



SINTEF

Report

Long term fate and behaviour of oil in an Arctic shoreline

Part 2, activities in 2024

Author(s):

Sigrid Hakvåg, Liv-Guri Faksness

Report No:

2024:01460 - Unrestricted

Client:

Norwegian Coastal Administration (Kystverket)

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KEYWORDS

Biodegradation, oil, chemical fingerprinting, microbial communities

VERSION

1.0

DATE

2024-12-12

AUTHOR(S)

Sigrid Hakvåg, Liv-Guri Faksness

CLIENT

Norwegian Coastal Administration (Kystverket)

CLIENT'S REFERENCE

Silje Berger

PROJECT NO.

302007903

NO. OF PAGES

26 + Appendices

SUMMARY

In this work, analysis of sediment samples collected from an oil-contaminated beach at Svalbard has been performed. Chemical fingerprinting analyses in combination with analysis of the microbial communities present have yielded insights into the long term-effect of an oil spill on a shoreline which has been left uncleaned for more than 25 years. In autumn 2023, sediment samples were collected from a beach by the Credner moraine where an experimental oil spill was performed in 1997. Chemical analyses of sediment samples collected in 1999-2011 indicated microbial degradation of the oil components. Samples collected in 2023 from both the oiled area and a nearby reference area have now been subjected to chemical and microbial analyses to characterize the long-term natural (bio)degradation of oil and the microbial community composition in a non-treated experimental oil spill in an Arctic shoreline.

PREPARED BY

Sigrid Hakvåg

 SIGNATURE
 

Sigrid Hakvåg (Dec 12, 2024 13:31 GMT+1)

CHECKED BY

Mari Creese

 SIGNATURE
 
APPROVED BY

Trond R. Størseth

 SIGNATURE
 

Trond R. Størseth (Dec 13, 2024 19:56 GMT+1)

REPORT NO.

2024:01460

ISBN

978-82-14-07078-1

CLASSIFICATION

Unrestricted

CLASSIFICATION THIS PAGE

Unrestricted

Document history

VERSION	DATE	VERSION DESCRIPTION
DRAFT	2024-11-11	First draft
1.0	2024-12-12	Final version

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Sammendrag

Målet med prosjektet 'Naturlig nedbrytning av oljeforurensning i et arktisk strandmiljø over lang tid' har vært å få bedre kunnskap om langtidsskjebnen for olje i en arktisk strandlinje. Prosjektet hadde også som mål å gi ny kunnskap om hvordan stedeodne mikroorganismer bidrar til å bryte ned forurensning i strandsedimentene. Et eksperimentelt oljeutslipp ble utført på tre strender ved Credner morenen i nærheten av Svea i 1997, hvorav ett felt ble etterlatt som referanse. Det er tatt prøver fra dette området i flere perioder og ved å sammenligne data fra 1997-2011 med nye prøver innhentet i 2023 er det ønskelig å få kunnskap om effekt av et oljesøl på strand, samt naturens evne til selvrensing av olje på strand ved bakteriell nedbrytning. Prøvetaking av sediment for kjemiske og mikrobielle analyser ble utført høsten 2023 (finansiert av Kystverket). Videre analyser, finansiert av Kystverket og Svalbard Miljøvernfond, har involvert DNA-baserte analyser, samt kjemisk analyse ved hjelp av GC-FID/GC-MS.

Forsvarlig oppfølging av strandsonen etter oljeforurensning forutsetter kunnskap om relevante strategier og metoder for opprensing. Kunnskap om naturens evne til selvrensing ved bakteriell nedbrytning av olje i sediment langs kysten vil derfor bidra til et bedre grunnlag for å velge ikke-invaderende metoder i en oljesøl-situasjon hvor oljen har strandet, og på den måten, om mulig, spare miljøet for store inngrep (for eksempel ved å redusere bruk av mekaniske opprensningsmetoder). Responsstrategier som involverer naturlig nedbrytning er særlig relevant for avsidesliggende områder i Arktis, men det er behov for mer kunnskap om biologisk nedbrytning i det arktiske miljø for å vurdere anvendelsesmulighetene.

I dette prosjektet er sedimentprøver innhentet fra det ubehandlede oljesøl-området (30 m x 3 m), samt prøver fra et nærliggende referanseområde på stranda. Analyser av disse er sammenlignet med tidligere analyserte prøver for å oppnå et mer helhetlig bilde av naturlig (bio)degradering av olje fra et ubehandlet oljeutslipp over lengre tid. Kjemiske analyser (total hydrokarbon-konsentrasjon (THC) av sediment innhentet i 2023 viser at olje fremdeles er til stede i sedimentene, men at det detekteres ulik konsentrasjon og grad av aldring av oljen i prøvene. En halvering av oljekonsentrasjonen er tidligere målt i prøver fra 1998 til 2011, men det ble ikke påvist ytterligere reduksjon i estimert oljekonsentrasjon i prøvene fra 2023. Resultatene indikerer at det har vært lite biodegradering av oljen de siste årene. Diagnostiske ratioer brukt i oljesølanalyser indikerer at ratioer for hopaner er robuste etter 26 år, men at noen av de triaromatiske stieranene og mesteparten av PAH ratioene påvirkes av biodegradering over såpass lang tid. DNA-baserte analyser av mikrobielle samfunn fra de samme sedimentene viser forskjeller mellom samfunnene assosiert med sediment i oljefeltet og sediment utenfor feltet. Majoriteten av taxa detektert i sediment fra oljefeltet er tidligere rapporterte å degradere ulike oljeforbindelser eller er assosiert med degradering av olje.

Observasjoner gjort i felt, samt evaluering av kjemisk- og mikrobiell- analyse av sedimentprøver fra 2023, indikerer at oljen trolig ikke vil ha noe ytterligere påvirkning på miljøet i Credner-morenen. Det forventes ingen videre risiko for spredning av oljen.

1 Background

SINTEF Ocean, Climate and Environment, together with the Norwegian Coastal Administration (NCA) have discussed the potential of studying the long-term fate and behaviour of oil in an Arctic shoreline. Chemical and microbial analyses were performed to achieve more data related to long-term effects of an oil spill on shoreline and the potential environmental impact if the clean-up of the contaminated site is left to natural attenuation. It was decided to perform a limited study, divided in two parts. The first part, financed by NCA, was performed in autumn 2023, and involved sampling of sediment from Svalbard. For execution of the second part of the project which involved analyses of the samples collected in 2023, the partners sought to receive financing from Svalbard Miljøvernfond and the NCA. The project was granted funding and is registered in the RiS-portal with RiS ID 12268.

The project aims to provide increased knowledge of Nature's ability to self-purge oil in coastal sediment by bacterial degradation. A better understanding of the natural attenuation can further provide a basis for choosing bioremediation methodology as a measure in an oil spill situation where the oil has stranded and potentially save the environment for major interventions by reducing the use of mechanical cleaning methods. Response strategies involving natural attenuation and non-invasive methods are particularly relevant for remote areas in the Arctic, however, more knowledge on biodegradation in the arctic environment is needed to evaluate the applicability of such response strategies.

Norway has a long coastline with a lot of marine traffic and offshore petroleum activities. There is therefore always a risk for accidental oil spills from vessel accidents, pipelines or offshore oil installations reaching shorelines. This will often result in a comprehensive, time consuming and expensive clean-up operation. Bioremediation is an alternative response option for oil in sediments, especially in remote and sensitive areas, such as the Arctic. In 1997, SINTEF and collaborating partners from USA and Canada carried out an experimental oil spill (IFO30) on three beaches close to Svea, Svalbard, to evaluate the effectiveness of in situ shoreline cleaning treatments to accelerate natural recovery. One site, located in Credner moraine, was left as a reference (no-treatment) to study the long-term fate and behaviour of a shoreline oil spill. Sampling has been performed almost every year from 1999 to 2008. A more comprehensive sampling to map and evaluate the environmental consequences of the remaining oil was performed in September 2011. Then the oil was located down to 80 cm below the shoreline surface. Chemical fingerprinting analyses were performed on all samples from 1999 to 2011, and the results were reported in Noreng (2012, Master thesis) and were presented on AMOP in 2012 (Faksness et al.) (Noreng 2012; Faksness, L.G., M.S. Noreng 2012b).

Following up on this work, sampling of sediment for further chemical analysis combined with analyses of the corresponding microbial communities present would yield a better understanding of the long-term effects of an oil spill on shoreline. This would also provide insights into any potential environmental impact if the contaminated site is left uncleaned and provide useful data for understanding the natural attenuation.

A new sampling of sediment was performed in 2023. Samples were collected from the non-treated oiled field (35 m x 3 m), and from a clean, nearby reference site of the shoreline. Samples were collected to allow chemical and microbial analysis to determine biodegradation of the oil and compare with historical chemical

data from previous samplings at the same site. The long-time impact of the spilled oil on the local microbial community has been studied by comparing microbial community compositions in samples from the oil spill with the reference samples from the same shoreline and provided valuable information of biodegradation performance. Results from the chemical analysis has been evaluated together with data from 1997-2011 and provided further details on the monitoring of biodegradation processes on oil compound level. Analysis of the chemical composition of remaining oil in combination of the microbial community has thus also allowed an evaluation of the bioremediation performance. In addition, some of the geochemical petroleum biomarkers that are used in oil spill forensics have been analysed and evaluated in selected samples using the methodology described in Kienhuis et al and EN (2023) (EN, 2023; Kienhuis, P., A.B. Hansen, L.G. Faksness, S.A. Stout, 2016). This methodology is applied by laboratories all over Europe for oil spill forensics and are mainly based on diagnostic ratios between components that are assumed to be relatively resistant to weathering. In the present study, several of the diagnostic ratios recommended in EN (2023) have been evaluated to investigate if weathering of the components have occurred, and the robustness of the recommended ratios.

The objectives of the project have been to

- Characterize the long-term natural (bio)degradation of oil and the microbial community composition in a non-treated experimental oil spill in an Arctic shoreline.
- Evaluate bioremediation as a response option for oil contaminated shorelines, especially as a low-footprint alternative in remote and sensitive areas, such as the Arctic.
- Achieve more data related to long-term effects of an oil spill on shoreline, and potential environmental impact if the contaminated site is left to cleanse naturally (or left to nature).

2 Materials and methods

2.1 Sampling and sample treatment

Sediment samples were collected at the Credner Moraine in Svea, at Svalbard, the 15.08.2023. The project was registered in the RiS-portal with RiS ID 12268. A detailed description of the sampling performed is presented in a memo (in Norwegian) made available to Kystverket after completion of part 1 of the project and included as Appendix A to this report. The non-treated oiled field (3m x 35 m) was indicated by a metal-rod marker at 0- and 35-meters with the following coordinates: 77°52'30.3"N 16°46'30.5"E; 77.875095, 16.775145; N77°52.5057 E16°46.5087 (0-meters) and 77°52'29.4"N 16°46'33.1"E; 77.874830, 16.775868; N77°52.4898 E16°46.5521 (35-meters).

A total of 48 samples were collected for chemical (20) and microbial (28) analyses, as shown in Table 1 below. Samples were collected both within the field, at a nearby reference site, and in the tidal zone below the field. To guide sampling, trenches were dug to visually determine the depth of the oil layer below the shoreline surface. For all samples, sampling was performed at approximately the depth of the oil layer (see Table 1). Each sediment sample consisted of approximately 100 g sediment. Water samples each consisted of 420 ml filtered seawater (Sterivex filters, 0,2 µm). 1 ml DNA/RNA shield (Zymo) was immediately added to each sample. Samples were collected at an air temperature of 5 °C, transported back to Longyearbyen in

the dark and at ambient temperature and stored at -20 °C until shipping to Trondheim. Shipping was performed over night, and the temperature in the shipping container was kept below 8 °C until arrival in Trondheim. At arrival, samples were stored at -20 °C until further processing.

Table 1: Overview of sediment samples collected at Svea, in 2023

Distance (m)		Depth	Sand layer	# samples		Comment
From 0m-mark*	From top of field	cm	cm	DNA	Chemistry	
17,5	1,9	125	40	3	3	
22,5	1,6	110	40	3	3	Semi-hard oil layer
22,5	2,1	110	40	3	3	
27,5	2	115	40	3	2	Hard oil layer
36,4	2	90	35	2	1	
39,5	2	130	40	3	1	
17,5	8	25	25	2	1	In tidal sone. Below oiled field
39,5	8	25	25	3	3	In tidal sone
62	8	25	25	3	3	In tidal sone
Seawater				3	0	3 samples á 420 ml
*Towards the right				28	20	

2.2 Chemical analysis and data treatment

2.2.1 Chemical extraction

Surrogate internal standards (SIS) (naphthalene-*d*8, phenanthrene-*d*10, chrysene-*d*12, and o-terphenyl) were added to sediment samples (approximately 10 g) before extraction. Samples for chemical analysis were extracted using dichloromethane (DCM), filtered through Bilsom and dried with Na₂SO₄. The extracts were diluted to 5 mL. A solvent exchange from DCM to hexane was performed on 1 mL of the extract prior to Solid Phase Extraction (SPE) using a silica column. The non-polar fraction was collected after SPE, concentrated to 1 mL, and added recovery internal (RIS)-standards (acenaphthene-*d*10, fluorene-*d*10 and 5 α -androstane). Extracts were stored at 4°C in the dark until analysis on GC-FID (gas chromatograph with flame ionization detector) for quantification of total hydrocarbons (THC) and on GC-MS (gas chromatography - mass spectrometry) for quantification of semi volatile organic compounds (SVOC, including PAHs and biomarkers).

2.2.2 GC-FID Analysis

Analysis of total hydrocarbons (THC) was performed on an Agilent 6890 GC-FID according to a modification of EPA Method 8015D (US EPA 2015). Quantification was done using the RRF from a 5-level external calibration curve (1 – 20 mg /mL) of the initial spilled IF30 after normalization to internal standard (5- α -androstane). The GC-FID analyses included THC, as defined by the boiling point range of normal alkanes nC10 – nC36, and calculation of the ratios between C₁₇/pristane and C₁₈/phytane.

2.2.3 GC-MS Analysis

Analysis of PAHs and biomarkers was performed using an Agilent 7890B GC coupled to an Agilent 5977A MS following a modified EPA Method 8270E (US EPA 2018). The ion source was operated in selected ion monitoring mode. Quantification was done using the abundance of the molecular ion and average response factor (RRF) of a 6-level calibration curve (0.01-1 µg/mL) after normalization to internal standard (fluorene-*d*10). The RRF from the parent PAHs were used for quantification of the respective homologues. The GC-MS target list is given in Table 3 (on page 23 of this report) and includes decalins, 2- to 6-ring polycyclic aromatic hydrocarbons (PAHs) including their alkyl homologues, the biomarker 17a(H),21b(H)-hopane (30ab or hopane) and other selected biomarkers (hopanes and triaromatic steranes).

