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Report

Bioremediation of oil on shorelines in Arctic conditions - A laboratory study

Author(s)
Liv-Guri Faksness
Dag Altin, Odd Gunnar Brakstad

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SINTEF Ocean AS
SINTEF Ocean AS
Address:
Postboks 4762 Torgarden
NO-7465 Trondheim
NORWAY
Switchboard: +47 46415000

Enterprise /VAT No:
NO 937 357 370 MVA

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AUTHOR(S)
Liv-Guri Faksness
Dag Altin, Odd Gunnar Brakstad

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ABSTRACT
The potential for using fertilizers to enhance microbial degradation of oil in shoreline sediments was studied in a sediment column system. A pre-weathered Ultra Low Sulphur Fuel Oil (2, 6 or 18 g oil/kg sediment) and a slow-releasing fertilizer (0, 1, 3 or 6 g/kg sediment) were applied in different combinations. The experiments demonstrated that fertilizers can be used to enhance biodegradation of oil in sediments at low temperature (5°C).

Oil degradation throughout the 56-day experiment was confirmed by chemical analyses in all sediments mixed with oil. The oil depletion varied from 0.2 g oil/kg sediment in the columns with highest oil concentration to 0.8 g oil/kg sediment in columns with 6 g oil. The chemical degradation was accompanied with a reduction in toxicity of tested sediments. However, the toxicity of sediments with the highest concentration of fertilizer exceeded that of corresponding sediments with low or no addition of nutrient. A toxicity test with only fertilizer indicated that the ammonia/ammonium was the main driver of toxicity of the fertilizer to Calanus early stages. Based on the current results, it is recommended that the amounts of the Plantagen slow-release fertilizer (NPK of 16:6:12) used on shoreline sediments should be maximum 3 g/kg sediment.

The microbial communities became rapidly predominated by alkane-degrading bacteria, which resulted in alkane biodegradation. Subsequent abundances of aromatic degraders were related to biodegradation of 2- to 3-ring PAH compounds which are associated with toxicity. These data substantiate the potentials for combining chemical and microbial analyses for describing the effectiveness of bioremediation and demonstrate the potential capacities of natural sediment for harbouring indigenous oil-degrading bacteria, which can be stimulated during bioremediation actions.

PREPARED BY	SIGNATURE
Liv-Guri Faksness	Liv-Guri Faksness (Apr 22, 2021 19:54 GMT+2)

CHECKED BY	SIGNATURE
Trond Nordtug	Trond Nordtug (Apr 22, 2021 12:50 GMT+2)

APPROVED BY	SIGNATURE
Atle Kleven	

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Sammendrag

Målet med prosjektet var å få bedre kunnskap om hvordan tilsats av næringsstoffer kan øke biodegraderingen (mikrobiell nedbrytning) av olje i strandsedimenter under Arktiske betingelser. Effekten av å tilsette næringsstoffer (bioremediering) for å fremskynde oljens nedbrytingsprosesser ble studert i sedimentkolonner med sjøvannsgjennomstrømning ved 5 °C. Forvitret ULSFO (Ultra Low Sulphur Fuel Oil 250°C+) og en langtidsvirkende gjødning for hage fra Plantasjen (NPK på 16:6:12) ble brukt. Kolonnene ble tilsatt 2, 6 eller 18 g olje/kg sediment og 0, 1, 3 eller 6 g gjødsel/kg sediment i ulike kombinasjoner. Forsøkene varte i 56 dager med prøvetakning etter 14, 28 og 56 dager. Det ble tatt prøver av sedimentet for oljeanalyser, giftighetstesting og karakterisering av mikrobielle samfunn, samt prøver av porevannet for næringsstoffanalyser.

Forsøkene viste at tilsetting av gjødsel økte biodegraderingen av olje i sedimentene ved lav temperatur (5 °C+). I kolonnene med høyeste oljekonsentrasjon (18 g/kg sediment) økte nedbrytingen med økt gjødselmengde, men gjødselmengden så ut til å bety mindre for reduksjon av oljekonsentrasjon i kolonner med mindre olje (2 og 6 g olje/kg sediment).

Forhåndstallene for C_{17}/pristan og C_{18}/fytan brukes ofte for å vurdere biodegradering av alkaner i olje, spesielt i miljøprøver som bl.a. olje-forurensede sedimenter. Lineære alkaner som C_{17} og C_{18} brytes fort ned, mens mer komplekse komponenter som pristan og fytan brytes langsommere ned. Forhåndstallene viste en nedgang i C_{17}/pristan og C_{18}/fytan i de fleste kolonnene. Imidlertid økte forhåndstallene fra dag 14 og utover i kolonnene med laveste oljekonsentrasjon (2g/kg sediment), noe som indikerte at bakteriene hadde startet nedbrytningen av pristan og fytan. En generell observasjon var at biodegraderingen var raskere etter 14 og 28 dager, spesielt i kolonner med 2 g og 6 g olje kombinert med 3 og 6 g gjødsel. Biodegraderingen fortsatte fra 28 til 56 dager, også i kolonnene uten gjødsel, noe som resulterte i mindre forskjeller i forhåndstallene mellom kolonner med og uten gjødsel når forsøkene ble avsluttet etter 56 dager. Det var omtrent samme oljedegradering i kolonner hvor gjødsel ble blandet i sedimentet som når det ble lagt på overflaten. Ingen nedbryting ble registrert i oljen som ble påført på overflaten uten gjødsel.

Innholdet av ammonium og fosfat i porevannet i de øverste 10 cm av sedimentet var mye høyere i kolonner med gjødsel gjennom hele forsøksperioden sammenlignet med de uten gjødsel. På slutten av forsøket var konsentrasjonene av nitrat og nitritt tilnærmet bakgrunnsnivå igjen. Den bakterielle aktiviteten indikerte ingen begrensning av næringsstoffer i de 56 dagene forsøkene varte.

Resultatene fra kolonnene med laveste oljekonsentrasjon (2 g/kg sediment) viste økt nedbryting ved tilsetting av gjødsel, men at nedgangen i PAH-konsentrasjonen ikke økte ved ytterligere tilsetting av gjødsel. Reduksjonen ved tilsetting av gjødsel var 95% for naftalenene, omtrent 50% for 2-3 rings PAH, og om lag 25% for 4-6 rings PAH. I kolonnene med høyeste oljekonsentrasjon (18 g/kg sediment) var den prosentvise reduksjonen lavere, men nedbrytingen økte med økende mengde gjødsel.

Tidligstadier (egg til nauplii, N2) av raudåte (*Calanus finmarchicus*) ble eksponert for små mengder sediment i 72 timer med registrering av klekkeraten og overlevelse. Klekkeraten var betydelig lavere i kolonner med olje enn i rent sediment. Testene indikerte høyere overlevelse etter 56 dager enn etter 14 og 28 dager. Det var en betydelig nedgang i overlevelse i kolonnene med høyeste gjødselmengde, noe som

kan indikere at gjødseltypen enten er giftig i seg selv, eller at den sammen med oljen blir mer giftig for raudåta.

Det ble derfor gjennomført giftighetstester med kun gjødsel ved at WAF (vannløselige fraksjoner) med 6 g næring/L sjøvann ble testet på tidligstadiet av raudåte. Samtidig ble det gjennomført en referansetest med ammoniumklorid for å estimere bidraget av ammonium/ammoniakk til giftigheten av gjødselen. Resultatene indikerte at ammonium/ammoniakk er hovedårsaken til den observerte toksisiteten i WAF av gjødselen. Ammoniumkonsentrasjonene i alle sedimentkolonnene var lavere enn LC_{50}-verdiene målt i giftighetstestene, men de målte konsentrasjonene i porevannet fra kolonner med høyeste gjødselmengde (6 g/kg sediment) var i området som kan bidra til dødelighet i tidligstadier av raudåte.

DNA-konsentrasjonene i sedimentet reflekterer en generell økning i mikrobiell biomasse under nedbrytningsprosessene. Det ble observert en stimulering av mikrobiell biomasse i alle kolonner med gjødsel, og konsentrasjonen av biomassen økte med økende gjødselmengde. I kolonnene med høyeste oljekonsentrasjon (18 g/kg) var det imidlertid en mindre økning i mikrobiell biomasse enn i kolonnene tilsatt 2 og 6 g olje/kg sediment, trolig på grunn av at toksiske effekter av oljen forsinket stimuleringen.

Karakteriseringen av de mikrobielle samfunnene (16S rDNA mikrobiom-analyser) viste signifikante forskjeller i sammensetning og diversitet av bakteriesamfunnene mellom referanseprøver (sedimentprøver med hhv 2, 6 og 18 g olje/kg sediment fra dag 0, dvs før gjødsling) og kolonneprøver som ble inkubert med olje over en periode på 14 til 56 dager. Sammensetningen i de mikrobielle samfunnene endret seg dessuten gradvis i løpet inkubasjonsperioden. Ulike oljekonsentrasjoner så imidlertid ikke ut til på påvirke bakteriesamfunnene i særlig grad. Tilsetting av gjødsel påvirket derimot endringene i de mikrobielle samfunnene sammenlignet med sedimenter med olje uten tilsats av gjødsel. Men det var større forskjeller i samfunnene fra sedimenter med ulike gjødselkonsentrasjoner. Identifisering av bakterie-gruppene viste høy tilstedeværelse av velkjente oljedegraderende bakterier, hvorav flere typer såkalte 'hydrocarbonoclastiske' (kan kun bruke hydrokarboner som organisk substrat). Sekvens-analyser av DNA i oljekontaminerte sedimenter viste en tidlig dominans av alkan-degraderende bakterier. Noen av disse kan assosieres med arktisk marint miljø og har blitt påvist som viktige for biodegradering av olje i sjøvann fra Svalbard. De alkan-degraderende bakteriene ble etterfulgt av sekundære alkan- og aromatdegraderende bakterier. De aromat-degraderende bakteriene kunne relateres til degradering av 2- to 3-ring PAHer, som igjen er assosiert med akutt giftighet. Spesielt etter 28 dagers inkubasjon var det positiv sammenheng mellom aromatdegraderende bakterier og gjødselkonentrasjon ved høye oljekonsentrasjoner, noe som er i overenstemmelse med øket nedbryting av naftalener i disse prøvene. Disse dataene viser potensialet for å kombinere kjemiske og mikrobielle analyser for å beskrive effektiviteten av bioremedieringsaksjoner. Dataene viser også at strandsedimenter kan være depot for naturlig forekommende oljedegraderende bakterier, som igjen kan stimuleres under en bioremedieringsaksjon.

Tidligstadier av raudåte har tidligere vist seg å være svært sensitive til forurensning, og forventes dermed å være mer sensitive enn de "vanlige" sedimentorganismene. Det anbefales at dersom den testede gjødselen (Plantasjen langtidsvirkende gjødsel, NPK 16:6:12) vurderes brukt i et mulig fremtidig feltforsøk, bør gjødselmengden begrenses til maksimalt 3 g/kg sediment. Resultatene indikerer at 2 g gjødsel/kg sediment bør være tilstrekkelig for å få en biodegraderingseffekt. Resultatene indikerer også at ved lavere oljekonsentrasjoner (<6 olje/kg sediment) kan mengde gjødsel muligens reduseres ytterligere uten en

vesentlig effekt på nedbrytningshastigheten. Eventuelt kan enda lavere gjødselmengde med gjentatte påføringer også vurderes.

I praksis vil en dosering på 2 g gjødsel/kg sediment tilsvare omtrent 320 g gjødsel/m^2 strand dersom det antas at sedimentet på 1 m^2 strand (10 cm dybde) veier 160 kg. For en forurenset strand på 100 m^2 vil dette si et forbruk av ca 30 kg gjødsel. Dersom oljefilmen har en tykkelse på ca 2 mm (ca 10 g olje/kg sediment), vil doseringsforholdet mellom gjødsel og olje bli 1 til 5.

I og med at giftighetstestene indikerte at ammonium ser ut til å være hovedårsaken til den observerte toksisiteten, bør en testing av andre gjødseltyper i laboratoriet vurderes før et eventuelt feltforsøk, fortrinnsvis gjødsel med andre nitrogenkilder enn ammonium.

1 Introduction

The present project is following up a study performed in 2018-2019, reported in Nordtug et al. (2019). In the previous study, sediment columns were used to study the potential for enhanced biodegradation of fuel oils in sediments at low temperatures. The results showed that adding a slow release fertilizer to the sediment increased the biodegradation of both the aliphatic and PAH compounds caused by increased growth of known aliphatic and PAH degrading bacteria in the fertilized sediment. However, the fertilizer and not the oil appeared to be the dominant cause of toxicity in fertilized sediments, probably caused by the experimental design which gave limited water exchange and wash-out of nutrients in the upper layer of the water column (Figure 1.1, left photo). It was suggested that further laboratory-based bioremediation studies with shoreline sediments and fertilizers in addition to tidal cycles should include controlled flushing of the sediment surface during high tide.

In the current project, the sediment column system was upgraded to improve the control of the water levels and flushing of the sediment surface (Figure 1.1, right photo). Pre-weathered ULSFO (Ultra Low Sulphur Fuel Oil, 250 °C+) and a slow-releasing fertilizer were used in different combinations to try to optimize the ratio between oil and fertilizer. The objective was to study how fertilizers can enhance biodegradation of oil in shoreline sediments at low temperature by describing temporal changes in oil chemistry, microbiology, toxicology, and nutrients.

Two add-on activities have been included: DNA sequencing with characterisation of the microbial communities and toxicity testing of the fertilizer used.

Figure 1.1 *Sediment column systems: Previous system (left photo) and modified system used in current project (right photo).*

2 Material and methods

2.1 Materials

The sediment used had a grain size of 2 to 6 mm and was washed in seawater before transferred to the columns. Pre-weathered Ultra Low Sulphur Fuel Oil (ULSFO 250°C+) was used in all columns, and as fertilizer a slow-releasing product from Plantagen (Figure 2.1).

The fertilizer used was "Plantagen langtidsvirkende gjødsel" (NPK(Mg)16-6-12 (+1.2)). This is a commercial slow-release fertilizer consisting of a granulate with nutrients incorporated in a matrix of undeclared composition. The composition of fertilizing agents given by the producer:

16.5% Total nitrogen
- 6.4 % Ammonia
- 4.7 % Nitrate
- 5.4 % Urea

6.2 % Phosphorous – soluble in neutral ammonium citrate
5.7 % Phosphorous – water soluble
11.9 % Water soluble potassium
1.2 % Water soluble manganese

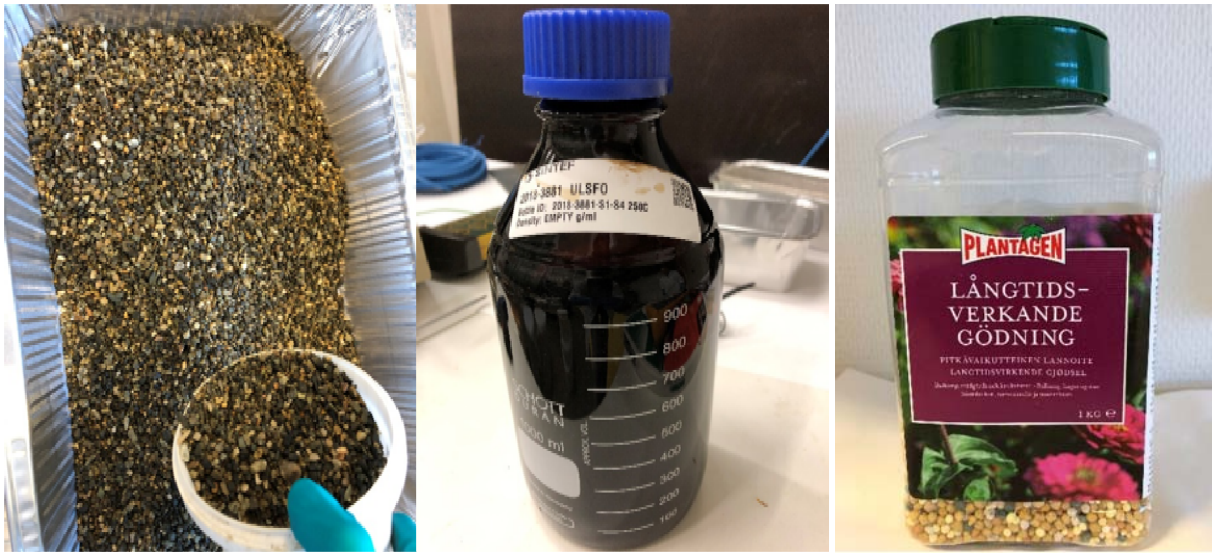

Figure 2.1 Sediment, oil and fertilized used in the column systems.

2.2 Equipment

The experimental work was carried out using a sediment column system with 16 columns at 5°C (Arctic summer temperature). The system stimulates coastal sediment influenced by tidal cycles and is shown in Figure 1.1 (right photo)). Seawater was filled and drained from the bottom according to a sinusoidal 12-hour tidal cycle, so that water repeatedly rising and draining through the sediment. The water level during high tide was approximately 10 cm above the sediment surface. The columns were completely drained at low tide to avoid possible cross-contamination between the columns.

The column system used in Nordtug et al. (2019) was modified to increase the water exchange on the sediment surface. During high tide, the volume above the sediment of each column (approx. 1 L) was flushed with clean seawater (20 L) drained at the top of the column through an overflow channel. This was done to simulate water movement and water exchange on the sediment surface.

The seawater used in the experiments was collected through a pipeline from 80 m depth in the Trondheim fjord and run through a sand filter to remove particles.

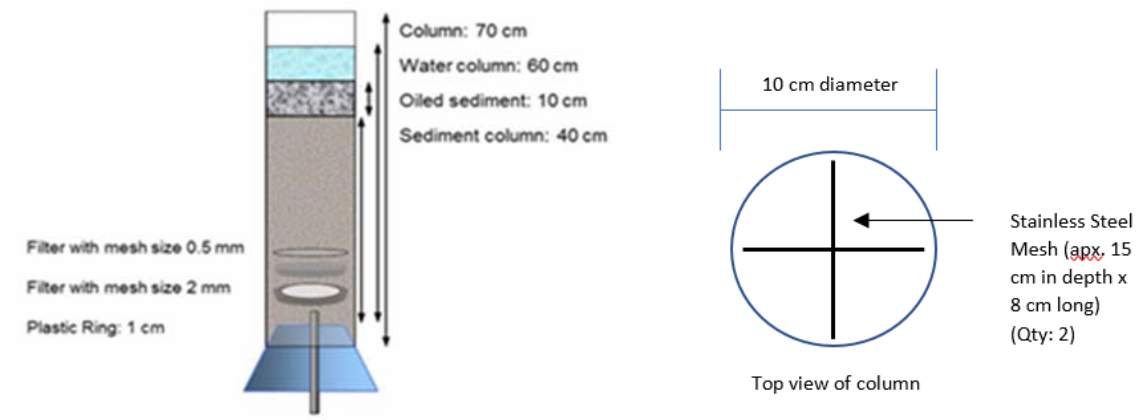

Figure 2.2 *Sketch of a sediment column (left figure) and top view of a column showing the subdivision of the contaminated sediment. Samples collected from the four sectors were replaced with clean sediment.*

2.3 Preparation of sediment columns

The sediment columns were first filled with approximately 50 cm of cleans sediment. Then surface seawater collected from the fjord was applied to inoculate the sediment with marine bacteria for two days, including renewal of the surface seawater after one day.

Sediment and oil were heated separately to 60°C. The hot mixture of oil and sediment was then transferred to a bucket with lid and shaken for 1 min to create a homogenous mixture. Fertilizer granules were then gently mixed into the sediment.

Oiled sediments with or without fertilizer in different concentration combinations were distributed as a 10 cm top layer in the columns as detailed in Table 2.1. The top layer was divided into 4 quadrants by a stainless steel mesh as shown in the examples of column surfaces with different combinations of oil and fertilizer in Figure 2.3.

Figure 2.3 *Surface of columns at day 0: From left to right: COL9 (18 g oil and 1 g fertilizer premixed with sediment), COL12 (18 g oil premixed with sediment and 3 g fertilizer applied on surface), and COL16 (6 g oil on surface, no fertilizer).*

Table 2.1 *Test matrix for the column experiments. Sampling after 14, 28, and 56 days.*

Col no.	Oil (g/kg sediment)	Oil application	Fertilizer (g/kg sediment)	Application fertilizer
1	2	Premix	6	Premix
2	2	Premix	3	Premix
3	2	Premix	1	Premix
4	6	Premix	6	Premix
5	6	Premix	3	Premix
6	6	Premix	1	Premix
7	18	Premix	6	Premix
8	18	Premix	3	Premix
9	18	Premix	1	Premix
10	2	Premix	3	Surface
11	6	Premix	3	Surface
12	18	Premix	3	Surface
13	2	Premix	None	
14	6	Premix	None	
15	18	Premix	None	
16	6	Surface	None	

2.4 Sampling

The sediment surface of the columns was divided into 4 quadrants using a stainless-steel mesh in the top 15 cm. The purpose of the mesh was to provide comparable sampling areas within each column. At each sampling, oiled sediment was sampled with a clean spoon from one quadrat of the column. The removed sediment was replaced with clean sediment to maintain the surface area. The sediment samples were split for chemical, microbial, and ecotoxicity analyses. Sampling was preformed after 14, 28, and 56 days. In addition, reference samples from day 0 with the three oil concentrations were collected.

Water samples for nutrient analysis were also collected. Water was sampled from the top of the column at high tide using a pipette.

An overview over the collected samples and analysis are provided in Table A 1 in Attachment A. All sediment and water samples were stored at -20°C until preparation and analysis.

2.5 Sample preparation and chemical analysis

All samples were added surrogate and recovery internal standards (SIS and RIS) for quantitative analysis on gas chromatograph with flame ionisation detector (GC/FID) and gas chromatograph with mass spectrometer (GC/MS). For GC/FID o-terphenyl (SIS) and 5α-androstane (RIS) were added, and for the GC/MS analysis SIS containing naphthalene-d_8, phenanthrene-d_{10}, and chrysene-d_{12}, and RIS containing fluorene-d_{10} and acenaphthene-d_{10} were added.

Sediment dry weight was quantified by drying about 5 g sediment over night at 50°C.

To the chemical analysis, approximately 10 g sediment was weight for extraction in a Pyrex-flask. The sediment was added 20 mL dichloro methane (DCM) and surrogate internal standards for analysis on GC/FID and GC/MS. The samples were extracted in an ultrasound bath with cold water for 5 min, added 2-3 teaspoons of sodium sulphate (Na_2SO_4) to remove water. The extract was filtrated carefully over Bilsom cotton to remove particles but allowing the sediment to remain in the flask. The same procedure was repeated twice, each time with 20 mL DCM. The DCM extracts were combined and concentrated using a Zymark® Turbovap 500 Concentrator. Samples with 2 g oil was concentrated to 1 mL, 6 g oil to 5 mL and 18 g oil to 10 mL. The final extract (approximately 1 mL) was spiked with RIS and analysed on GC/FID and GC/MS.

All samples were analyzed for total extractable organic materials (THC) using GC/FID and selected samples for semi volatile organic components (SVOC including decalins, PAHs and hopane) using GC/MS. A list of all target analytes is shown in Appendix D (Table D 1). This list includes the recommended analytes given by Singer et al. (2000) and is a typical standard list for the target compounds used during post-oil spill damage assessments.

The GC/FID analyses were performed according to a modification of EPA Method 8015D (US EPA, 2003). TPH (resolved plus unresolved TPH) was quantified by the method of internal standards using the baseline corrected total area of the chromatogram and the average relative response factor from a calibration curve prepared of ULSFO 250°C+ (Figure 2.4) In addition, the ratios C_{17}/pristane, C_{18}/phytane, and pristane/phytane were calculated to get an indication on the biodegradation over time.

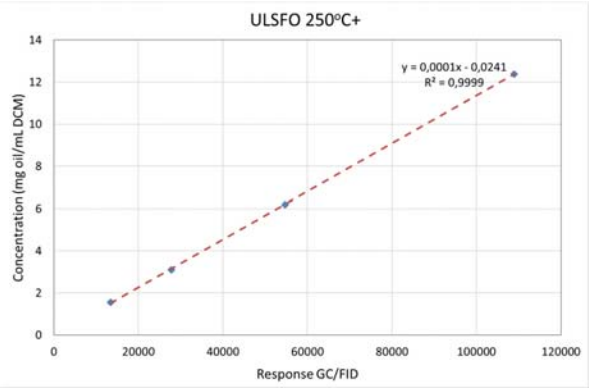

Figure 2.4 *Calibration curve for ULSFO 250°C+ used for calculation of total extractable organic materials in the sediment columns.*

The semi-volatiles were quantified by modifications of EPA Method 8270D (US EPA, 2007). The mass spectrometer was operated in the selective ion monitoring mode to achieve optimum sensitivity and specificity. The quantification of target compounds was performed by the method of internal standards, using average response factors (RF) for the parent compounds. The PAH alkyl homologues were quantified using the straight baseline integration of each level of alkylation and the RF for the respective parent PAH compound. The response factors were generated for all targets and surrogates versus fluorene-d_{10}. Concentrations of were normalized to the non-degradable biomarker hopane (17α(H),21β(H)-hopane) to get an indication of biodegradation of PAHs over time.

As hopane has been assumed to be a "conservative internal marker within the oil" and 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; Douglas et al., 1996), the concentrations for the individual SVOC components were calculated both quantitatively (in g analyte/kg oil) and semi-quantitatively (normalize to hopane (17α(H), 21β(H)-hopane)).

Douglas et al. (2012) used the following equation to determine percent depletion of individual compontents and component groups in the sediment samples:

$$\% \text{ analyte depletion} = [1-((C_1/C_0) \times (H_0/H_1))] \times 100\% \qquad \text{(Eq. 1)}$$

Where C_1 and H_1 are the concentrations of the target analyte and hopane in the exposed samples, respectively, and C_0 and H_0 are the concentrations of the target analyte and hopane in the initial samples from day 0.

2.6 Toxicity testing

Early stages of copepods (*Calanus finmarchicus*) were exposed to a standard amount of sediment in water and mortality was recorded with time. The test includes hatching and the two firs nauplii stages (NI and NII) of *C. finmarchicus*.

A test developed by BioTrix and SINTEF previously used for toxicity testing chemicals (EOR polymers; Farkas et al. 2020) was slightly modified to include sediment. The test includes hatching and the two first nauplii stages (NI and NII) of *C. finmarchicus*. The test medium was seawater in contact with contaminated or clean sediment to simulate a pore-water like solution.

Due to limitations in the number of tests that could be performed, groups with medium concentration (6 g/kg) were prioritized together with selected samples from the first (day 0) and terminal day (day 56) of the experiment (Table A 1 in Attachment A).

Testing was performed at 10˚C on samples previously frozen at -30˚C. Each sediment sample was tested in 6 replicates by distributing the sediment in 6-well plates with 2 g of sediment in each well. The wells were then supplied with 8 ml seawater and left for 24 hours to equilibrate. After the equilibration period, fertilized eggs from *C. finmarchicus* (age approx. 16 hours prior to hatch) were added in 0.5 ml water. The copepods were observed every 24 hours for 72 hours whereafter the test was terminated and the final number of hatched eggs and surviving nauplii were recorded.

2.7 Microbial studies

Microbial analysis and changes over time with next generation sequencing technology provide information about the relative distribution of individual taxa in a sample. This is a suitable parameter for indication of active biodegradation by determining the successional changes in the microbial population as a result of pollutant degradation. Microbial community characteristics may also suggest when biodegradation has terminated, and when the original microbial community structure has returned to a pre-exposure state. This method does not require cultivation of bacteria, and it can capture most known taxa in a sample.

Total DNA from sediment samples was isolated and 16S rRNA gene amplicons were sequenced on an Illumina platform for detailed characterisation of microbes and changes over time. A FastDNA SPIN kit for Soil was used to extract the DNA from the sediment samples. The DNA extracts will give an indication of the total amount of DNA.

Total DNA from sediment samples were sent to Beijing Genomics Institute (BGI) for 16S rDNA microbiome sequencing. In brief, 30 ng qualified DNA template and the 16S rRNA fusion primers were added for Polymerase chain reaction (PCR). All PCR products were purified by Agencourt AMPure XP beads, dissolved in Elution Buffer and eventually labeled to finish library construction. Library size and concentration were detected by Agilent 2100 Bioanalyzer. Qualified libraries were sequenced on a HiSeq platform according to their insert size. Raw data were filtered to obtain high-quality clean data, after which clean reads that can overlap with each other were merged to tags. Raw data are filtered to generate high quality clean reads as follows:

1) truncate primmer and adapter contamination with cutadapt v2.6;
2) truncate reads whose average phred quality values are lower than 20 over a 30 bp sliding window then remove reads whose length are %75 of their original lengths after truncation;
3) Remove reads with ambiguous base;
4) Remove low-complexity reads (default:reads with 10 consecutive same base).

Sequence data received by SINTEF were treated on a bioinformatics platform as follows:

1. Plotting of rarefication curves to show sequencing depths and observed species in the sample DNA extracts. The species are described as operational taxonomic units (OTUs)
2. Determination of alpha diversities (diversity within each sample), where species numbers are taken into account (Chao1) and where species abundance (richness) and evenness (how close in numbers each species in an environment is) are take into account (Simpson diversity index)
3. Determination of beta diversities (diversities between samples) depicted as two-dimensional principal component analyses (PCoA) plots, where each point represents the total community structure of a sample.
4. PLS-discriminant analyses to discriminate microbial communities in samples from different treatments.
5. Visualization of microbial community distributions in the different samples. In this report, we have shown the relative abundances of genera with a 5% cut-off.

2.8 Fertilizer/mineral nutrient analyses

Seawater samples (50 ml) were collected from above the sediment at "high tide", i.e. when water level was above the sediment surface and before the surface of the sediment was flushed by clean seawater. Seawater samples were frozen at -20°C for fertilizer/mineral nutrient analyses.

Mineral nutrient analyses in the water were performed according to standardized methods by a commercial laboratory (ALS Laboratory Group Norway AS). Concentrations of nitrate-nitrite, ammonium plus ammonia, and ortho-phosphate were measured.

2.9 Toxicity of "slow-releasing" fertilizer

Toxicity tests of oiled sediment from degradation experiments in the SINTEF sediment column system indicated increased toxicity of sediments added 6g/kg Plantagen "Slow release fertilizer". The current toxicity testing of the mixture components released form the fertiliser was initiated to test if the addition of nutrient to the sediment may have contributed to the observed increase in toxicity.

A water accommodated fraction was made from 6 mg/L fertilizer in seawater and tested for toxicity on early stages of the copepod *Calanus finmarchicus*. A reference test with ammonium chloride was used to estimate the contribution of ammonium/ammonia to the observed toxicity of the fertilizer.

Description of the methodology used are detailed in Attachment G.

3 Results and discussion

3.1 Biodegradation and oil concentrations estimated from GC-analysis

The water contents were determined in all sediment samples, but the water contents were low (between 2-3%).

