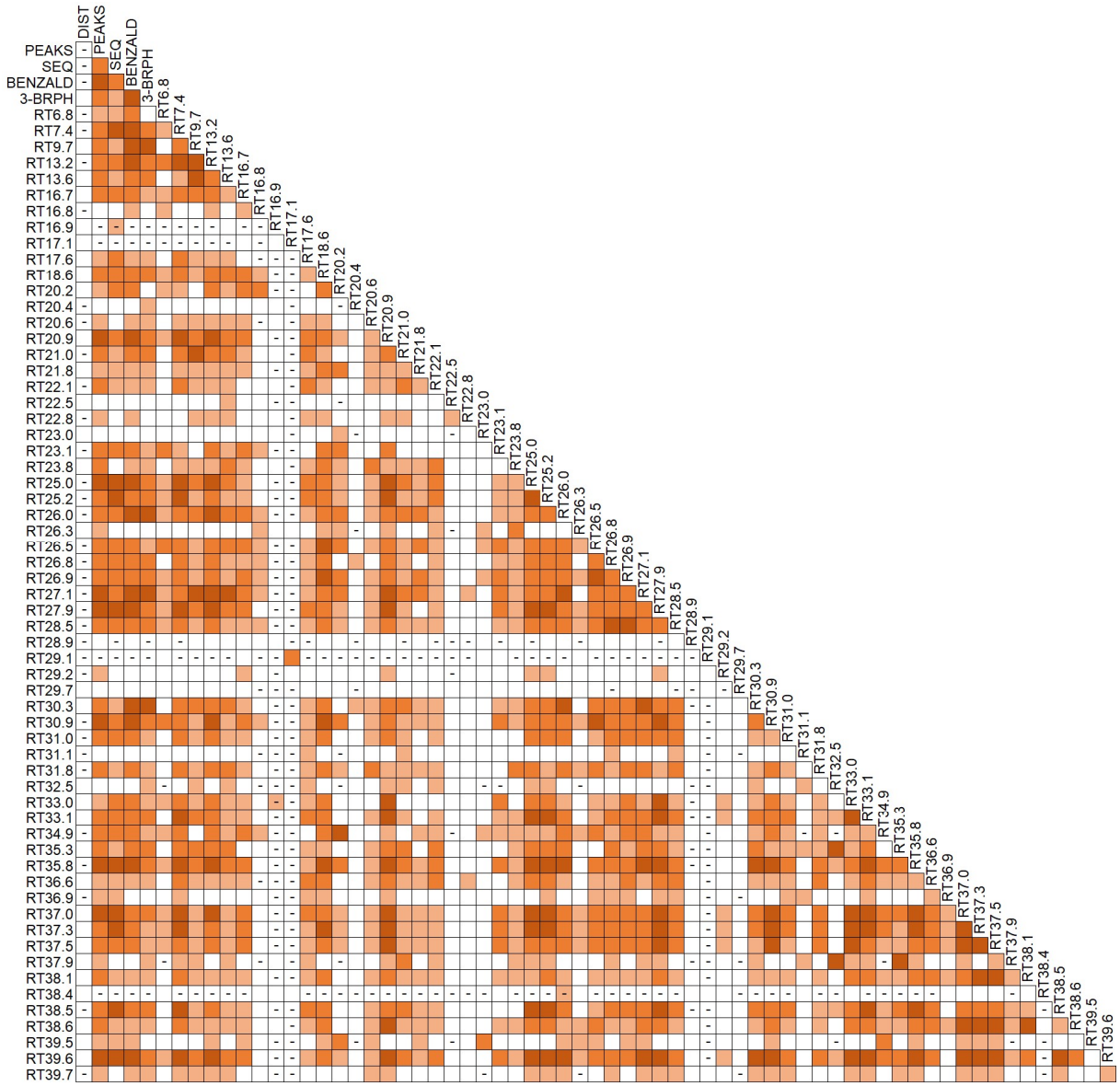

Figure A.5 Spearman correlations for organic analyses. Distance from installation (DIST) is included. Legend for color codes are given in Table 5.7.