In addition to quantitative analysis, the PAHs were also normalized to 30 ab hopane (hopane). Hopane has been assumed to be a "conservative internal marker within the oil" and the use of hopane as an internal GC-MS standard is well-established in connection to weathering processes such as evaporation and biodegradation (e.g. Prince et al. 1994). The quantification method used has previously been described (Prince, Owens, and Sergy 2002; Stout et al. 2016) to indicate the degree of weathering (depletion) in field-collected oils after e.g. the Deepwater Horizon incident. The following formula was used to calculate percent depletion of any given fraction (e.g. THC or total PAHs (TPAH)) or individual compounds (e.g. naphthalene) in the samples:

$$\% \text{depletion (or \% loss) of A} = \left[\frac{(A_0/H_0) - (A_s/H_s)}{(A_0/H_0)} \right] \times 100 \quad (1)$$

Where A_s and H_s are the concentrations of the target analyte and hopane in the field samples, respectively, and A_0 and H_0 are the concentrations of the target analyte and hopane in the initially spilled oil.

2.2.4 Evaluation of biomarkers

GC-MS is an important tool in oil spill forensics, and the components and diagnostic ratios (DR) evaluated here (Table 3), are also included in EN 2023. DRs are the ratios between the peak height or peak area of single compounds or compound groups selected by their diversity in chemical composition and on their known behavior in weathering processes. To estimate an acceptable difference between two analytical results, EN (2023) uses the standard deviation of the analysis method (ISO 1994b; 1994a). Repeatability, r , is applied as the test method to compare individual ratios, assuming that the two samples to be compared originated from the same source. In this case, DRs in the IFO30 from 1997 were used as reference and source oil, and DRs in the samples collected over the years were compared with it to get an indication of the robustness of the DRs over time. The repeatability limit is calculated from the relative standard deviation, RSD. In EN 2023 RSD of 5 % is recommended. For the normal distribution, the critical difference between two values at 95 % confidence level ($r_{95\%} = 2.8 \times \text{RSD} = 2.8 \times 5\% = 14\%$) is 14% if an RSD of 5 % is used (ISO 1994b). Consequently, any measured difference in a DR between two samples (i.e. the absolute difference in percentage based on the mean of the two ratios) higher than 14% should be regarded as significant, meaning that these two ratios are statistically different. In our study, this indicates that this specific DR has been influenced by weathering since the oil was spilled in 1997. If the critical difference is <14% for some DR, it indicates that this DR is more robust and has not been affected by weathering, such as evaporative loss, photo oxidation, or biodegradation.

2.3 Microbial analysis and data treatment

2.3.1 DNA extraction

FastDNA Spin kit for soil (MP Biomedicals) was used for extraction of genomic DNA from the sediment samples, according to the producers' manuals. Each sample consisted of ~1,5 g of sediment. DNA was extracted from the filters of the three seawater samples (each 420 ml filtered water) using the ZymoBIOMICS DNA MiniPrep kit according to the producer's manuals. DNA extracted from samples using both kits were eluted in 60 μ L. Concentrations of the extracted DNA were quantified using a NanoDrop 1000 Spectrophotometer (ThermoFisher Scientific), and a Qubit 3.0 Fluorometer (Invitrogen) with dsDNA High Sensitivity kit (ThermoFisher Scientific), according to the producer's manuals. Extracted DNA (25 μ L of each sample) was sent to BGI Tech Solutions (Hong Kong) for further analysis. Shipping was performed at ambient temperature, for a total duration of 2 days.

2.3.2 Sequencing and data treatment (bioinformatic analyses)

Sequencing was performed at BGI Tech Solutions in Hong Kong. The variable v3-v4 regions of the bacterial 16S rRNA gene were amplified by PCR at BGI, using the primers 338F (ACTCCTACGGGAGGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT). Further procedure at BGI was as follows: All PCR products were purified by Agencourt AMPure XP beads, dissolved in Elution Buffer and labelled for library construction. Library size and concentration are detected by Agilent 2100 Bioanalyzer, and qualified libraries are sequenced by utilizing an DNBSEQ sequencing strategy to produce 300 base pairs paired end reads. Raw data are filtered to generate high quality clean reads. Primers and adapters are removed using the cutadapt v2.6. Truncation of reads was performed for reads with average phred quality values lower than 20 over a 30 bp sliding window, followed by removing reads whose length are less than 75 % of their original lengths after truncation. Reads with ambiguous base and low-complexity reads (reads with 10 consecutive same base) was removed. A consensus sequence is generated by FLAS (Fast Length Adjustment of Short reads, V1.2.11) for paired-end reads overlapping with each other (minimum 15 bp), and with a mismatching ration of the overlapping region of $\leq 0,1$. Fq-files of merged reads were then shared with SINTEF Ocean by BGI.

Files received at SINTEF Ocean was further processed using QIAGEN CLC Genomics Workbench 24.0.2 Operational taxonomic unit (OTU) clustering was performed, and chimeric and filtered reads removed. Paired reads were merged and trimmed. Taxonomy was assigned to the OUT's using the SILVA SSU 99% (138.1) reference database. OTU's taxonomically assigned as chloroplasts were removed, together with OTU's with abundancies less than 5 in the complete dataset. The OTU table was rarefied to 15,571 reads.

3 Results and discussion

Sampling of sediments have been performed from the non-treated oiled field and reference site at Svea, more than 25 years after the spill. Samples from the oiled field were taken from the visible oil layer. Chemical fingerprinting has been performed and the results compared to historical data from the same site (1997-2011). The chemical data has been combined with microbial analyses of all new sediment samples to investigate biodegradation and the effect of natural attenuation.

3.1 Chemical analysis

Chemical analysis of the sediment samples has been performed using GC/FID and GC/MS. The analysis was intended to give a more detailed monitoring of biodegradation processes on oil compound level, and to be used to indicate to which extent the oil has been degraded. In addition, an evaluation of the robustness of some of the geochemical biomarkers and diagnostic ratios related to oil spill forensics recommended in EN (2023) have been performed,

The oil was applied on the shoreline in August 1997, and approximately 30% of the oil was lost in the first 5 days, possibly due to evaporative loss and washout (Owens and Lee 2003). However, no further reduction was observed one year later. After evaporation of the lighter components, biodegradation will be the most dominant weathering process. The microorganisms degrade the straight-chain alkanes before the branched alkanes in the oil, and the ratios between the peak heights of C₁₇/pristane and C₁₈/phytane have been used for decades as an indication of biodegradation (e.g. Atlas 1981). The results from the GC/FID analysis show that C₁₇ and C₁₈ were completely gone in all samples collected after 2003, and that also pristane and phytane had disappeared in many of the samples collected in 2004 and later (Faksness, L.G., M.S. Noreng 2012a). In the samples collected in 2023, pristane, phytane, and n-alkanes from C₂₄ and higher were detected in some of the samples. GC chromatograms of the initial spilled oil and selected samples from 2023 are shown in Figure 1. All GC chromatograms are shown in Appendix B (Figure B 1 to Figure B 18).

The GC analyses were also used to estimate the oil concentration (Total Hydrocarbon Concentration (THC)) in the samples (Table B 1 in Appendix B). As mentioned above, the sampling was performed in the visible oil layer, collecting 3 or 4 parallel samples at each site. The average oil concentration (13 samples) was 12.0 g/kg sediment (± 7.6 g/kg), varying from 3.0 to 24.6 g/kg. The oil content in the samples collected at 17.5 m were considerably lower than at the other sites (3.48 g oil/kg sediment (± 0.5 g/kg)), but this could be due to that this area of the spill had been sampled more often over the years. At 22.5 m, the concentration varied from 7.3 to 20.4 g oil/kg sediment (12.8 g oil/kg sediment (± 5.4 g/kg)), and at 27.5 m, the concentration varied from 9.87 to 24.6 g oil/kg sediment (17.3 g oil/kg sediment (± 8.3 g/kg)). In 2011, 45 samples were collected from the entire spill site (0 to 35 m), and the average concentration was 7.5 g oil/kg sediment (± 6.2 g/kg), varying from 0.1 to 20.2 g oil/kg sediment (Faksness et al., 2012). Owens et al. (2003) reported that the mean oil concentration in 1998 was 14 g oil/kg sediment, which suggests that the mean oil concentration was halved from 1998 to 2011. From 2011 to 2023, there seems to be no significant reduction in the estimated oil concentration.

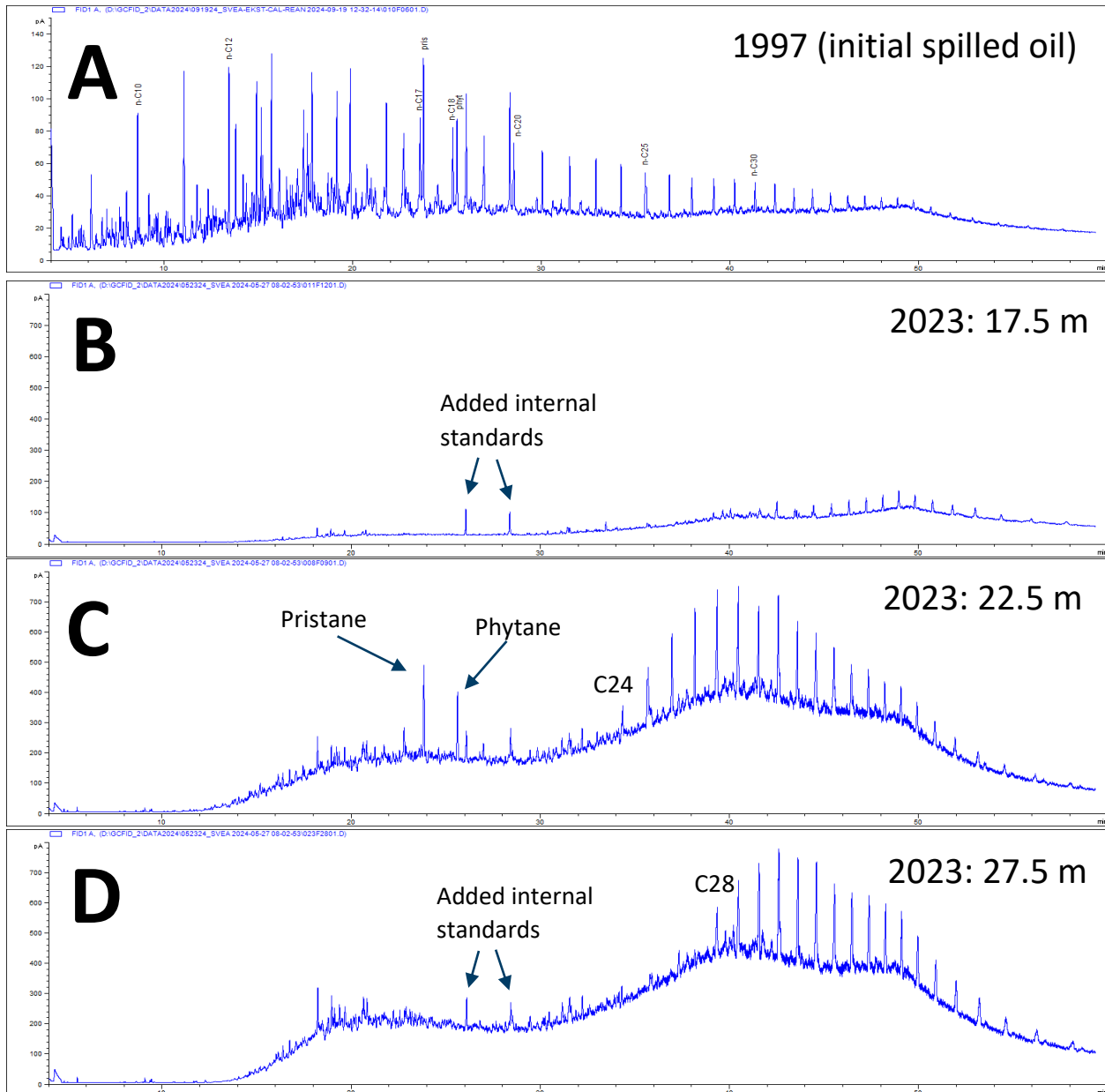


Figure 1: GC chromatograms of the initial oil (A, 2011-0277), and selected samples from 2023 from different locations in the site: 17.5 m (B, 2023-4235), 22.5 m (C, 2023-4231), and 27.5 m (D, 2023-4244).

GC/MS analysis was applied to get more detailed information on single components as PAHs and biomarkers (components detailed in Table 3). Petroleum biomarkers to study e.g. biodegradation oil in post-spill studies have been used for years, and especially hopane (17 α (H), 21 β (H)-hopane), which has been known to be resistant towards all weathering processes (e.g. Prince et al., 1994).

As most of the samples were highly weathered, the individual PAH components were normalized to hopane (detailed results given in Table B 2 and Table B 3). Figure 2 illustrates that the non-alkylated PAHs were biodegraded earlier than the alkylated, and that the biodegradation of the 2-3 ring PAHs were faster than the larger and more complex 4-6 ring PAHs. These results confirm the findings from the GC-FID, that the samples collected at 17.5 m are more weathered than the samples from 22.5 and 27.5 m.