All samples, including reference samples, were analysed on GC/FID, and the GC chromatograms are given in Attachment C. The oil concentration in the sediments, expressed as amount of total extractable organic materials (TPH), were estimated using the calibration curve of ULSFO shown in Figure 2.4 (Table B 1). In addition, the ratios nC_{17}/pristane and nC_{18}/phytane were quantified to get an indication of biodegradation over time (Table B 2). Ratios between the rapidly degraded n-alkanes nC_{17} and nC_{18} and the less degradable isoprenoid compounds pristane and phytane have been used for decades for determination of n-alkane biodegradation in oil-polluted environments (e.g. Douglas et al., 1996). Since pristane and phytane have similar boiling and evaporation properties as their n-alkane analogues, selective depletion of the n-alkanes will be the result of biodegradation (not evaporation).

As shown in Table B 1, the oil concentrations were higher in some of the exposed samples than in the reference samples from day 0. This could be caused by inhomogeneity in the premixed oil and sediment or analytical issues. The reduction in oil concentrations is calculated, both in mg oil/kg sediment and in percent reduction and is presented in Figure 3.1. The highest percentage reduction in oil concentration was observed in columns with 2 g oil (approximately 40%) and the lowest in columns with 18 g oil (approximately 5%). On weight basis, the loss was approximately the same in columns with 2 and 18 g oil (approximately 0.4 g oil/kg sediment) in columns with 2 and 18 g oil. The largest loss was measured in columns with 6 g oil (up to 0.8 oil/kg sediment).

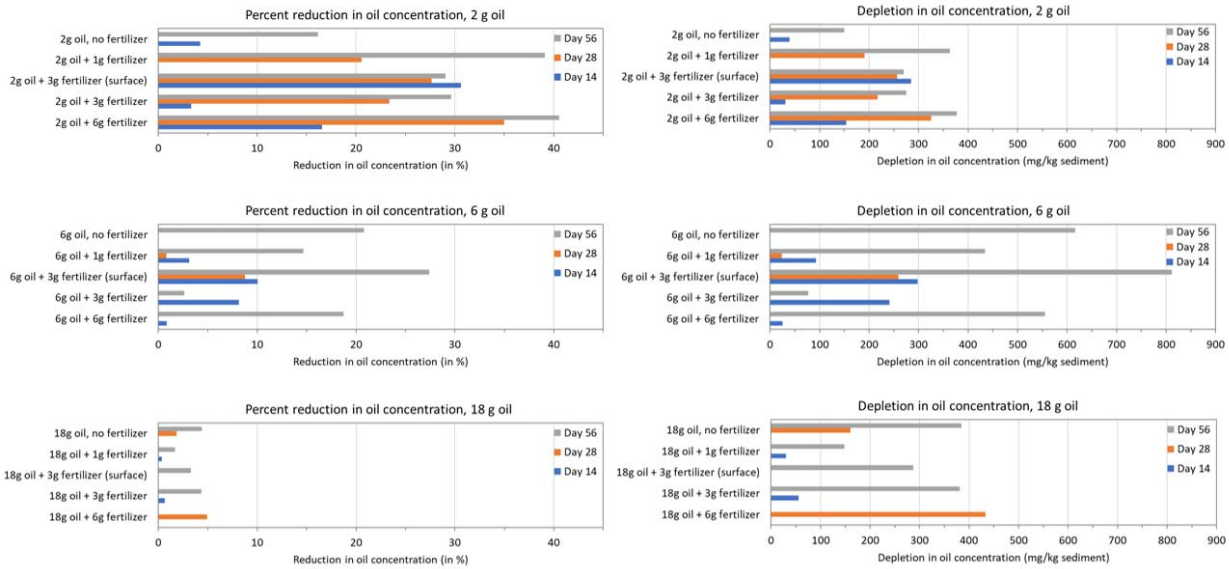

Figure 3.1 *Percentage reduction in oil concentration (left graphs) and reduction in oil concentration (in mg oil/kg) in the sediment samples over time, related to day 0.*

As mentioned above, to get an indication of biodegradation over time, the ratios C_{17}/pristane and C_{18}/phytane were calculated (Table B 2) and are shown in Figure 3.2. A decrease in the ratios was observed in most of the columns. However, in the columns added 2 g oil an increase in the C_{17}/pristane ratio was observed from day 14 to 56 with 6 g fertilizer and from day 28 to 56 in the columns with 1 and 3 g fertilizer. The pristane/phytane ratio gives an indication of biodegradation of pristane and/or phytane. In the reference oils the ratio was between 1.9 and 2.1, but in the samples on day 56 it was reduced to between 1.4 and 1.7, indicating that the bacteria have started to degrade pristane and phytane as well. The biodegradation increased with increased amount of fertilizer in columns with 6 g oil and 18 g oil, and the reduction in the ratios were less in columns with 18 g oil. No biodegradation was observed in the column with no fertilizer and 6 g oil applied on the sediment surface. Overall, the degradation was faster after 14 and 28 days, especially in columns with 2 and 6 g oil and 3 and 6 g fertilizer. Also, in the columns without fertiliser the biodegradation continued between 28 and 56 days, resulting in less difference between fertilized and non-fertilized oil when the experiments were terminated on day 56.

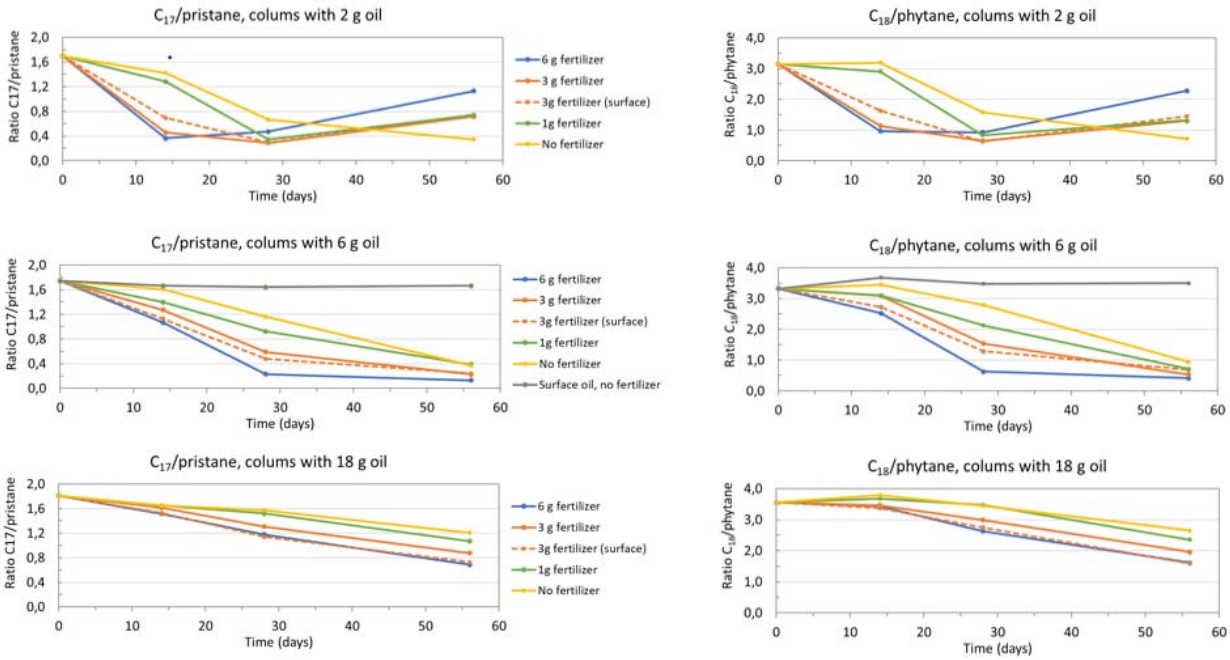

Figure 3.2 Ratios between C_{17}/pristane and C_{18}/phytane over time.

3.2 Quantification and depletion of semi-volatile organic compounds

GCMS-analysis were performed on approximately 30 samples, which included the samples from day 0 and day 56. In addition, all samples from columns with 6 g oil were analysed to study depletion over time. Table B 3 (Attachment B) summarizes the concentrations of SVOC in the sediment samples, and more detailed results are provided in Table B 4 to Table B 8.

Eq. (1) given in Section 2.5 was used to determine percent depletion of individual components and component groups in the sediment samples by comparing the exposed samples with sediment samples

from day 0 premixed with 2, 6 and 18 g oil. Percent depletion for the component groups are summarized in Table B 9 and shown in Figure 3.3. As also seen in the GC/FID-analysis, the SVOC concentrations were higher in some of the exposed samples than in the reference samples from day 0 (no bars shown for these component groups).

Figure 3.3 shows that the depletion in SVOC in columns with 2 g oil with fertilizer, seems to be independent of the amount of fertilizer, and that the depletion is less in the column with no fertilizer. The results indicated a reduction in the concentration of naphthalenes of 95%, 2-3 ring PAH of approximately 50% and close to 25% for 4-6 ring PAH. In the columns with the highest oil concentration (18 g oil/kg sediment), the depletion increased with increased amount of fertilizer. No depletion of 2-6 ring PAHs was observed. The reduction in the concentrations of decalins and naphthalenes was 20 and 35%, respectively. Thus, the amount of fertilizer seems to have least impact in the columns with the lowest oil concentration (2 and 6 g oil/kg sediment).

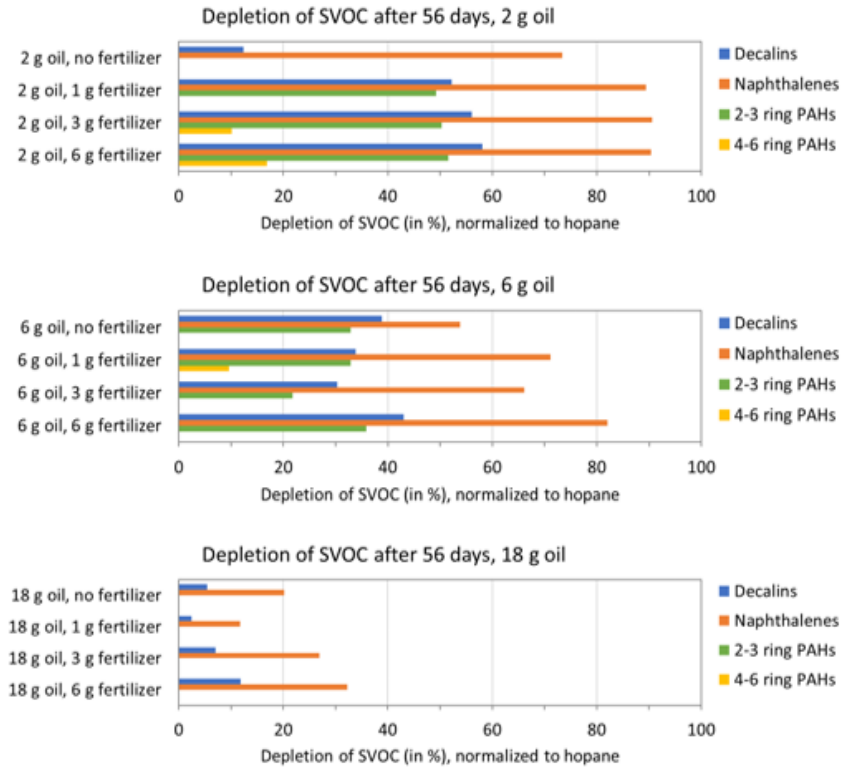

Figure 3.3 *Depletion in SVOC (normalized to hopane) from day 0 to day 56 with regard to oil concentration and amount of fertilizer. The component groups are described in Table D 1.*

In Figure 3.4 the depletion on day 0 was compared with day 16, 28 and 56 in the columns with 6 g oil/kg sediment. The results are presented as percent reduction in concentration and are not normalized to hopane. Normalizing the data to hopane show the same trend, but a more limited numbers of data were shown as the concentration in several of the columns were increasing instead of depleting. This may indicate that hopane is not as robust for biodegradation as assumed in this type of experiments where

nutrients are added. The results show that there was a decrease in concentration of decalins, naphthalene and 2-3 ring PAH also in the columns without fertilizer, and that the amount of fertilizer seem to be less important for the reduction in concentration. The reduction increases with time in all columns, and no significant difference was observed between columns with premixed fertilizer and columns with fertilizer applied on the surface sediment.

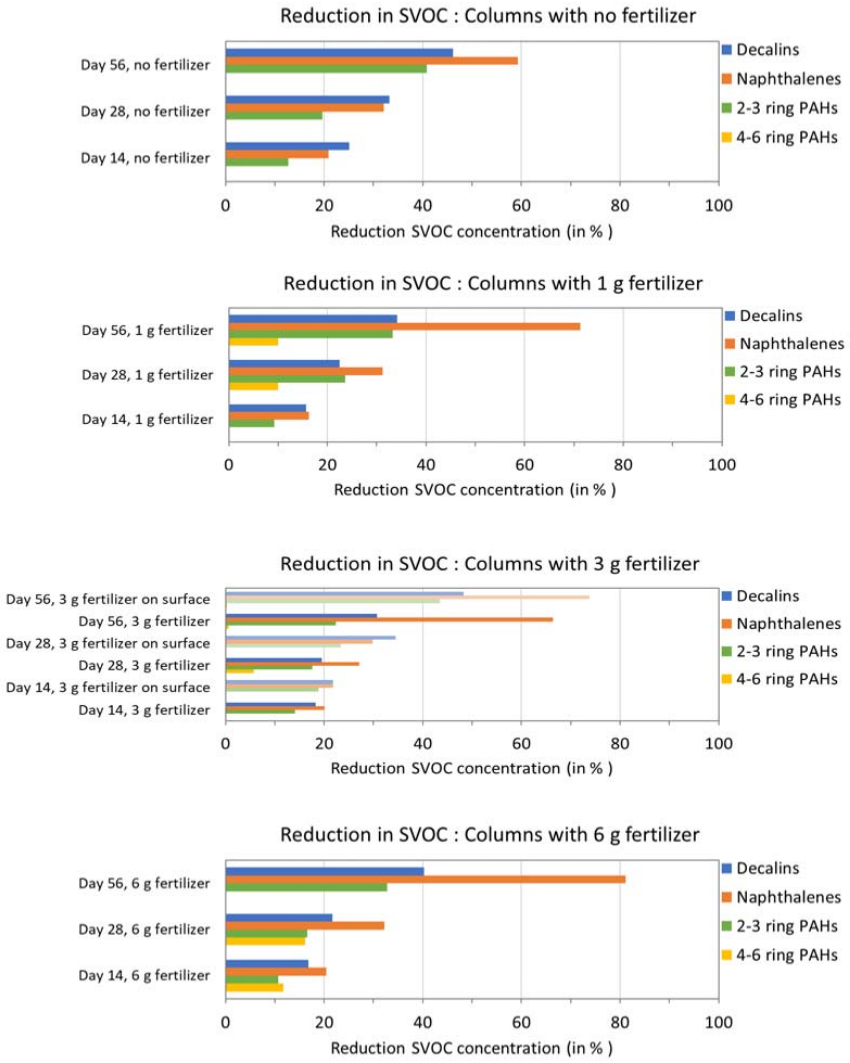

Figure 3.4 *Columns with 6 g oil/kg sediment sampled on day 14, 28 and 56. Depletion in SVOC concentration from day 0 with regard to sampling time and amount of fertilizer. The component groups are described in Table D 1.*

3.3 Toxicity testing

Selected sediment samples tested for toxic effects are given in Table A 1. Early stage (nauplii, N2) of *Calanus finmarchicus* were exposed to small amounts of sediment for 72 hours using hatching rate and survival as endpoints.

The average hatching rate varied from 93.2 (± SD = 2.7) in the control group (clean sediment) to 87.1 (± SD = 3.9) in all exposed groups, except for the sediment with 18 g oil on day 0 (64.3 ± SD = 7.3). The hatching rate was significantly lower than the controls in all exposed groups ($p < 0.01$) except for the groups 6-0 and 6S-0 on day 28 and 6-0, 6-1 and 6-3 on day 56.

Survival in copepods exposed to the selected sediments for 72 hours are given in Figure 3.5.There was a statistically significant increase in survival from day 14 to day 56 for all tested groups (Ordinary one-way ANOVA, Turkey's multiple comparison test). However, the sediment of the two groups with the highest concentration of oil (18-3, 18 g oil and 3 g fertilizer) and the fertilizer (6-6, 6 g oil and 6 g fertilizer) were significantly more toxic at day 56 than the other groups. This indicate that the concentration of both oil and fertilizer impact the detoxification process. The only sediment with 6 g oil/kg sediment having the same survival rate as the control groups on day 56 (and significantly higher than the other treated sediments) was the unfertilized sediment.

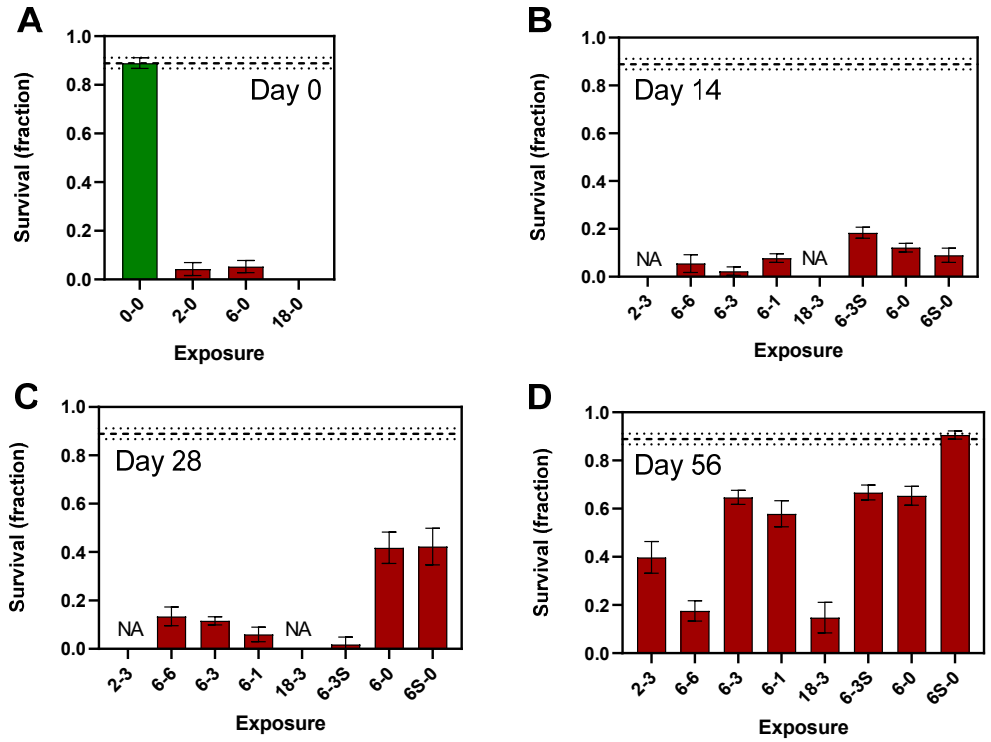

Figure 3.5 *Survival of* C. finmarchicus *early stages exposed to sediment with different combinations of oil and fertilizer at different times during the experiment. X-axis labels show the amount of oil and nutrient mixed into the sediment (oil (g/kg sediment) – nutrient (g/kg sediment)) or applied to the sediment surface (marked with S). Control group (0 – 0) values based on 24 replicates and exposed groups based on 6 replicates. Horizontal broken line indicates control average with ± SD indicated by dotted lines. Vertical bars indicate ± SD. NA indicate no data available (not tested).*

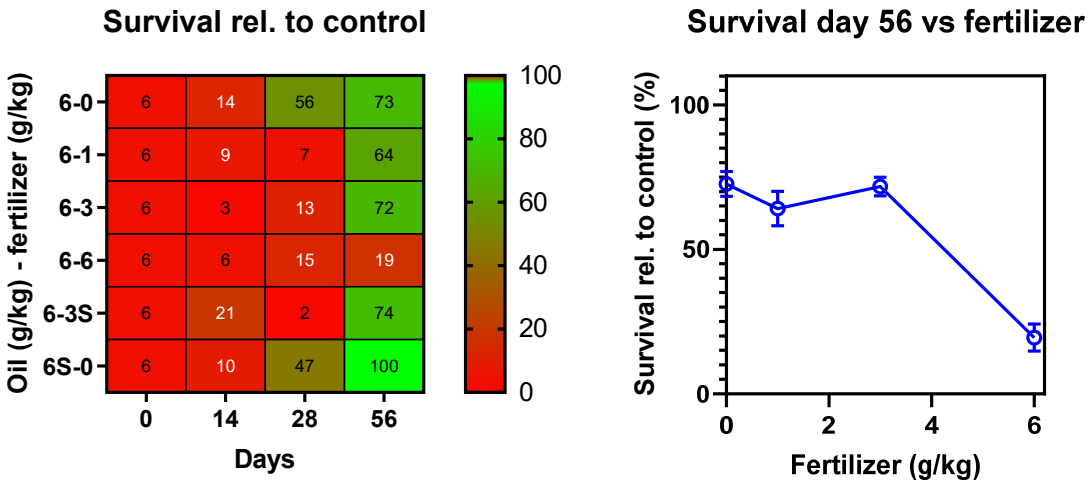

Figure 3.6 *Survival of* Calanus finmarchicus *early stages exposed to sediment with 6 g oil/kg sediment and varying concentrations of fertilizer. Left figure: Heat plot showing the percentage of surviving copepods exposed to sediments degraded for different time periods relative to the control groups (clean sediment). Labels on the y-axis indicate concentrations of oil (g/kg) and nutrient (g/kg). Values marked with "S" (e.g. 6S-3) indicate that the addition was made on the sediment surface. In all other instances the oil and/or fertilizer were mixed into the sediment. Values at day 0 are from sediment before the fertilizer was added. Right figure: Relation between mortality and the amount of nutrient mixed into sediment with 6 g oil/kg sediment on day 56. 6 replicate tests for each sediment and SD indicated by vertical bars.*

From previous experiment using 10 g fertilizer/kg sediment, it was concluded that toxic effects mediated by the fertilizer had a large impact on the observed mortality (Nordtug et al., 2019). It was further suggested that this result was biased by the experimental design where the water is supplied and drained through the sediment column without any surface flushing of the sediment causing accumulation of dissolved nutrients in the upper part of the moving water column. In the current experiment, controlled flushing of the sediment surface was therefore introduced, and the amounts of added fertilizer were reduced. In spite of this, there is still a significantly decreased survival in the groups with the highest concentrations of fertilizer (Figure 3.6).

The current results therefore contradict the previous assumption and suggests that the negative impact of the fertilizer on the copepods is due to interactions with the oil in the sediment independent of surface water exchange.

3.4 DNA extractions and community analyses

3.4.1 DNA quantifications

DNA was extracted from the sediment and quantified using Qbit analyses. The results are summarized in Figure 3.7 and show that the DNA concentrations reflects an increase of the microbial biomass during the degradation processes. The results are tabulated in Appendix E (Table E1) Stimulation rate of microbial biomass increased from 2 g oil/kg sediment to 6 g oil/kg, but a reduction in stimulation rate was observed in samples with 18 g oil/kg. This could be caused by a delayed stimulation due to toxic effect. A distinct stimulation of microbial biomass was measured in all columns added fertilizer, and it increased with increasing concentration of fertilizer. No effect of applying the fertilizer on the surface vs premix with the oiled sediment was detected.

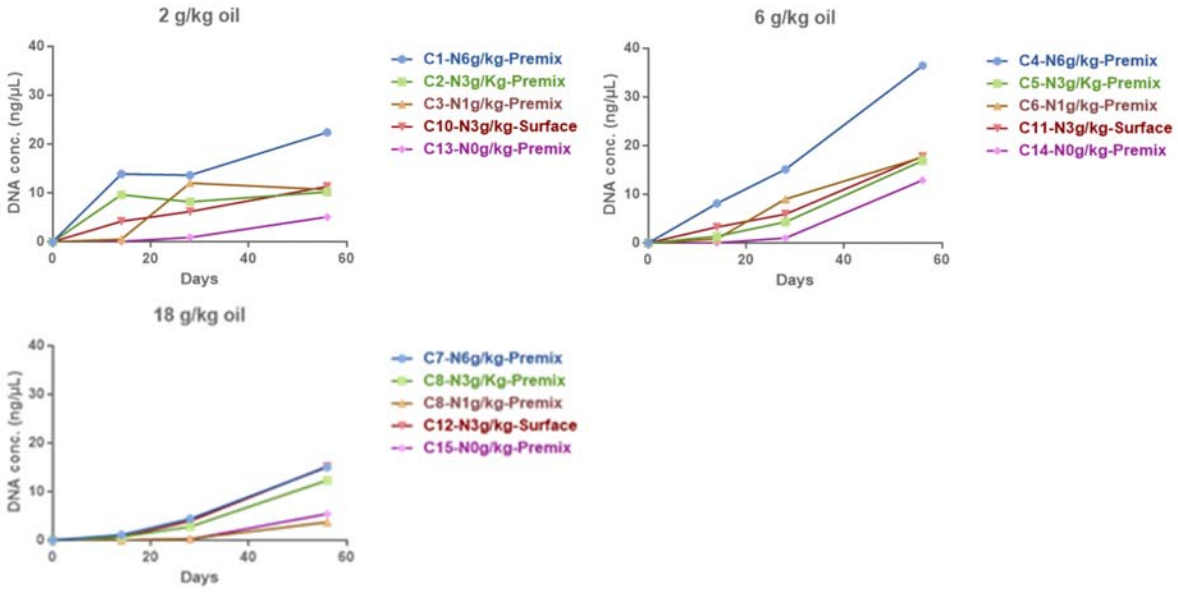

Figure 3.7 DNA concentrations (in ng/µL) in sediment samples over time.

Comparison of the DNA concentration to the results of the chemical analyses (Figure 3.1) and toxicity testing (Figure 3.5) show agreement in relation to oil concentration since the highest oil concentration (18 g oil/kg sediment) showed both poor degradation when determined as % reduction in concentration and persistence of toxicity. However, as DNA concentrations increased between day 28 and 56, oil became depleted and toxicity decreased (Figure 3.1 and Figure 3.5). The relations between DNA concentrations and fertilizer concentrations agreed well with the nC_{17}/Pristane and nC_{18}/phytane results, particularly in the columns with 6 g oil/kg sediment (Figure 3.2), where both DNA concentrations and n-alkane degradation increased with fertilizer concentrations. In sediment columns with 2 g oil/kg sediment, fast n-alkane degradation was also related to fertilizer concentrations, in agreement with the DNA concentrations, although degradation of pristane and phytane between 28 and 56 days seemed to occur (Figure 3.2).

3.4.2 Characterisation of microbial communities

Microbial communities were characterized by 16S rDNA microbiome analyses. A number of 51 samples were analysed, representing a total of 696 taxa (Attachment F, Table F1). A total of 52 samples were originally DNA-extracted. Of these contained 7 samples very low DNA concentration (see Table E1) and it was decided to use a whole genome amplification (WGA) method to increase the DNA concentration, as shown in Table E1. However, the 16S rDNA sequencing of the WGA extracts contained only a few taxa compared to the original low-DNA extracts (not shown), so data from the original DNA extracts were used instead. Only one of the 52 samples could not be successfully amplified (Ky10; Figure F1), representing sediment column 6 (see Table 2.1), with a sample of 6 g oil/kg sediment with 1 g fertilizer/kg sediment collected after 15 days of incubation.

The *alpha-diversities* were determined in all treatments, describing the microbial diversity within each sample. Environmental exposure to oil pollution will usually results in reduced microbial diversity, since a few taxa will become predominant. Many of these will be so-called *hydrocarbonoclastic* bacteria, specialized to degrade hydrocarbons. In addition, the oil may be toxic to other taxa, thereby reducing the diversity further. Comparison of reference samples (from day 0) and samples from the columns (day 14 to 56), showed that the diversities in the column samples (irrespective of treatment and time of sampling) did not differ significantly from the diversities in reference samples (P=0.47) when only numbers of species were taken into account, as shown by Chao1 diversity (Figure F2). However, when evenness was accounted for, the treatments differed significantly (P<0.03), as shown (Figure 3.8). Evenness describes the phylogenetic similarities between the taxa in a sample, and these results demonstrated that the taxa in the oil-containing samples were more phylogenetically similar than the taxa in the reference samples.

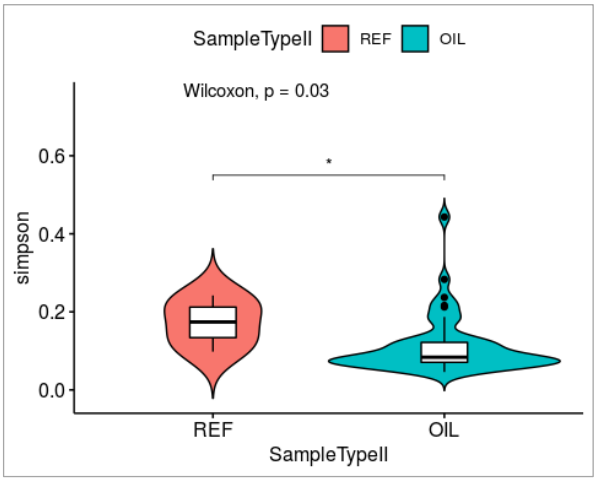

Figure 3.8 *Alpha-diversity between reference (REF samples from day 0 (in red) and oil amended samples (OIL in green) shown as Simpson Diversity Index (y-axis). The diversities are based on numbers of species and evenness (phylogenetic similarities between taxa in the sample).*

However, when the different oil treatments were compared (different oil concentrations), or nutrient treatments (different fertilizer concentrations, or oil without fertilizer), the differences in diversities were significantly different, neither by Chao1 or by Simpson methods (Figure F3 and F4). The incubation time in

the columns therefore seemed to be the driver for changes in the microbial diversities, rather than the fertilizer treatment, or different oil concentrations.

The *beta diversity* a measure of diversity differences between samples, and the results are shown as two-dimensional Principal Component Analyses (PCoA) plots. Samples with similar community compositions will cluster together, while differences will result in distances between samples on the plots. Figure 3.9, it is separated between community differences with respect to sampling day (A), oil concentration (B) and nutrient concentration (C). In all figures, it is shown that communities in reference samples (day 0) located distantly from the communities in the column samples (from day 14 to 56) on the PCoA plots. There is an obvious development in the community successions with time in the column samples, as shown by the "movement" from left to right of samples in Figure 3.9A. These differences were further substantiated by analyses of significance, showing that differences between the samples were mainly significant ($P<0.05$; Table F2) The microbial community dynamics is therefore influenced by incubation time in the columns. Comparison of oil concentrations did not result in specific community differences (Figure 3.9B), with the exception of a sample with oil applied on the sediment surface, and without any fertilizer (yellow circles). For the different nutrient concentration, no distinct patterns or differences between the samples were observed (Figure 3.9C).