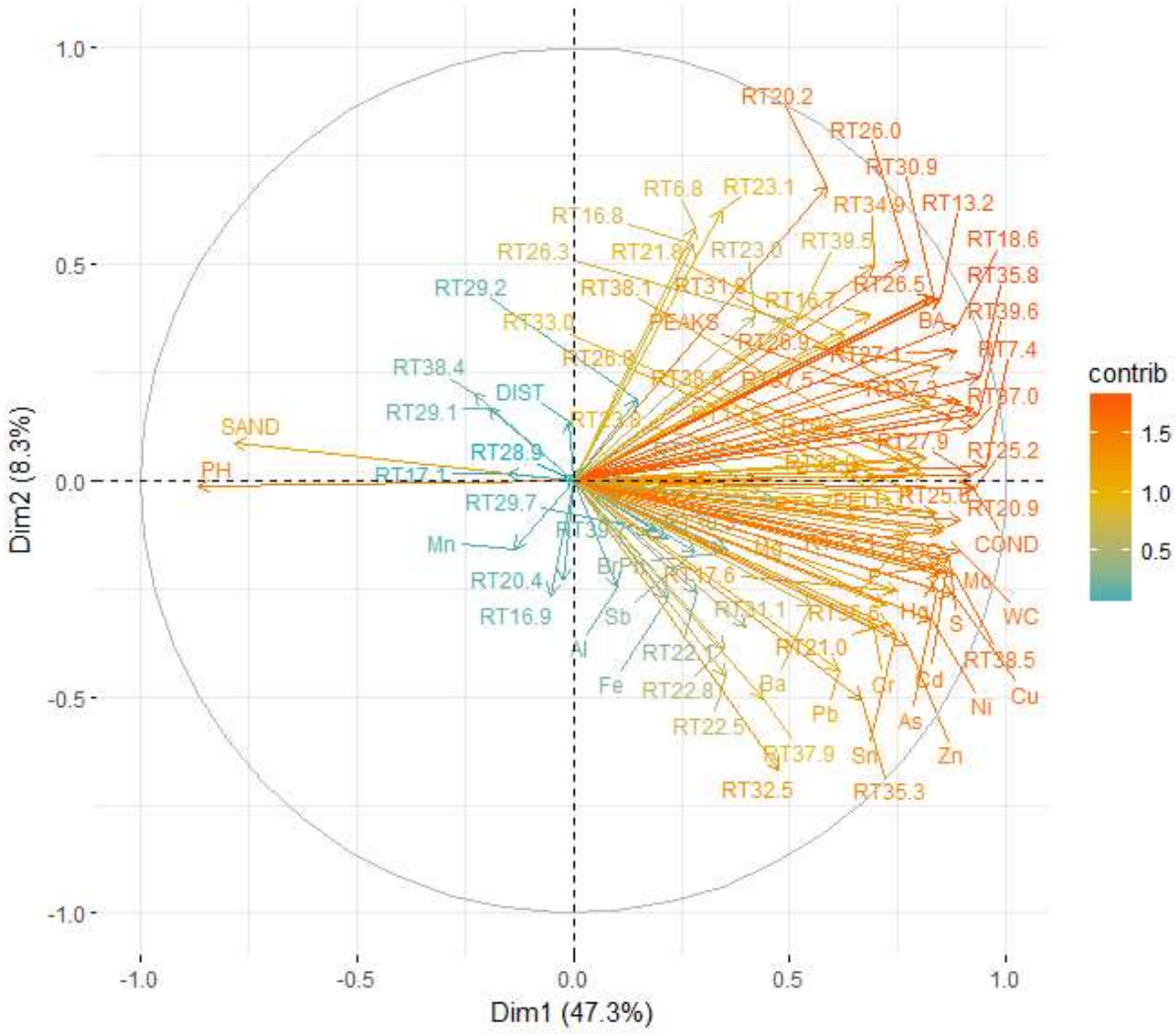

Figure A.6 Loadings plot for the combined dataset visualizing all 90 variables. Color codes represent the variables' contribution to the first two principal components, given by the legend in the figure (contrib). *N* = 52.

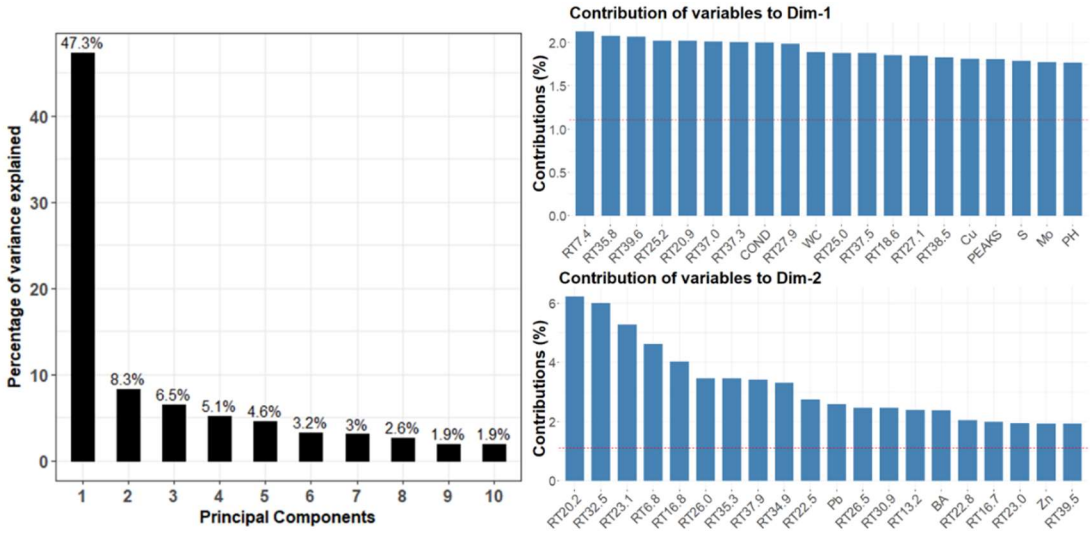

Figure A.7 The left plot shows the percentage of the variation in the combined dataset described by the first ten principal components. The bars to the right shows the top 20 variables that contributes the most to PC1 and PC2. The red line marks the average contribution from all variables. *N* = 52.