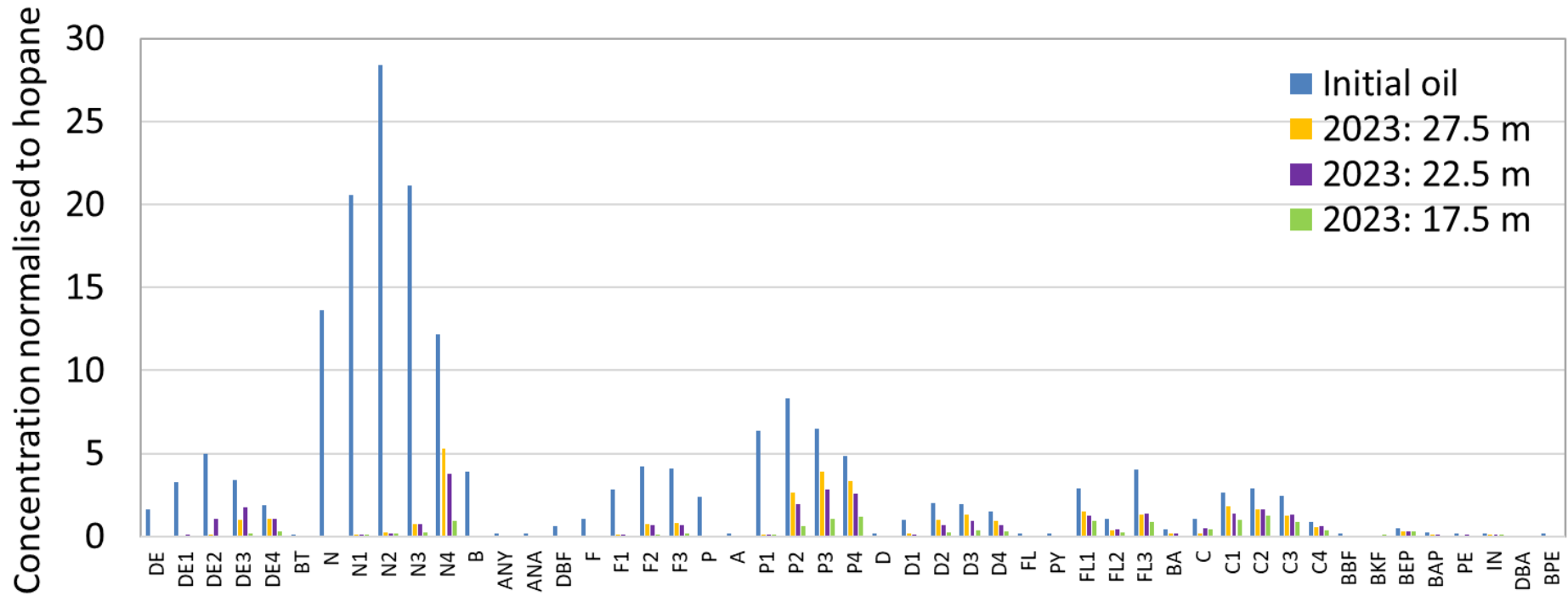


Figure 2: SVOC in the initial oil (blue bars), and samples from 17.5 m (green bars), 22.5 m (purple bars), and 27.5 m (orange bars). Abbreviations on the x-axis are described in **Table 3** and the results are detailed in Appendix B (Table B2 and Table B3).

The reduction in the total content of PAHs (TPAH) have been estimated in all analysed samples since 1999 using the quantification method described by e.g. Prince, Owens, and Sergy, 2002. The results are summarized in Figure 3, and the decrease in TPAH content has been estimated by comparison with the reference oil (Eq. 1). The TPAH content was reduced with more than 65% already in 1999, and the average depletion in PAHs in the samples collected in 2023 was 89%, with only 11% PAHs left in the remaining oil. The results indicate that the total content of PAHs have not changed significantly since 2001, which probably was due to that the more complex PAHs are left and that they make a smaller part of the total PAH content in the oil. Similar results have also previously been described, and Atlas and Hazen (2011) indicated that the estimated TPAH depletion was more than 70% in samples collected 19 years after the Exxon Valdez spill (Ronald M Atlas and Hazen 2011). Biodegradation of the oil has been a continuous process, but the process has slowed down as the more complex PAHs and the even more complex asphaltenes and resins are left in the oil.

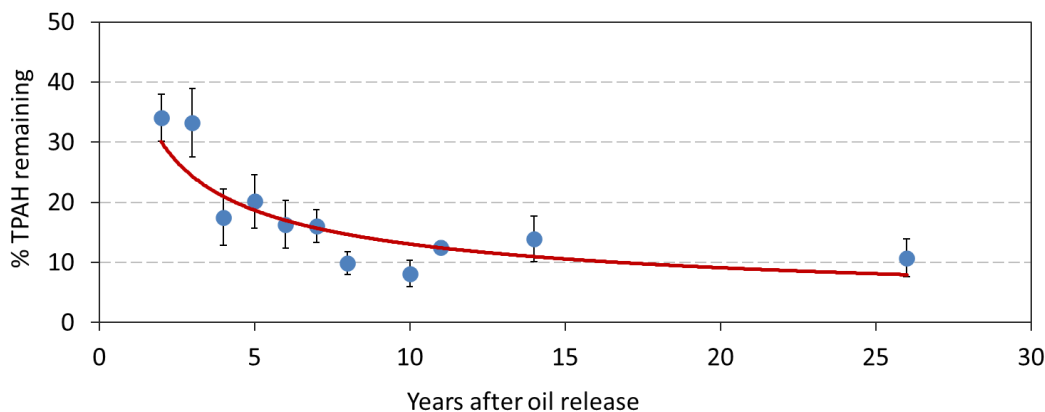


Figure 3: Percentage of TPAH (44 comp) remaining in the sediment (with standard deviation) from each sampling year since 1999. All samples are compared with the initial oil and Eq. (1) has been used for the calculations.

To get an indication of the robustness of some of the biomarkers and diagnostic ratios used in oil spill forensics, the samples have been compared with oil that was initially spilled in 1997. Visual differences in the ion chromatograms of the initial oil compared with a sample collected 26 years later (in 2023) are shown in Figure 4. In the ion chromatograms of the C1-phenanthrenes, it can be seen that the two first peaks and the two last peaks have been reduced in the sample from 2023, and that these four peaks clearly have been influenced by weathering, most likely biodegradation, while the methyl anthracene (peak in the middle) does not seem to be as affected. In the ion chromatograms of the C1-fluoranthenes/pyrenes/benzofluoranthenes (m/z 216), especially the three first labelled peaks were influenced by weathering. The peak patterns of the hopanes (m/z 191) do not appear to be changed over the 26 years period in the sediment. However, the peak heights of the two first peaks (C20TA and C21TA) in the ion chromatogram of triaromatic steranes (m/z 231), indicate that these peaks have been influenced by weathering over the years. This is also reflected in Figure 5.

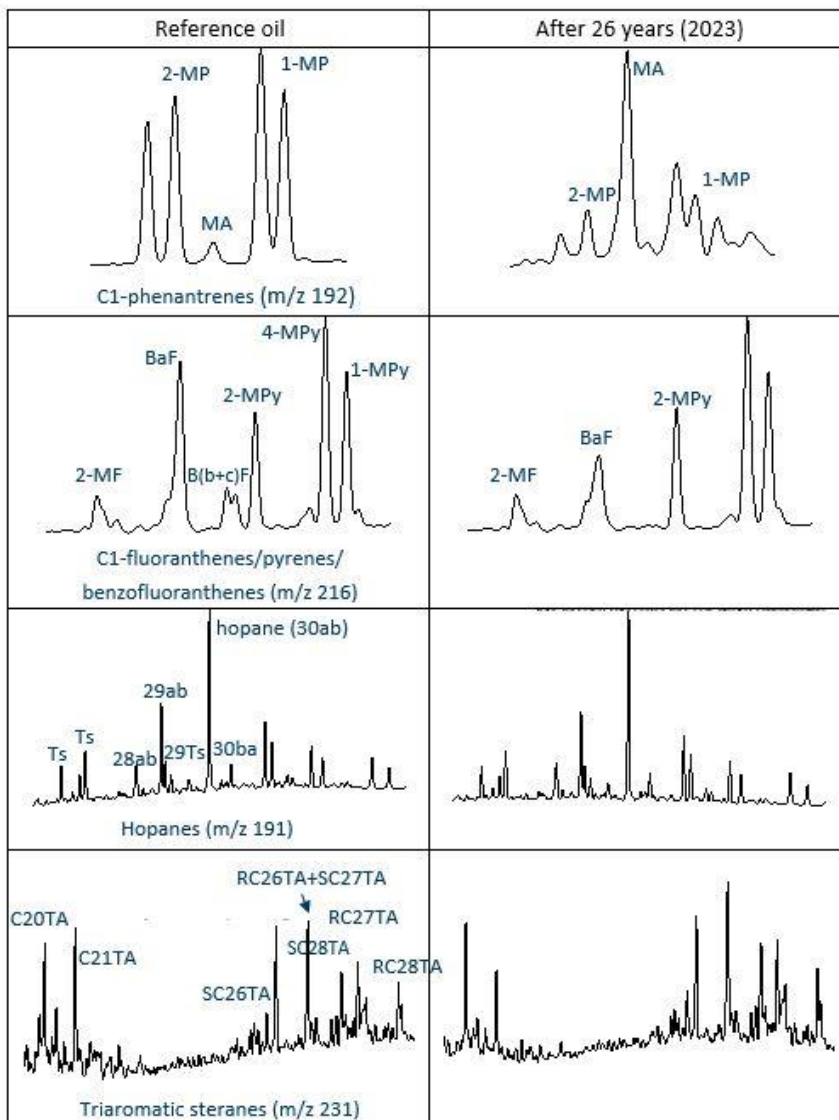


Figure 4: Comparing selected ion chromatograms from the initial oil (2011-0277) with a sample from 2023 (2023-4232) collected after 26 years in the shoreline sediment.

Selected DRs from EN (2023) have been calculated and evaluated. This was done by the repeatability method, meaning that the ratios must not differ more than 14% relatively as critical difference (CD). A difference <14% indicates that the ratio is not significantly influenced by weathering after the oil was spilled. Figure 5 illustrates the robustness of the diagnostic ratios. The calculations confirm what is observed visually in Figure 4: All ratios calculated from the hopanes have a CD<14%, and some of the ratios calculated from the triaromatic steranes (m/z 231) have CD>14%. The DR for the PAHs are not so robust, and several of them have a CD >14%. The DRs of selected samples from 2023 are compared with the initial oil in Figure B 19 (Appendix B), and even though the CD for some of the DR varies among the samples, CDs for all DR with hopanes are 14% or lower. This confirmed that the calculated DRs for hopanes still are robust after 26 years, but that some of the triaromatic steranes (C20TA/C21TA and C21TA/(RC26+SC27)) and most of the PAH ratios were exposed to biodegradation over the years.

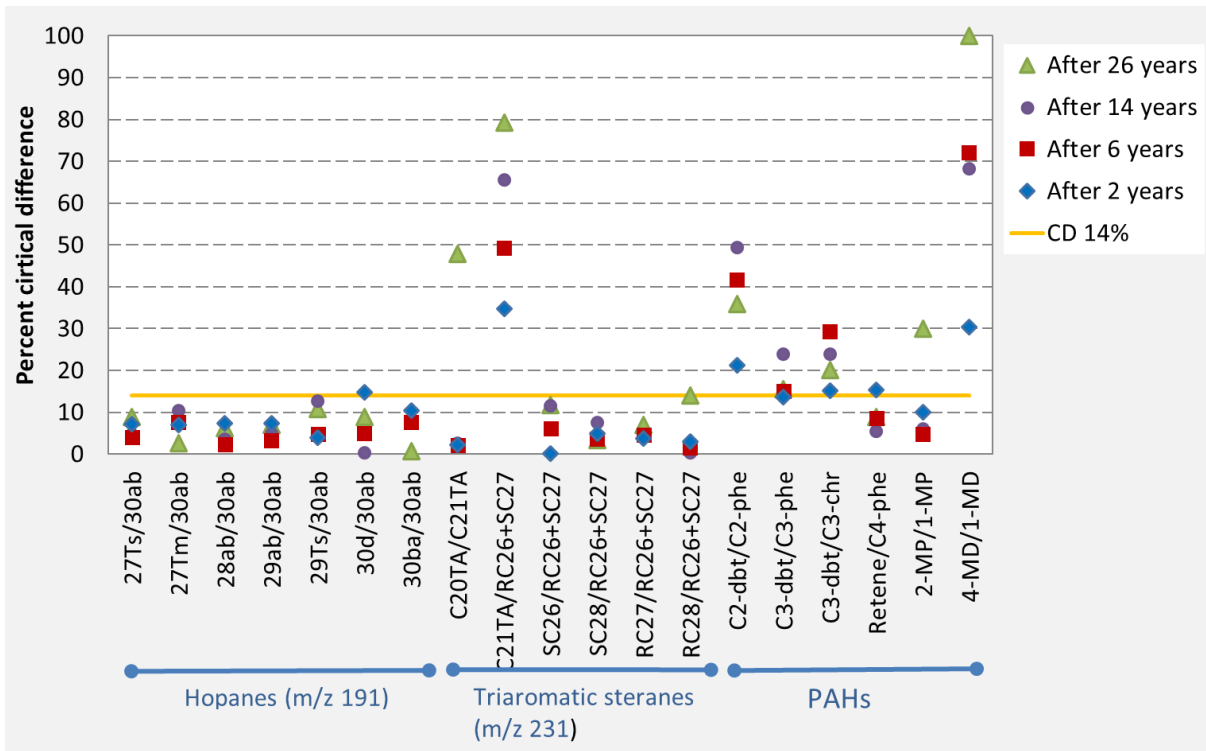


Figure 5: Robustness of diagnostic ratios: Difference in DR between the reference oil (initially spilled oil) and samples collected after 2, 6, 14 and 26 years. The critical difference of 14% is indicated with a yellow line. Abbreviations for PAHs and biomarkers given in Table 3, and examples of ion chromatograms are shown in Figure 4.

The sediment sample labelled 36.4m-REF (2023-4217) was collected approximately 1.4 m from the initially spilled area. However, heavier components are detected in the GC-chromatogram (Figure B 14), and the ion chromatograms of hopanes and triaromatic steranes (Figure B 20) show the same peak patterns as in the samples collected within the spilled area. Calculated DRs for hopanes are also within a CD<14% (Figure B 19) when compared with the initially spilled oil and confirmed that this was the same oil. The oil concentration was lower than in the spill samples, but higher than in the other reference samples collected outside the spilled area (Table B 1). Biomarkers were not detected in the other reference samples.

3.2 Microbial community analysis

Total genomic DNA was extracted from the sediment and seawater samples. For all samples, obtained concentrations of extracted DNA was sufficient for further analyses, indicating growth of organisms in the sediment samples. In general, higher concentrations of DNA was obtained from samples taken from the oiled field than from the reference area(s) outside the field. The extracted DNA was sent to BGI Tech Solutions (Hong Kong) Co., Limited for 16S rRNA gene amplicons sequencing, and further studies of the bacterial communities were performed at SINTEF Ocean.

To study the microbial communities, the 28 samples were divided into sample groups based on their sampling location, as described in Table 2 below, yielding a total of 5 sample groups with varying number of samples. The alpha and beta diversity of the samples has been studied. The alpha diversity is a measure of the diversity within a single sample, or group of samples, whereas beta diversity is a measure of the diversity between samples or groups of samples. The diversity within samples, (alpha diversity) reveals the number (richness) and abundance (evenness) of the different taxa present in a sample or sample group.

Table 2: Grouping of samples based on sampling location

Sampling location	Sample group	# of samples
Within oiled field	Oil	12
Close to oiled field	Close	2
Reference site outside oiled field	Out	3
Low tide, Outside oiled field	Below	2
Low tide, Below oiled field	Below	6
Seawater	SW	3

Chao 1 diversity index indicates richness whereas the Shannon entropy metric estimates both richness and evenness in the same equation. Higher Shannon entropy hence indicates higher degree of unordered, shown in Figure 6 below. Differences between groups of samples can be observed, however a statistical comparison can only be performed between samples from the ‘oil’ group and the ‘below’ group, as these have sufficient amounts of samples. Sample group ‘Below’ constituting samples from the tidal sone, shows higher richness within the sample group than the ‘oil’ sample group. The ‘oil’ sample group has a more ordered composition than the ‘below’ group. The difference observed between sample groups ‘oil’ and ‘below’ are significant (data not shown).