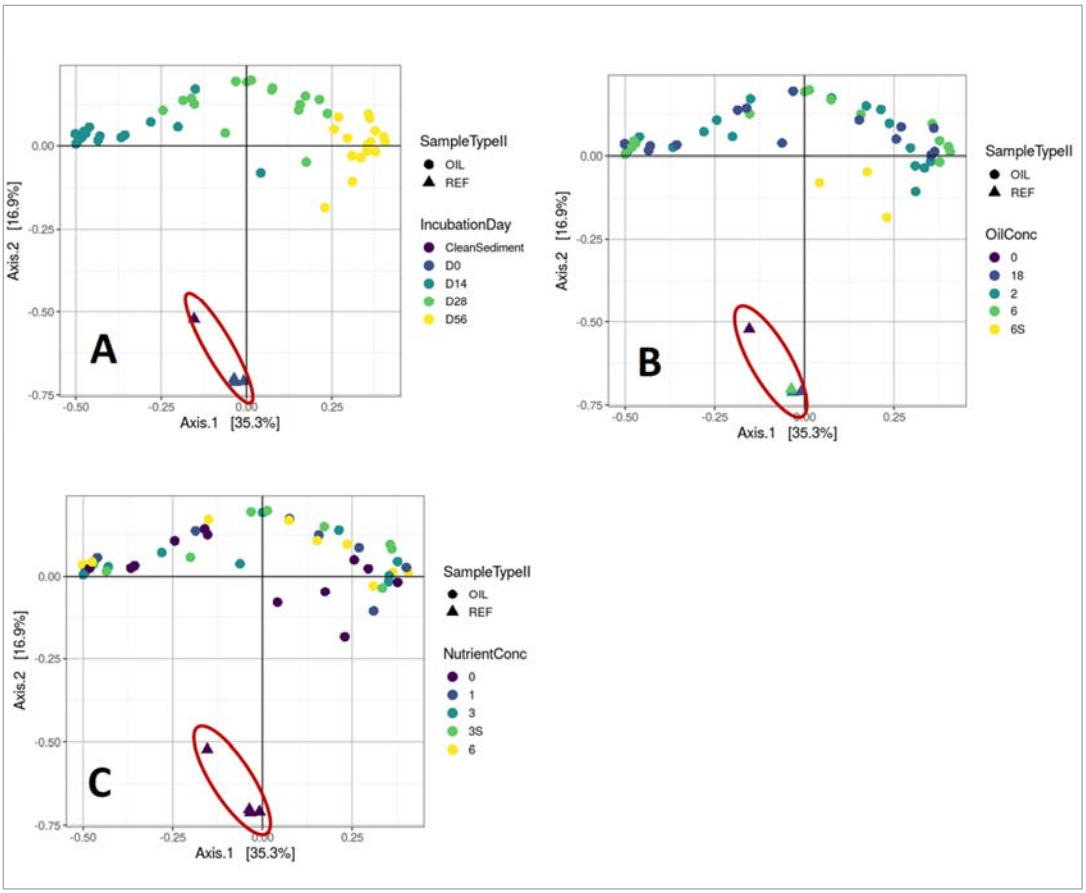

Figure 3.9 *Beta-diversity of complete dataset coloured as incubation days (A), oil concentration (B), and nutrient concentration (C). The red circles show the clustering of the communities from reference samples (day 0).*

Closer examination of beta diversities with different oil or fertilizer concentrations for different sampling days showed some differences between oil treatments, mainly between the communities where the oil was mixed into the sediment or applied on the sediment surface (Figure 3.10 and Table F3).

Different fertilizer concentrations did not seem to influence community dynamics at any of selected sampling days (Figure 3.11 and Table F3). However, there was an increasing difference between oil not treated and oil treated with fertilizer, increasing from P=0.49 when non-treated oil was compared to a treatment of 1 g/kg fertilizer, to P=0.06 and P=0.05 when untreated oil was compared to 3 g/kg and 6 g fertilizer/kg sediment, respectively (Table F3). This emphasized an effect on the microbial communities by the application of fertilizer, although this effect was not statistically significant.

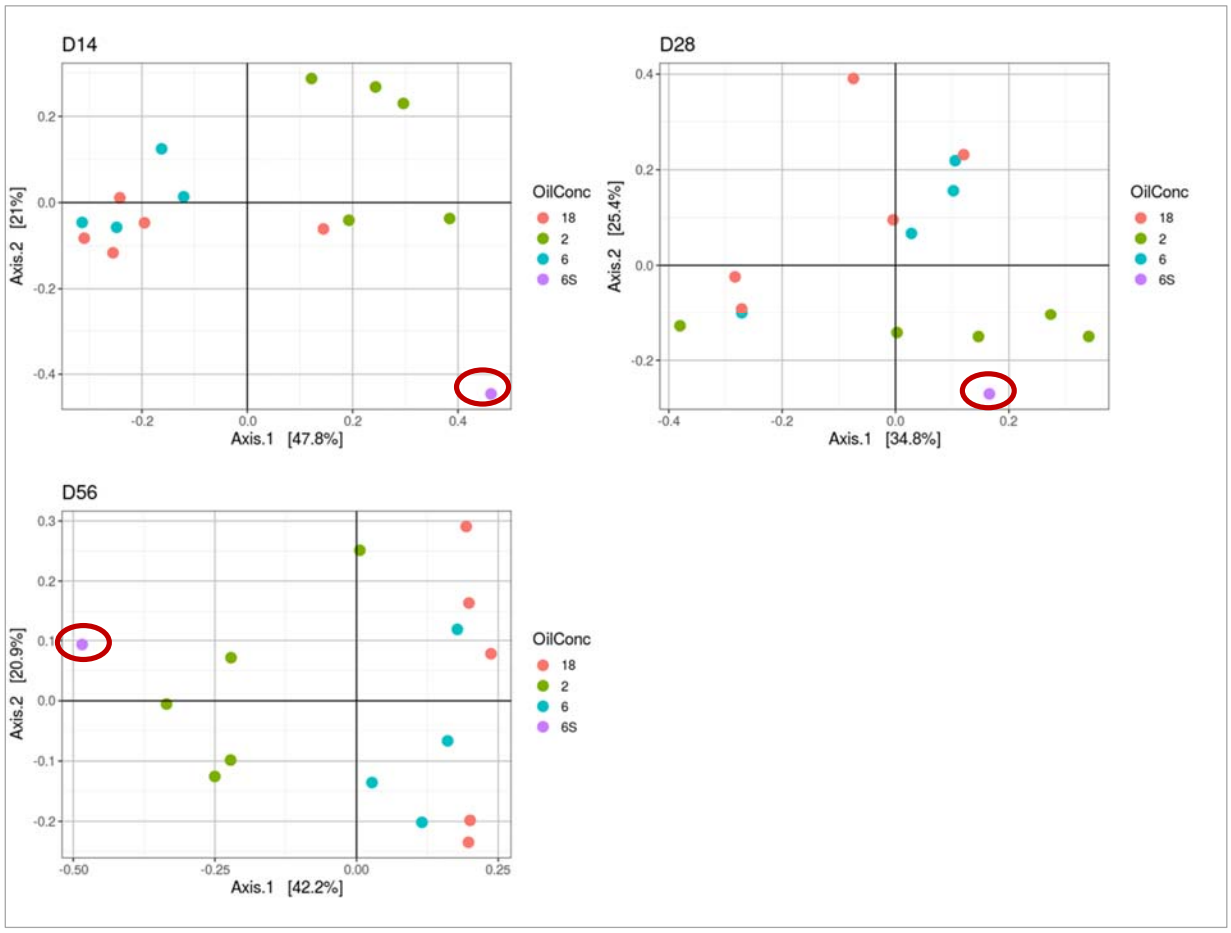

Figure 3.10 *Beta-diversity of total dataset broken down to distinct incubation time and indicated after oil concentrations. The red circle shows the sediment samples with oil applied on the surface (no fertilizer applied).*

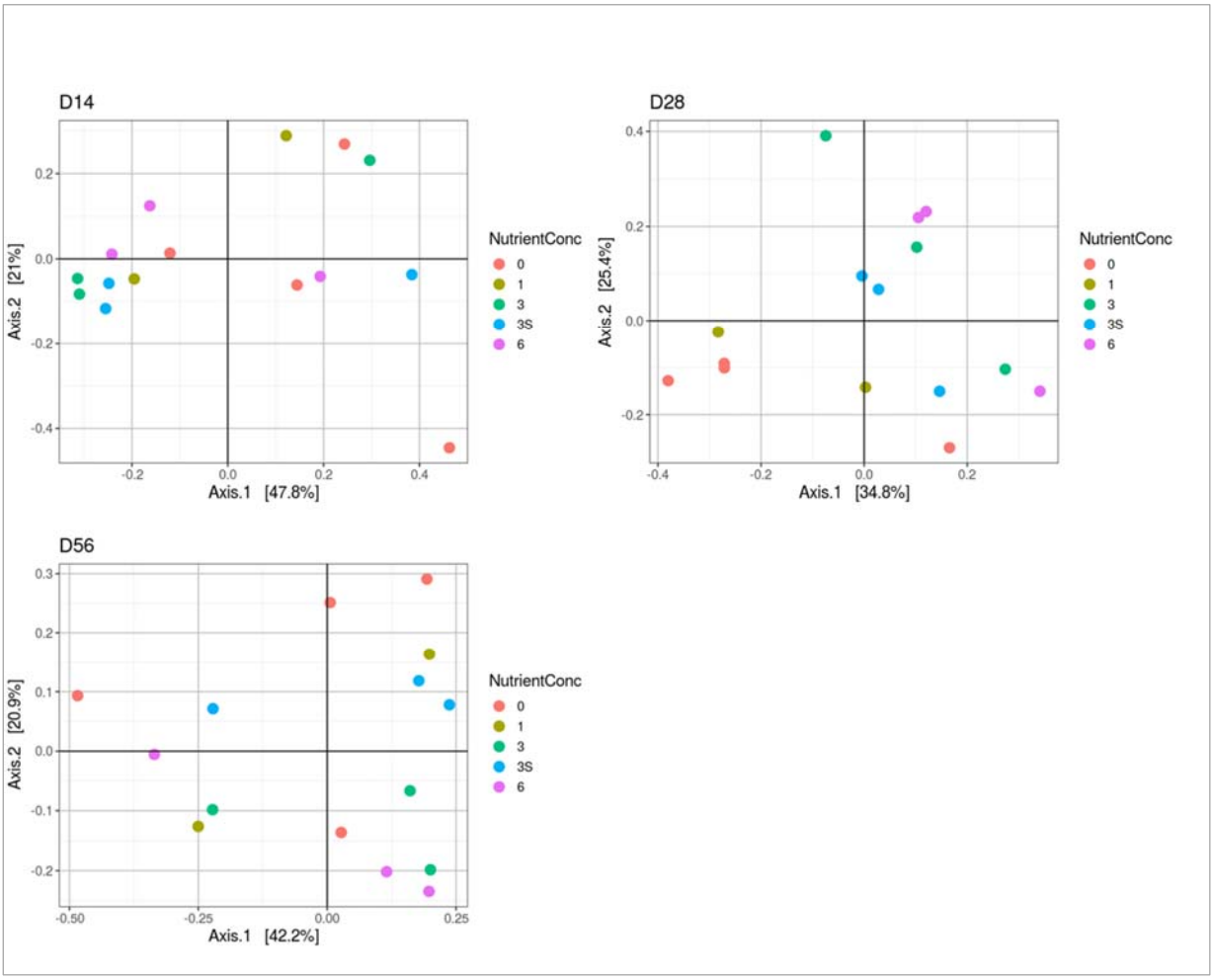

Figure 3.11 *Beta-diversity of total dataset broken down to distinct incubation time and indicated after nutrient concentrations.*

The most abundant taxa, with a cut-off level of 5% (representing 5% or more of the total community) are shown in Figure 3.12 and in Table F4. At the start of the bioremediation study, several bacterial taxa associated with oil biodegradation were abundant in the sediments, including the genera *Marinobacter*, *Alcanivorax* and *Oleispira* (Head et al.2006; Yakimov et al. 2007). However, after 14 days of incubation, the sediments became predominated by the obligate hydrocarbonoclastic genera *Oleibacter* and *Oleispira*. These bacteria can only utilize hydrocarbons as carbon source and are associated with degradation of alkanes. Usually, *Oleibacter* prefers higher seawater temperatures than *Oleispira* (Lofthus et al, 2018), and species of *Oleispira* are often associated with oil biodegradation in Arctic or Antarctic marine environments (Yakimov et al., 2003). During biodegradation of oil in seawater from Svalbard, we observed *Oleispira* as a key-player in the degradation of n-alkanes (Ribicic et al., 2018). Only one sample (KyV20 – 6 g oil/kg sediment applied on sediment surface; no fertilizer applied) differed from the other samples, with a predominance of *Alcanivorax*, which also is considered to be an hydrocarbonoclastic alkane-degrader.

As shown from the degradation curves of n-alkane (C_{17}/Pristane; Figure 3.2), the n-alkane degradation varied after 14 days, mainly in relation to oil concentration. However, the abundances of alkane-degrading bacteria showed the potentials for degradation at all oil concentrations, even in sediments with rather low n-alkane degradation. This may reflect reduced oil bioavailability, limitation of oxygen, or a concentration-independent degradation (zero-order degradation rates) at the highest oil concentrations (assuming the fertilizer treatments resulted in adequate nutrient capacities in the sediments). Oil dilution and increased bioavailability, in combination with avoiding oxygen limitations, may therefore be essential.

These data also demonstrate that *biostimulation* (stimulation of the indigenous oil-degrading microbes) may be a better strategy than *bioaugmentation* (adding exogenous oil-degrading bacteria), since high numbers of indigenous oil-degrading bacteria may be stimulated by sediment fertilization. Later in the incubations, these alkane-degraders became less predominant. However, after 28 days alkane-degraders were still the predominant taxa in sediments without fertilizer (KyV33, KyV34, KyV35) and with high oil concentration (18 g/kg sediment) and low fertilizer concentration (1 g/kg; KyV29)(Figure 3.12 and Table E1). This corresponds well with the delayed alkane degradation in these sediments compared to the sediments treated with fertilizer (Figure 3.2). In the other sediments, taxa of *Sphingomonadaceae*, *Colwellia* and *Alcanivorax* became more abundant.

After 56 days of incubation, members of *Porticoccaceae* and *Cycloclasticus* increased in abundances, although alkane-degraders were still abundant in the sediments with high oil concentrations (18 g oil/kg), and in sediments with no or low (1 g/kg) fertilizer (KyV45 and KyV51) (Figure 3.12 and Table E1). The abundances of the alkane-degraders in these sediments corresponded with slow alkane degradation in these samples (Figure 3.2). Species of *Porticoccaceae* have been identified as obligate hydrocarbonoclastic bacteria (Gutierrez et al., 2015) and has been associated with secondary alkane degradation (Ribicic et al., 2018). *Cycloclasticus* represents the typical aromatic degraders and were typically most abundant in sediments with fertilizer applied on the sediment surface after 56 days of incubation (KyV46-KyV48), increasing in abundance by increased oil concentrations from 9 to 20% abundance from 2 to 18 g oil/kg sediment.

In sediments not treated by fertilizer (KyV49-KyV51), the abundance of *Cycloclasticus* was particularly high in the highest oil concentration of 18 g/kg (KyV51) (Figure 3.12 and Table E1). This is reasonable. Since high concentrations of soluble naphthalenes and PAHs were released from this oil (Table B3). The reductions of naphthalenes and smaller PAHs were also relatively high in the sediments with surface-applied fertilizer (Figure 3.4). However, for the oils treated with fertilizer mixed in the sediment, a correlation between abundance of *Cycloclasticus* and oil concentration was not observed.

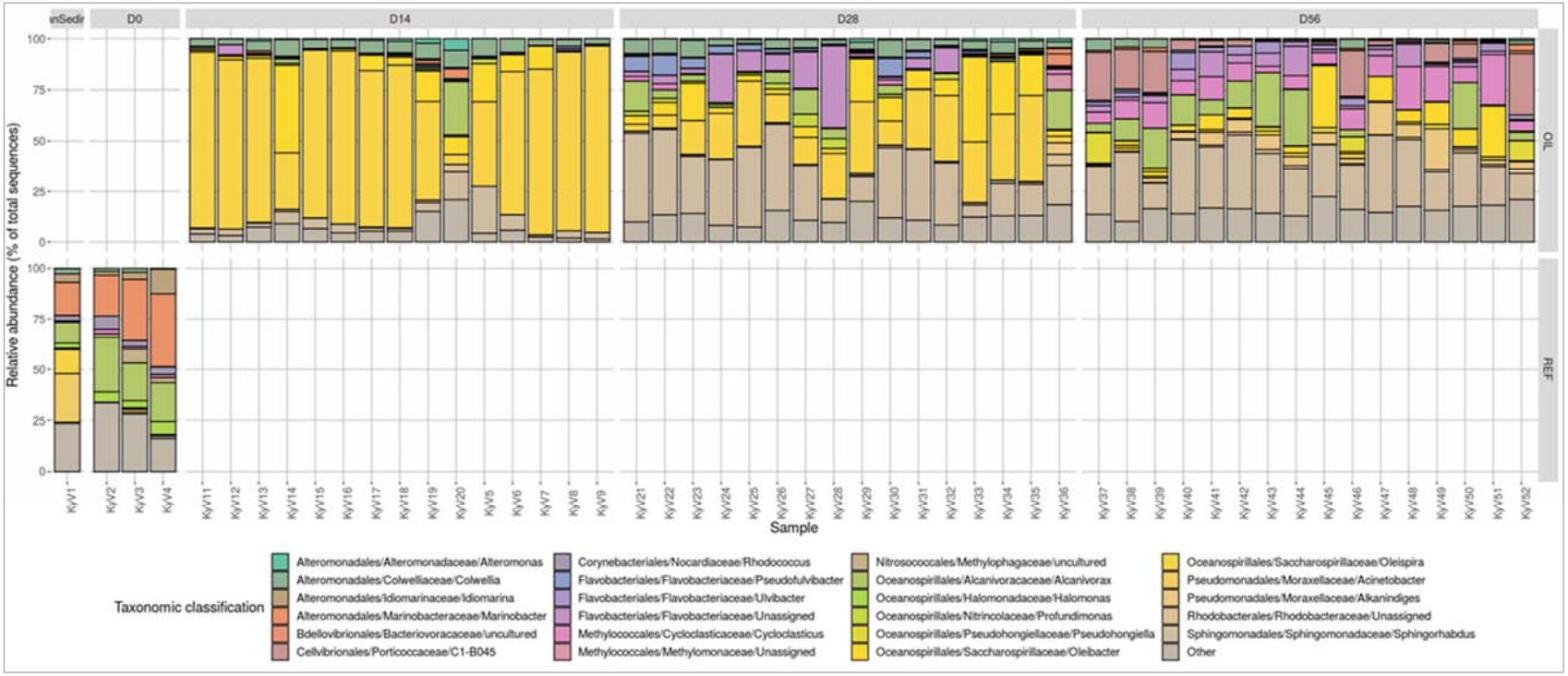

Figure 3.12 *Microbial community composition at 5% cut-off shown for sediment samples collected on day 14 to 56 (upper panel) and reference samples from day 0 (lower panel). The explanation of the sample identities is shown in Table E1.*

3.5 Nutrient analysis

Water samples for the nutrient analyses were collected at the surface of the sediment at rising water levels before flushing the surface water volume. The measured concentrations are thus assumed to represent the concentrations in the pore water of the upper part of the sediment. All the analysed nutrients declined during the experiment (Figure 3.8A). Ammonia and phosphate remained considerably higher than in the unfertilised groups throughout the whole experiment (56 days). Nitrate + nitrite, however, approached the background values towards the end of the experiment. Figure 3.8 (B – C) show the average concentrations from groups added 1, 3 and 6 g fertilizer, respectively from days 1, 14 and 56. There was no apparent impact of oil concentration on the pattern of decline of the nutrients. Compared to the previous experiment with 10 g fertilizer/kg sediment (Nordtug et al. 2019), the decline in water nutrient concentrations with time is not very different. Despite the surface flushing with clean water during each high tide the measured water concentrations for all nutrients in the 6 g/kg fertilized groups were about 50 % of those in the previous experiment.

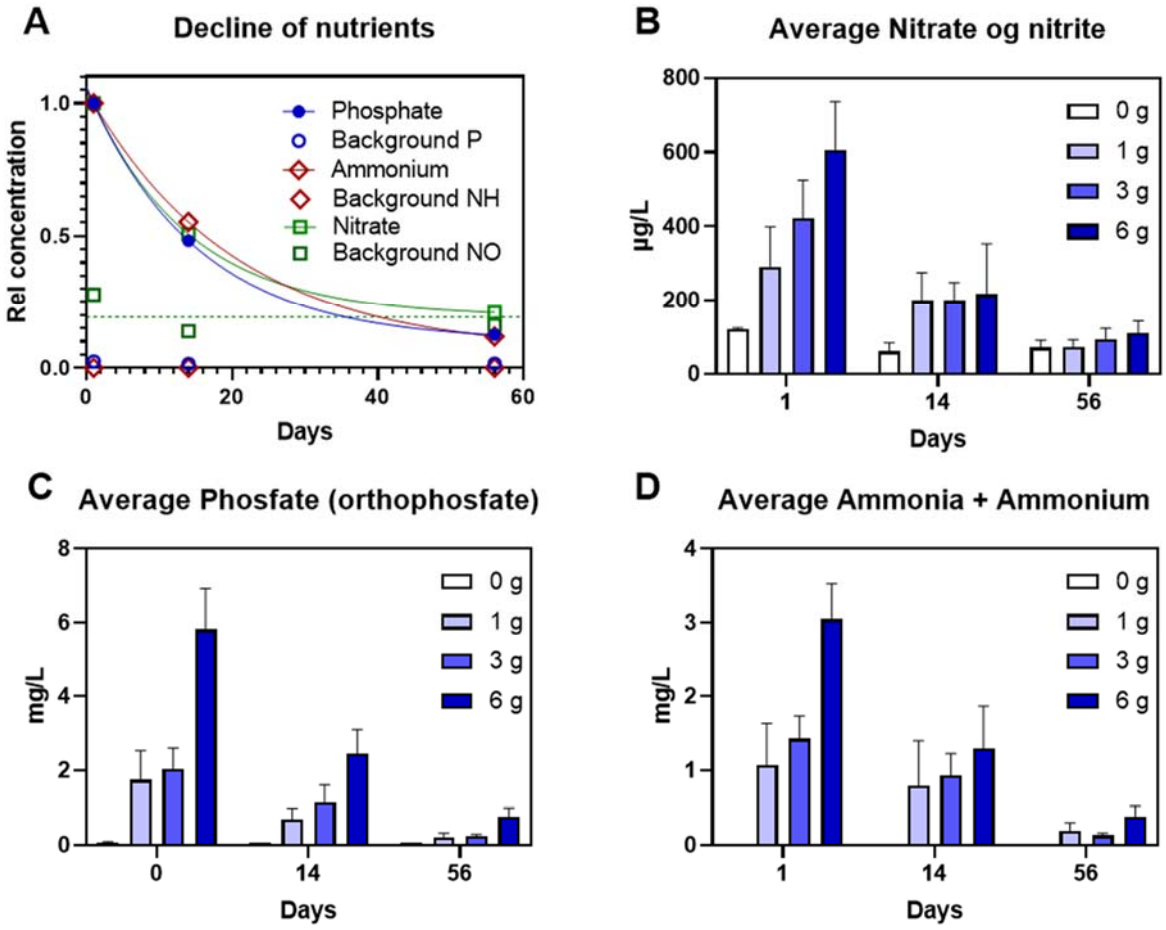

Figure 3.8. *Concentrations of nutrients in pore water sampled above the sediment during flooding. A; Decline in nutrient concentrations over time (average of all samples) B – C: Average water concentration of nutrients in columns added equal amounts of fertilizer (SD indicated by vertical bars).*

3.6 Toxicity of "slow-releasing fertilizer"

Concentrations of nutrients in the exposure solutions and the highest values recorded in the sediment column experiments are shown in Table 3.1. The concentrations in the current WAF from the fertilizer, except for the phosphate, was much higher than those in the surface water in the column experiment (presumably pore water) collected from the columns with 6g fertilizer/kg sediment. The results are discussed in more detail in Attachment G.

Table 3.1 Analyses of exposure solutions

	Fertilizer (mg/L)	NH_4Cl (mg/L)	Seawater (mg/L)	Columns 6g fertilizer day 1
Ammonium + Ammonia as NH_4^+	140 (± 21)	26 (± 3,9)	0,013 (± 0,010)	3,05 (± 0,96)
Phosphate (orthophosphate)	2,55 (± 0,38)		0,067 (± 0,030)	5.83 (± 2,21)
Nitrate and nitrite	100 (± 15)		0,120 (± 0,018)	0,605 (± 0,264)

The toxicity of the ammonium chloride was similar to that of the fertilizer WAF when related to ammonia + ammonium (Table 3.2 and Figure 3.9). The equilibrium distribution between NH_4^+ and NH_3 is determined by temperature, salinity, and pH. Based on measured parameters the fraction of NH_3 relative to NH_4^+ estimated in the exposure solutions were in the range 1 to 2.2%. The toxicities (LC_{10} and LC_{50}) related to ammonium and ammonia are shown in Table 3.2.

The toxicity (LC_{50}) related to both NH_4^+ and estimated NH_3 was found to be very similar in the two tested solutions (Figure 3.9) indicating that the ammonia/ammonium complex is the main driver of toxicity observed in the water soluble fraction of the "slow release fertilizer".

Table 3.2 LC_{50} related to ammonia (NH_4^+ and NH_3) in the exposure solutions

	72 hours LC_{10} (mg/L)		72 hours LC_{50} (mg/L)	
	NH_4^+	NH_3	NH_4^+	NH_3
Fertilizer	3,16 (2,52- 3,97)	0,074 (0,059 - 0,093)	9,47 (8,52 - 10,1)	0,210 (0,189 - 0,233)
Ammonium chloride	1,75 (1,21 – 2,53)	0,038 (0,026 - 0,056)	6,74 (5,71 - 7,96)	0,151 (0,179 - 0,128)

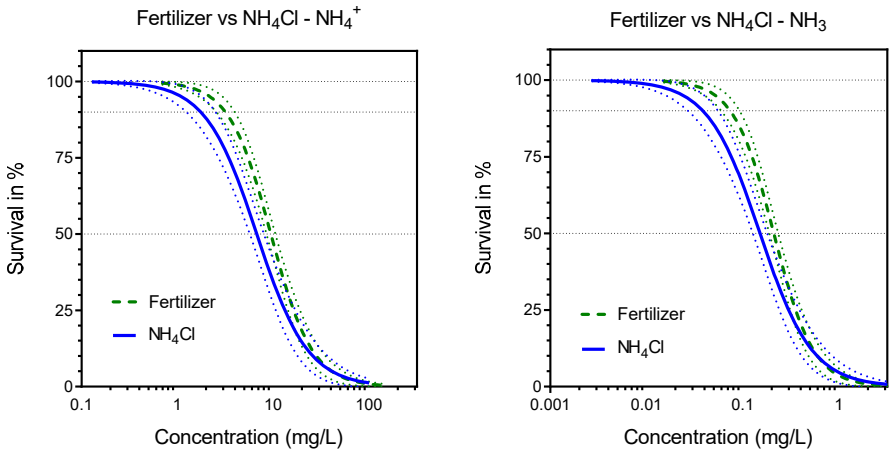

Figure 3.9 Comparison of the toxicity of fertilizer WAF and ammonium chloride related to ammonium (NH_4^+) and ammonia (NH_3). Dotted lines indicate the 75% confidence interval.

The results confirm that that the fertilizer at the highest concentration used in the degradation test (6g/kg sediment) may contribute to the observed toxicity, and that this toxicity most likely related to the concentration of ammonium/ammonia.

Early stages of *C. finmarchicus* have previously been shown to be rather sensitive to contaminants, and thus expected to be more sensitive than "the average" sediment organisms. The concentrations of ammonium/ammonia in samples from the previously conducted degradation experiments are all below the LC_{50}-values recorded in the toxicity test. The concentrations in the pore water of the columns added 6 g fertilizer/kg sediment are in a range that may contribute to mortality in early stages of *C. finmarchicus*. We thus recommend that the amount of the current fertilizer (Plantagen slow release) used on shoreline sediment should be limited to 3 g/kg sediment.

4 Summary and conclusions

The potential of using fertilizers to enhance microbial degradation of oil in shoreline sediments was studied in a sediment column system. Pre-weathered ULSFO (Ultra Low Sulphur Fuel Oil, 250 °C+) and a slow-release fertilizer were applied in different combinations to investigate the effect on oil biodegradation at a temperature of 5°C. Initial amounts of oil were 2, 6 or 18 g /kg sediment and the amounts for fertilizer were 0, 1, 3 or 6 g/kg sediment.

The experiments demonstrated that the biodegradation of oil in sediments at the low temperature can be enhanced by adding a slow-release fertilizer (NKP 16:6:12). In the columns with 18 g oil/kg sediment, the depletion increased with increased fertilizer concentration, but the added amount of fertilizer seems to be less important for the reduction in oil concentration in columns added 2 and 6 g oil/kg sediment.

The ratios between rapidly biodegradable n-alkanes and the less degradable isoprenoids (C_{17}/pristane and C_{18}/phytane) were calculated to get an indication of n-alkane biodegradation over time, and a decrease in the ratios was observed in most of the columns. However, in the columns added 2 g oil/kg, an increase in the ratios were observed from day 14 to day 56 indicating that the bacteria had started to degrade also pristane and phytane. Overall, the degradation rate was faster up to 28 days than from 28 to 56 days, especially in columns with 2 and 6 g oil and 3 and 6 g fertilizer. The biodegradation continued between 28 and 56 days, also in the columns without fertilizer, resulting in less difference in the ratios between fertilized and non-fertilized oil when the experiments were terminated on day 56 than those observed on day 28.

Nutrient analyses were made on water sampled above the sediment at rising tide, and these nutrient concentrations were assumed to represent pore water in the upper part of the sediment. Ammonia and phosphate remained considerably higher than in the unfertilized columns throughout the whole experiment (56 days). Nitrate and nitrite approached background values towards the end of the experiment. However, the bacterial activity did not indicate that a nutrient limitation appeared during the experiments.

The results suggested that the depletion in PAH concentration in columns with 2 g oil/kg sediment was independent of the amount of fertilizer, but faster than in columns with no fertilizer. The results showed a 95% reduction in the concentration of naphthalenes, approximately 50% reduction for 2-3 ring PAH, and close to 25% reduction for 4-6 ring PAHs. In the columns with the highest oil concentration (18 g oil/kg sediment), the depletion increased with increased amount of fertilizer added.

Hatching rate and survival were applied as endpoints in toxicity testing of sediment samples with early stages (egg - N2) of *Calanus finmarchicus*. The hatching rate was significantly lower in the columns with oil compared with in the clean sediment. There was a significantly reduced survival in the groups with the highest concentration of fertilizer which may indicate that the fertilizer either displays toxicity by itself or interacts with the oil to make it more toxic to the copepods.

The concentrations of DNA extracted from the sediment showed a distinct increase in columns added fertilizer and was further increased by increased fertilizer concentration. DNA used as a measure of microbial biomass therefore demonstrated that fertilizer stimulated the biomass in a concentration dependent manner. However, a reduction in stimulation rate was observed in samples with 18 g oil/kg sediment, probably caused by temporary toxic effects of the oil on the microbial biomass.