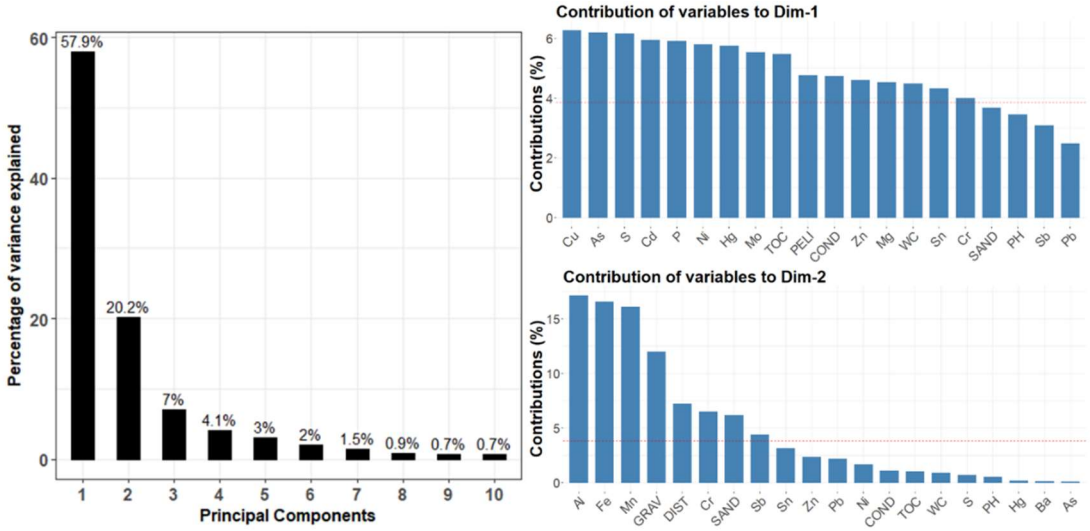

Figure A.8 The left plot shows the percentage of the variation among the selected elements described by the first ten principal components. The bars to the right shows the top 20 variables that contributes the most to PC1 and PC2. The red line marks the average contribution from all variables. $N = 30$.

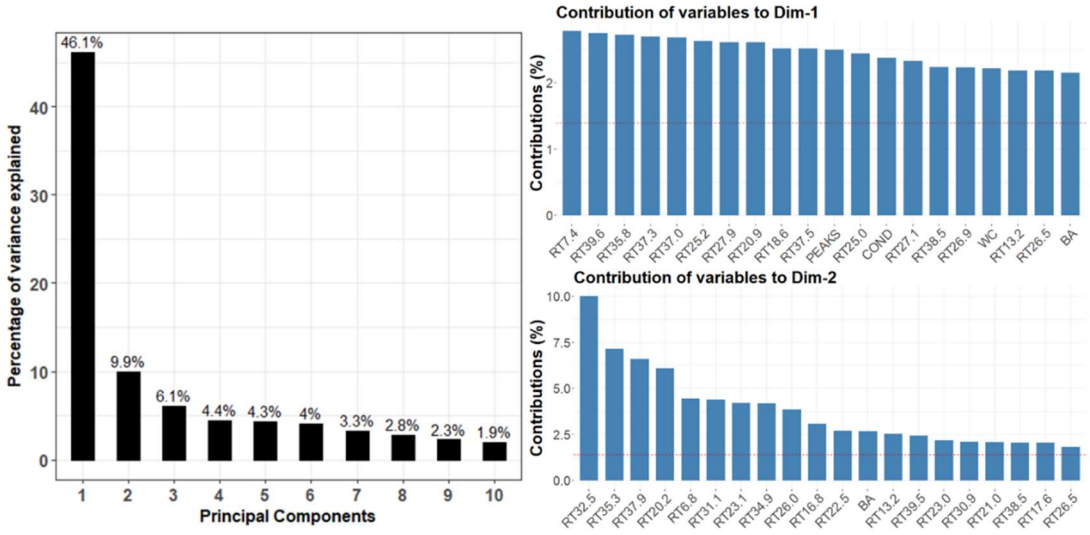

Figure A.9 The left plot shows the percentage of the variation among the organic analyses described by the first ten principal components. The bars to the right shows the top 20 variables that contributes the most to PC1 and PC2. The red line marks the average contribution from all variables. $N = 44$.