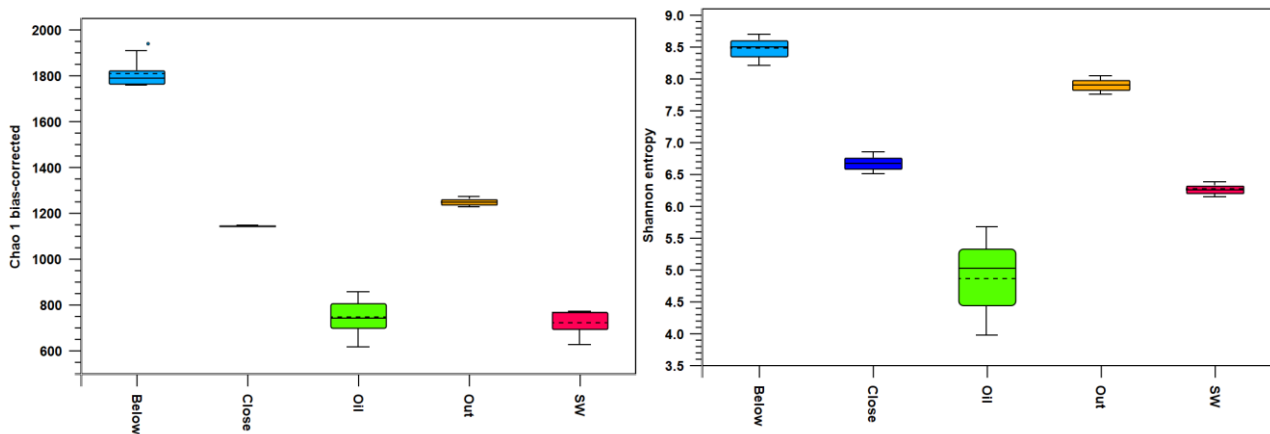


Figure 6: Comparison of alpha diversities of different sample groups, as indicated on the x-axis. Left panel displays the sample richness (Chao 1 diversity index) and the right panel displays the Shannon entropy.

Beta diversity analyses of the microbial communities in sediment and water samples are presented as a PCoA plot based on Bray-Curtis dissimilarity index, shown in Figure 7, where colours indicates the different sample groups. In this analysis, clustering of samples can be observed for all defined sample groups; oil, close, out, below, and SW. The clustering indicates that the community in the sea water samples are different from the sediment samples, and that there is a shift in the microbial communities from the samples collected from outside the oiled field ('out'), via the samples collected close to the oiled field ('close') and to the samples collected in the oiled field ('oil'). Sediment samples collected in the tidal zone below the oiled field and a nearby reference site, are further distinguished from the other samples. There is however no sub-clustering within the samples collected in the tidal zone, indicating that there is no influence of the oiled field on the sediment in the tidal zone below the field. PERMANOVA analyses further indicated that the observed differences between 'oil' samples and 'below' samples are significantly different ($p < 0.01$). No statistical analyses were performed including the remaining samples, due to the lower number of replicates from these sample groups.

The composition of the microbial communities at order and genus level is shown in Figure 8 and Figure 9 respectively. The abundances are displayed with a filter level of 10, allowing the 10 most abundant features from each sample to be shown in all columns. The less abundant features are grouped as 'other', shown as white fields on the top of the abundance bars. Sea water samples are not included in the genus overview in Figure 9, but are included in the summary at order level shown in Figure 8.

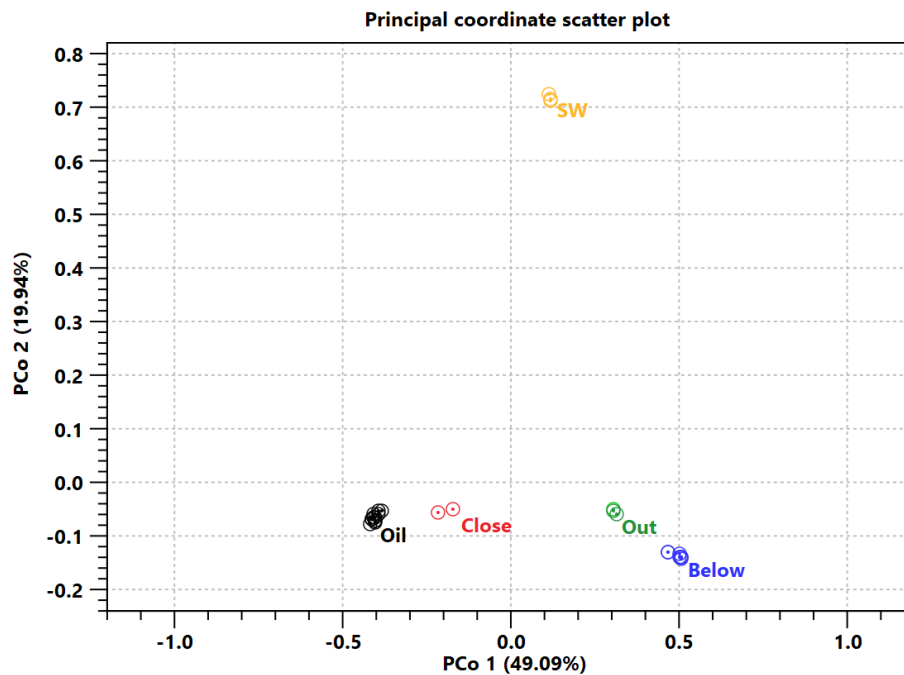


Figure 7: Beta-diversity of microbial communities in sediment and water samples represented by PCoA plot and based on Bray-Curtis dissimilarity index. Colours indicates the different sample groups.

The microbial community compositions, at both order and genus level, indicate that there are different dominant taxa between the sample groups. Differences between sample groups are also visualised by the PCoA-plot in Figure 7. The samples collected outside the oiled field ('out'), and the samples collected in the low tide all have varying abundances of taxa as *Illumatobacter*, uncultured genera of the order Actinomarinales and the family *Microtrichaceae*, in addition to *Aquibacter*, *Nocardioides* and *Sva0996* marine group. Together these taxa constitute approximately 20% of the total communities in the samples, whereof all representatives of all taxa are previously reported marine taxa (Hameed et al. 2014; Delpech et al. 2021; Miksch et al. 2021; Cong et al. 2023). Both *Illumatobacter* and *Sva0996* are described as indicator organisms of August water in an Arctic Fjord (Isfjorden, Svalbard) (Delpech et al. 2021), and Actinobacteriota of uncultured Actinomarinales and *Microtrichales* have been reported as key heterotrophs for carbon mineralization at Svalbard (Isfjorden) (Miksch et al. 2021). An unclassified genus of *MBAE14* is also present (~5-10%) in the low-tide samples but not in the reference samples collected outside the oiled field. As the taxa detected in the reference samples outside the oiled field are also previously detected in arctic marine environments, this suggests that oil or its components at the time of sampling are not affecting the microbial communities outside the oiled field.

In the oil-samples *Mycobacterium* is the dominating taxa in all samples, with relative abundance of between 30-60% (bottom orange color in Figure 9). *Rhodococcus* (light green) and *Williamsia* (blue/grey) are also highly abundant in the oil samples, with relative abundance between 2-40% and 4-17% respectively. In total, at least 70-80% of the taxa detected in the oil related samples are previously reported aerobic alkane- and PAH degraders, and/or to be associated with degradation of oil (Cébron et al. 2022; Kim et al. 2008; Blanco-Enríquez et al. 2018; Villela et al. 2019; Hackbusch et al. 2020). The communities are distinctly different from what is observed in the nearby area of the beach which has not been exposed to oil.

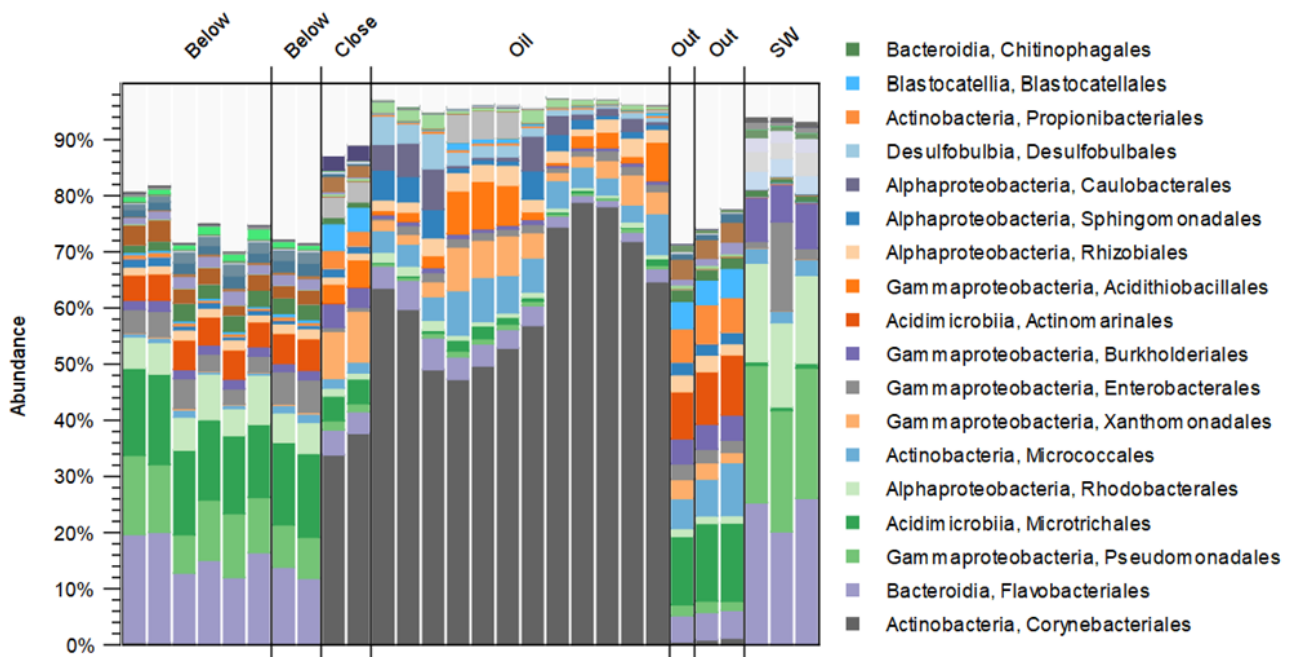


Figure 8: Microbial community composition at order level, most abundant features shown. Samples are listed according to sample group as indicated on top of the figure. The ten most abundant features from each sample are shown in all columns. The less abundant features are grouped as ‘other’, shown as white fields on the top of the abundance bars. Legend showing 18 taxa. The colour marking in the legend is shown in the same order as they would appear in the community composition columns, bottom to top.

Samples collected close to the oiled field displays similarities to samples from the oil. Both sample groups display high abundance of *Mycobacterium* and *Rhodococcus*. The two sample groups also constitute varying abundances of the genera *KCM-B-112*. *Williamsia*, found in the oil samples, on the other hand is almost non-existent (less than 0,5% abundance) in the samples collected close to, and outside the oiled field. *KCM-B-112* and *Williamsia* are both suggested as possible indicator taxa related to the hydrocarbon level in long term petroleum contaminated forest soils and salt marshes (Cébron et al. 2022; Cagle et al. 2024).

Nocardioides have been isolated from different environments, including the arctic (e.g. Deng et al. 2015). This taxon, although also reported to potentially be associated with oil degradation both in Arctic shorelines (Ellis et al. 2022; Kundu, Harrison, and Ghoshal 2023) and in desert samples (Ali et al. 2020), was in this project mainly found in samples close to (sample group ‘close’) and outside the oiled field (sample group ‘out’). It should be noted that a limited number of samples were analysed in this project, and that only one timepoint is included in the microbial analyses. To obtain a better understanding of, and to evaluate to what extent the different taxa identified can be used as indicators and related to the remaining hydrocarbon level in the sediments, a more extensive sampling campaign will be needed.

Although there are similarities between the microbial communities associated with samples collected close to- and within- the oiled field, beta diversity analyses as shown in the PcoA-plot in Figure 7, suggests that there might be a shift in the community compositions that could be related to the presence of oil. Low amounts of oil were detected in samples collected close to the oiled field (~1% of the average THC in samples from the oiled field), however the concentration of DNA obtained (ng/μl) were similar to concentrations obtained from samples collected within the oiled field; both sample types with higher obtained concentrations of DNA than samples collected outside the oiled field (see Table B 1 in Appendix B).

After the Exxon Valdez Spill in Prince William Sound in 1989, it was debated whether bioremediation, e.g. by addition of nutrients, should be reapplied to treat residual subsurface oil, as reviewed by Atlas and Hazen (Ronald M Atlas and Hazen 2011). As remaining oil after over 20 years were highly weathered, and might not reach sensitive biota, the value of any treatment was assumed to be limited. Based on field trials performed in Prince William Sound in 2011 and 2012 it was found that low oxygen concentrations were the major factor causing oil persistence, and that bioremediation by injection of oxygen and nutrients into the submerged oil greatly increased biodegradation (Boufadel, Geng, and Short 2016). Despite this, Boufadel et al questioned the benefit of the treatment as it comes with an environmental cost. As also discussed by Atlas and Hazen (Ronald M Atlas and Hazen 2011), analyses of the sediments collected at Svea in this work have indicated that the microbial communities outside the oiled field (similar depth on the beach) are not affected by oil or its components at the time of sampling (2023). It would however be of interest to study the microbial communities at different depths (above/below the oil layer) within the oiled field to further evaluate the impact of the remaining oil.

4 Conclusion

In this work, analysis of sediment samples collected from an oil-contaminated beach at Svalbard has been performed. Chemical fingerprinting analyses in combination with analysis of the microbial communities present have yielded insights into the long term-effect of an oil spill on a shoreline, that has been left uncleaned for more than 25 years.

As both visually observed during sampling, and indicated by THC analyses, oil is still present in the sediments. Varying concentrations and degrees of weathering of the oil were however found in the different samples. Previous analyses indicated that oil concentrations in the oiled field was halved from 1998 to 2011, however no further reduction in the estimated oil concentration could be detected based on analyses of the sediments in 2023. The total concentration of PAH's also has not changed since 2001, although there is a shift towards the more complex PAH's, in addition to the more complex asphaltenes and resins. In sum, the results indicates that biodegradation of the oil has slowed down in recent years. Selected diagnostic ratios (DR) used in oil spill forensics have been calculated and indicated that the DRs for hopanes still are robust after 26 years, but that some of the triaromatic steranes and most of the PAH ratios were influenced by biodegradation over the years.