Microbial community characterization was performed as 16S rDNA microbiome analyses. Comparison of the community compositions between the various samples showed that the compositions in the reference samples (sediment samples with 2, 6 or 18 g oil/kg sediment from day 0 (not fertilized)) differed significantly from the compositions in the samples incubated with oil for 14 to 56 days, and that there also partly significant changes in the communities by time, although greater resemblance was shown during the incubation period than the reference samples at day 0. Different oil concentrations did not seem to influence on community compositions significantly, with one exception (a sediment where oil was applied on the sediment surface, without any fertilizer added). Comparison of communities in sediments with and without fertilizer showed higher differences than communities in sediments with different fertilizer concentrations. The results therefore showed that incubation with oil and the introduction of fertilizers had greater impacts on the microbial community composition than oil and fertilizer concentrations. Identification of bacterial taxa showed high abundances of well-known oil-degrading bacteria, of which several may be typical 'hydrocarbonoclastic' (i.e. digesting hydrocarbons as their only organic food source). The successions in the oil-contaminated sediments showed early predominance of alkane-degrading bacteria, some of these associated with cold marine environment, follow by secondary alkane and aromatic degraders. The compositions of the communities and the relative abundances showed correlations to the hydrocarbon degradation patterns, particularly for the degradation of alkanes. The depletion of 2- to 3-ring PAHs, related to acute toxicity of oil, were also linked to the appearances of bacteria associated with degradation of these compounds. These results show that indigenous microbes have a potential for removing these compounds from oil-polluted sediments at low temperature. It was also interesting to note that some of the oil-degrading bacteria are associated with cold environments and have been found in abundances in oil-contaminated seawater from Svalbard. These data substantiate the potentials for combining chemical and microbial analyses for describing the effectiveness of bioremediation actions. The data also demonstrate the potential capacities of natural sediment for harbouring indigenous oil-degrading bacteria, which can be stimulated during bioremediation actions.

As concluded in Nordtug et al. (2019), the slow-releasing fertilizer used may have contributed to toxic effect on the tested organisms at the highest fertilizer concentrations tested. Therefore, a toxicity test with the pure fertilizer was performed. A WAF was made from 6 g fertilizer/L seawater and tested for toxicity on early stages of the copepod *Calanus finmarchicus*. A reference test with ammonium chloride was used to estimate the contribution of ammonium/ammonia to the observed toxicity of the fertilizer. The toxicity (LC_{50}) related to both NH_4^+ and estimated NH_3 was found to be very similar in the two tested solutions indicating that the ammonia/ammonium complex is the main driver of toxicity observed in the water-soluble fraction of the "slow release" fertilizer.

The concentrations of ammonium/ammonia in samples from the sediment column experiments are all below the LC_{50}-values recorded in the toxicity test. The concentrations in the pore water of the columns added 6 g fertilizer/kg sediment are in a range that may contribute to mortality in early stages of *C. finmarchicus*. However, early stages of *C. finmarchicus* have previously been shown to be rather sensitive to contaminants, and thus expected to be more sensitive than "the average" sediment organisms.

Based on the current results, we recommend that the amounts of the Plantagen slow-release fertilizer (NPK 16:6:12) used on shoreline sediments must be less than 3 g/kg sediment. However, our results indicate that 2 g fertilizer/kg sediment should be appropriate to enhance the oil biodegradation. The results also indicate that with low oil concentrations (less than 6 g oil/kg sediment), the amount of fertilizer probably could be further reduced with a limited impact on the biodegradation rate. In a future field experiment, an option could be to apply low dosage of fertilizer, possibly followed by repeated fertilizer application.

An example of practical use on an oiled shoreline: A dosage of 2 g fertilizer/kg sediment is equal to approximately 320 g fertilizer/m^2 shoreline, assuming that the sediment on 1 m^2 shore (depth of 10 cm) weighs 160 kg. This means that approximately 30 kg fertilizer are needed to a contaminated shoreline of 100 m^2. If the oil film thickness is 2 mm (approx. 10 g oil/kg sediment), the dosage ratio will be 1 to 5 between fertilizer and oil.

The results from the toxicity testing indicate that the ammonia/ammonium complex is the main driver of toxicity observed in the water-soluble fraction of the "slow release" fertilizer. Therefore, testing other fertilizers in the laboratory prior to the field experiment should be considered, preferably fertilizers with other N-sources than ammonium.

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A Attachment A Overview collected samples and analyses performed

An overview of the test matrix of the column experiment was given in Table 2.1. To make the sample identification easier, a sample code has been applied and can be explained by a few examples:

COL1-2-6-D14
COL1: Column 1
2: 2 g oil (premixed in sediment)
6: 6 g fertilizer (premixed in sediment)
D14: Sampled on day 14

COL10-2-3S-D28
COL10: Column 10
2: 2 g oil (premixed in sediment)
3S: 3 g fertilizer applied on the surface
D14: Sampled on day 28

COL16-6S-0-D56
COL16: Column 16
6S: 6 g oil applied on sediment surface
0: No fertilizer applied
D14: Sampled on day 56

Table A 1 Overview collected samples and the analysed performed.

LIMS ID	Description	GC/FID	GC/MS	Toxicity	DNA extraction	Nutrients (water)
2020-5109	Clean sediment	x	x	x	x	
2020-4919	REF-2-D0	x	x	x	x	
2020-4920	REF-6-D0	x	x	x	x	
2020-4921	REF-18-D0	x	x	x	x	
2020-4922	COL1-2-6-D14	x			x	
2020-4923	COL2-2-3-D14	x			x	
2020-4924	COL3-2-1-D14	x			x	
2020-4925	COL4-6-6-D14	x	x	x	x	
2020-4926	COL5-6-3-D14	x	x	x	x	
2020-4927	COL6-6-1-D14	x	x	x	x	
2020-4928	COL7-18-6-D14	x			x	
2020-4929	COL8-18-3-D14	x			x	
2020-4930	COL9-18-1-D14	x			x	
2020-4931	COL10-2-3S-D14	x			x	
2020-4932	COL11-6-3S-D14	x	x	x	x	
2020-4933	COL12-18-3S-D14	x			x	
2020-4934	COL13-2-0-D14	x			x	
2020-4935	COL14-6-0-D14	x	x	x	x	
2020-4936	COL15-18-0-D14	x			x	
2020-4937	COL16-6S-0-D14	x	x	x	x	

LIMS ID	Description	GC/FID	GC/MS	Toxicity	DNA extraction	Nutrients (water)
2020-4938	COL1-2-6-D28	x			x	
2020-4939	COL2-2-3-D28	x			x	
2020-4940	COL3-2-1-D28	x			x	
2020-4941	COL4-6-6-D28	x	x	x	x	
2020-4942	COL5-6-3-D28	x	x	x	x	
2020-4943	COL6-6-1-D28	x	x	x	x	
2020-4944	COL7-18-6-D28	x			x	
2020-4945	COL8-18-3-D28	x			x	
2020-4946	COL9-18-1-D28	x			x	
2020-4947	COL10-2-3S-D28	x			x	
2020-4948	COL11-6-3S-D28	x	x	x	x	
2020-4949	COL12-18-3S-D28	x			x	
2020-4950	COL13-2-0-D28	x			x	
2020-4951	COL14-6-0-D28	x	x	x	x	
2020-4952	COL15-18-0-D28	x			x	
2020-4953	COL16-6S-0-D28	x	x	x	x	
2020-4954	COL1-2-6-D56	x	x		x	
2020-4955	COL2-2-3-D56	x	x	x	x	
2020-4956	COL3-2-1-D56	x	x		x	
2020-4957	COL4-6-6-D56	x	x	x	x	
2020-4958	COL5-6-3-D56	x	x	x	x	
2020-4959	COL6-6-1-D56	x	x	x	x	
2020-4960	COL7-18-6-D56	x	x		x	
2020-4961	COL8-18-3-D56	x	x	x	x	
2020-4962	COL9-18-1-D56	x	x		x	
2020-4963	COL10-2-3S-D56	x	x		x	
2020-4964	COL11-6-3S-D56	x	x	x	x	
2020-4965	COL12-18-3S-D56	x	x		x	
2020-4966	COL13-2-0-D56	x	x		x	
2020-4967	COL14-6-0-D56	x	x	x	x	
2020-4968	COL15-18-0-D56	x	x		x	
2020-4969	COL16-6S-0-D56	x	x	x	x	
	Tot samples	52	32	24	52	

B Attachment B Results from chemical analysis

Table B 1 *Total extractable material (TPH) from sediment columns calculated from GC/FID-analysis. The concentrations are given in g THC/kg sediment (dry weight). Oil and fertilizer weights labelled S indicate that oil and fertilizer were applied on the surface and not premixed with the sediment.*

Sample ID	Oil g/kg	Fertilizer g/kg	Day 0 g/kg	Day 14 g/kg	Day 28 g/kg	Day 56 g/kg
Clean sediment	0	0	0,015			
REF-2-0	2	0	2,32			
COL1-2-6	2	6		1,93	1,51	1,38
COL2-2-3	2	3		2,24	1,78	1,63
COL10-2-3S	2	3 S		1,61	1,68	1,64
COL3-2-1	2	1		2,43	1,84	1,41
COL13-2-0	2	0		2,22	2,40	1,94
REF-6-0	6	0	7,38			
COL4-6-6	6	6		7,32	7,72	6,00
COL5-6-3	6	3		6,78	8,10	7,19
COL11-6-3S	6	3 S		6,64	6,74	5,36
COL6-6-1	6	1		7,15	7,32	6,30
COL14-6-0	6	0		7,46	8,02	5,85
COL16-6S-0	6 S	0		2,25	8,30	26,0
REF-18-0	18	0	21,8			
COL7-18-6	18	6		22,3	20,7	22,9
COL8-18-3	18	3		21,7	24,0	20,9
COL12-18-3S	18	3 S		22,5	23,8	21,1
COL9-18-1	18	1		21,7	24,7	21,4
COL15-18-0	18	0		25,2	21,4	20,9

Table B 2 *Calculated ratios based on peak areas from GC/FID analysis: C_{17}/pristane, C_{18}/phytane, and pristane/phytane. Sample description in e.g. Table B 1.*

	C_{17}/pristane				C_{18}/phytane				Pristane/phytane			
Sample ID	D0	D14	D28	D56	D0	D14	D28	D56	D0	D14	D28	D56
REF-2-0-D0	1,70				3,15				1,90			
COL1-2-6		0,36	0,47	0,90		0,96	0,96	1,98		2,06	1,79	1,77
COL2-2-3		0,46	0,29	0,70		1,14	0,64	1,28		2,06	2,02	1,56
COL10-2-3S		0,69	0,30	0,71		1,63	0,63	1,29		1,94	1,84	1,42
COL3-2-1		1,28	0,34	0,61		2,90	0,83	1,14		2,05	2,07	1,56
COL13-2-0		1,42	0,66	0,35		3,19	1,58	0,72		2,07	2,02	1,80
REF-6-0-D0	1,74				3,32				2,03			
COL4-6-6		1,07	0,23	0,13		2,52	0,63	0,41		2,08	2,17	1,80
COL5-6-3		1,27	0,59	0,23		3,09	1,53	0,53		2,21	2,12	1,84
COL11-6-3S		1,13	0,48	0,24		2,73	1,29	0,69		2,07	2,10	1,95
COL6-6-1		1,40	0,92	0,39		3,10	2,13	0,71		2,11	2,12	2,10
COL14-6-0		1,61	1,16	0,37		3,45	2,79	0,94		1,98	2,09	2,11
COL16-6S-0		1,66	1,64	1,66		3,68	3,48	3,50		2,02	2,09	2,10
REF-18-0-D0	1,81				3,56				2,05			
COL7-18-6		1,52	1,18	0,68		3,43	2,63	1,61		2,02	1,91	1,97
COL8-18-3		1,61	1,31	0,88		3,46	2,99	1,96		2,00	2,12	2,05
COL12-18-3S		1,53	1,14	0,73		3,38	2,74	1,60		2,07	2,22	2,01
COL9-18-1		1,65	1,52	1,07		3,68	3,48	2,36		2,07	2,17	2,03
COL15-18-0		1,65	1,57	1,21		3,79	3,45	2,65		2,22	2,05	2,10

Table B 3 *Summary of the SVOC results in the sediment columns given in mg analytes/kg sediment. The component groups are described in Table D 1.*

Sample ID	Sum SVOC	Decalins	Naphthalenes	2-3 ring PAHs	4-6 ring PAHs
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Clean sediment	0,14	0,01	0,01	0,07	0,04
REF-2-0-D0	**39,9**	**2,96**	**3,55**	**18,3**	**14,2**
COL1-2-6-D56	20,6	1,10	0,31	7,91	10,5
COL2-2-3-D56	23,3	1,24	0,32	8,70	12,2
COL10-2-3S-D56	22,5	1,05	0,23	8,08	12,7
COL3-2-1-D56	24,8	1,33	0,35	8,74	13,5
COL13-2-0-D56	31,5	1,54	0,56	11,5	17,4
REF-6-0-D0	**133**	**11,8**	**12,1**	**60,3**	**46,4**
COL4-6-6-D14	116	9,84	9,59	53,8	41,0
COL4-6-6-D28	109	9,26	8,18	50,2	38,9
COL4-6-6-D56	103	7,07	2,27	40,5	50,6
COL5-6-3-D14	126	9,67	9,63	51,8	52,3
COL5-6-3-D28	114	9,52	8,80	49,7	43,8
COL5-6-3-D56	108	8,19	4,06	46,8	46,2
COL11-6-3S-D14	127	9,24	9,43	48,9	57,7
COL11-6-3S-D28	119	7,75	8,46	46,2	54,6
COL11-6-3S-D56	92,1	6,12	3,16	34,1	46,3
COL6-6-1-D14	135	9,97	10,1	54,7	58,2
COL6-6-1-D28	107	9,17	8,30	46,0	41,8
COL6-6-1-D56	95,9	7,80	3,47	40,2	41,8
COL14-6-0-D14	146	8,86	9,53	52,6	73,4
COL14-6-0-D28	130	7,90	8,19	48,5	63,2
COL14-6-0-D56	103	6,37	4,91	35,6	53,6
COL16-6S-0-D14	45,7	2,57	3,14	15,8	23,2
COL16-6S-0-D28	163	9,19	11,4	57,5	82,4
COL16-6S-0-D56	499	26,6	33,5	172	260
REF-18-0-D0	**335**	**31,2**	**31,2**	**142**	**124**
COL7-18-6-D56	338	26,3	20,2	148	138
COL8-18-3-D56	325	24,4	19,2	138	138
COL12-18-3S-D56	372	26,0	17,3	143	181
COL9-18-1-D56	342	25,4	23,0	137	151
COL15-18-0-D56	408	26,3	22,2	144	210

Table B 4 *Semi-volatiles in sediments samples with 18 g oil premixed with sediment and fertilized, except in COL12 (2020-4965), where the fertilizer was applied on the surface. No fertilized added to the reference sample, all other samples collected on day 56.*

SINTEF ID	2020-4921	2020-4960	2020-4961	2020-4965	2020-4962	2020-4968
Sample ID	REF-18-0-D0	COL7-18-6-D56	COL8-18-3-D56	COL12-18-3S-D56	COL9-18-1-D56	COL15-18-0-D56
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Decalin	1,08	0,39	0,41	0,43	0,46	0,49
C1-decalins	3,71	2,20	2,11	2,32	2,29	2,32
C2-decalins	5,87	4,57	4,26	4,68	4,47	4,60
C3-decalins	8,59	7,85	7,08	7,56	7,26	7,51
C4-decalins	12,0	11,3	10,5	11,0	10,9	11,3
Benzo(b)thiophene	ND	ND	ND	ND	ND	ND
Naphthalene	0,83	0,01	0,01	0,01	0,02	0,02
C1-naphthalenes	3,12	0,16	0,21	0,08	0,76	0,37
C2-naphthalenes	7,00	2,61	2,68	1,61	4,49	3,67
C3-naphthalenes	10,6	8,15	7,55	6,73	8,87	8,85
C4-naphthalenes	9,64	9,29	8,71	8,92	8,83	9,28
Biphenyl	0,10	0,02	0,02	0,01	0,03	0,03
Acenaphthylene	0,08	0,03	0,02	0,03	0,06	0,04
Acenaphthene	0,15	0,11	0,10	0,10	0,09	0,10
Dibenzofuran	0,06	0,04	0,04	0,03	0,04	0,04
Fluorene	0,56	0,32	0,34	0,28	0,39	0,37
C1-fluorenes	1,91	1,65	1,48	1,41	1,62	1,64
C2-fluorenes	6,54	6,12	5,76	5,94	5,67	6,00
C3-fluorenes	8,80	8,65	8,19	8,48	8,07	8,49
Phenanthrene	2,25	1,27	1,17	0,86	1,80	1,59
Anthracene	0,50	0,40	0,35	0,27	0,43	0,42
C1-phenanthrenes/anthracenes	11,1	10,7	9,81	9,59	10,5	10,8
C2-phenanthrenes/anthracenes	33,0	35,1	32,8	34,2	32,1	34,2
C3-phenanthrenes/anthracenes	42,6	46,5	43,7	45,6	42,3	44,6
C4-phenanthrenes/anthracenes	28,0	30,0	27,9	29,0	27,0	28,5
Dibenzothiophene	0,10	0,08	0,06	0,06	0,09	0,07
C1-dibenzothiophenes	0,86	0,82	0,76	0,77	0,79	0,83
C2-dibenzothiophenes	1,77	1,77	1,68	1,76	1,68	1,77
C3-dibenzothiophenes	2,42	2,46	2,38	2,49	2,34	2,49
C4-dibenzothiophenes	1,68	1,88	1,75	1,80	1,69	1,82
Fluoranthene	0,65	0,57	0,63	0,89	0,69	1,04
Pyrene	4,05	3,79	4,19	6,47	4,98	7,94
C1-fluoranthrenes/pyrenes	17,0	16,3	17,2	25,2	19,6	31,4
C2-fluoranthenes/pyrenes	4,84	5,14	5,39	7,71	6,06	9,46
C3-fluoranthenes/pyrenes	26,9	31,9	33,1	45,6	36,9	54,2
Benz(a)anthracene	2,81	4,13	3,95	5,89	4,57	7,03
Chrysene	3,27	3,97	3,98	5,68	4,37	6,62
C1-chrysenes	16,2	19,6	20,0	26,3	21,9	30,2
C2-chrysenes	21,2	24,1	23,5	28,3	24,9	31,6
C3-chrysenes	15,5	16,8	15,7	17,3	16,0	18,6
C4-chrysenes	6,04	5,96	5,43	5,50	5,30	5,73
Benzo(b)fluoranthene	0,71	0,71	0,64	0,77	0,82	0,84
Benzo(k)fluoranthene	0,06	0,10	0,09	0,11	0,16	0,12
Benzo(e)pyrene	1,91	2,29	2,19	2,47	2,39	2,69
Benzo(a)pyrene	1,36	1,44	1,25	1,38	1,52	1,51
Perylene	0,61	0,57	0,46	0,54	0,67	0,63
Indeno(1,2,3-c,d)pyrene	ND	ND	ND	ND	ND	ND
Dibenz(a,h)anthracene	0,34	0,22	0,20	0,23	0,20	0,19
Benzo(g,h,i)perylene	0,79	0,40	0,40	0,34	0,38	0,35
30 ab hopane	6,09	5,83	5,12	5,08	5,08	5,42

SINTEF ID	2020-4921	2020-4960	2020-4961	2020-4965	2020-4962	2020-4968
Sample ID	REF-18-0-D0	COL7-18-6-D56	COL8-18-3-D56	COL12-18-3S-D56	COL9-18-1-D56	COL15-18-0-D56
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Sum SVOC	335	338	325	372	341	408
Decalins	31,2	26,3	24,4	26,0	25,4	26,3
Naphthalenes	31,2	20,2	19,2	17,3	23,0	22,2
2-3 ring PAHs	142	148	138	143	137	144
4-6 ring PAHs	124	138	138	181	151	210

Table B 5 *Semi-volatiles in sediments samples with 2 g oil premixed with sediment and fertilized, except in COL10 (2020-4963), where the fertilizer was applied on the surface. No fertilized added to the reference sample, all other samples collected on day 56.*

SINTEF ID	2020-4919	2020-4954	2020-4955	2020-4963	2020-4956	2020-4966
Sample ID	REF-2-0-D0	COL1-2-6-D56	COL2-2-3-D56	COL10-2-3S-D56	COL3-2-1-D56	COL13-2-0-D56
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Decalin	0,07	ND	ND	ND	ND	0,01
C1-decalins	0,28	0,03	0,03	0,03	0,03	0,05
C2-decalins	0,52	0,09	0,09	0,09	0,11	0,20
C3-decalins	0,85	0,28	0,33	0,30	0,40	0,47
C4-decalins	1,23	0,70	0,79	0,63	0,78	0,81
Benzo(b)thiophene	ND	ND	ND	ND	ND	ND
Naphthalene	0,07	ND	ND	ND	ND	ND
C1-naphthalenes	0,28	ND	ND	ND	ND	ND
C2-naphthalenes	0,74	0,03	0,03	0,02	0,03	0,04
C3-naphthalenes	1,31	0,08	0,07	0,05	0,08	0,13
C4-naphthalenes	1,15	0,20	0,22	0,16	0,24	0,39
Biphenyl	0,01	ND	ND	ND	ND	ND
Acenaphthylene	0,01	ND	ND	ND	ND	ND
Acenaphthene	0,01	ND	ND	ND	ND	ND
Dibenzofuran	0,01	ND	ND	ND	ND	ND
Fluorene	0,07	ND	ND	ND	ND	ND
C1-fluorenes	0,23	0,02	0,02	0,01	0,02	0,03
C2-fluorenes	0,70	0,18	0,19	0,15	0,23	0,34
C3-fluorenes	1,08	0,44	0,59	0,52	0,55	0,71
Phenanthrene	0,27	0,01	0,01	0,01	0,02	0,03
Anthracene	0,05	ND	ND	ND	ND	0,01
C1-phenanthrenes/anthracenes	1,39	0,14	0,13	0,12	0,17	0,36
C2-phenanthrenes/anthracenes	4,39	0,84	0,91	1,03	1,23	2,30
C3-phenanthrenes/anthracenes	5,63	3,24	3,66	3,27	3,56	4,28
C4-phenanthrenes/anthracenes	3,53	2,64	2,74	2,56	2,51	2,92
Dibenzothiophene	0,01	ND	ND	ND	ND	ND
C1-dibenzothiophenes	0,11	0,02	0,02	0,02	0,02	0,03
C2-dibenzothiophenes	0,23	0,05	0,06	0,06	0,07	0,12
C3-dibenzothiophenes	0,34	0,16	0,20	0,17	0,19	0,22
C4-dibenzothiophenes	0,25	0,16	0,16	0,15	0,15	0,17
Fluoranthene	0,07	0,01	0,02	0,02	0,03	0,06
Pyrene	0,47	0,06	0,07	0,12	0,14	0,43
C1-fluoranthrenes/pyrenes	1,86	1,11	1,37	1,38	1,82	2,42
C2-fluoranthenes/pyrenes	0,62	0,54	0,58	0,55	0,70	0,77
C3-fluoranthenes/pyrenes	3,07	2,40	2,74	3,37	3,13	4,52
Benz(a)anthracene	0,35	0,18	0,27	0,31	0,38	0,49
Chrysene	0,34	0,29	0,30	0,37	0,40	0,59

SINTEF ID	2020-4919	2020-4954	2020-4955	2020-4963	2020-4956	2020-4966
Sample ID	REF-2-0-D0	COL1-2-6-D56	COL2-2-3-D56	COL10-2-3S-D56	COL3-2-1-D56	COL13-2-0-D56
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
C1-chrysenes	1,87	1,43	1,66	2,02	1,95	2,66
C2-chrysenes	2,38	1,85	2,18	2,17	2,25	2,64
C3-chrysenes	1,75	1,47	1,67	1,42	1,54	1,67
C4-chrysenes	0,68	0,63	0,68	0,51	0,59	0,59
Benzo(b)fluoranthene	0,07	0,06	0,07	0,07	0,08	0,08
Benzo(k)fluoranthene	0,01	0,01	0,01	0,01	0,01	0,01
Benzo(e)pyrene	0,25	0,20	0,24	0,17	0,24	0,20
Benzo(a)pyrene	0,14	0,08	0,14	0,11	0,13	0,13
Perylene	0,06	0,04	0,06	0,04	0,06	0,05
Indeno(1,2,3-c,d)pyrene	ND	ND	ND	ND	ND	ND
Dibenz(a,h)anthracene	0,04	0,03	0,03	0,02	0,02	0,02
Benzo(g,h,i)perylene	0,14	0,09	0,07	0,04	0,05	0,04
30 ab hopane	0,88	0,78	0,84	0,47	0,83	0,52
Sum SVOC	39,9	20,6	23,3	22,5	24,8	31,5
Decalins	2,96	1,10	1,24	1,05	1,33	1,54
Naphthalenes	3,55	0,31	0,32	0,23	0,35	0,56
2-3 ring PAHs	18,3	7,91	8,70	8,08	8,74	11,5
4-6 ring PAHs	14,2	10,5	12,2	12,7	13,5	17,4

Table B 6 *Semi-volatiles in sediments samples with 6 g oil premixed with sediment and fertilized, except in COL16 (2020-4969) and COL11 (2020-4964), where the oil and fertilizer were applied on the surface, respective. No fertilized added to the reference sample, all other samples collected on day 56.*

SINTEF ID	2020-4920	2020-4957	2020-4958	2020-4959	2020-4964	2020-4967	2020-4969
Sample ID	REF-6-0-D0	COL4-6-6-D56	COL5-6-3-D56	COL6-6-1-D56	COL11-6-3S-D56	COL14-6-0-D56	COL16-6S-0-D56
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Decalin	0,37	0,02	0,04	0,04	0,03	0,05	0,19
C1-decalins	1,33	0,23	0,46	0,45	0,35	0,41	1,32
C2-decalins	2,23	0,97	1,34	1,30	0,97	1,07	3,95
C3-decalins	3,25	2,23	2,49	2,42	1,86	1,92	8,13
C4-decalins	4,64	3,64	3,85	3,58	2,91	2,94	13,0
Benzo(b)thiophene	ND	ND	ND	ND	ND	ND	ND
Naphthalene	0,31	ND	ND	ND	ND	ND	0,19
C1-naphthalenes	1,18	0,02	0,02	0,02	0,01	0,04	2,31
C2-naphthalenes	2,75	0,10	0,24	0,17	0,15	0,61	7,43
C3-naphthalenes	4,13	0,50	1,32	1,09	1,02	1,97	12,2
C4-naphthalenes	3,69	1,66	2,47	2,19	1,97	2,29	11,3
Biphenyl	0,04	ND	ND	ND	ND	ND	0,10
Acenaphthylene	0,04	ND	0,01	ND	ND	0,01	0,07
Acenaphthene	0,06	ND	0,02	0,01	0,01	0,01	0,19
Dibenzofuran	0,02	ND	0,01	ND	ND	0,01	0,08
Fluorene	0,20	0,01	0,04	0,03	0,02	0,06	0,64
C1-fluorenes	0,71	0,17	0,32	0,29	0,24	0,36	2,24
C2-fluorenes	2,58	1,35	1,83	1,58	1,43	1,58	7,30
C3-fluorenes	3,52	2,50	2,90	2,55	2,26	2,27	9,94
Phenanthrene	0,84	0,07	0,17	0,11	0,14	0,33	2,61
Anthracene	0,13	0,03	0,07	0,06	0,06	0,09	0,55
C1-phenanthrenes/anthracenes	4,58	1,19	2,38	2,01	2,08	2,67	13,6

SINTEF ID	2020-4920	2020-4957	2020-4958	2020-4959	2020-4964	2020-4967	2020-4969
Sample ID	REF-6-0-D0	COL4-6-6-D56	COL5-6-3-D56	COL6-6-1-D56	COL11-6-3S-D56	COL14-6-0-D56	COL16-6S-0-D56
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
C2-phenanthrenes/anthracenes	14,0	8,57	9,98	9,40	8,35	8,63	40,3
C3-phenanthrenes/anthracenes	18,5	15,3	16,5	14,0	11,0	11,0	52,3
C4-phenanthrenes/anthracenes	12,1	9,49	10,4	8,34	6,81	6,85	33,7
Dibenzothiophene	0,04	ND	0,01	0,01	0,01	0,01	0,13
C1-dibenzothiophenes	0,35	0,12	0,19	0,16	0,17	0,20	1,04
C2-dibenzothiophenes	0,72	0,42	0,52	0,46	0,45	0,46	2,10
C3-dibenzothiophenes	0,97	0,78	0,84	0,70	0,60	0,61	2,94
C4-dibenzothiophenes	0,82	0,53	0,59	0,48	0,41	0,43	2,17
Fluoranthene	0,24	0,18	0,18	0,20	0,24	0,31	1,34
Pyrene	1,55	1,39	1,47	1,36	1,50	1,93	10,2
C1-fluoranthrenes/pyrenes	6,48	7,59	6,46	6,08	6,42	7,85	40,0
C2-fluoranthenes/pyrenes	1,94	2,14	1,76	1,74	2,02	2,40	11,9
C3-fluoranthenes/pyrenes	10,3	11,7	10,2	9,01	11,3	13,4	68,0
Benz(a)anthracene	1,02	1,49	1,09	1,15	1,55	1,90	8,41
Chrysene	0,99	1,43	1,23	1,10	1,38	1,55	8,49
C1-chrysenes	6,17	7,31	6,32	5,74	6,37	7,25	36,8
C2-chrysenes	7,71	8,58	7,70	6,74	7,25	7,88	38,4
C3-chrysenes	5,48	5,20	5,62	4,75	5,00	5,36	22,7
C4-chrysenes	2,14	1,76	2,19	1,84	1,84	1,90	6,76
Benzo(b)fluoranthene	0,26	0,24	0,26	0,26	0,23	0,26	0,98
Benzo(k)fluoranthene	0,04	0,04	0,03	0,04	0,02	0,02	0,17
Benzo(e)pyrene	0,82	0,74	0,78	0,77	0,61	0,73	3,07
Benzo(a)pyrene	0,58	0,37	0,53	0,44	0,30	0,46	1,67
Perylene	0,27	0,16	0,18	0,23	0,11	0,19	0,58
Indeno(1,2,3-c,d)pyrene	ND	ND	ND	ND	ND	ND	ND
Dibenz(a,h)anthracene	0,13	0,08	0,06	0,10	0,08	0,06	0,24
Benzo(g,h,i)perylene	0,34	0,17	0,12	0,22	0,14	0,13	0,35
30 ab hopane	2,60	2,72	2,58	2,58	2,43	2,29	6,36
Sum SVOC	133	103	108	95,9	92,1	103	499
Decalins	11,8	7,07	8,19	7,80	6,12	6,37	26,6
Naphthalenes	12,1	2,27	4,06	3,47	3,16	4,91	33,5
2-3 ring PAHs	60,3	40,5	46,8	40,2	34,1	35,6	172
4-6 ring PAHs	46,4	50,6	46,2	41,8	46,3	53,6	260