DNA-based analyses of the microbial communities associated with the same sediment samples indicated distinct differences between the microbial communities associated with the sediments in the oiled field and outside the oiled field. The majority of the taxa detected in the samples collected from the oiled field, are previously reported degraders of different oil compounds, or to be associated with degradation of oil, suggesting that the communities are still affected by the oil present. Taxa identified in the current sampling, could potentially also be used as indicator taxa related to any further degradation of the remaining oil.

From the observations in the field and evaluation of the chemical and microbial analyses, it is not recommended to remove the oil, as it is located relatively deep into the sediment, likely posing no further impact on the environment and wildlife in the Credner moraine. No risk for further spreading of the oil is expected.

Correlations between the chemical and microbial analysis performed on samples collected in this work can be observed, however more samples and analyses are needed to draw any further conclusions on nature's ability (and time needed) to self-purge. E.g. detection and quantification of functional genes in the active biomass, associated with oil degradation would contribute to our understanding of the degradation processes. In particular studies of the microbial communities at different depths within the oiled field would be interesting after any new sampling. Following up on this work with more samples and samplings in the years to come would hence provide valuable information on the microbial communities, their ability to further degrade the remaining oil in the area and the communities' reconstitution.

Table 3: Quantified SVOCs (left column), and biomarkers and PAHs (right column) used to calculate selected diagnostic ratios ((with abbreviations (abb)).

Compounds (quantified)	Abb	Compound (biomarkers and PAHs for DR)	Abb
Decalin	DE	17 α (H),21 β (H)-hopane	30 ab
C1-decalins	DE1	18 α (H)-22,29,30-trisnorhopane	27 Ts
C2-decalins	DE2	17 α (H)-22,29,30-trisnorhopane	27 Tm
C3-decalins	DE3	17 α (H),21 β (H)-28,30-bisnorhopane	28ab
C4-decalins	DE4	17 α (H),21 β (H)-25-norhopane	25nor30ab
Benzo(b)thiophene	BT	17 α (H),21 β (H)-30-norhopane	29ab
Naphthalene	N	18 α (H)-30-norneohopane	29Ts
C1-naphthalenes	N1	15 α -methyl-17 α (H)-27-norhopane (diahopane)	30d
C2-naphthalenes	N2	17 β (H),21 α (H)-30-norhopane (normoretane)	29ba
C3-naphthalenes	N3	18 α (H)-oleanane	30O
C4-naphthalenes	N4	17 β (H),21 α (H)-hopane	30ba
Biphenyl	B	17 α (H),21 β (H), 22S-homohopane	31abS
Acenaphthylene	ANY	17 α (H),21 β (H), 22R-homohopane	31abR
Acenaphthene	ANA	Gammacerane	30G
Fluorene	F	17 α (H),21 β (H), 22S-bishomohopane	32abS
C1-fluorenes	F1	17 α (H),21 β (H), 22R-bishomohopane	32abR
C2-fluorenes	F2	C20-triaromatic sterane	C20TA
C3-fluorenes	F3	C21-triaromatic sterane	C21TA
Phenanthrene	P	C26, 20S-triaromatic sterane	SC26TA
Anthracene	A	C26, 20R+ C27, 20S-triaromatic steranes	RC26TA+SC27TA
C1-phenanthrenes/anthracenes	P1	C28, 20S-triaromatic sterane	SC28TA
C2-phenanthrenes/anthracenes	P2	C27, 20R-triaromatic sterane	RC27TA
C3-phenanthrenes/anthracenes	P3	C28, 20R-triaromatic sterane	RC28TA
C4-phenanthrenes/anthracenes	P4		
Dibenzothiophene	D	2-methyl phenanthrene	2-MP
C1-dibenzothiophenes	D1	1-methyl phenanthrene	1-MP
C2-dibenzothiophenes	D2	4-methyl dibenzothiophene	4-MD
C3-dibenzothiophenes	D3	1-methyl dibenzothiophene	1-MD
C4-dibenzothiophenes	D4	retene	
Fluoranthene	FL		
Pyrene	PY		
C1-fluoranthenes/pyrenes	FL1		
C2-fluoranthenes/pyrenes	FL2		
C3-fluoranthenes/pyrenes	FL3		
Benzo[a]anthracene	BA		
Chrysene	C		
C1-chrysenes	C1		
C2-chrysenes	C2		
C3-chrysenes	C3		
C4-chrysenes	C4		
Benzo[b]fluoranthene	BBF		
Benzo[k]fluoranthene	BKF		
Benzo[e]pyrene	BEP		
Benzo[a]pyrene	BAP		
Perylene	PE		
Indeno[1,2,3-c,d]pyrene	IN		
Dibenz[a,h]anthracene	DBA		
Benzo(g,h,i)perylene	BPE		

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Appendix A Memo. Sediment sampling in Svea, 15.08.2023 (copy)

Sediment sampling in Svea, 15.08.2023.

Long term fate and behaviour of oil in an Arctic shoreline

PERSON RESPONSIBLE / AUTHOR

Sigrid Hakvåg

PROJECT NO / FILE CODE

302007903

DATE

21/08/2023

CLASSIFICATION

Unrestricted

Dette notater beskriver innhenting av prøver fra felt, utført uke 33 (15.08.2023) som en del av prosjektet 'Long term fate and behavior of oil in an Arctic shoreline).

Deltagere i felt: Liv-Guri Faksness (SO), Bror Johansen, Jann-Egil Gjerde (KyV), Knut Eide (Isbjørnvakt fra SNSK) og Sigrid Hakvåg (SO). Med til Credner morenen i Svea var også skipper og maskinist (KyV) ombord i losbåten 'Elling Carlsen'. Disse ble ikke med i land ved Svea. Det ble i forkant av utreise i felt (dagen før) gjennomført en times sikkerhets-briefing i regi av UNIS for alt personell som skulle i land i Svea.

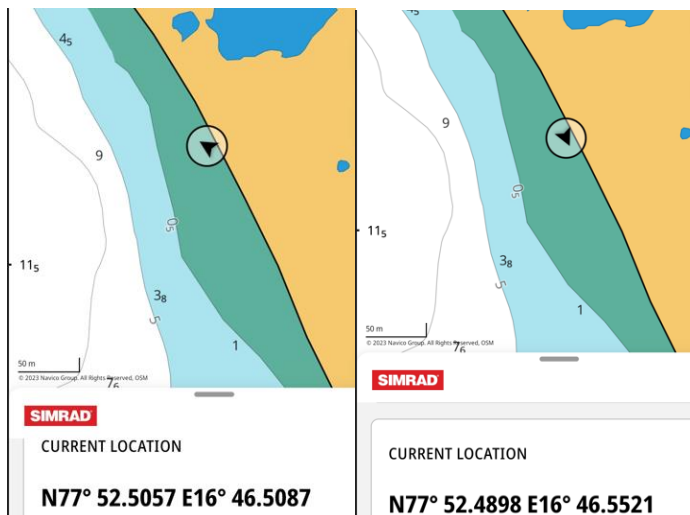
Varighet i felt:	05:55 (avreise med båt fra Longyearbyen) til 20:30 (ankomst Longyearbyen, retur)
Varighet sampling:	Ca kl 11:00 til 17:15 (~6 timer)
Tid fra sampling til innfrysing:	~opp til 10 timer (arbeid avsluttet kl 21:30)
Lufttemperatur:	5 °C
# prøver:	20 + 25/28 prøver til hhv kjemisk og mikrobiologisk analyse

Losbåt Elling Carlsen ble benyttet til transport fra Longyearbyen til Credner. Båttransport ble arrangert av Kystverket. Båtturen ut tok ca 4,5 timer grunnet noe urolig vær. En medbragt gummibåt med påhengsmotor ble benyttet for transport av folk og utstyr til stranda. Retur fra Svea ca kl 18, og grunnet mindre vind tok hjemturen ca 3 timer.

GPS-koordinater for feltet var ikke tilgjengelig, men første jernstang (0-markør) ble funnet raskt (ved bål plass og gapahuk). For andre jernstang (35-meters markering) ble det først feilaktig tatt utgangspunkt i to tettstående stenger, se Figur 3 . Etter påbegynt graving (grøft 4 og 5 i Tabell 1 under) ble det oppdaget en annen jernstang som stakk opp ca 3 cm, 7,5 meter nærmere 0-markeringa. Denne nye stanga er den korrekte 35-meters markeringen. Det gjøres oppmerksom på at de to tettstående stengene også var plassert lengre opp på stranden/lengre vekk fra vannet enn de korrekte markeringene. Koordinater for 0-markør og 35-meters markering er gitt under, samt i Figur 2. Bilder av stengene er gitt i Figur 2.

0-markør: <https://goo.gl/maps/Exkt92N7eK85SWnZA>
Koordinater: 77°52'30.3"N 16°46'30.5"E; 77.875095, 16.775145; N77°52.5057 E16°46.5087

35-meters markør: <https://goo.gl/maps/Jd82MY7E1bdjuqcG7>
Koordinater: 77°52'29.4"N 16°46'33.1"E; 77.874830, 16.775868; N77°52.4898 E16°46.5521



Figur 1 Koordinater til 0- og 35meters-markører, fra SIMRAD



Figur 2 Stenger som markerer ytterkanter av felt, 0- og 35meters-markører



Figur 3 Jernstenger, ca 7,5 meter sørøst for 35-meters markør. Trolig brukt til feste av lense under oppstart av forsøket i 1997.

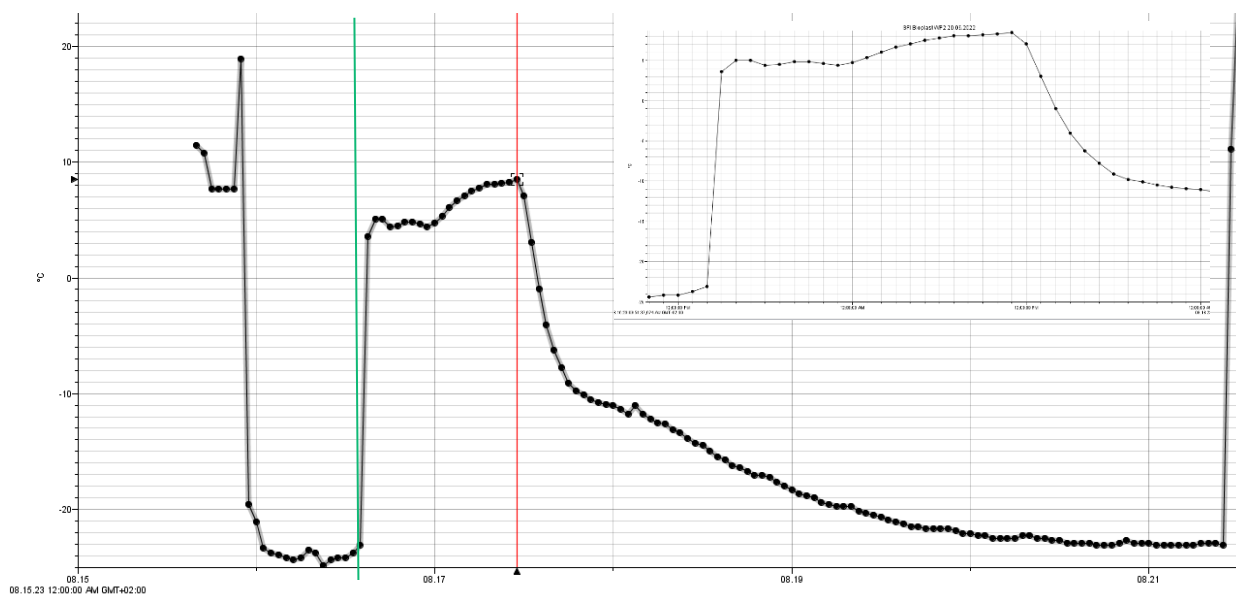
Det ble først gravd grøfter ved ca 40 og 35 meter. (Grøt 4 og grøft 5, hhv ca 2,5 og 5 m fra de to stengene). Det var vasket opp store mengder flate runde steiner opp til 7-8 cm i diameter som måtte fjernes med krafse (Figur 5). Ved graving raste steinene ned i hullet, og det tok følgelig mye tid å fjerne de. Laget med de større steinene var opptil 40-50 cm, deretter lå et tilsvarende tykt lag med grov grus før sanden kom til syne. Det ble benyttet spade for å grave i sanda, og det ble laget et hull på opptil 40-50 cm dybde som det ble tatt prøver fra. Det var ingen tegn til oljeforurensning i disse første grøftene (da de var utenfor det opprinnelige olje-feltet), og prøver ble tatt for referanse og merket REF 2 og REF 1. Ved videre graving ble det brukt drivved som "forskaling" for å redusere utrasingen av større steiner ned i grøfta, se Figur 6. To og to personer byttet på å jobbe med en grøft om gangen, for mest effektiv jobbing.

På grunn av laget med større steiner tok gravingen mer tid enn beregnet, og det ble derfor innhentet færre prøver enn planlagt. I tillegg til referanseprøvene, ble det tatt prøver fra 3 grøfter, fra ca 17,5 m 22,5 m (to hull), og 27,5 m. For prøver tatt mellom 0- og 35-meters markering ble prøvene forsøkt tatt fra selve oljelaget (Figur 7). I tillegg til referanseprøver og oljeprøver ble det tatt prøver fra fjæra, både rett nedenfor feltet, samt referanseprøver (fjære) bortenfor feltet. Til slutt ble det tatt prøver av vannet, og vannprøver (3 prøver á 420 ml) ble filtrert (Sterivex, 0,2 um) umiddelbart etter prøvetaking, tilsatt 1 ml DNA/RNA shield (Zymo) og lagret mørkt og kjølig (lufttemperatur på 5 °C) fram til retur Longyearbyen. En fullstendig oversikt over prøver er gitt i Tabell 1 under. Se ellers lenket excel-dokument for LIMS-nummerering (2023-4197 til 2023-4267). Sample list Svea for LIMS_08.08.2023.xlsx

Tabell 1 Oversikt over sedimentprøver tatt ved sampling ved Credner morenen. Prøver hentet 8 meter fra topplinjen er tatt ut i fjæra nedenfor selve feltet. 'M-' og 'C'-prøver angir hhv prøver til mikrobiologiske og kjemiske analyser.