Table B 7 *Semi-volatiles in sediments samples with 6 g oil premixed with sediment and fertilized, except in COL16 (2020-4953) and COL11 (2020-4948), where the oil and fertilizer were applied on the surface, respective. No fertilized added to the reference sample, all other samples collected on day 28.*

SINTEF ID	2020-4920	2020-4941	2020-4942	2020-4943	2020-4948	2020-4951	2020-4953
Sample ID	REF-6-0-D0	COL4-6-6-D28	COL5-6-3-D28	COL6-6-1-D28	COL11-6-3S-D28	COL14-6-0-D28	COL16-6S-0-D28
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Decalin	0,37	0,15	0,13	0,14	0,12	0,13	0,14
C1-decalins	1,33	0,71	0,70	0,73	0,60	0,62	0,64
C2-decalins	2,23	1,53	1,55	1,56	1,29	1,31	1,47
C3-decalins	3,25	2,76	2,80	2,73	2,31	2,36	2,76
C4-decalins	4,64	4,12	4,33	4,01	3,43	3,49	4,17
Benzo(b)thiophene	ND	ND	ND	ND	ND	ND	ND
Naphthalene	0,31	0,01	0,03	0,02	ND	0,01	0,15
C1-naphthalenes	1,18	0,35	0,55	0,49	0,38	0,38	1,00
C2-naphthalenes	2,75	1,61	1,81	1,74	1,85	1,71	2,59
C3-naphthalenes	4,13	3,11	3,24	3,08	3,20	3,11	3,99
C4-naphthalenes	3,69	3,09	3,17	2,97	3,02	2,98	3,67
Biphenyl	0,04	0,02	0,02	0,02	0,02	0,02	0,03
Acenaphthylene	0,04	0,02	0,02	0,02	0,03	0,03	0,03
Acenaphthene	0,06	0,03	0,04	0,03	0,04	0,03	0,06
Dibenzofuran	0,02	0,02	0,01	0,01	0,02	0,02	0,02
Fluorene	0,20	0,14	0,15	0,15	0,14	0,14	0,20
C1-fluorenes	0,71	0,59	0,59	0,56	0,58	0,55	0,72
C2-fluorenes	2,58	2,00	2,05	1,89	1,95	1,91	2,42
C3-fluorenes	3,52	2,84	2,93	2,68	2,59	2,70	3,32
Phenanthrene	0,84	0,66	0,69	0,64	0,64	0,65	0,89
Anthracene	0,13	0,16	0,16	0,15	0,14	0,15	0,18
C1-phenanthrenes/anthracenes	4,58	3,69	3,73	3,48	3,59	3,66	4,52
C2-phenanthrenes/anthracenes	14,0	11,8	11,7	10,8	11,0	11,4	13,5
C3-phenanthrenes/anthracenes	18,5	15,6	15,4	14,3	14,3	15,2	17,7
C4-phenanthrenes/anthracenes	12,1	10,4	10,0	9,25	9,06	9,60	11,1
Dibenzothiophene	0,04	0,03	0,03	0,03	0,03	0,03	0,04
C1-dibenzothiophenes	0,35	0,25	0,26	0,24	0,26	0,27	0,34
C2-dibenzothiophenes	0,72	0,57	0,58	0,53	0,54	0,57	0,70
C3-dibenzothiophenes	0,97	0,80	0,79	0,73	0,77	0,85	1,02
C4-dibenzothiophenes	0,82	0,60	0,56	0,52	0,55	0,61	0,70
Fluoranthene	0,24	0,17	0,19	0,22	0,24	0,33	0,45
Pyrene	1,55	1,21	1,44	1,38	1,77	2,24	3,24
C1-fluoranthrenes/pyrenes	6,48	5,12	5,96	5,79	7,28	9,35	12,6
C2-fluoranthenes/pyrenes	1,94	1,47	1,60	1,59	2,26	2,82	3,78
C3-fluoranthenes/pyrenes	10,3	8,36	9,49	9,18	13,4	16,0	21,5
Benz(a)anthracene	1,02	0,87	0,95	0,88	1,90	2,24	2,85
Chrysene	0,99	1,00	1,17	1,05	1,75	1,98	2,60
C1-chrysenes	6,17	5,17	5,88	5,61	7,80	8,83	11,3
C2-chrysenes	7,71	6,64	7,50	7,09	8,67	9,43	11,8
C3-chrysenes	5,48	4,90	5,47	5,09	5,49	5,78	7,17
C4-chrysenes	2,14	1,92	2,10	1,93	1,92	2,02	2,38
Benzo(b)fluoranthene	0,26	0,24	0,24	0,25	0,27	0,30	0,35
Benzo(k)fluoranthene	0,04	0,04	0,04	0,04	0,05	0,05	0,06
Benzo(e)pyrene	0,82	0,67	0,66	0,66	0,84	0,88	1,07
Benzo(a)pyrene	0,58	0,50	0,51	0,50	0,49	0,53	0,65
Perylene	0,27	0,24	0,23	0,23	0,22	0,25	0,30
Indeno(1,2,3-c,d)pyrene	ND	ND	ND	ND	ND	ND	ND
Dibenz(a,h)anthracene	0,13	0,10	0,11	0,09	0,07	0,06	0,07

SINTEF ID	2020-4920	2020-4941	2020-4942	2020-4943	2020-4948	2020-4951	2020-4953
Sample ID	REF-6-0-D0	COL4-6-6-D28	COL5-6-3-D28	COL6-6-1-D28	COL11-6-3S-D28	COL14-6-0-D28	COL16-6S-0-D28
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Benzo(g,h,i)perylene	0,34	0,25	0,25	0,21	0,12	0,12	0,13
30 ab hopane	2,60	1,98	2,10	1,98	1,80	1,82	2,15
Sum SVOC	133	109	114	107	119	130	163
Decalins	11,8	9,26	9,52	9,17	7,75	7,90	9,19
Naphthalenes	12,1	8,18	8,80	8,30	8,46	8,19	11,4
2-3 ring PAHs	60,3	50,2	49,7	46,0	46,2	48,5	57,5
4-6 ring PAHs	46,4	38,9	43,8	41,8	54,6	63,2	82,4

Table B 8 *Semi-volatiles in sediments samples with 6 g oil premixed with sediment and fertilized, except in COL16 (2020-4932) and COL11 (2020-4937), where the oil and fertilizer were applied on the surface, respective. No fertilized added to the reference sample, all other samples collected on day 14.*

SINTEF ID	2020-4920	2020-4926	2020-4927	2020-4925	2020-4932	2020-4935	2020-4937
Sample ID	REF-6-0-D0	COL5-6-3-D14	COL6-6-1-D14	COL4-6-6-D14	COL11-6-3S-D14	COL14-6-0-D14	COL166S-0-D14
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Decalin	0,37	0,15	0,18	0,16	0,15	0,14	0,03
C1-decalins	1,33	0,75	0,82	0,76	0,73	0,68	0,15
C2-decalins	2,23	1,61	1,70	1,61	1,54	1,44	0,38
C3-decalins	3,25	2,97	2,98	2,91	2,75	2,65	0,76
C4-decalins	4,64	4,18	4,30	4,40	4,08	3,96	1,25
Benzo(b)thiophene	ND	ND	ND	ND	ND	0,00	0,00
Naphthalene	0,31	0,11	0,10	0,07	0,06	0,05	0,04
C1-naphthalenes	1,18	0,87	0,86	0,82	0,75	0,64	0,28
C2-naphthalenes	2,75	2,10	2,30	2,06	2,13	2,09	0,66
C3-naphthalenes	4,13	3,33	3,54	3,37	3,36	3,48	1,13
C4-naphthalenes	3,69	3,22	3,31	3,26	3,13	3,28	1,03
Biphenyl	0,04	0,03	0,03	0,03	0,03	0,02	0,01
Acenaphthylene	0,04	0,03	0,03	0,03	0,03	0,03	0,01
Acenaphthene	0,06	0,05	0,05	0,04	0,04	0,04	0,01
Dibenzofuran	0,02	0,01	0,02	0,02	0,02	0,02	0,01
Fluorene	0,20	0,17	0,17	0,17	0,16	0,15	0,06
C1-fluorenes	0,71	0,64	0,64	0,63	0,62	0,62	0,21
C2-fluorenes	2,58	2,14	2,28	2,20	2,01	2,15	0,71
C3-fluorenes	3,52	2,89	3,22	3,00	2,73	2,96	0,97
Phenanthrene	0,84	0,76	0,72	0,81	0,74	0,72	0,26
Anthracene	0,13	0,17	0,13	0,18	0,17	0,13	0,06
C1-phenanthrenes/anthracenes	4,58	3,84	4,04	4,06	3,67	4,07	1,31
C2-phenanthrenes/anthracenes	14,0	12,1	12,8	12,4	11,3	12,4	3,78
C3-phenanthrenes/anthracenes	18,5	16,0	17,0	16,6	15,3	16,4	4,70
C4-phenanthrenes/anthracenes	12,1	10,6	11,1	11,1	9,77	10,3	2,88
Dibenzothiophene	0,04	0,04	0,03	0,04	0,04	0,03	0,01
C1-dibenzothiophenes	0,35	0,29	0,31	0,30	0,28	0,31	0,12
C2-dibenzothiophenes	0,72	0,59	0,65	0,61	0,57	0,63	0,20
C3-dibenzothiophenes	0,97	0,86	0,92	0,88	0,85	0,92	0,28
C4-dibenzothiophenes	0,82	0,63	0,66	0,68	0,63	0,67	0,19
Fluoranthene	0,24	0,27	0,33	0,18	0,26	0,40	0,13
Pyrene	1,55	1,85	2,19	1,28	1,89	2,83	0,87
C1-fluoranthrenes/pyrenes	6,48	7,64	8,89	5,46	7,72	11,4	3,50

SINTEF ID	2020-4920	2020-4926	2020-4927	2020-4925	2020-4932	2020-4935	2020-4937
Sample ID	REF-6-0-D0	COL5-6-3-D14	COL6-6-1-D14	COL4-6-6-D14	COL11-6-3S-D14	COL14-6-0-D14	COL166S-0-D14
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
C2-fluoranthenes/pyrenes	1,94	2,21	2,47	1,58	2,43	3,39	1,09
C3-fluoranthenes/pyrenes	10,3	12,0	13,8	8,95	14,4	18,9	5,97
Benz(a)anthracene	1,02	1,32	1,48	0,94	1,85	2,63	0,88
Chrysene	0,99	1,41	1,59	1,04	1,77	2,44	0,76
C1-chrysenes	6,17	7,11	8,09	5,42	7,98	9,98	3,02
C2-chrysenes	7,71	8,48	9,00	6,99	9,09	10,5	3,27
C3-chrysenes	5,48	5,89	6,11	5,14	5,93	6,66	2,20
C4-chrysenes	2,14	2,09	2,11	2,04	2,13	2,16	0,76
Benzo(b)fluoranthene	0,26	0,26	0,29	0,22	0,31	0,25	0,11
Benzo(k)fluoranthene	0,04	0,04	0,05	0,02	0,05	0,02	0,02
Benzo(e)pyrene	0,82	0,73	0,78	0,69	0,90	0,89	0,30
Benzo(a)pyrene	0,58	0,49	0,53	0,47	0,56	0,50	0,19
Perylene	0,27	0,21	0,23	0,20	0,27	0,20	0,08
Indeno(1,2,3-c,d)pyrene	ND	ND	ND	ND	ND	0,00	0,00
Dibenz(a,h)anthracene	0,13	0,08	0,07	0,11	0,07	0,06	0,03
Benzo(g,h,i)perylene	0,34	0,19	0,14	0,26	0,12	0,12	0,06
30 ab hopane	2,60	2,23	2,36	2,09	1,98	2,03	0,91
Sum SVOC	133	126	135	116	127	146	45,6
Decalins	11,8	9,67	9,97	9,84	9,24	8,86	2,57
Naphthalenes	12,1	9,63	10,1	9,59	9,43	9,53	3,14
2-3 ring PAHs	60,3	51,8	54,7	53,8	48,9	52,6	15,8
4-6 ring PAHs	46,4	52,3	58,2	41,0	57,7	73,4	23,2

Table B 9 *Depletion of SVOC component groups, normalized to hopane, given as % depletion compared to sample from day 0. In addition, the hopane concentrations are shown (in mg/kg sediment). Description of component groups in Attachment E.*

SINTEF ID	Sample ID	Decalins	Naphthalenes	2-3 ring PAHs	4-6 ring PAHs	Sum SVOC	30ab hopane
		% depleted	% depleted	% depleted	% depleted	% depleted	mg/kg sediment
2020-4919	**REF-2-0-D0**	0,0	0,0	0,0	0,0	0,0	0,88
2020-4954	COL1-2-6-D56	58,1	90,3	51,6	16,9	42,0	0,78
2020-4955	COL2-2-3-D56	56,1	90,5	50,3	10,1	38,9	0,84
2020-4963	COL10-2-3S-D56	32,8	87,7	16,8	-68,8	-6,5	0,47
2020-4956	COL3-2-1-D56	52,2	89,4	49,2	-1,6	33,8	0,83
2020-4966	COL13-2-0-D56	12,3	73,4	-6,3	-106	-33,3	0,52
2020-4920	**REF-6-0-D0**	0,0	0,0	0,0	0,0	0,0	2,60
2020-4925	COL4-6-6-D14	-3,5	1,1	-11,1	-9,7	-8,6	2,09
2020-4941	COL4-6-6-D28	-2,9	10,9	-9,6	-10,2	-7,1	1,98
2020-4957	COL4-6-6-D56	43,0	82,1	35,9	-3,9	26,2	2,72
2020-4926	COL5-6-3-D14	4,7	6,9	-0,1	-31,3	-9,9	2,23
2020-4942	COL5-6-3-D28	0,4	9,7	-2,0	-16,7	-5,8	2,10
2020-4958	COL5-6-3-D56	30,3	66,1	21,8	-0,1	18,5	2,58
2020-4932	COL11-6-3S-D14	-2,6	-2,7	-6,5	-63,3	-25,5	1,98
2020-4948	COL11-6-3S-D28	5,6	-1,1	-10,5	-69,5	-28,6	1,80
2020-4964	COL11-6-3S-D56	44,7	72,0	39,6	-6,6	26,1	2,43
2020-4927	COL6-6-1-D14	7,1	7,7	0,0	-38,2	-12,0	2,36
2020-4943	COL6-6-1-D28	-1,5	9,9	0,0	-17,8	-5,4	1,98
2020-4959	COL6-6-1-D56	33,8	71,1	32,9	9,6	27,7	2,58
2020-4935	COL14-6-0-D14	4,2	-1,1	-11,6	-102	-40,6	2,03
2020-4951	COL14-6-0-D28	4,6	3,0	-14,8	-94,3	-38,9	1,82
2020-4967	COL14-6-0-D56	38,8	53,8	32,9	-31,0	12,4	2,29
2020-4937	COL16-6S-0-D14	38,4	26,1	25,7	-42,1	2,7	0,91
2020-4953	COL16-6S-0-D28	6,1	-14,2	-15,2	-114	-47,5	2,15
2020-4969	COL16-6S-0-D56	8,1	-13,3	-16,5	-129	-52,9	6,36
2020-4921	**REF-18-0-D0**	0,0	0,0	0,0	0,0	0,0	6,09
2020-4960	COL7-18-6-D56	11,8	32,3	-8,5	-15,9	-5,4	5,83
2020-4961	COL8-18-3-D56	7,0	27,0	-15,6	-32,4	-15,5	5,12
2020-4965	COL12-18-3S-D56	0,0	33,4	-20,1	-74,2	-33,0	5,08
2020-4962	COL9-18-1-D56	2,4	11,7	-15,1	-46,1	-22,2	5,08
2020-4968	COL15-18-0-D56	5,4	20,2	-13,5	-90,0	-36,7	5,42

C Attachment C GC chromatograms

Chromatograms from day 0

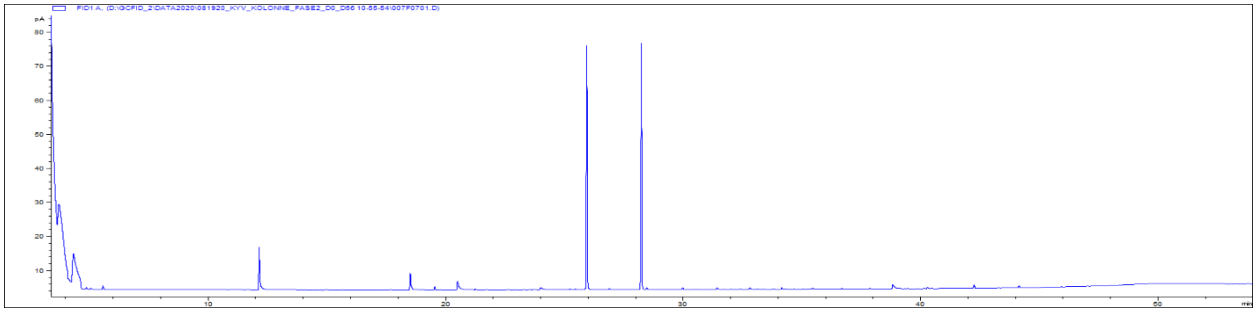

Figure C 1 *GC chromatogram of clean sediment: Peaks at approx 26 and 28 min are added internal standards.*

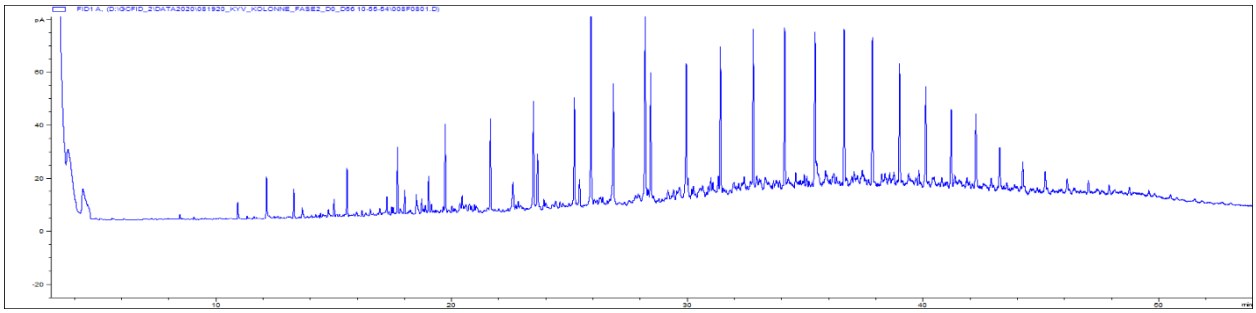

Figure C 2 *GC chromatogram of REF-2-D0: Reference sample containing sediment mixed with 2 g oil (SINTEF ID 2020-4919). Sampled on day 0. Peaks at approximately 26 and 28 min are added internal standards.*

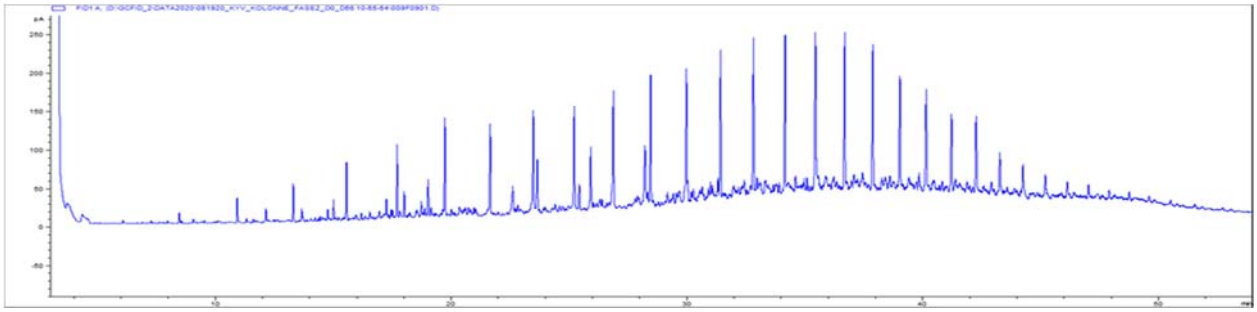

Figure C 3 *GC chromatogram of REF-6-D0: Reference sample containing sediment mixed with 6 g oil (SINTEF ID 2020-4920). Sampled on day 0.*

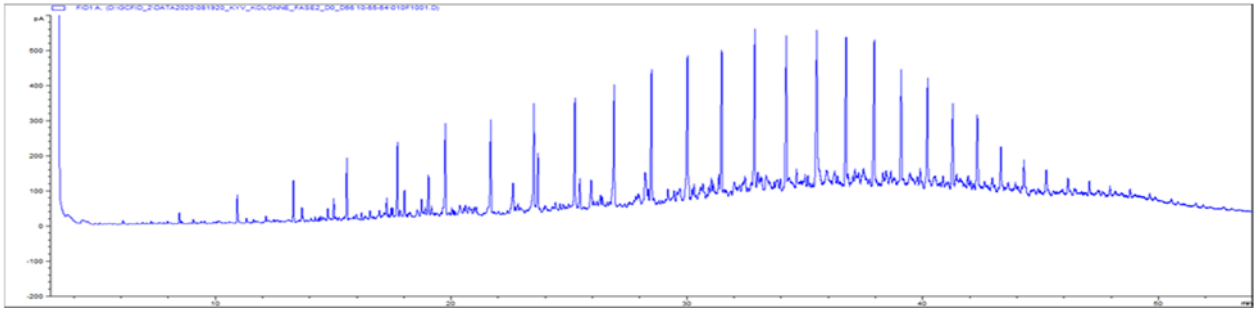

Figure C 4 *GC chromatogram of REF-18-D0: Reference sample containing sediment mixed with 18 g oil (SINTEF ID 2020-4921). Sampled on day 0.*

Chromatograms from day 14

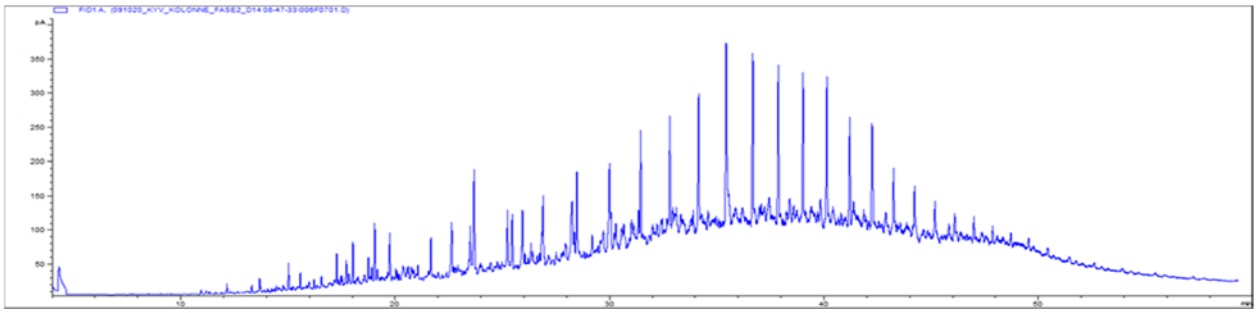

Figure C 5 *COL1-2-6-D14: Column 1, sediment premixed with 2 g oil and 6 g fertilizer. Sampled on day 14 (SINTEF ID 2020-4922).*

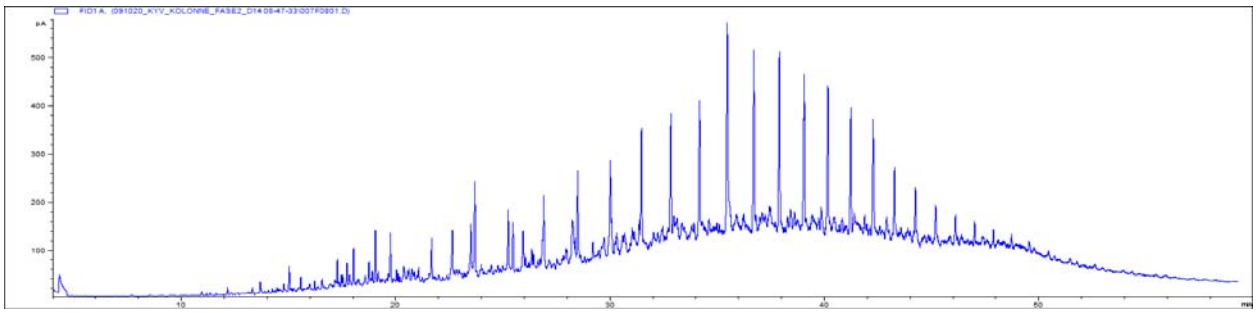

Figure C 6 *COL2-2-3-D14: Column 2, sediment premixed with 2 g oil and 3 g fertilizer. Sampled on day 14 (SINTEF ID 2020-4923).*

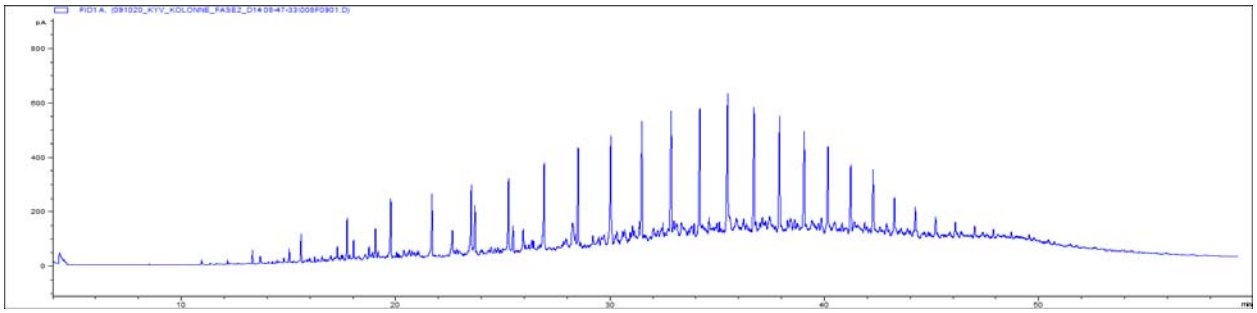

Figure C 7 *COL3-2-1-D14: Column 3, sediment premixed with 2 g oil and 1 g fertilizer. Sampled on day 14 (SINTEF ID 2020-4924).*

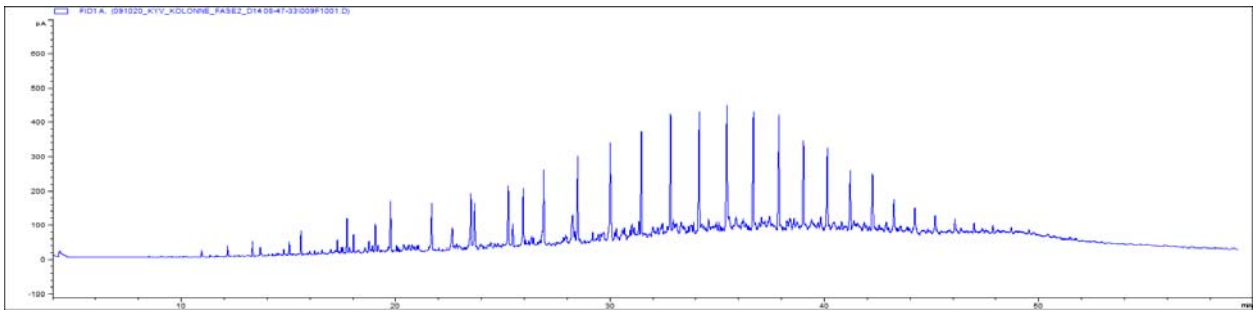

Figure C 8 *COL4-6-6-D14: Column 4, sediment premixed with 6 g oil and 6 g fertilizer. Sampled on day 14 (SINTEF ID 2020-4925).*

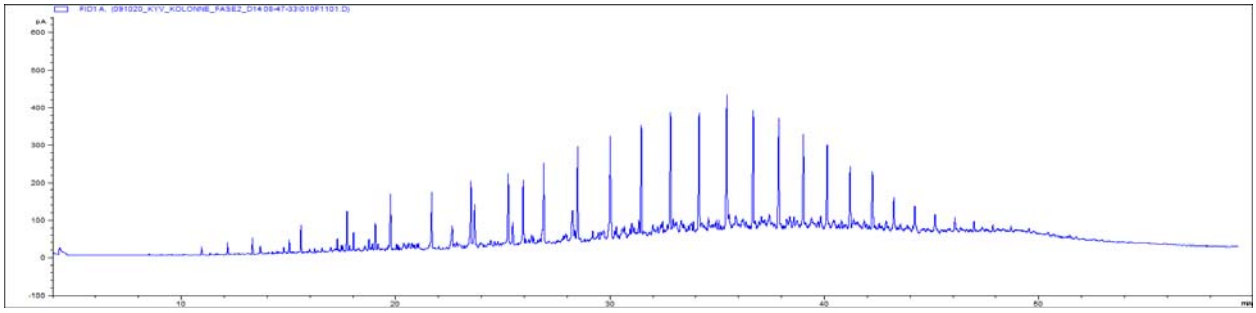

Figure C 9 *COL5-6-3-D14: Column 5, sediment premixed with 6 g oil and 3 g fertilizer. Sampled on day 14 (SINTEF ID 2020-4926).*

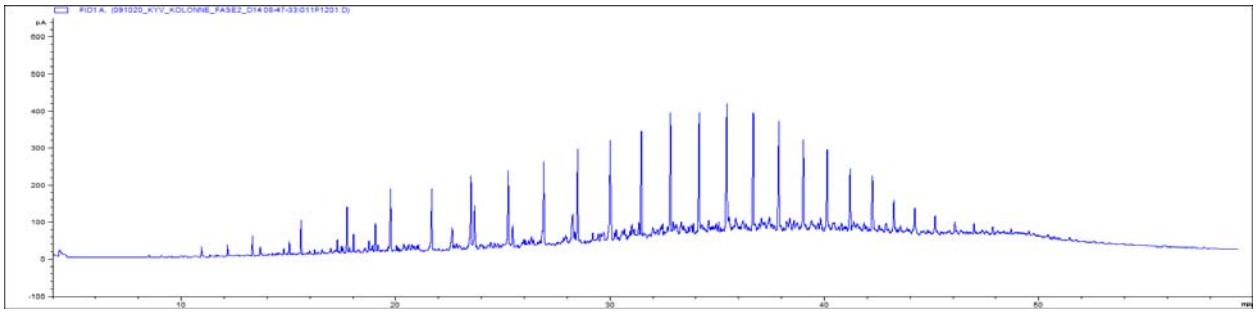

Figure C 10 *COL6-6-1-D14: Column 6, sediment premixed with 6 g oil and 1 g fertilizer. Sampled on day 14 (SINTEF ID 2020-4927).*

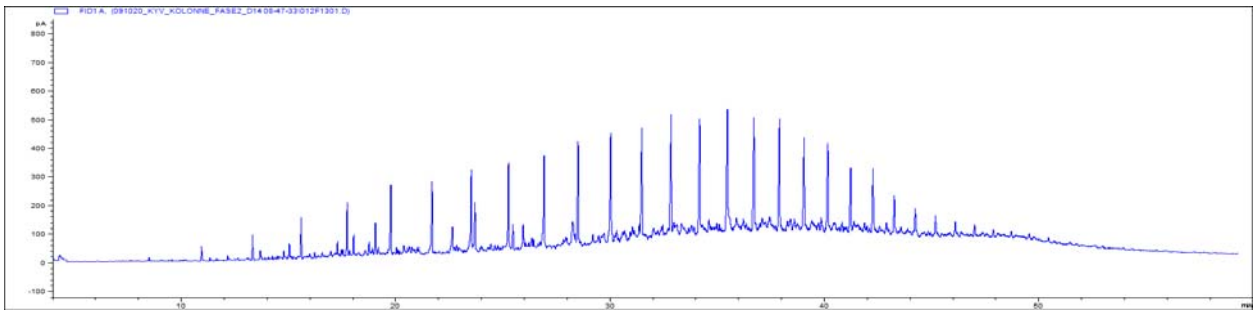

Figure C 11 *COL7-18-6-D14: Column 7, sediment premixed with 6 g oil and 6 g fertilizer. Sampled on day 14 (SINTEF ID 2020-4928).*

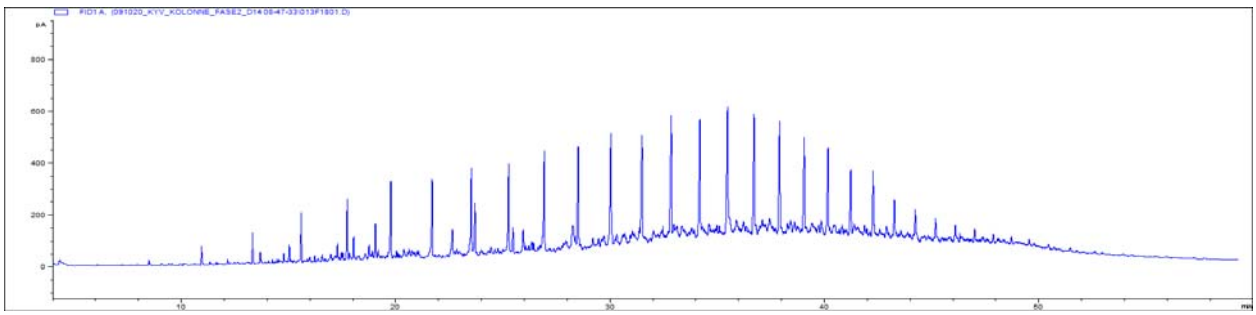

Figure C 12 *COL8-18-3-D14: Column 8, sediment premixed with 6 g oil and 3 g fertilizer. Sampled on day 14 (SINTEF ID 2020-4929).*

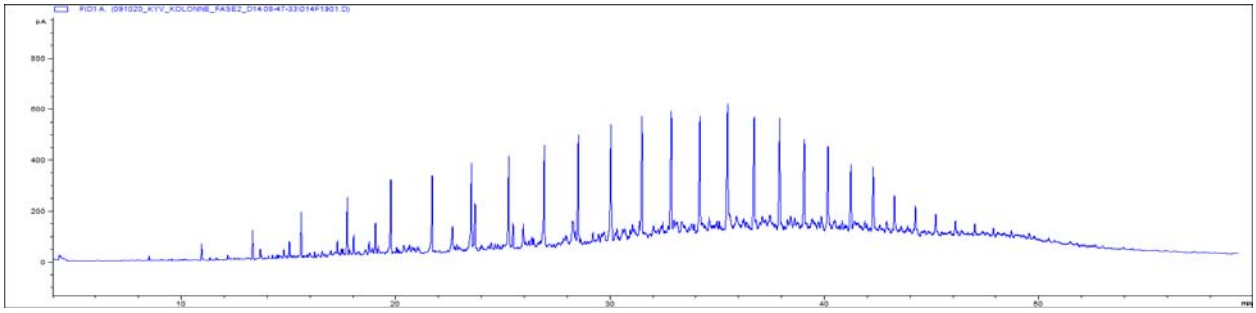

Figure C 13 *COL9-18-1-D14: Column 9, sediment premixed with 6 g oil and 1 g fertilizer. Sampled on day 14 (SINTEF ID 2020-4930).*

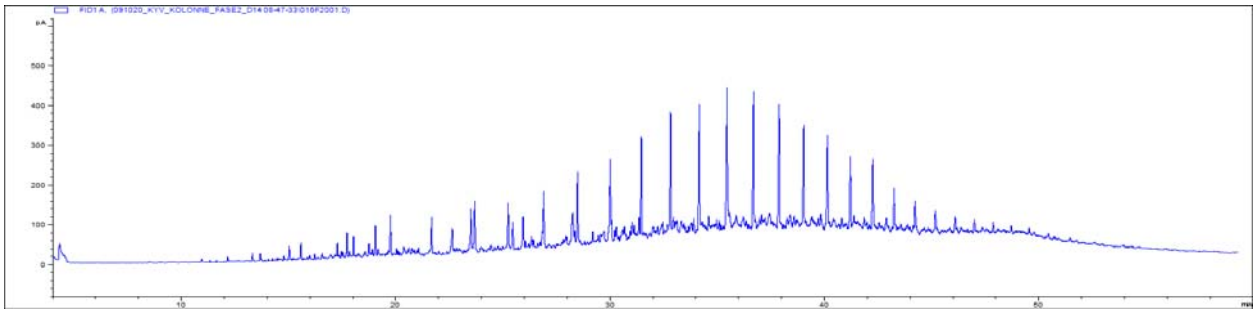

Figure C 14 *COL10-2-3S-D14: Column 10, sediment premixed with 2 g oil, 3 g fertilizer on surface. Sampled on day 14 (SINTEF ID 2020-4931).*

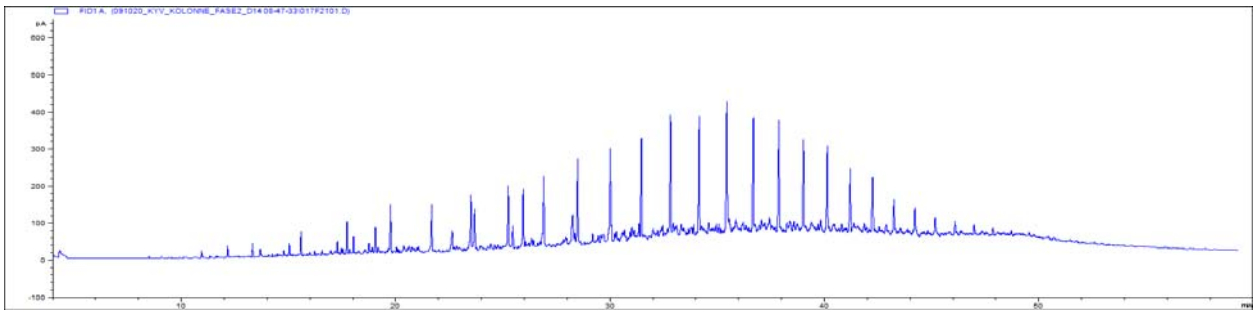

Figure C 15 *COL11-6-3S-D14: Column 11, sediment premixed with 6 g oil, 3 g fertilizer on surface. Sampled on day 14 (SINTEF ID 2020-4932).*

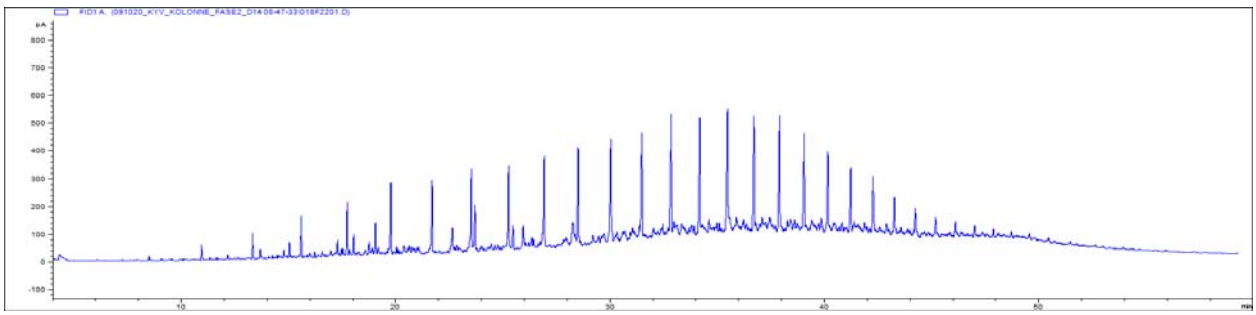

Figure C 16 *COL12-18-3S-D14: Column 12, sediment premixed with 18 g oil, 3 g fertilizer on surface. Sampled on day 14 (SINTEF ID 2020-4933).*

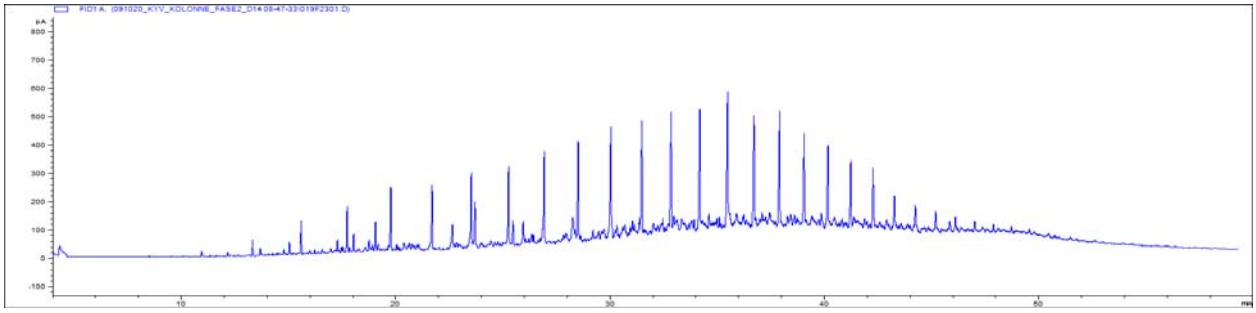

Figure C 17 *COL13-2-0-D14: Column 13, sediment premixed with 2 g oil, no fertilizer added. Sampled on day 14 (SINTEF ID 2020-4934).*

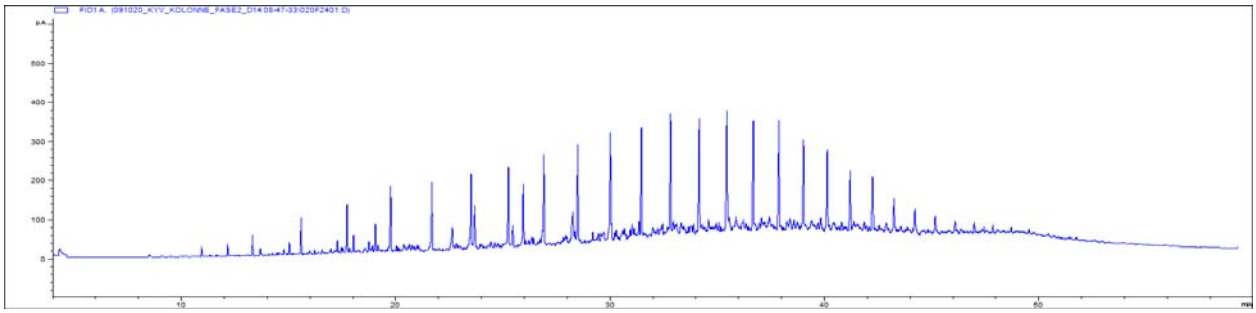

Figure C 18 *COL14-6-0-D14: Column 14, sediment premixed with 6 g oil, no fertilizer added. Sampled on day 14 (SINTEF ID 2020-4935).*

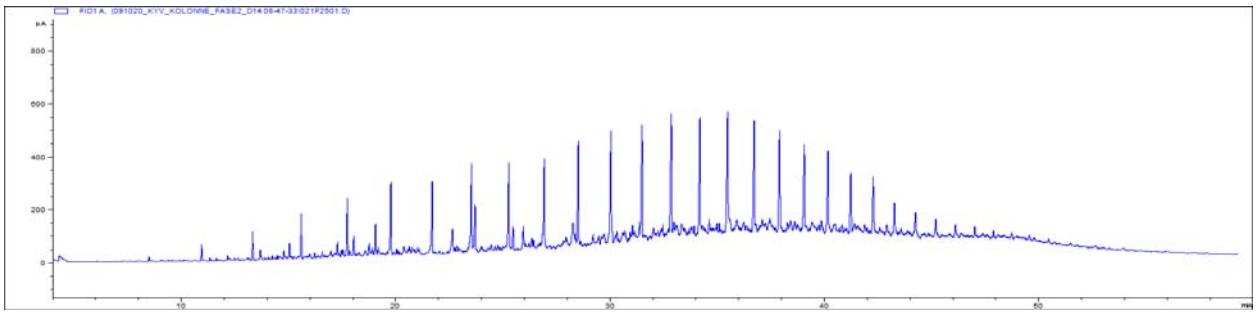

Figure C 19 *COL15-18-0-D14: Column 15, sediment premixed with 18 g oil, no fertilizer added. Sampled on day 14 (SINTEF ID 2020-4936).*

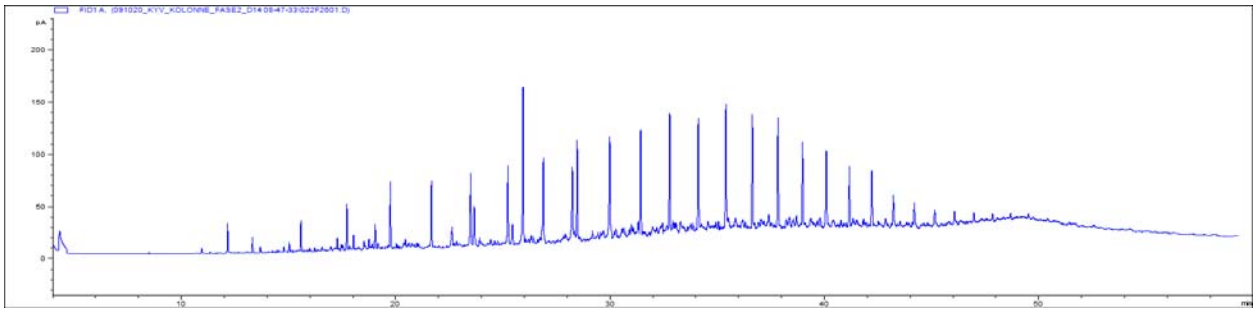

Figure C 20 *COL16-6S-0-D14: Column 16, oil on sediment surface, no fertilizer added. Sampled on day 14 (SINTEF ID 2020-4937).*

Chromatograms from day 28.

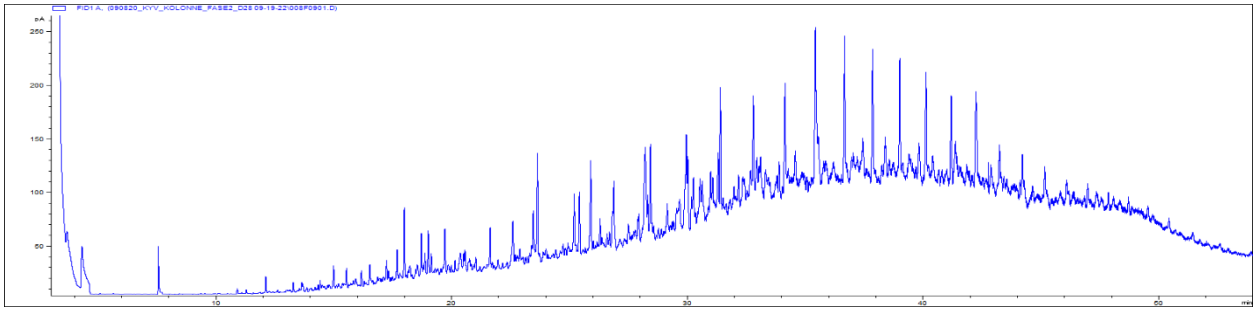

Figure C 21 *COL1-2-6-D28: Column 1, sediment premixed with 2 g oil and 6 g fertilizer. Sampled on day 28 (SINTEF ID 2020-4938).*

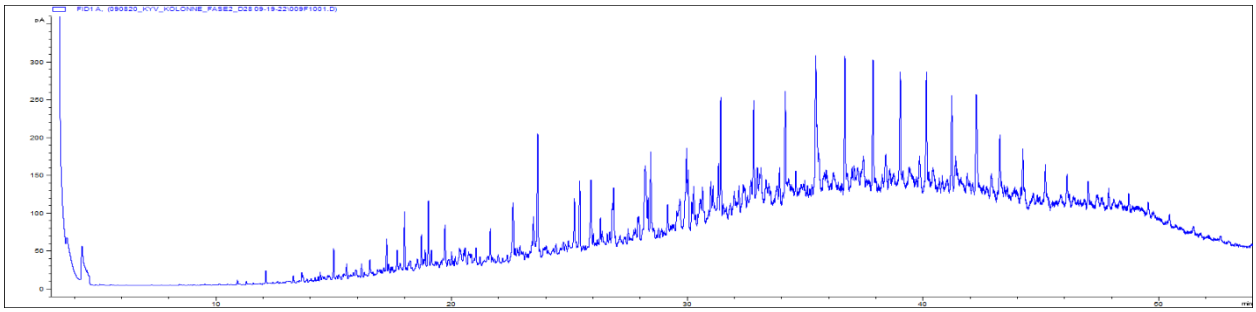

Figure C 22 *COL2-2-3-D28: Column 2, sediment premixed with 2 g oil and 3 g fertilizer. Sampled on day 28 (SINTEF ID 2020-4939).*

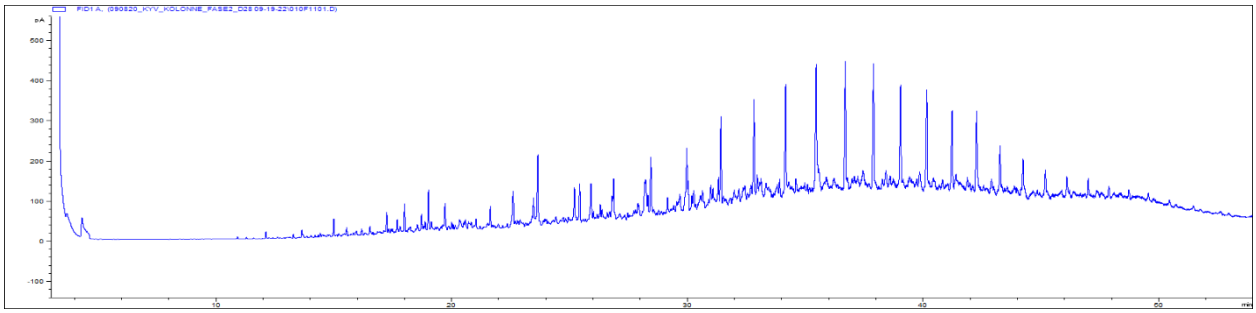

Figure C 23 *COL3-2-1-D28: Column 3, sediment premixed with 2 g oil and 1 g fertilizer. Sampled on day 28 (SINTEF ID 2020-4940).*

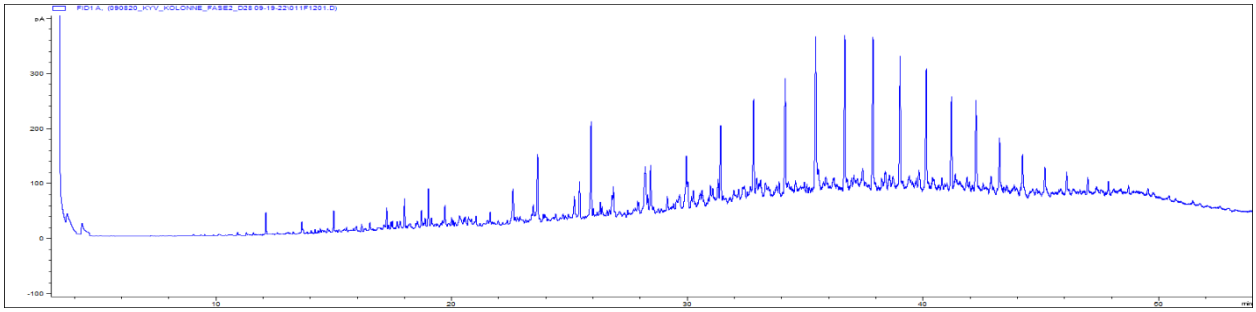

Figure C 24 *COL4-6-6-D28: Column 4, sediment premixed with 6 g oil and 6 g fertilizer. Sampled on day 28 (SINTEF ID 2020-4941).*

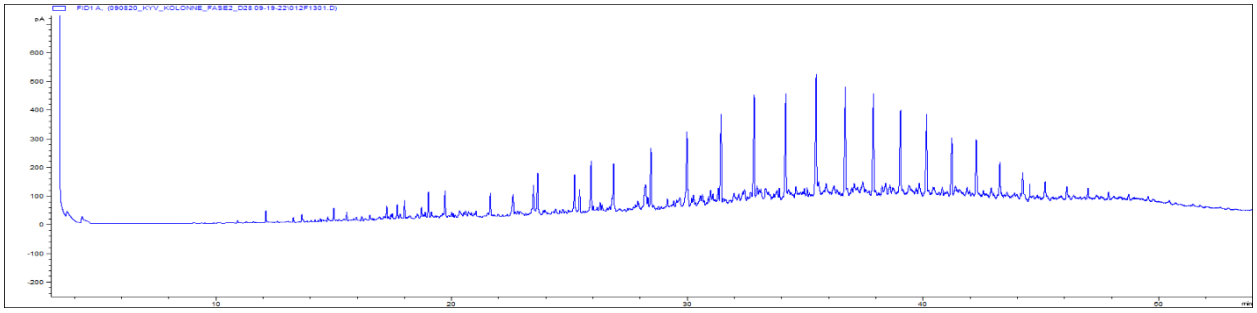

Figure C 25 *COL5-6-3-D28: Column 5, sediment premixed with 6 g oil and 3 g fertilizer. Sampled on day 28 (SINTEF ID 2020-4942).*

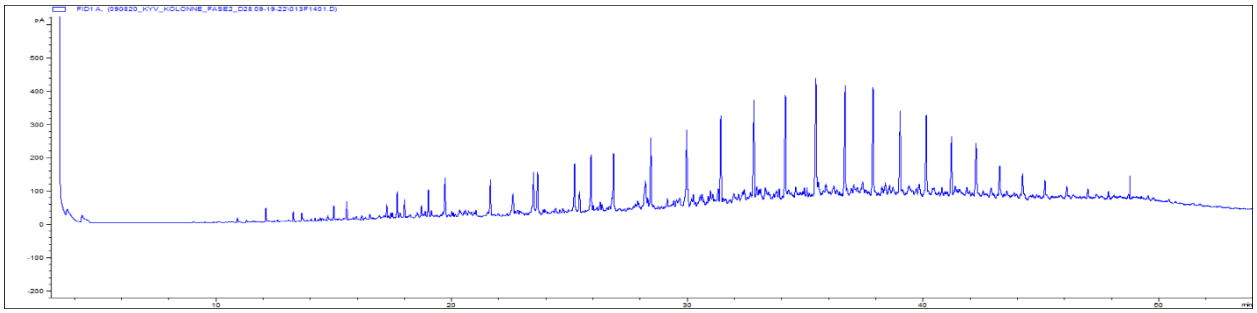

Figure C 26 *COL6-6-1-D28: Column 6, sediment premixed with 6 g oil and 1 g fertilizer. Sampled on day 28 (SINTEF ID 2020-4943).*

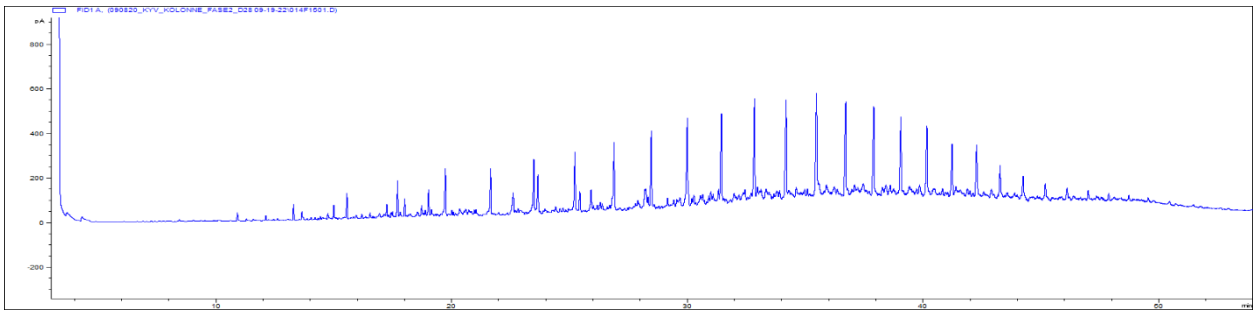

Figure C 27 *COL7-18-6-D28: Column 7, sediment premixed with 6 g oil and 6 g fertilizer. Sampled on day 28 (SINTEF ID 2020-4944).*

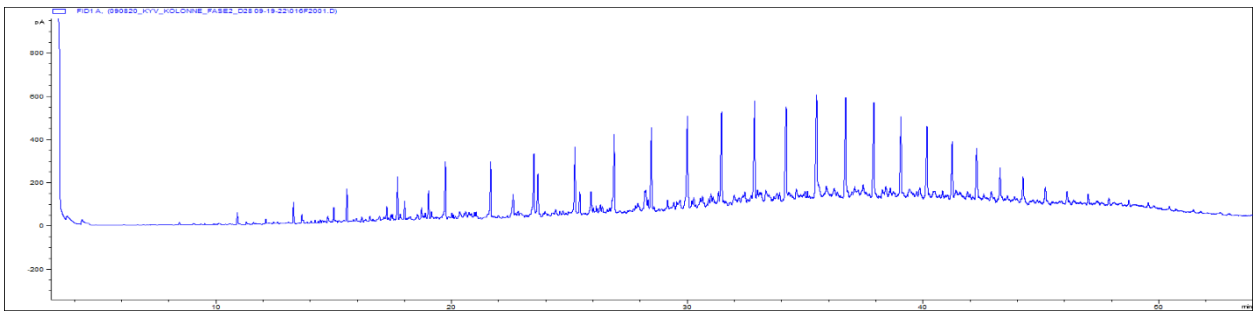

Figure C 28 *COL8-18-3-D28: Column 8, sediment premixed with 6 g oil and 3 g fertilizer. Sampled on day 28 (SINTEF ID 2020-4945).*

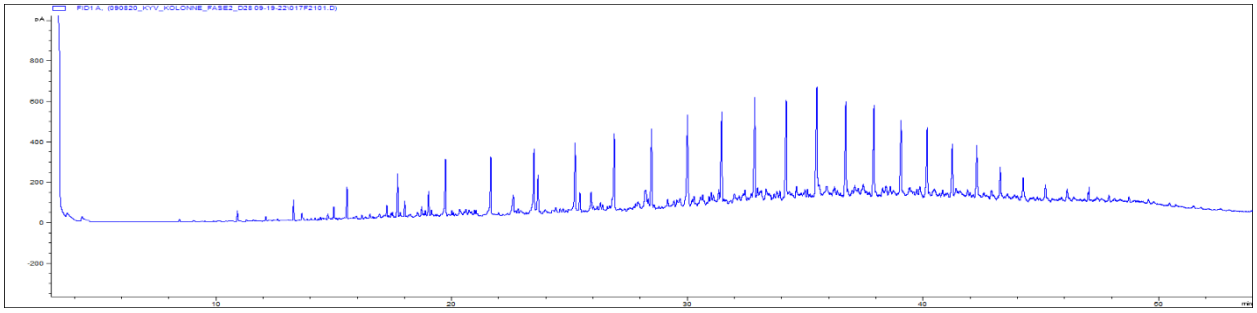

Figure C 29 *COL9-18-1-D28: Column 9, sediment premixed with 6 g oil and 1 g fertilizer. Sampled on day 28 (SINTEF ID 2020-4946).*

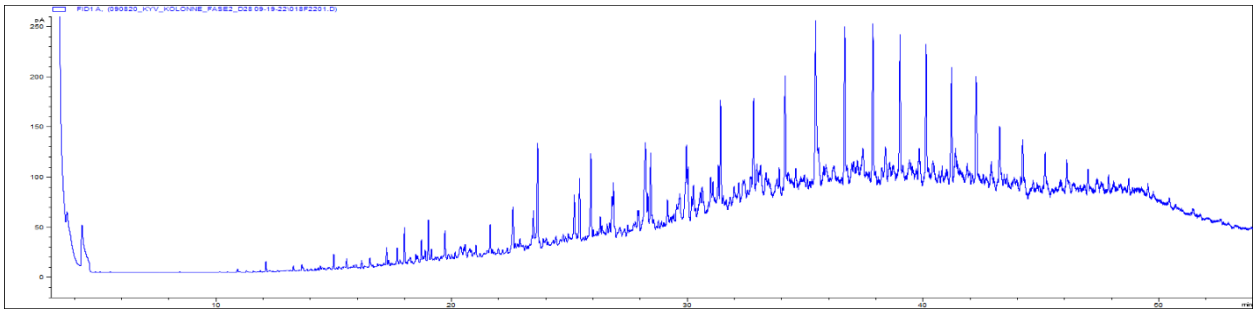

Figure C 30 *COL10-2-3S-D28: Column 10, sediment premixed with 2 g oil, 3 g fertilizer on surface. Sampled on day 28 (SINTEF ID 2020-4947).*

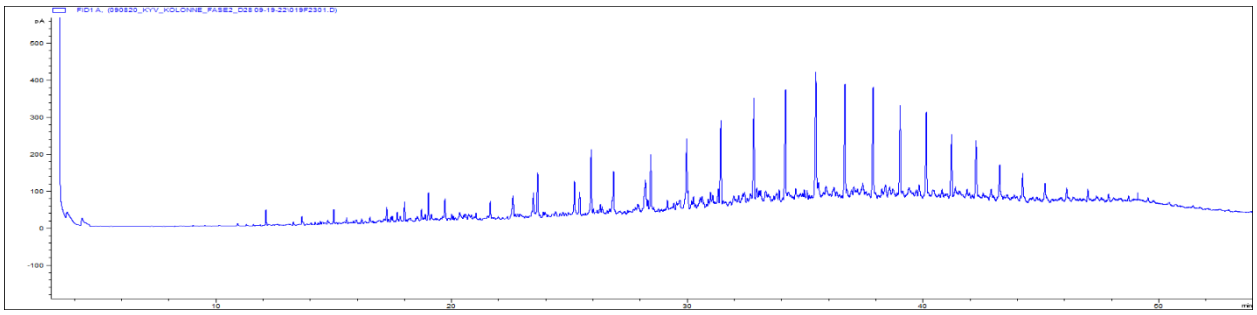

Figure C 31 *COL11-6-3S-D28: Column 11, sediment premixed with 6 g oil, 3 g fertilizer on surface. Sampled on day 28 (SINTEF ID 2020-4948).*