	Avstand (i meter)		Dybde cm	Sandsjikt cm	# prøver		Kommentar
	Fra 0-pinne*	Fra topplinje			M-prøver	C-prøver	
Grøft 1	17,5	1,9	125	40	3	3	Trebjelke på tvers i grøfta
Grøft 2	22,5	1,6	110	40	3	3	Ikke så hardt oljelag
	22,5	2,1	110	40	3	3	
Grøft 3	27,5	2	115	40	3	2	Hardt oljelag
Grøft 4: REF2	36,4	2	90	15	2	1	
Grøft 5: REF 1	39,5	2	130	40	3	1	
Grøft 1	17,5	8	25	25	2	1	En M-prøve avsatt til C-analyse
Grøft 5: REF 1	39,5	8	25	25	3	3	
Grøft 6: REF 3	62	8	25	25	3	3	
Sjøvann					3	0	3 prøver á 420 ml filtrert.
	* Mot høyre				28	20	

Alle prøver ble ved ankomst Longyearbyen lagret ved -20 °C over natt. Transport ble bestilt med Jetpak. Prøver ble plassert i hvit isolert transportkasse (Pelican), sammen med tre kjøleelement og bobleplast. Sending ble hentet av JetPak kl 15, onsdag 16.08.2023 og var framme ved Brattørkaia torsdag 17.08.2023 ca. kl.11, hvor kassen umiddelbart ble plassert i fryserom ved -20 °C. Temperaturlogger viste at temperaturen ikke steg over 8 °C under sending. Logget temperatur er vist i Figur 4.



Figur 4 Logget temperatur ved lagring av prøver etter sampling. Område mellom grønn og rød vertikal linje indikerer temperatur under transport med JetPak. Utsnitt fra graf i høyre hjørne viser logget temperatur i tidsrommet rett før til rett etter transport.



Figur 5 Graving av grøft uten forskaling



Figur 6 Forskaling rundt grøft for å hindre utrasing av steiner ved graving



Figur 7 Oljesjikt i grøft



Figur 8 Transport av personell og utstyr mellom land fra losbåt

Alle bilder: Liv-Guri Faksness, Bror Johansen og Sigrid Hakvåg

Appendix B Additional results

Table B 1 Summary of results from chemical analysis (THC in g/kg sediment and SVOC in mg/kg sediment), and overview of extracted DNA in ng/μl. TPAH is the sum of all SVOCs.

SINTEF ID	Sample ID	Short name	THC (g/kg)	TPAH (mg/kg)	Decalins (mg/kg)	Naphthalenes (mg/kg)	2-3 ring PAHs (mg/kg)	4-6 ring PAHs (mg/kg)	DNA extracted (ng/μl)*
2023-4235	17.5m-1	Svea 4	3.58	22.6	7.34	1.02	4.56	9.65	63.6
2023-4236	17.5m-2	Svea 5	2.96	18.5	5.12	0.65	3.22	9.50	67.0
2023-4237	17.5m-3	Svea 6	3.92	23.3	6.53	1.28	5.39	10.1	49.1
2023-4239	22.5m-1A	Svea 7	10.6	68.9	30.4	5.63	17.2	15.6	16.5
2023-4240	22.5m-2A	Svea 8	7.30	44.3	18.3	3.86	11.8	10.4	26.5
2023-4241	22.5m-3A	Svea 9	7.95	61.1	26.0	3.57	14.8	16.8	49.8
2023-4231	22.5m-1B	Svea 1	20.4	124	50.4	8.09	37.5	27.8	22.4
2023-4232	22.5m-2B	Svea 2	12.3	75.4	28.8	5.06	21.1	20.4	24.4
2023-4233	22.5m-3B	Svea 3	18.4	83.0	32.3	5.77	25.6	19.3	26.6
2023-4243	27.5m-1	Svea 10	10.3	82.0	20.8	5.30	25.4	30.4	56.0
2023-4244	27.5m-2	Svea 11	24.6	72.3	22.2	5.34	23.8	21.0	40.4
2023-4244	27.5m-2-New		24.4	129	31.7	10.6	44.9	41.6	
2023-4245	27.5m-3	Svea 12	9.87	31.3	7.00	1.84	8.90	13.6	64.0
2023-4217	36.4m-ref	Svea 24	0.13	0.693	0.055	0.146	0.216	0.277	18.2
2023-4213	39.5m-ref	Svea 15	0.08	0.271	0.028	0.086	0.109	0.047	1.11
2023-4246	17.5m-1-below ht	Svea 13	0.68	0.280	0.029	0.084	0.128	0.040	7.12
2023-4247	17.5m-2-below ht	Svea 14	0.30	0.147	0.012	0.026	0.080	0.029	6.35
2023-4250	39.5m-ref-2	Svea 16							1.31
2023-4251	39.5m-ref-3	Svea 17							1.78
2023-4252	39.5m-1-below ht	Svea 18							8.56
2023-4253	39.5m-2-below ht	Svea 19							8.55
2023-4254	39.5m-3-below ht	Svea 20							7.28
2023-4255	62m-1-below ht	Svea 21							8.18
2023-4256	62m-2-below ht	Svea 22							8.00
2023-4257	62m-3-below ht	Svea 23							6.24
2023-4259	36.4m-2-ref	Svea 25							21.3

*From ~1,5 gram sediment pr sample

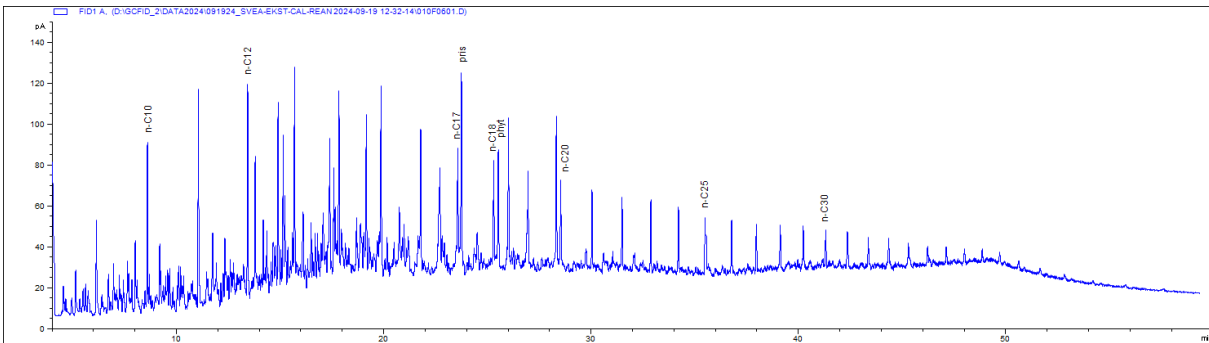


Figure B 1 GC chromatogram of initially spilled IF30 oil (SINTEF ID 2011-0277 or 2024-5414)

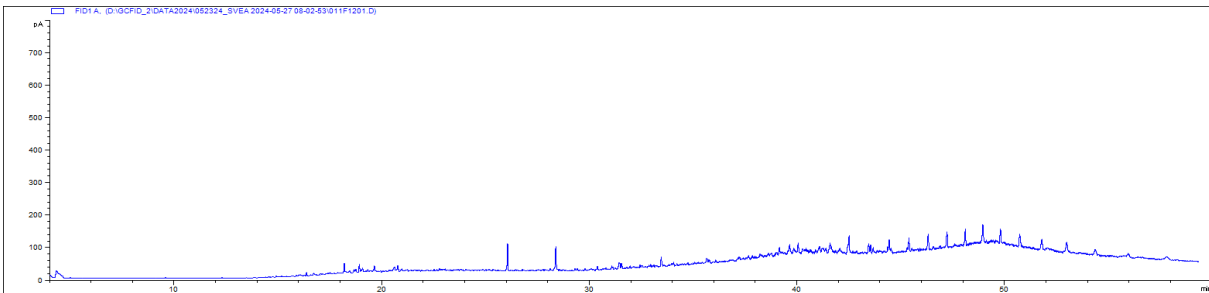


Figure B 2 GC chromatogram of sample 17.5m-1 (SINTEF ID 2023-4235)

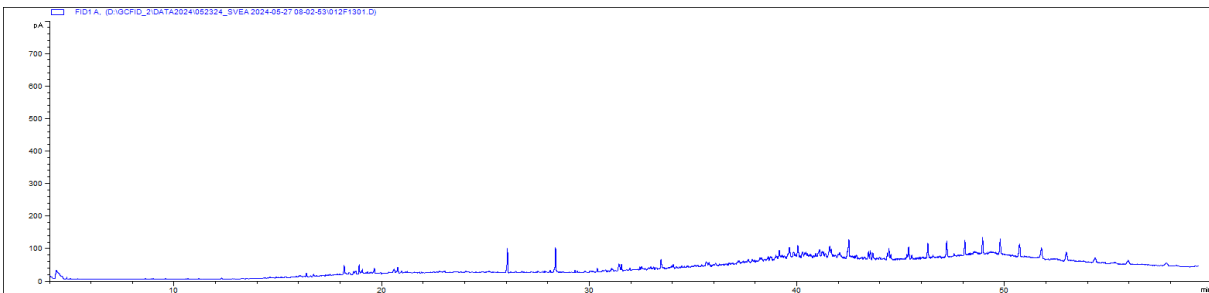


Figure B 3 GC chromatogram of sample 17.5m-2 (SINTEF ID 2023-4236)

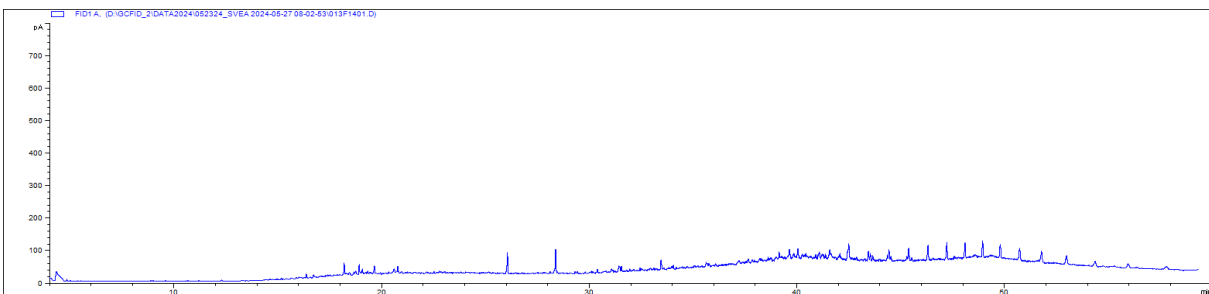


Figure B 4 GC chromatogram of sample 17.5m-3 (SINTEF ID 2023-4237)

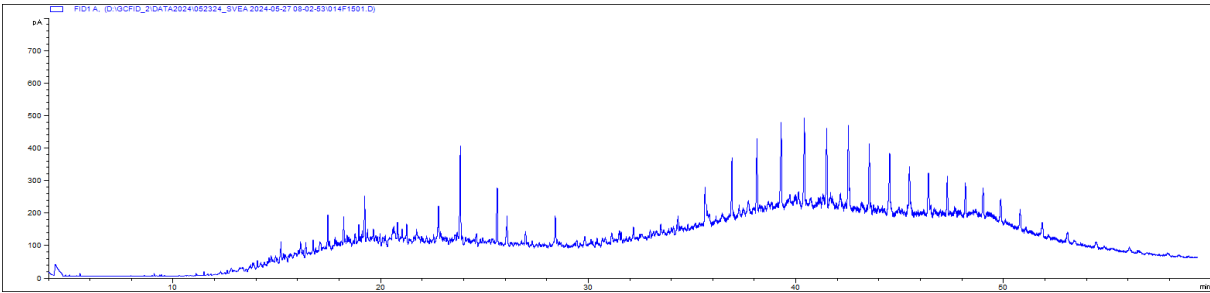


Figure B 5 GC chromatogram of sample 22.5m-1A (SINTEF ID 2023-4239)

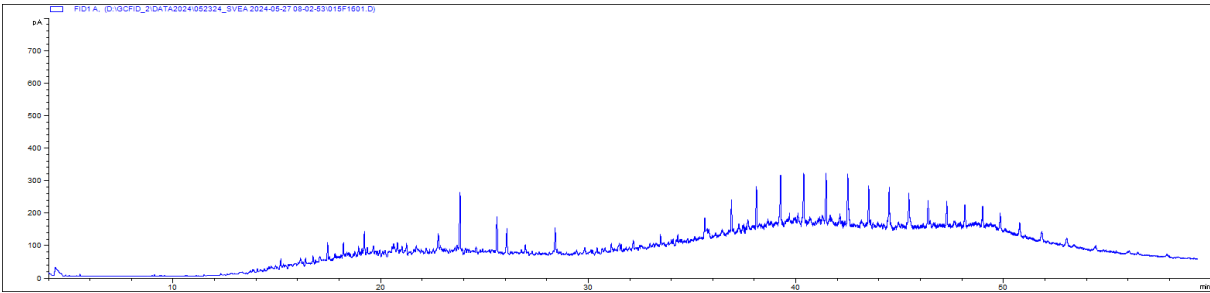


Figure B 6 GC chromatogram of sample 22.5m-2A (SINTEF ID 2023-4240)

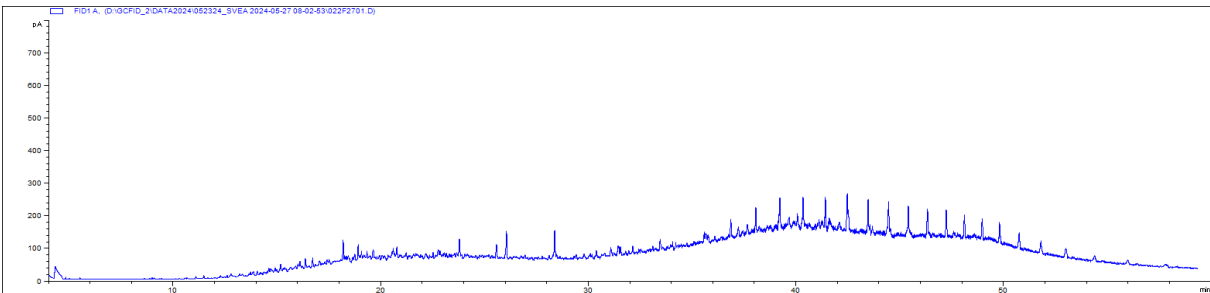


Figure B 7 GC chromatogram of sample 22.5m-3A (SINTEF ID 2023-4241)