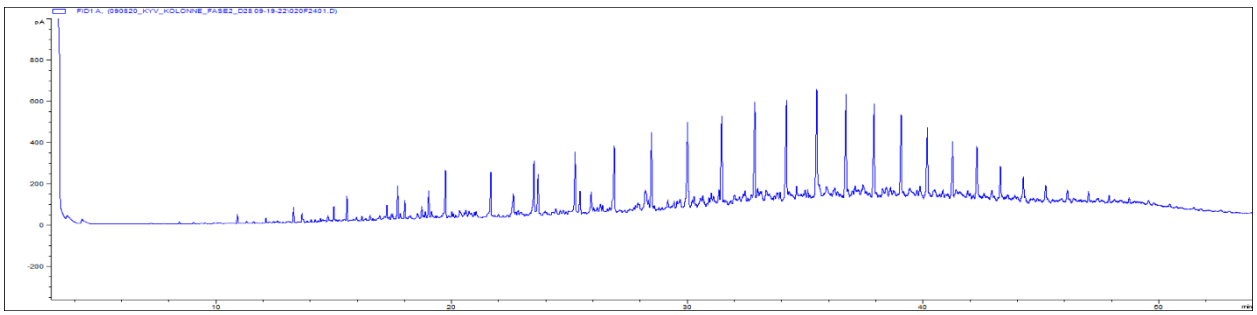

Figure C 32 *COL12-18-3S-D28: Column 12, sediment premixed with 18 g oil, 3 g fertilizer on surface. Sampled on day 28 (SINTEF ID 2020-4949).*

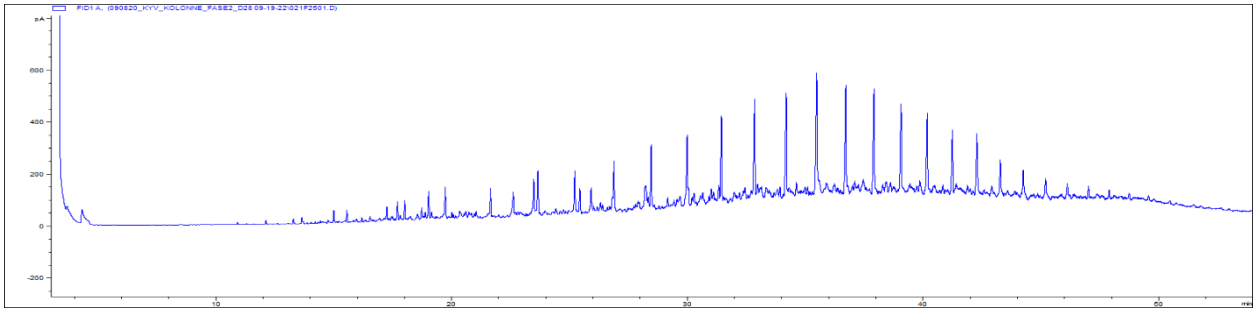

Figure C 33 *COL13-2-0-D28: Column 13, sediment premixed with 2 g oil, no fertilizer added. Sampled on day 28 (SINTEF ID 2020-4950).*

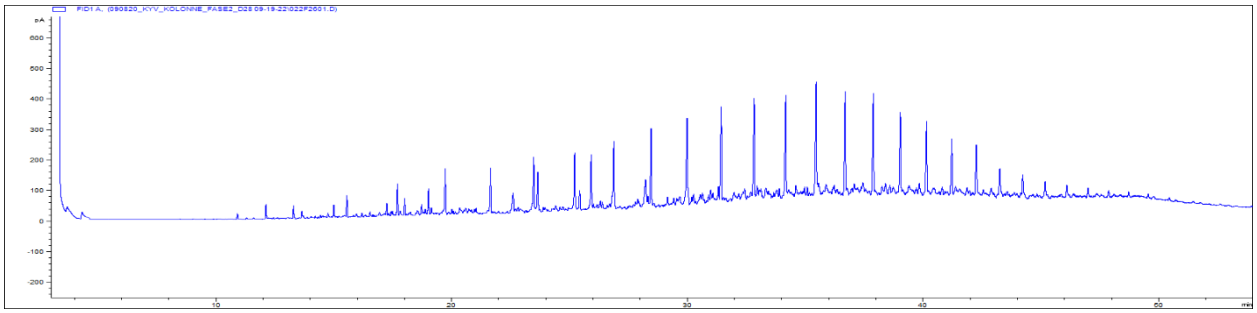

Figure C 34 *COL14-6-0-D28: Column 14, sediment premixed with 6 g oil, no fertilizer added. Sampled on day 28 (SINTEF ID 2020-4951).*

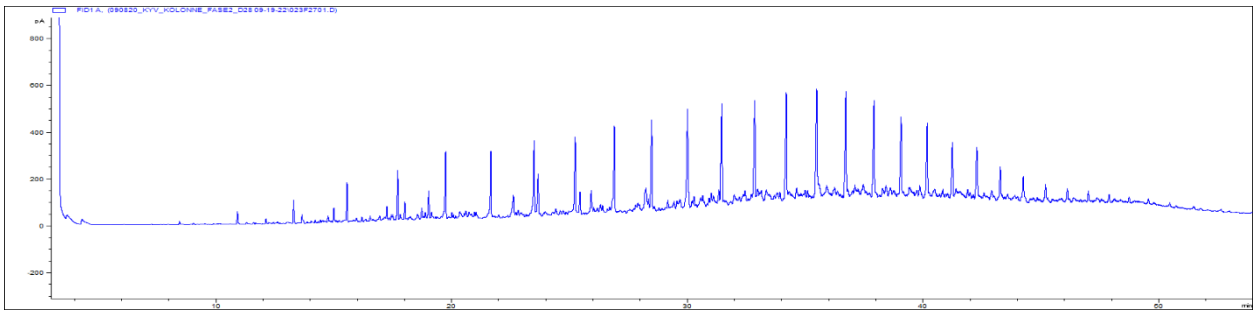

Figure C 35 *COL15-18-0-D28: Column 15, sediment premixed with 18 g oil, no fertilizer added. Sampled on day 28 (SINTEF ID 2020-4952).*

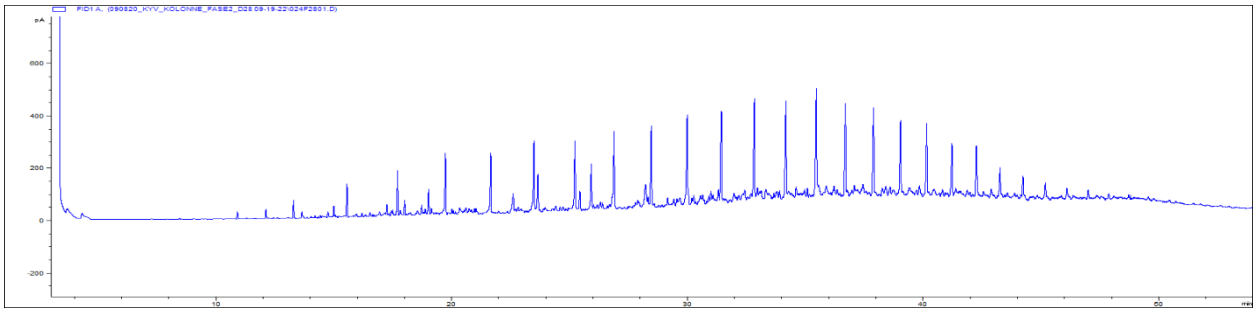

Figure C 36 *COL16-6S-0-D28: Column 16, oil on sediment surface, no fertilizer added. Sampled on day 28 (SINTEF ID 2020-4953).*

Chromatograms from day 56

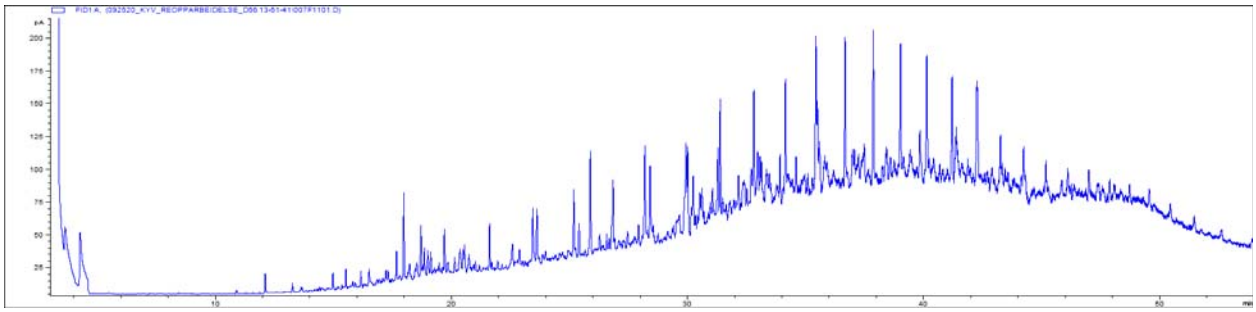

Figure C 37 *COL1-2-6-D56: Column 1, sediment premixed with 2 g oil and 6 g fertilizer. Sampled on day 56 (SINTEF ID 2020-4954).*

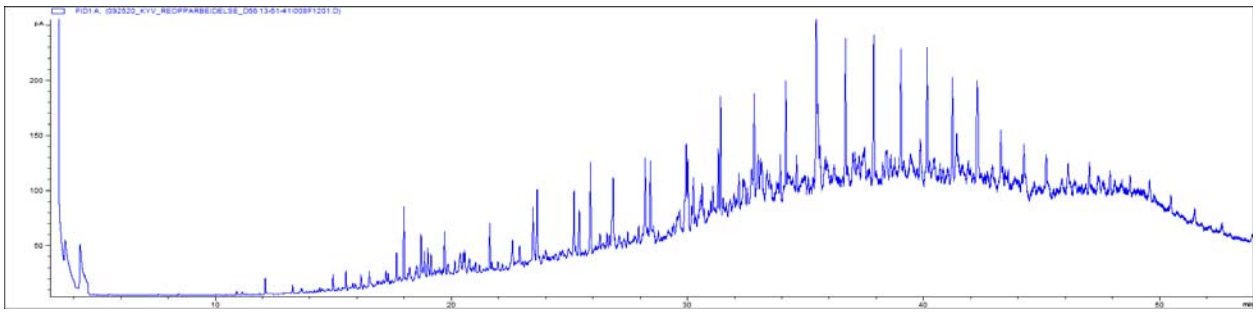

Figure C 38 *COL2-2-3-D56: Column 2, sediment premixed with 2 g oil and 3 g fertilizer. Sampled on day 56 (SINTEF ID 2020-4955).*

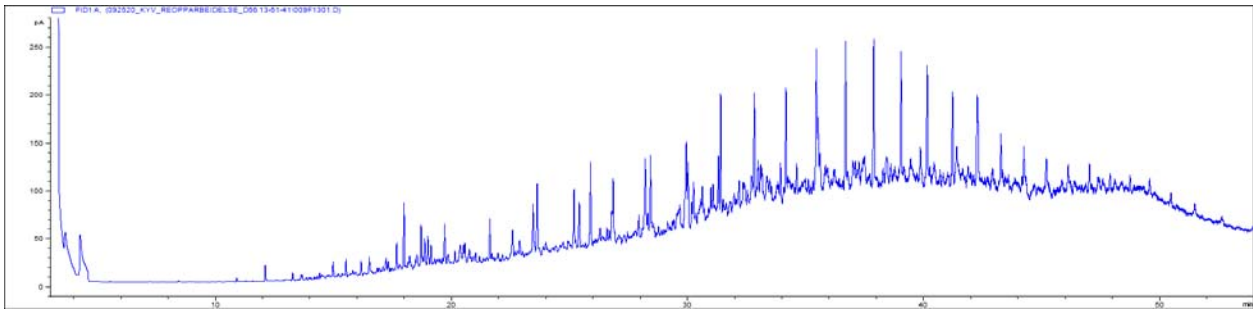

Figure C 39 *COL3-2-1-D56: Column 3, sediment premixed with 2 g oil and 1 g fertilizer. Sampled on day 56 (SINTEF ID 2020-4956).*

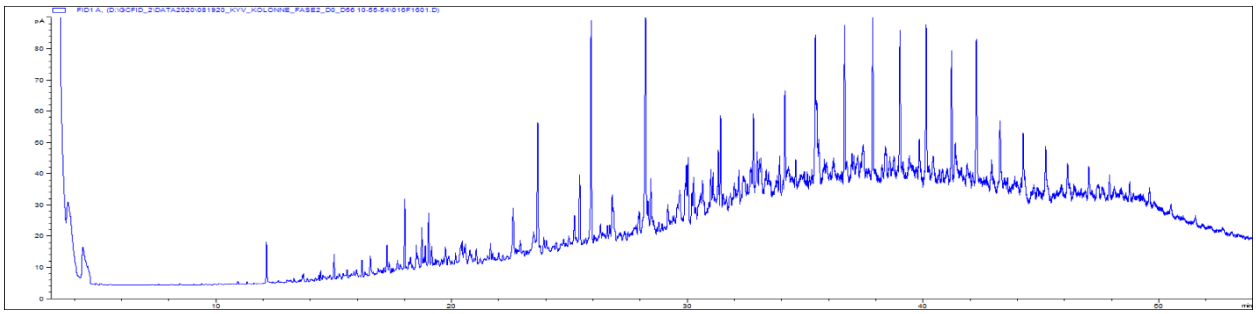

Figure C 40 *COL4-6-6-D56: Column 4, sediment premixed with 6 g oil and 6 g fertilizer. Sampled on day 56 (SINTEF ID 2020-4957).*

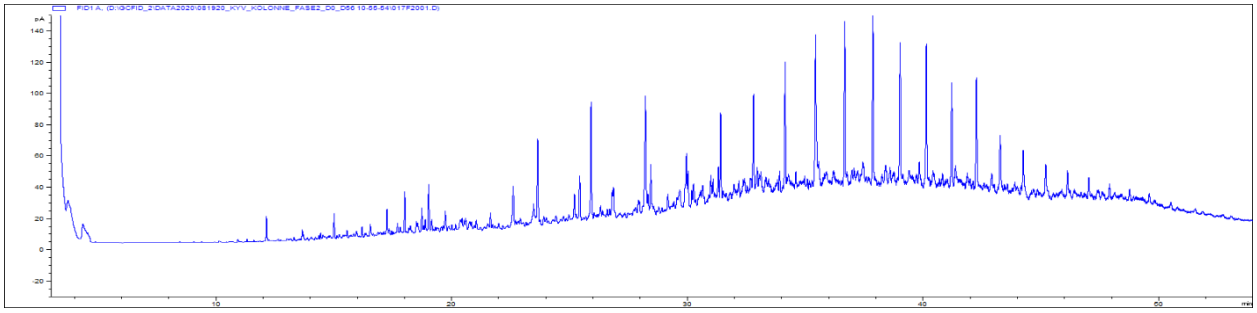

Figure C 41 *COL5-6-3-D56: Column 5, sediment premixed with 6 g oil and 3 g fertilizer. Sampled on day 56 (SINTEF ID 2020-4958).*

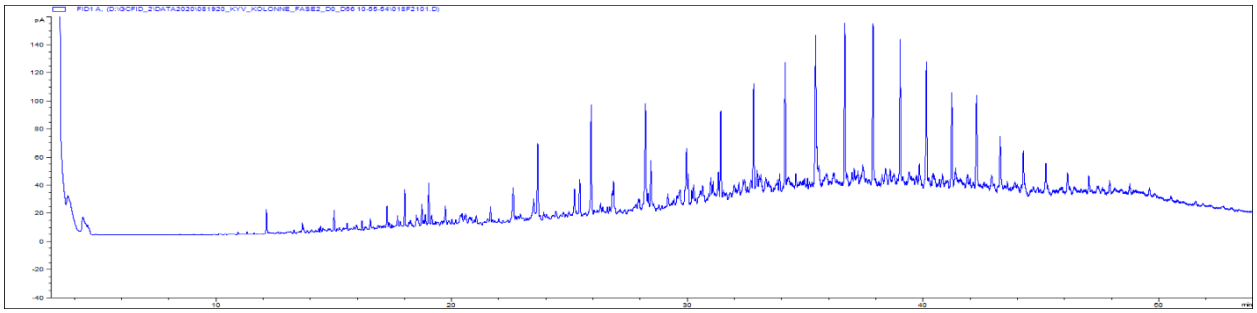

Figure C 42 *COL6-6-1-D56: Column 6, sediment premixed with 6 g oil and 1 g fertilizer. Sampled on day 56 (SINTEF ID 2020-4959).*

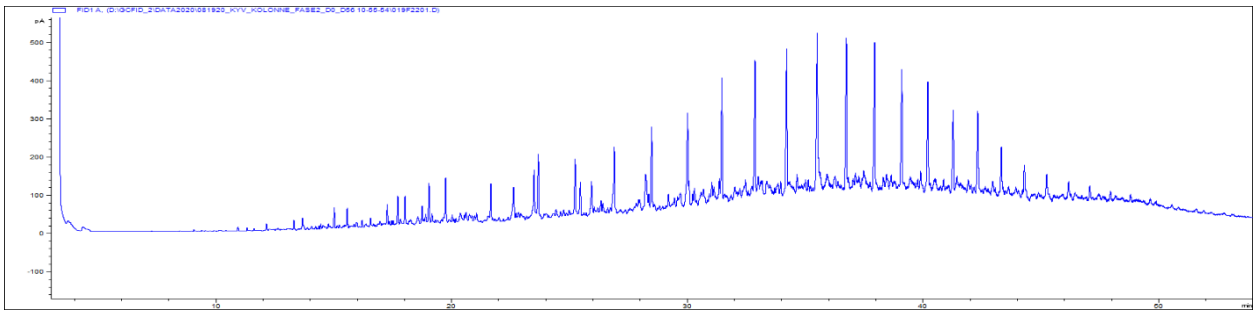

Figure C 43 *COL7-18-6-D56: Column 7, sediment premixed with 6 g oil and 6 g fertilizer. Sampled on day 56 (SINTEF ID 2020-4960).*

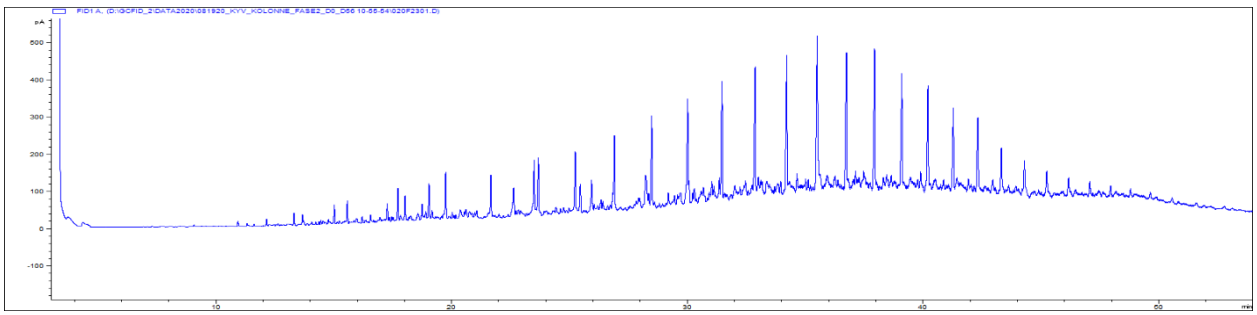

Figure C 44 *COL8-18-3-D56: Column 8, sediment premixed with 6 g oil and 3 g fertilizer. Sampled on day 56 (SINTEF ID 2020-4961).*

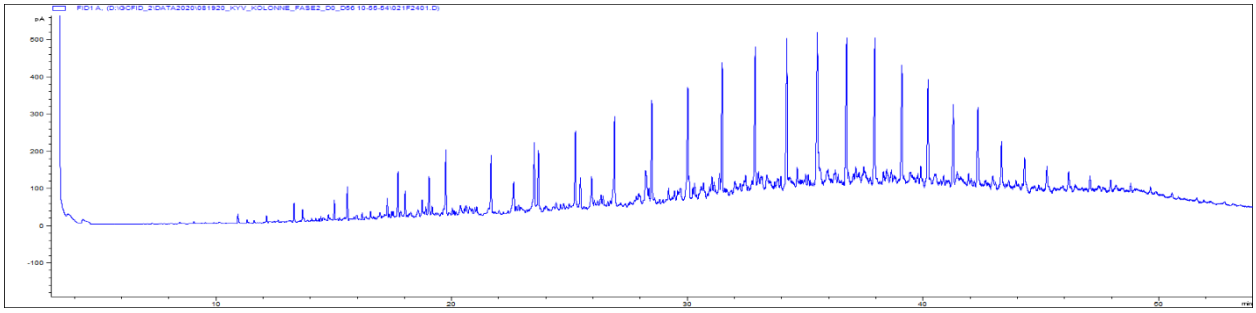

Figure C 45 *COL9-18-1-D56: Column 9, sediment premixed with 6 g oil and 1 g fertilizer. Sampled on day 56 (SINTEF ID 2020-4962).*

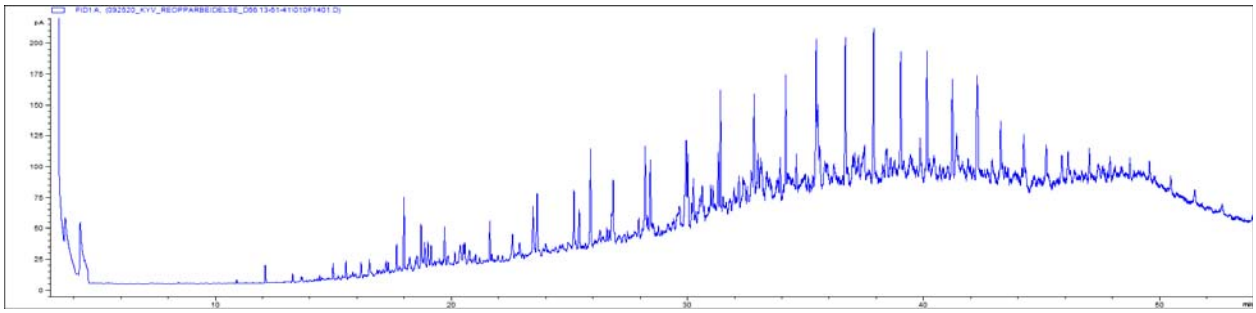

Figure C 46 *COL10-2-3S-D56: Column 10, sediment premixed with 2 g oil, 3 g fertilizer on surface. Sampled on day 56 (SINTEF ID 2020-4963).*

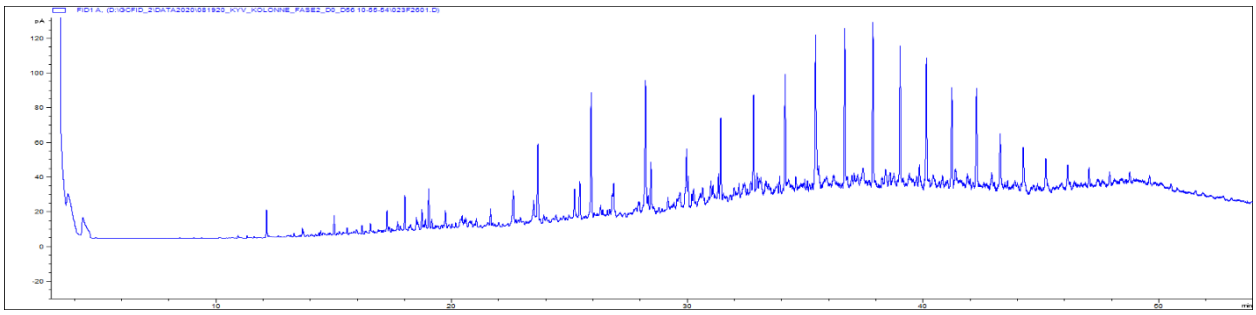

Figure C 47 *COL11-6-3S-D56: Column 11, sediment premixed with 6 g oil, 3 g fertilizer on surface. Sampled on day 56 (SINTEF ID 2020-4948).*

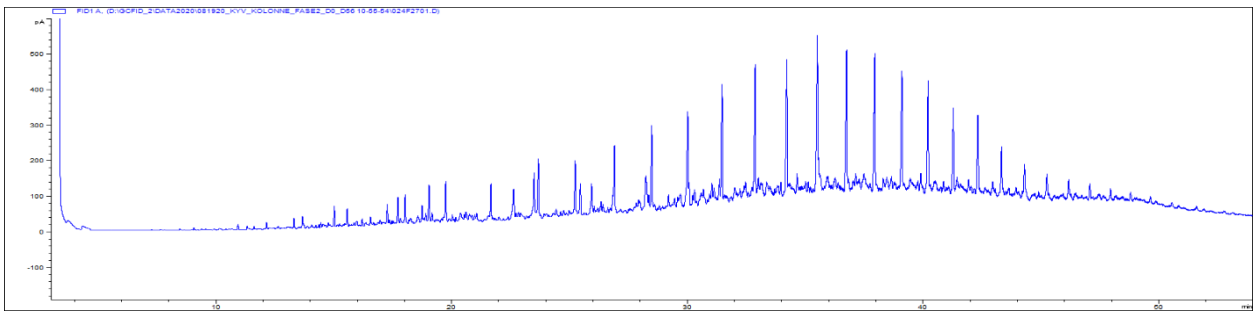

Figure C 48 *COL12-18-3S-D56: Column 12, sediment premixed with 18 g oil, 3 g fertilizer on surface. Sampled on day 56 (SINTEF ID 2020-4964).*

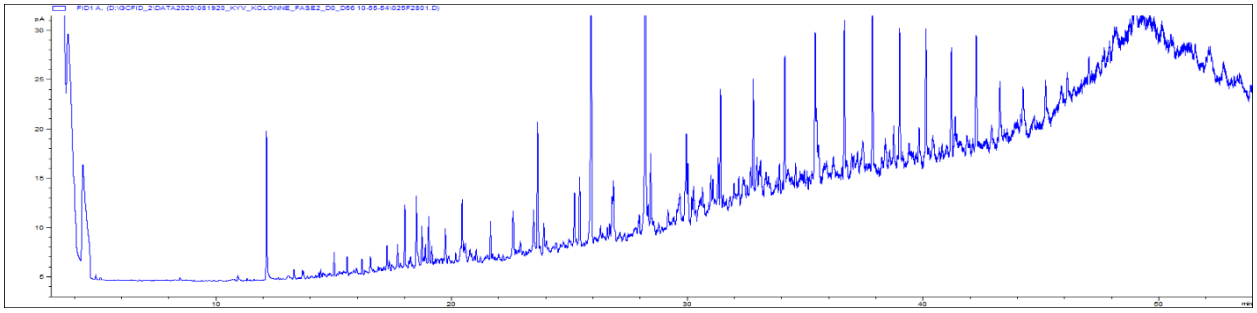

Figure C 49 *COL13-2-0-D56: Column 13, sediment premixed with 2 g oil, no fertilizer added. Sampled on day 56 (SINTEF ID 2020-4965).*

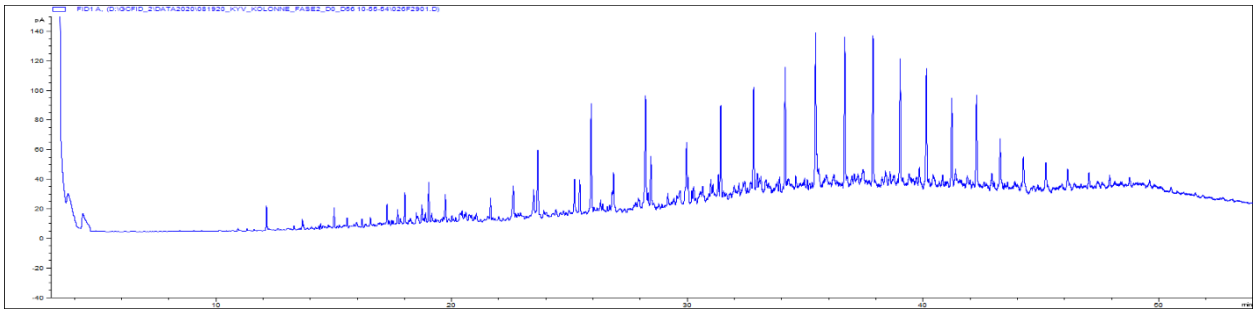

Figure C 50 *COL14-6-0-D56: Column 14, sediment premixed with 6 g oil, no fertilizer added. Sampled on day 56 (SINTEF ID 2020-4966).*

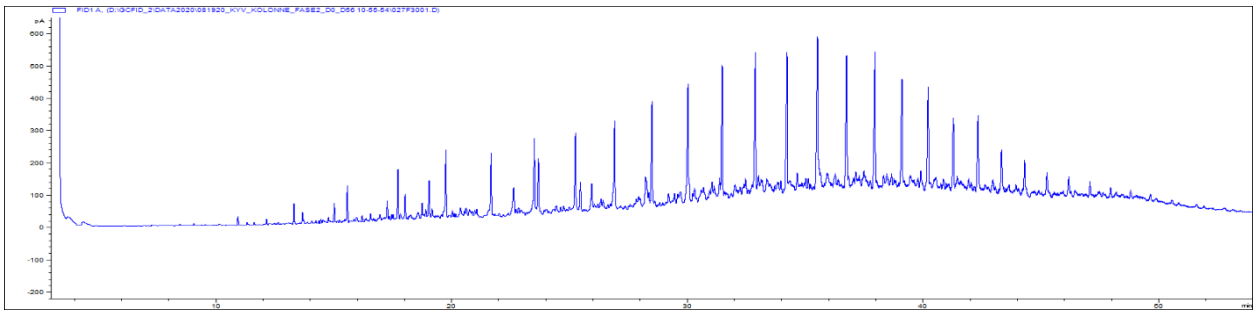

Figure C 51 *COL15-18-0-D56: Column 15, sediment premixed with 18 g oil, no fertilizer added. Sampled on day 56 (SINTEF ID 2020-4967).*

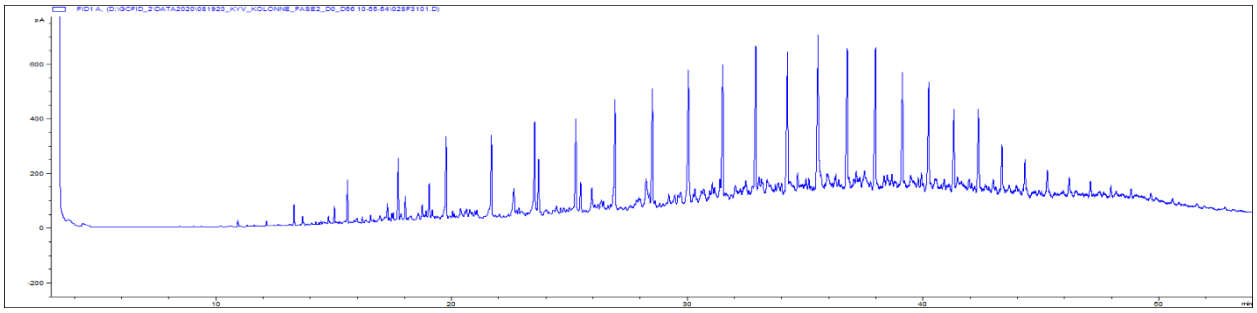

Figure C 52 *COL16-6S-0-D56: Column 16, oil on sediment surface, no fertilizer added. Sampled on day 56 (SINTEF ID 2020-4968).*