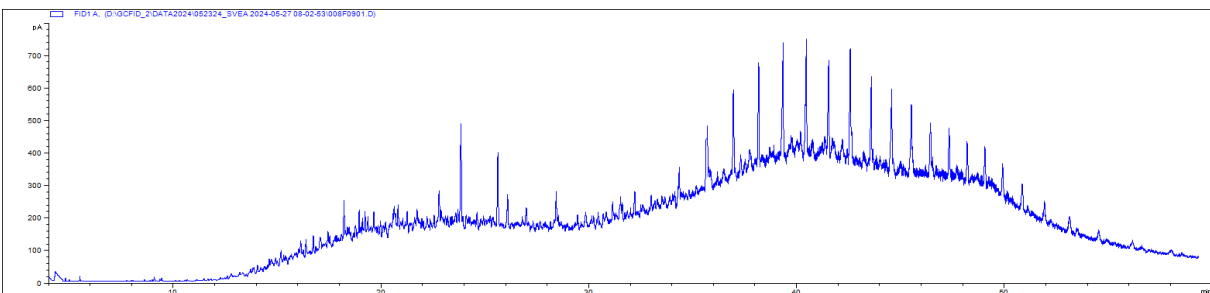


Figure B 8 GC chromatogram of sample 22.5m-1B (SINTEF ID 2023-4231)

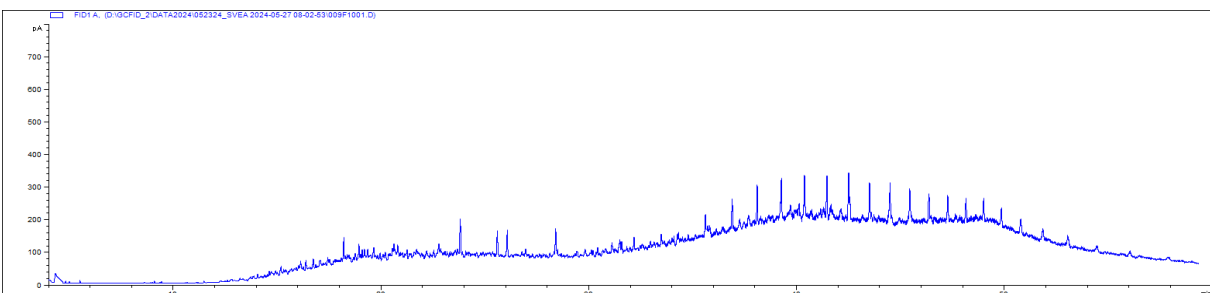


Figure B 9 GC chromatogram of sample 22.5m-2B (SINTEF ID 2023-4232)

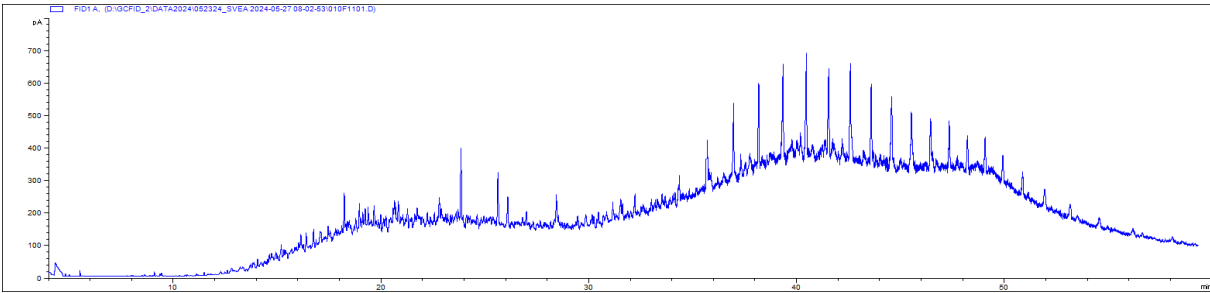


Figure B 10 GC chromatogram of sample 22.5m-3B (SINTEF ID 2023-4233)

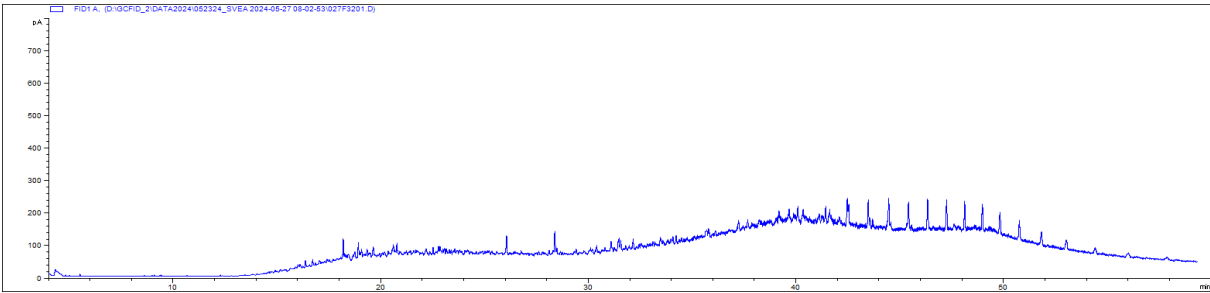


Figure B 11 GC chromatogram of sample 27.5m-1 (SINTEF ID 2023-4243)

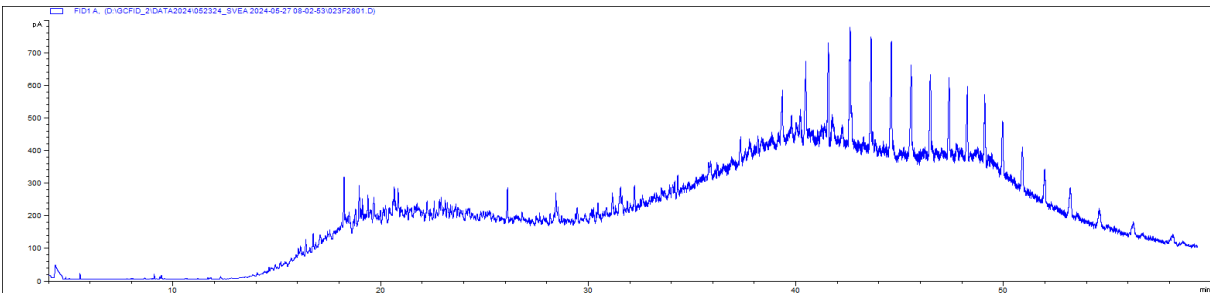


Figure B 12 GC chromatogram of sample 27.5m-2 (SINTEF ID 2023-4244)

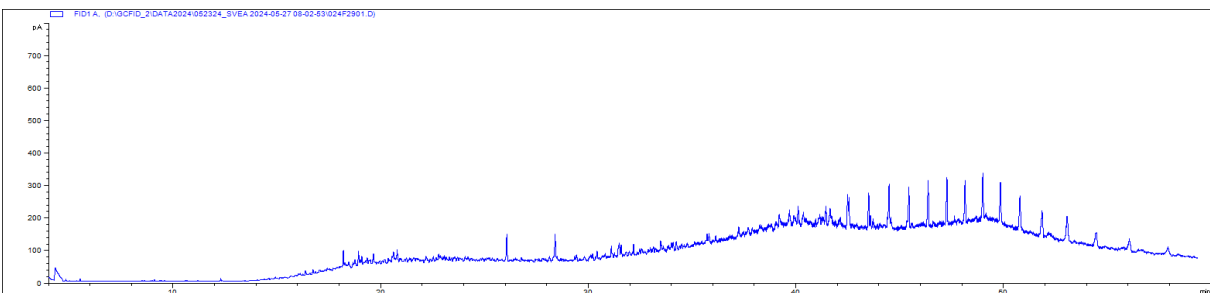


Figure B 13 GC chromatogram of sample 27.5m-3 (SINTEF ID 2023-4245)

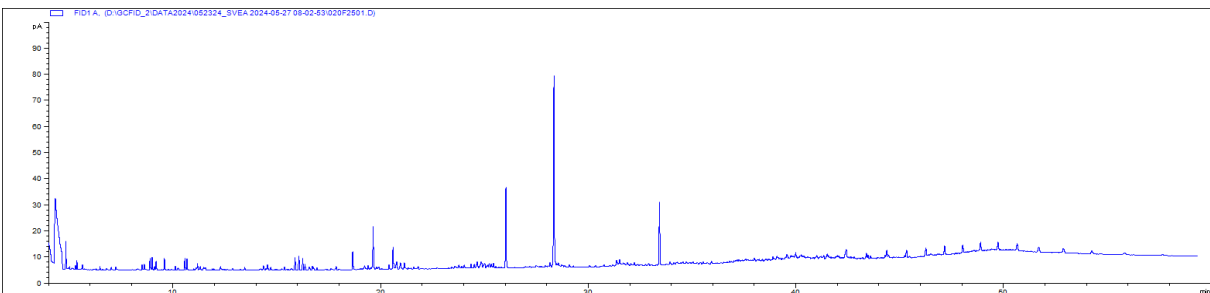


Figure B 14 GC chromatogram of reference sample at 36.4m (SINTEF ID 2023-4217)

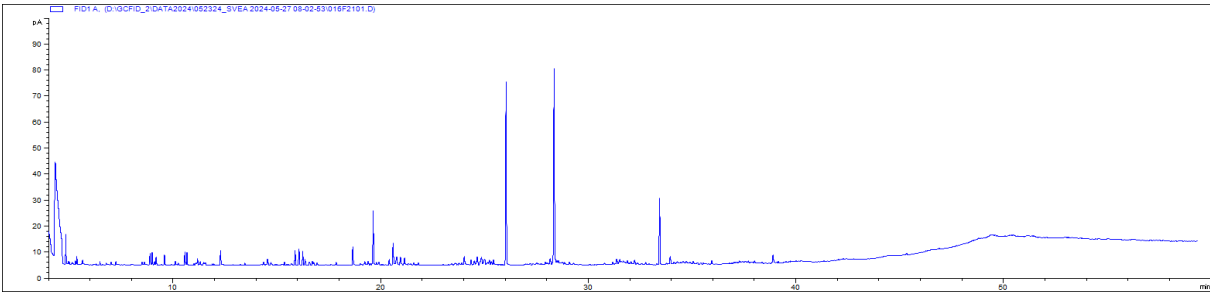


Figure B 15 GC chromatogram of reference sample at 39.5m (SINTEF ID 2023-4213)

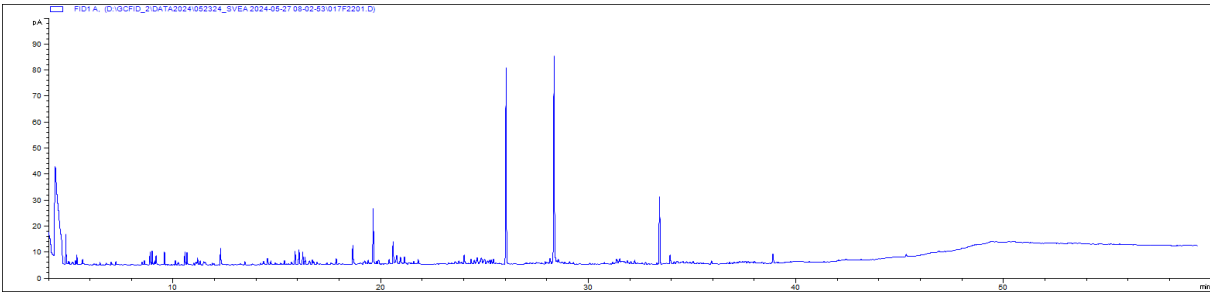


Figure B 16 GC chromatogram of reference sample 39.5m-ht1, collected below high tide (SINTEF ID 2023-4214)

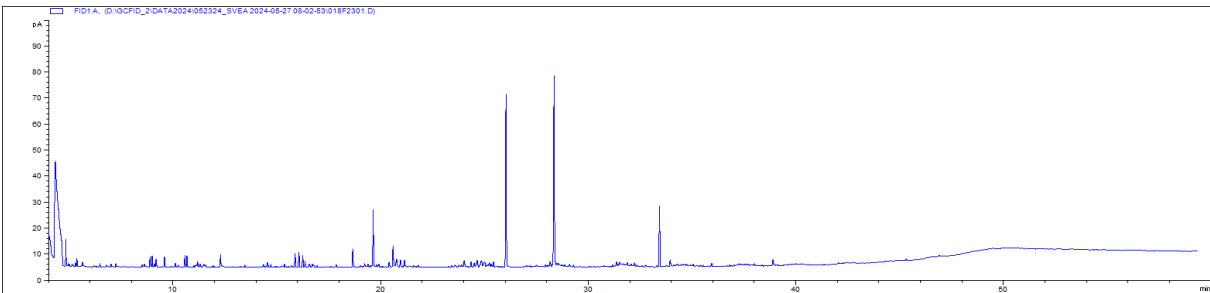


Figure B 17 GC chromatogram of reference sample 39.5m-ht1, collected below high tide (SINTEF ID 2023-4215)

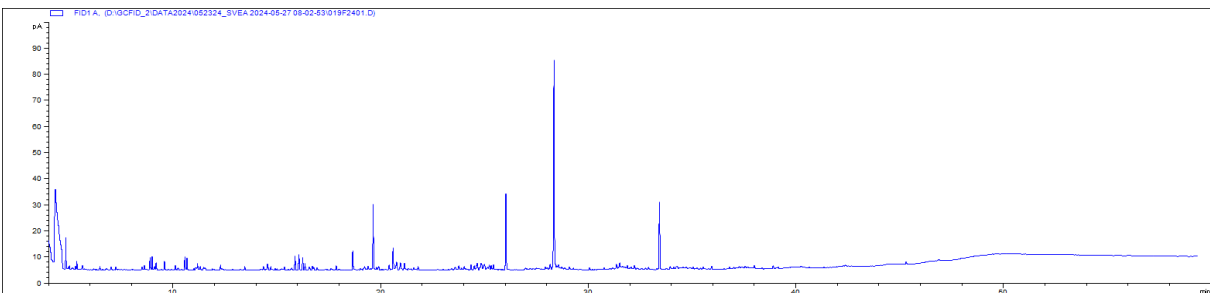


Figure B 18 GC chromatogram of reference sample 39.5m-ht2, collected below high tide (SINTEF ID 2023-4216)

Table B 2 **Content of SVOCs, normalised to hopane: Initial oil and sediment samples from locations at 17.5 m and 22.5m (two sampling spots at 22.5 m, spot A given here). TPAH depletion is also calculated.**

SINTEF ID	2011-0277	2023-4235	2023-4236	2023-4237	2023-4239	2023-4240	2023-4241
Sample description	Initial oil	17.5m-1	17.5m-2	17.5m-3	22.5m-1A	22.5m-2A	22.5m-3A
Naphthalene	13.6	0.041	0.062	0.098	0.127	0.119	0.083
C1-naphthalenes	20.6	0.079	0.089	0.133	0.214	0.202	0.144
C2-naphthalenes	28.4	0.153	0.150	0.233	0.346	0.362	0.243
C3-naphthalenes	21.2	0.244	0.200	0.382	1.44	1.46	0.699
C4-naphthalenes	12.2	0.921	0.415	1.695	6.14	6.17	3.76
Biphenyl	3.92	0.021	0.024	0.037	0.036	0.043	0.032
Acenaphthylene	0.167	0.023	0.025	0.040	0.079	0.087	0.064
Acenaphthene	0.175	ND	ND	ND	ND	ND	ND
Dibenzofuran	0.633	0.012	0.013	0.015	0.018	0.019	0.014
Fluorene	1.03	0.007	0.006	ND	0.096	0.099	0.028
C1-fluorenes	2.83	0.036	0.030	0.055	0.163	0.176	0.107
C2-fluorenes	4.19	0.102	0.068	0.138	0.747	0.712	0.392
C3-fluorenes	4.07	0.145	0.078	0.184	0.702	0.681	0.422
Phenanthrene	2.39	0.037	0.048	0.036	0.033	0.039	0.044
Anthracene	0.142	0.004	0.005	0.004	0.014	0.011	0.007
C1-phenanthrenes/anthracenes	6.33	0.086	0.079	0.074	0.145	0.138	0.118
C2-phenanthrenes/anthracenes	8.32	0.598	0.303	0.851	2.14	2.19	1.70
C3-phenanthrenes/anthracenes	6.50	1.03	0.812	1.663	3.14	3.20	2.84
C4-phenanthrenes/anthracenes	4.84	1.15	0.981	1.710	2.77	2.80	2.65
Dibenzothiophene	0.169	0.009	0.010	0.013	0.021	0.022	0.017
C1-dibenzothiophenes	1.01	0.056	0.049	0.070	0.144	0.150	0.121
C2-dibenzothiophenes	2.02	0.216	0.103	0.303	0.866	0.894	0.680
C3-dibenzothiophenes	1.97	0.359	0.279	0.555	1.06	1.10	1.03
C4-dibenzothiophenes	1.49	0.324	0.270	0.480	0.786	0.790	0.790
Fluoranthene	0.191	0.007	0.006	0.010	0.017	0.019	0.013
Pyrene	0.199	ND	ND	ND	ND	ND	ND
C1-fluoranthrenes/pyrenes	2.86	0.924	0.893	1.143	1.40	1.37	1.36
C2-fluoranthrenes/pyrenes	1.08	0.230	0.259	0.309	0.445	0.449	0.354
C3-fluoranthrenes/pyrenes	4.05	0.844	0.888	0.984	1.39	1.37	1.32
Benz(a)anthracene	0.418	0.075	0.045	0.103	0.195	0.205	0.182
Chrysene	1.06	0.431	0.427	0.501	0.588	0.600	0.623
C1-chrysenes	2.61	1.00	0.974	1.25	1.51	1.56	1.65
C2-chrysenes	2.86	1.23	1.19	1.39	1.64	1.69	1.84
C3-chrysenes	2.46	0.893	0.870	0.936	1.09	1.20	1.28
C4-chrysenes	0.871	0.362	0.343	0.383	0.577	0.519	0.522
Benzo(b)fluoranthene	0.191	0.006	0.007	0.007	0.012	0.006	0.008
Benzo(k)fluoranthene	0.076	0.112	0.109	0.115	0.115	0.116	0.134
Benzo(e)pyrene	0.456	0.302	0.296	0.280	0.238	0.260	0.306
Benzo(a)pyrene	0.235	0.068	0.050	0.065	0.097	0.107	0.106
Perylene	0.143	0.059	0.046	0.053	0.059	0.068	0.074

SINTEF ID	2011-0277	2023-4235	2023-4236	2023-4237	2023-4239	2023-4240	2023-4241
Sample description	Initial oil	17.5m-1	17.5m-2	17.5m-3	22.5m-1A	22.5m-2A	22.5m-3A
Indeno(1,2,3-c,d)pyrene	0.196	0.110	0.113	0.104	0.105	0.104	0.120
Dibenz(a,h)anthracene	0.039	0.033	0.034	0.031	0.034	0.038	0.033
Benzo(g,h,i)perylene	0.196	ND	ND	ND	ND	ND	ND
Sum all SVOC	168	12.4	10.6	16.4	30.8	31.2	25.9
Naphthalenes	96.0	1.44	0.92	2.54	8.27	8.31	4.93
2-3 ring PAHs	52.3	4.22	3.18	6.23	13.0	13.2	11.1
4-6 ring PAHs	20.2	6.69	6.55	7.67	9.52	9.69	9.92
% TPAH depletion		92.7	93.7	90.3	81.8	81.5	84.6

Table B 3 Content of SVOCs, normalised to hopane: Initial oil and sediment samples from locations at 22.5 m (spot B), 27.5 m, and 36.4 m (1.4 m outside the contaminated site). TPAH depletion is also calculated.

SINTEF ID	2011-0277	2023-4231	2023-4232	2023-4233	2023-4243	2023-4244	2023-4245	2023-4217
Sample description	Initial oil	22.5m-1B	22.5m-2B	22.5m-3B	27.5m-1	27.5m-2	27.5m-3	36.4m-REF
Naphthalene	13.6	0.062	0.085	0.065	0.082	0.076	0.086	0.253
C1-naphthalenes	20.6	0.103	0.129	0.102	0.147	0.133	0.133	0.333
C2-naphthalenes	28.4	0.180	0.225	0.158	0.306	0.225	0.245	0.462
C3-naphthalenes	21.2	0.725	0.845	0.789	0.635	0.759	0.534	0.691
C4-naphthalenes	12.2	3.77	4.35	4.07	3.83	5.27	2.99	0.210
Biphenyl	3.92	0.019	0.031	0.016	0.042	0.035	0.035	0.059
Acenaphthylene	0.167	0.075	0.078	0.062	0.068	0.074	0.062	0.010
Acenaphthene	0.175	ND	ND	ND	ND	ND	ND	0.002
Dibenzofuran	0.633	0.010	0.010	ND	0.013	0.007	0.014	0.073
Fluorene	1.03	0.037	0.040	0.041	ND	0.042	0.008	0.016
C1-fluorenes	2.83	0.110	0.127	0.119	0.112	0.106	0.085	0.038
C2-fluorenes	4.19	0.697	0.749	0.759	0.639	0.762	0.504	0.055
C3-fluorenes	4.07	0.702	0.739	0.769	0.705	0.822	0.522	0.054
Phenanthrene	2.39	0.013	0.021	0.013	0.046	0.028	0.047	0.222
Anthracene	0.142	0.009	0.010	0.011	0.012	0.011	0.010	0.003
C1-phenanthrenes/anthracenes	6.33	0.118	0.117	0.109	0.160	0.133	0.104	0.352
C2-phenanthrenes/anthracenes	8.32	1.92	1.96	1.94	2.09	2.61	1.50	0.414
C3-phenanthrenes/anthracenes	6.50	2.84	3.00	2.85	3.27	3.87	2.81	0.295
C4-phenanthrenes/anthracenes	4.84	2.60	2.66	2.62	2.84	3.34	2.68	0.285
Dibenzothiophene	0.169	0.010	0.016	0.019	0.019	0.033	0.021	0.014
C1-dibenzothiophenes	1.01	0.106	0.136	0.115	0.154	0.163	0.163	0.080
C2-dibenzothiophenes	2.02	0.674	0.717	0.716	0.740	0.967	0.497	0.056
C3-dibenzothiophenes	1.97	0.905	0.978	0.929	1.094	1.317	0.947	0.061
C4-dibenzothiophenes	1.49	0.671	0.722	0.655	0.834	0.917	0.769	0.080
Fluoranthene	0.191	0.014	0.015	0.011	0.016	0.015	0.015	0.025

SINTEF ID	2011-0277	2023-4231	2023-4232	2023-4233	2023-4243	2023-4244	2023-4245	2023-4217
Sample description	Initial oil	22.5m-1B	22.5m-2B	22.5m-3B	27.5m-1	27.5m-2	27.5m-3	36.4m-REF
Pyrene	0.199	ND	ND	ND	ND	ND	ND	0.009
C1-fluoranthrenes/pyrenes	2.86	1.22	1.34	1.17	1.46	1.51	1.48	0.414
C2-fluoranthrenes/pyrenes	1.08	0.421	0.436	0.380	0.419	0.338	0.410	0.128
C3-fluoranthrenes/pyrenes	4.05	1.39	1.41	1.29	1.54	1.29	1.496	0.751
Benz(a)anthracene	0.418	0.189	0.200	0.180	0.232	0.202	0.188	0.023
Chrysene	1.06	0.511	0.548	0.502	0.654	0.202	0.625	0.180
C1-chrysenes	2.61	1.40	1.41	1.34	1.76	1.84	1.68	0.273
C2-chrysenes	2.86	1.65	1.58	1.50	1.91	1.64	1.85	0.352
C3-chrysenes	2.46	1.30	1.24	1.20	1.34	1.24	1.32	0.361
C4-chrysenes	0.871	0.640	0.579	0.612	0.542	0.544	0.543	0.259
Benzo(b)fluoranthene	0.191	ND	ND	ND	0.006	ND	0.007	0.036
Benzo(k)fluoranthene	0.076	ND	0.116	ND	0.132	ND	0.137	0.008
Benzo(e)pyrene	0.456	0.279	0.266	0.262	0.301	0.276	0.311	0.294
Benzo(a)pyrene	0.235	0.122	0.111	0.121	0.121	0.117	0.115	0.036
Perylene	0.143	0.078	0.073	0.077	0.079	0.064	0.076	0.063
Indeno(1,2,3-c,d)pyrene	0.196	0.093	0.104	0.095	0.122	0.111	0.111	0.151
Dibenz(a,h)anthracene	0.039	0.024	0.031	0.035	0.040	0.036	0.038	0.029
Benzo(g,h,i)perylene	0.196	ND	ND	ND	ND	ND	ND	0.151
Sum all SVOC	168	25.7	27.2	25.7	28.5	31.1	25.2	7.66
Naphthalenes	96.0	4.84	5.63	5.18	5.00	6.46	3.99	1.95
2-3 ring PAHs	52.3	11.5	12.1	11.8	12.8	15.2	10.8	2.17
4-6 ring PAHs	20.2	9.33	9.46	8.77	10.7	9.43	10.4	3.54
% TPAH depletion		84.8	83.9	84.8	83.1	81.6	85.1	95.5

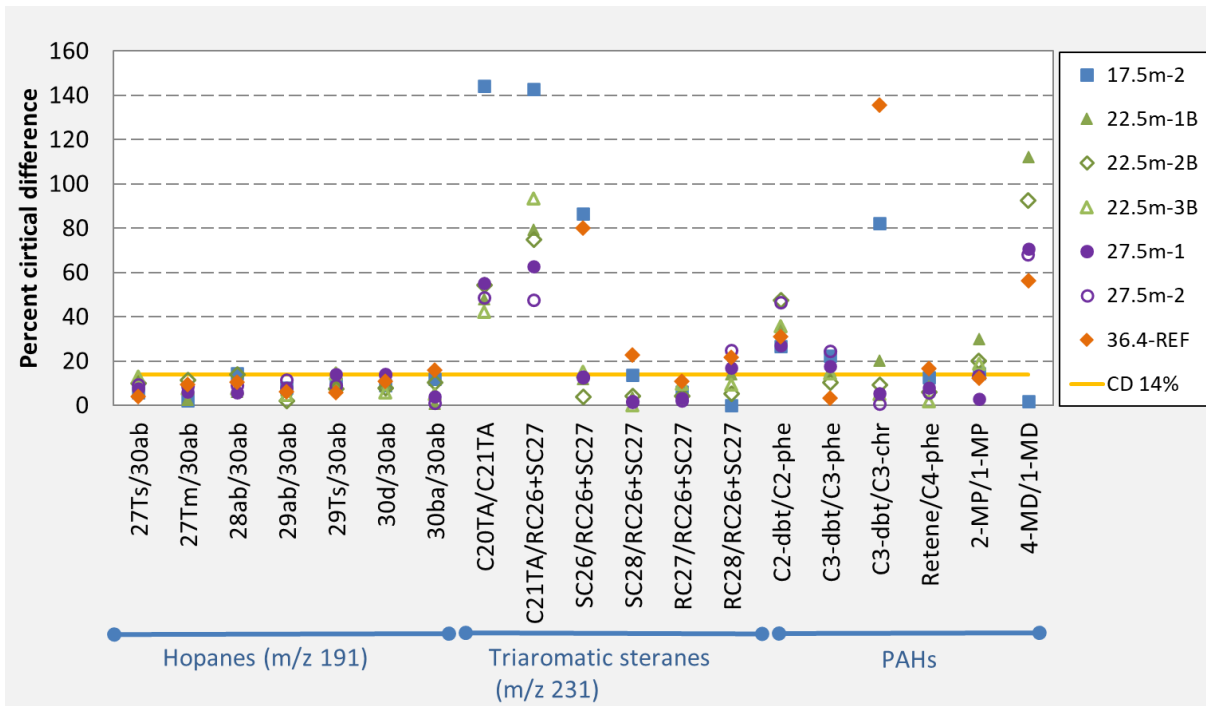


Figure B 19 Difference in diagnostic ratios between the initially spilled oil and samples collected after 26 years. The method described in EN (2023) is used to calculate the percent critical difference (CD), and CD of 14% is indicated with a yellow line. Abbreviations for PAHs and biomarkers are given in Table 3 and examples of ion chromatograms are shown in Figure 4.

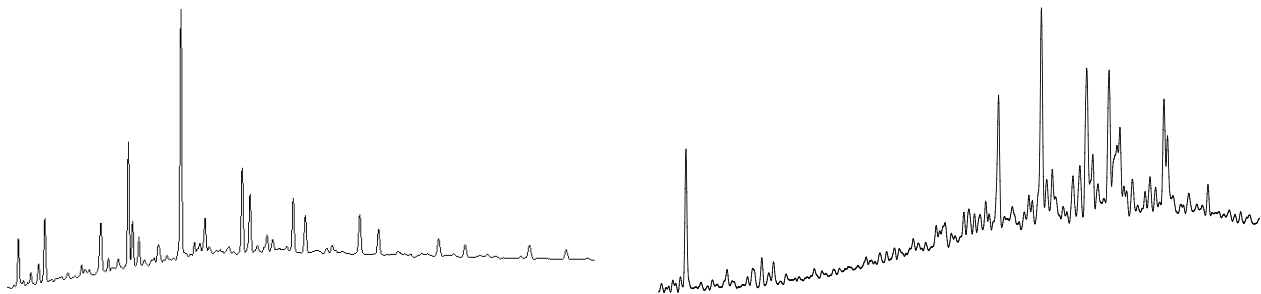


Figure B 20 Ion chromatograms from sample 36.4m-REF (2023-4217): Left chromatogram of hopanes m/z 191) and right chromatogram of the triaromatic steranes (m/z 231).