D Attachment D Overview of target analytes and component groups

Table D 1 *Overview target analytes with abbreviation (SVOC: Semi volatile organic compounds and THC: Total extractable organic material, UCM. Unresolved organic materials).*

Group	Compound	Abb	Group	Compound	Abb
Decalins	Decalin	DE	4-6 ring PAHs	Fluoranthene	FL
	C1-decalins	DE1		Pyrene	PY
	C2-decalins	DE2		C1-fluoranthrenes/pyrenes	FL1
	C3-decalins	DE3		C2-fluoranthenes/pyrenes	FL2
	C4-decalins	DE4		C3-fluoranthenes/pyrenes	FL3
Naphthalenes	Naphthalene	N		Benz[*a*]anthracene	BA
	C1-naphthalenes	N1		Chrysene	C
	C2-naphthalenes	N2		C1-chrysenes	C1
	C3-naphthalenes	N3		C2-chrysenes	C2
	C4-naphthalenes	N4		C3-chrysenes	C3
2-3 ring PAHs	Benzo(b)thiophene	BT		C4-chrysenes	C4
	Biphenyl	B		Benzo[*b*]fluoranthene	BBF
	Acenaphthylene	ANY		Benzo[*k*]fluoranthene	BKF
	Acenaphthene	ANA		Benzo[*e*]pyrene	BEP
	Dibenzofuran	DBF		Benzo[*a*]pyrene	BAP
	Fluorene	F		Perylene	PE
	C1-fluorenes	F1		Indeno[*1,2,3-c,d*]pyrene	IN
	C2-fluorenes	F2		Dibenz[*a,h*]anthracene	DBA
	C3-fluorenes	F3		Benzo(g,h,i)perylene	BPE
	Phenanthrene	P	Hopane	17α(H),21β(H)-hopane (C30)	HOP
	Anthracene	A			
	C1-phenanthrenes/anthracenes	P1	THC	C_{10}-C_{36}	
	C2-phenanthrenes/anthracenes	P2		C_{17}	
	C3-phenanthrenes/anthracenes	P3		Pristane	
	C4-phenanthrenes/anthracenes	P4		C_{18}	
	Dibenzothiophene	D		Phytane	
	C1-dibenzothiophenes	D1			
	C2-dibenzothiophenes	D2	UCM	THC - SVOC	
	C3-dibenzothiophenes	D3			
	C4-dibenzothiophenes	D4			

E Attachment E DNA concentrations

Table E 1 *Overview of samples for DNA analyses, and DNA concentrations measured Qubit and Nanodrop analyses. Nanodrop analyses also included ratios 260/280 and 260/230 for determination of sample DNA quality. The DNA content in some samples with low DNA concentrations were subject to amplification by whole genome amplification (WGA), as shown under Comment.*

No.	Sample ID	Qubit		NanoDrop			
		Date	ng/µl	Date	ng/µl	260/280	260/230
KyV1	Clean-Sediment	7.9.2020	Too low	4.9.2020	9,8	2,24	0,02
KyV2	REF-2-D0	7.9.2020	Too low	4.9.2020	10,1	1,92	0,02
KyV3	REF-6-D0	7.9.2020	Too low	4.9.2020	7,9	2,64	0,01
KyV4	REF-18-D0	7.9.2020	Too low	4.9.2020	10,1	1,67	0,02
KyV5	COL1-2-6-D14	7.9.2020	13,90	4.9.2020	21,0	1,86	0,04
KyV6	COL2-2-3-D14	7.9.2020	9,64	4.9.2020	19,6	1,74	0,03
KyV7	COL3-2-1-D14	7.9.2020	0,49	4.9.2020	9,8	2,12	0,02
KyV8	COL4-6-6-D14	7.9.2020	8,12	4.9.2020	18,5	1,87	0,03
KyV9	COL5-6-3-D14	7.9.2020	1,37	4.9.2020	11,1	1,92	0,02
KyV10	COL6-6-1-D14	7.9.2020	0,75	4.9.2020	9,9	2,42	0,02
KyV11	COL7-18-6-D14	7.9.2020	1,14	4.9.2020	11,2	1,87	0,02
KyV12	COL8-18-3-D14	7.9.2020	0,74	4.9.2020	13,6	1,77	0,03
KyV13	COL9-18-1-D14	7.9.2020	0,02	4.9.2020	8,2	1,92	0,01
KyV14	COL10-2-3S-D14	7.9.2020	4,20	4.9.2020	11,6	2,38	0,02
KyV15	COL11-6-3S-D14	7.9.2020	3,26	4.9.2020	15,9	1,48	0,02
KyV16	COL12-18-3S-D14	7.9.2020	0,58	4.9.2020	7,9	1,83	0,02
KyV17	COL13-2-0-D14	7.9.2020	0,08	4.9.2020	8,5	0,86	0,01
KyV18	COL14-6-0-D14	7.9.2020	0,06	4.9.2020	8,8	0,66	0,01
KyV19	COL15-18-0-D14	7.9.2020	0,03	4.9.2020	7,3	0,36	0,02
KyV20	COL16-6S-0-D14	7.9.2020	0,01	4.9.2020	7,9	2,17	0,01
KyV21	COL1-2-6-D28	7.9.2020	13,60	4.9.2020	20,7	2,07	0,03
KyV22	COL2-2-3-D28	7.9.2020	8,16	4.9.2020	13,9	1,94	0,02
KyV23	COL3-2-1-D28	7.9.2020	12,00	4.9.2020	16,6	1,94	0,03
KyV24	COL4-6-6-D28	7.9.2020	15,10	4.9.2020	22,0	1,94	0,04
KyV25	COL5-6-3-D28	9.9.2020	4,32	9.9.2020	12,1	1,83	0,02
KyV26	COL6-6-1-D28	9.9.2020	8,96	9.9.2020	17,5	1,89	0,03
KyV27	COL7-18-6-D28	9.9.2020	4,36	9.9.2020	12,9	1,73	0,02
KyV28	COL8-18-3-D28	9.9.2020	2,82	9.9.2020	11,3	1,59	0,02
KyV29	COL9-18-1-D28	9.9.2020	0,32	9.9.2020	9,4	1,46	0,02
KyV30	COL10-2-3S-D28	9.9.2020	6,72	9.9.2020	18,3	1,86	0,03
KyV31	COL11-6-3S-D28	9.9.2020	5,88	9.9.2020	20,1	1,75	0,03
KyV32	COL12-18-3S-D28	9.9.2020	3,91	9.9.2020	19,1	1,56	0,03
KyV33	COL13-2-0-D28	9.9.2020	0,94	9.9.2020	11,9	1,63	0,02
KyV34	COL14-6-0-D28	9.9.2020	0,97	9.9.2020	14,0	1,65	0,02
KyV35	COL15-18-0-D28	9.9.2020	0,22	9.9.2020	12,3	1,65	0,02
KyV36	COL16-6S-0-D28	9.9.2020	Too low	9.9.2020	10,7	1,42	0,02
KyV37	COL1-2-6-D56	9.9.2020	22,40	9.9.2020	39,5	1,77	0,06
KyV38	COL2-2-3-D56	9.9.2020	10,20	9.9.2020	24,3	1,73	0,40
KyV39	COL3-2-1-D56	9.9.2020	10,70	9.9.2020	24,5	1,76	0,04
KyV40	COL4-6-6-D56	9.9.2020	36,40	9.9.2020	56,8	1,86	0,09
KyV41	COL5-6-3-D56	9.9.2020	16,90	9.9.2020	33,4	1,77	0,05
KyV42	COL6-6-1-D56	9.9.2020	17,60	9.9.2020	31,3	1,92	0,05
KyV43	COL7-18-6-D56	9.9.2020	15,00	9.9.2020	30,5	1,65	0,05
KyV44	COL8-18-3-D56	9.9.2020	12,30	9.9.2020	27,5	1,66	0,04
KyV45	COL9-18-1-D56	9.9.2020	3,67	9.9.2020	14,1	1,74	0,03
KyV46	COL10-2-3S-D56	9.9.2020	11,30	9.9.2020	23,0	1,78	0,04
KyV47	COL11-6-3S-D56	9.9.2020	17,80	9.9.2020	33,1	1,65	0,05
KyV48	COL12-18-3S-D56	9.9.2020	15,20	9.9.2020	33,5	1,60	0,06
KyV49	COL13-2-0-D56	9.9.2020	5,08	9.9.2020	16,9	1,71	0,03
KyV50	COL14-6-0-D56	9.9.2020	12,90	9.9.2020	26,1	1,80	0,04
KyV51	COL15-18-0-D56	9.9.2020	5,44	9.9.2020	15,7	1,58	0,03
KyV52	COL16-6S-0-D56	9.9.2020	0,02	9.9.2020	8,3	1,56	0,01
WGA1	Clean-Sediment	13.11.2020	540	13.11.2020	2080,7	1,88	1,94
WGA2	REF-2-D0	13.11.2020	972	13.11.2020	1920,7	1,94	2,09
WGA3	REF-6-D0	13.11.2020	981	13.11.2020	2128,0	1,87	1,98
WGA4	REF-18-D0	13.11.2020	765	13.11.2020	1728,2	1,89	2,01
WGA20	COL16-6S-0-D14	13.11.2020	796	13.11.2020	1738,7	1,74	1,90
WGA36	COL16-6S-0-D28	13.11.2020	692	13.11.2020	1627,6	1,73	1,94
WGA52	COL16-6S-0-D56	13.11.2020	780	13.11.2020	1508,2	1,86	1,96

F Attachment F - 16S rRNA amplicon analyses report

Table F1 *Analysed sample information*

```
OTU Table:          [ 696 taxa and 51 samples ]
Sample Data:        [ 51 samples by 10 sample variables ]
Taxonomy Table:     [ 696 taxa by 7 taxonomic ranks ]
Phylogenetic Tree: [ 696 tips and 692 internal nodes ]
DNAStringSet:       [ 696 reference sequences ]
```

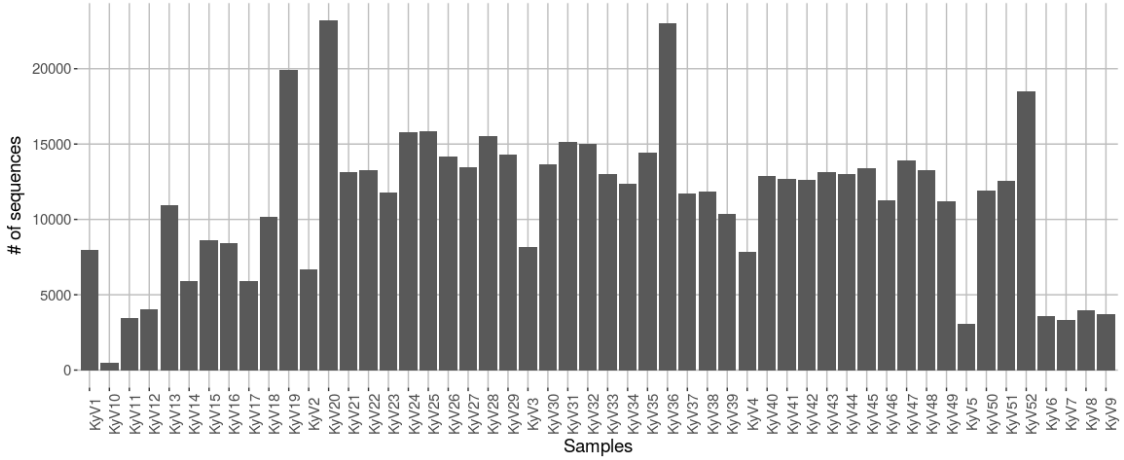

Figure F1 *Sequencing depth*

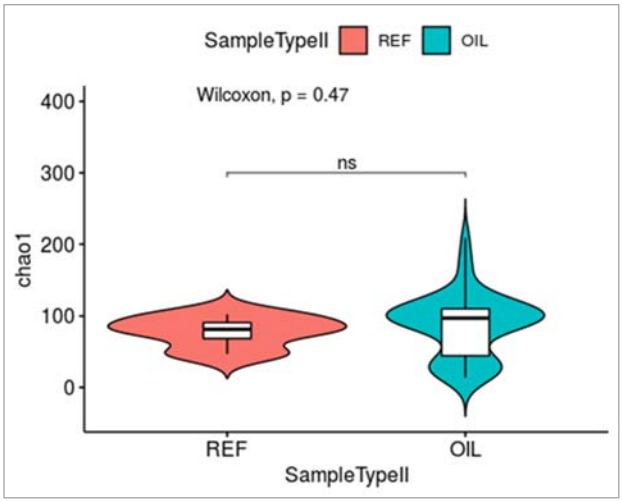

Figure F2 *Alpha-diversity between reference (clean sediment and REF samples from day 0) and oil amended samples. The diversity is based numbers of species (Chao1).*

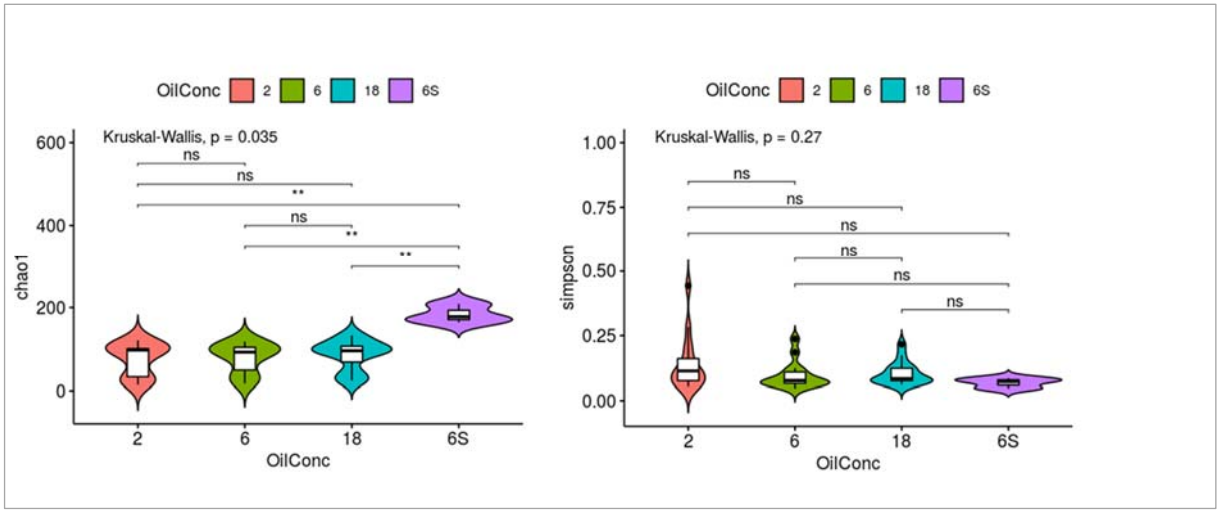

Figure F3 *Alpha-diversity between treatments amended with different oil concentrations*

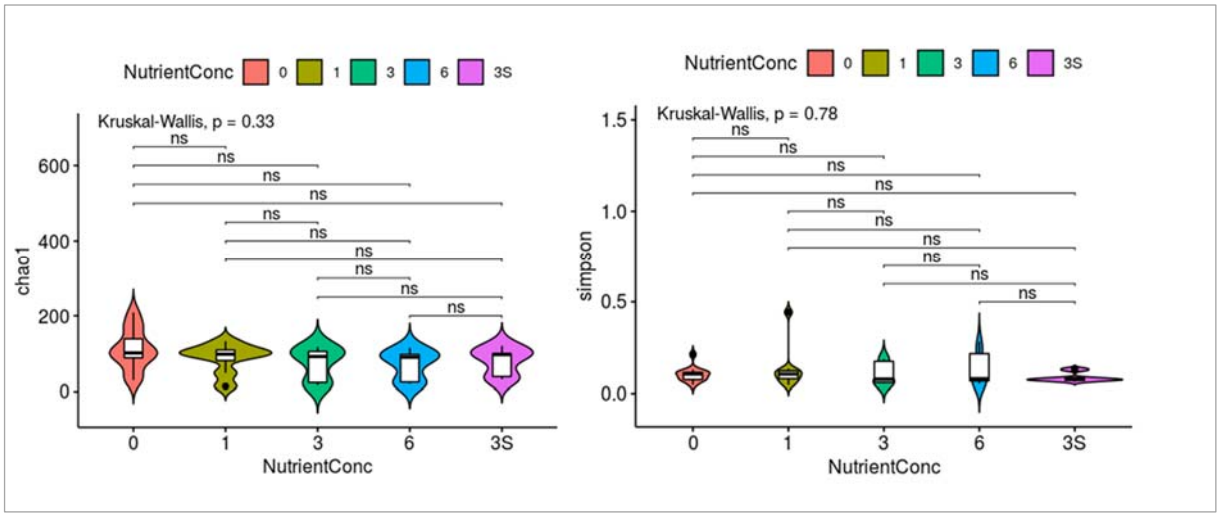

Figure F4 *Alpha-diversity between treatments amended with different nutrient concentrations*

Table F2 *ADONIS and BETADISPER output of beta-diversity significance test for different oil amendment and incubation times.*

ADONIS	p-value	BETADISPER
REF vs OIL	0.001***	0.004***
D14 vs D28	0.001***	0.88
D14 vs D56	0.001***	
D28 vs D56	0.001***	

Table F3 *ADONIS and BETADISPER output of beta-diversity significance test for different oil and nutrient amendment.*

ADONIS	p-value	BETADISPER	ADONIS	p-value	BETADISPER
Oil treatments			Nutrient treatments		
18 vs 2	0.037*	0.30	6 vs 3	0.97	0.99
18 vs 6	0.49		6 vs 1	0.65	
18 vs 6S	0.006**		6 vs 3S	0.53	
2 vs 6	0.065		6 vs 0	0.05	
2 vs 6S	0.053*		3 vs 1	0.71	
6 vs 6S	0.009**		3 vs 3S	0.69	
			3 vs 0	0.06	
			1 vs 3S	0.75	
			1 vs 0	0.49	
			3S vs 0	0.14	

Table F4A The most discriminant taxa in reference treatment

ASVs	comp1	comp2	Order	Family	Genus
912733cf376ea8b4d1c654032e072c5c	0.19563352	0.000000000	Cellvibrionales	Spongiibacteraceae	Zhongshania
0869fad5736b149bc352577b9588d74e	0.18588023	0.000000000	Alteromonadales	Marinobacteraceae	Marinobacter
c5ffee926feb3aefea98e593da6035e7	0.18421516	0.000000000	Salinisphaerales	Salinisphaeraceae	Salinisphaera
be36e96fe24adebd5c5629ed4247985b	0.18390096	0.000000000	Oceanospirillales	Halomonadaceae	Halomonas
5adfcdf34deaa637ab4262e3f7bfaf34	0.17602425	0.000000000	Corynebacteriales	Nocardiaceae	Rhodococcus
4eb998cb85806f2000c03c4a213af927	0.15871275	0.000000000	Alteromonadales	Idiomarinaceae	Idiomarina
d7f7d470407525c6f8742f81b4d2fd36	0.15612768	0.000000000	Pseudomonadales	Pseudomonadaceae	Pseudomonas
887bce01b30aad5a07c25741f18c3907	0.14836400	0.109898060	Betaproteobacteriales	Burkholderiaceae	Ralstonia
78475488a87b86a642507db9870da747	0.14030994	0.000000000	Nitrosococcales	Methylophagaceae	uncultured
f50212a7073572ba2248eebd4ec76c5f	0.13566856	0.000000000	Oceanospirillales	Halomonadaceae	Salinicola
3ffe019fe9d74cfc755bfa931dfaf002	0.13366670	0.000000000	Rhizobiales	Beijerinckiaceae	Methylobacterium
cdcb3f413d0b9e3b7dd753610dfe0008	0.13359433	-0.003096683	Salinisphaerales	Solimonadaceae	Polycyclovorans
65d861d1b000389cb425a9c124821149	0.13218355	0.000000000	Enterobacteriales	Enterobacteriaceae	Serratia
f22cf6867cce24297d1d9e244fe39617	0.13189620	0.000000000	Nitrosococcales	Methylophagaceae	Methylophaga
d2bd64c25841896798d5e0feb2859a25	0.13000338	0.000000000	Rhodobacterales	Rhodobacteraceae	Pseudophaeobacter
96270810873813f11d23891e18ccd07f	0.12810362	0.000000000	Oceanospirillales	Saccharospirillaceae	Oceanobacter
d59be9118876dadb2c344a4bf1363c05	0.12734911	0.000000000	Propionibacteriales	Propionibacteriaceae	Cutibacterium
baef4cdc02b661015cfbd27d5cd8f003	0.12677597	0.000000000	Campylobacterales	Arcobacteraceae	Arcobacter
af637cd761d28f9946f605ee82b064bb	0.12647387	0.000000000	Caulobacterales	Caulobacteraceae	Brevundimonas
ba6475616b221791a76298cd85c83355	0.12316991	0.000000000	Betaproteobacteriales	Methylophilaceae	Methylotenera
ba06be5d9fc616d3f792e1dc2e9640a6	0.11712376	0.000000000	Oceanospirillales	Saccharospirillaceae	Oceaniserpentilla
4c37ff08892b05c95cc466cd09811873	0.11450193	0.000000000	Vibrionales	Vibrionaceae	Vibrio
d10d1dd9c568b8fb68232b62b55c45c9	0.11271323	0.000000000	Rhodobacterales	Rhodobacteraceae	uncultured
a98a1fdf0a306cbae119204712376e1d	0.11262904	0.000000000	Micrococcales	Micrococcaceae	Renibacterium
9f887a05a57c4baae8797cd130fb3c98	0.11197587	-0.203917271	Pseudomonadales	Moraxellaceae	Enhydrobacter
47711caa1a5c7e601cfdb17358c0040b	0.11061119	0.000000000	Sphingomonadales	Sphingomonadaceae	Sphingomonas
75f66a6da86aeded2bf6e2322b3e790c	0.10765676	0.000000000	Cellvibrionales	Porticoccaceae	Porticoccus
81d8c71ccd177a080957b62aacc49bcf	0.10563103	0.000000000	Parvibaculales	PS1 clade	uncultured bacterium
5bed72441d42faea56f412e4ef09739b	0.10243802	0.000000000	Bacillales	Staphylococcaceae	Staphylococcus
b8ecf6ec5cc4215ab3954b72f41f0912	0.09890797	0.000000000	Corynebacteriales	Corynebacteriaceae	Corynebacterium 1
cf1524f5edb2ff04837decf92db1cc03	0.09605897	0.000000000	Pseudomonadales	Moraxellaceae	Acinetobacter

Table F4B Most discriminant taxa in oil treatment

ASVs	comp1	comp2	Order	Family	Genus
5f9567973d95c48047eadf47a9021b52	-0.07377726	0.02762797	Sphingomonadales	Sphingomonadaceae	Sphingorhabdus
53a96445cd58bf0a186afc143e155427	-0.06169153	0.00000000	Rhodobacterales	Rhodobacteraceae	Celeribacter
1317a9e09148ecdf60b876467e5e3715	-0.05620415	0.00000000	Alteromonadales	Pseudoalteromonadaceae	Pseudoalteromonas
9b60aff542bd32473c70ec7657ed856d	-0.05319521	0.08982010	Caulobacterales	Hyphomonadaceae	Hyphomonas
6456d76356e3558f049e0747d4932f87	-0.05022865	0.05216969	Rhodobacterales	Rhodobacteraceae	Halocynthiibacter
52c6016f6082944ca63feecc10b171a7	-0.04979714	0.00000000	Alteromonadales	Colwelliaceae	Colwellia
ed671393e1b982c5edc8eb61c8d3c7b1	-0.04894271	0.10347096	Rhodobacterales	Rhodobacteraceae	Sulfitobacter
57baee6dcb1818b19b2e4b406c2065de	-0.04633976	0.00000000	Oceanospirillales	Saccharospirillaceae	Oleibacter
1bfad8ba57a5b81d0d65a2ea48b9b3e6	-0.04451493	-0.35600173	Oceanospirillales	Saccharospirillaceae	Oleispira
55ae6d40702296801ad34c31232fa486	-0.04207407	0.00000000	Rhodobacterales	Rhodobacteraceae	Sedimentitalea
b76d650604e0e6fc85c3830379806222	-0.04046771	0.00000000	Rhizobiales	Rhizobiaceae	Cohaesibacter
9da210e48863c0d2b476b1febeda07a7	-0.03977941	0.00000000	Cellvibrionales	Halieaceae	Halioglobus
3fdb1a778cecc49ddec883894e4839f3	-0.03514237	0.00000000	Flavobacteriales	Flavobacteriaceae	Dokdonia
eea5e5b09420f692877b2f94a2fa6f4d	-0.03451057	0.01594404	Rhodobacterales	Rhodobacteraceae	Roseobacter clade NAC11-7 lineage
d821ae302f87b3bb0c7ccd184bfa8e27	-0.03414224	0.00000000	Oceanospirillales	Marinomonadaceae	Marinomonas
6edb0ae0d8bf71f3bb45db471c58ed1f	-0.03401748	0.00000000	Alteromonadales	Alteromonadaceae	Alteromonas
58ad6e7cdece5a44d2b2462665aefeaf	-0.03359745	0.00000000	Flavobacteriales	Flavobacteriaceae	Lutibacter
577afd67775f9fdd64ef1ae47119814c	-0.03292480	0.00000000	Flavobacteriales	Flavobacteriaceae	Muricauda
8b0a8d601e3a1b36faf3b3cfed560dd2	-0.03175403	0.00000000	Flavobacteriales	Flavobacteriaceae	[Polaribacter] huanghezhanensis
e73bb78966ee7a257013a78ff1596daa	-0.03052723	0.00000000	Flavobacteriales	Flavobacteriaceae	Ulvibacter
8741deb7a5d2efa1eb6dfd2f3d70e1b0	-0.02992066	0.00000000	Flavobacteriales	Flavobacteriaceae	Pseudofulvibacter
e7eed14495dd93dc00587b5e0c0681c8	-0.02983314	0.00000000	Oceanospirillales	Nitrincolaceae	Profundimonas
f09576ae6bc580f5b6092095b8572c58	-0.02967693	0.00000000	Rhodospirillales	Terasakiellaceae	uncultured
2782e52b407074d5b187943851f09e6e	-0.02838222	0.00000000	Pseudomonadales	Moraxellaceae	Alkanindiges
1f1ab49d87d8d40d459080aabc4773e0	-0.02763608	0.01129000	Flavobacteriales	Flavobacteriaceae	Maribacter
6075113d168159558ad7e1bd972bce78	-0.02708664	0.00000000	Micavibrionales	Micavibrionaceae	uncultured
e43b75e08891747e369eed42f01ef337	-0.02674689	0.00000000	Flavobacteriales	Flavobacteriaceae	Jejudonia
eac92b511bee99a49252c0b87edd0457	-0.02525190	0.00000000	Campylobacterales	Thiovulaceae	Sulfurimonas
453bf81d61730f7bef135c38545ac290	-0.02476300	0.00000000	Rhodobacterales	Rhodobacteraceae	Planktotalea
05b61d27b2bdad505a376a0554b0be67	-0.02451864	0.00000000	Chlamydiales	Simkaniaceae	Candidatus Fritschea
eac0f15f44db0281d0f1bb08b2154e72	-0.02441355	0.03783469	Methylococcales	Cycloclasticaceae	Cycloclasticus

G Toxicity of "slow-releasing fertilizer"

[Report number] - **Restricted**

Internal Report

Toxicity of "slow-releasing fertilizer"

Author(s)
Trond Nordtug
Dag Altin

SINTEF Ocean AS

2021-01-29

SINTEF Ocean AS

Address:
Postboks 4762 Torgarden
NO-7465 Trondheim
NORWAY

Switchboard: +47 46415000

Enterprise /VAT No:
NO 937 357 370 MVA

Internal Report

Toxicity of "slow-releasing fertilizer"

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AUTHOR(S)
Trond Nordtug
Dag Altin

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ABSTRACT

Laboratory experiments with degradation of oil in sediment including slow-release fertilizer indicated that the fertilizer contributed to the observed toxicity of sediment samples. A toxicity test with the pure fertilizer was therefore initiated. A water accommodated fraction was made from 6 g/L fertilizer in seawater and tested for toxicity on early stages of the copepod *Calanus finmarchicus*. A reference test with ammonium chloride was used to estimate the contribution of ammonium/ammonia to the observed toxicity of the fertilizer. The results confirm that that the fertilizer at the highest concentration used in the degradation test (6 g/kg sediment) may contribute to the observed toxicity, and that this toxicity most likely related to the concentration of ammonium/ammonia.

Early stages of *C. finmarchicus* have previously been shown to be rather sensitive to contaminants, and thus expected to be more sensitive than "the average" sediment organisms. Based on the current results, however, we recommend that the amounts of the Plantagen slow-release fertilizer used on shoreline sediments should be maximum 3 g/kg sediment.

PREPARED BY	CHECKED BY	APPROVED BY
Trond Nordtug	Liv-Guri Faksness	

SINTEF

Table of Contents

A Background

Toxicity tests of oiled sediment from degradation experiments in the SINTEF sediment column system indicated increased toxicity of sediments added 6g/kg Plantagen "Slow release fertilizer". The current toxicity testing of the mixture components released form the fertiliser was initiated to test if the addition of nutrient to the sediment may have contributed to the observed increase in toxicity.

B Materials and methods

B.1 Test substance

The tested substance was "Plantagen langtidsvirkende næring" (NPK(Mg)16-6-12 (+1,2)). This is a commercial slow-release fertilizer consisting of a granulate with nutrients incorporated in a matrix of undeclared composition.

Composition of fertilizing agents given by the producer:
16.5% total nitrogen
- 6.4 % Ammonia
- 4.7 % Nitrate
- 5.4 % Urea

6.2 % Phosphorous – soluble in neutral ammonium citrate
5.7 % Phosphorous – water soluble
11.9 % Water soluble potassium
1.2 % Water soluble manganese

B.2 Test organisms

The organisms tested were early stages of the marine copepod *Calanus finmarchicus*. Fertilized eggs of the copepods were collected during a period of 12 hours before they were transferred to the exposure system at a developmental stage of approx. 16 (± 6) hours before hatching. The test period was 72 hours starting with eggs and covered hatching and the first moult from nauplii 1 to nauplii 2 stage. These stages are not feeding; thus, no food was supplies during the test.

B.3 Generation and verification of exposure solutions:

The initial test solution (stock solution) was generated as a water accommodated fraction (WAF) of the fertilizer. 6 g/L of fertilizer was added in 2 litres of sterile filtered (0.2 µm) seawater in a bottle and stirred without vortex (low energy stirring) for 20 hours at 20 °C in darkness (Figure 1). The solution was then left to for 4 hours to allow particles to settle. The water phase was then collected and distributed to nutrient analyses and toxicity testing.

Samples collected:
For toxicity testing: 2 x 200 mL
For nutrient analyses: 2 x 200 mL (NH_4^+), 1 x ca 50 mL (PO_4^{3-}), 1 x ca 50 mL (NO_3^-)

Nutrient analyses were performed by ALS Laboratory Group (Oslo).

Nutrient:	Method
Ammonium + Ammonia as NH_4^+	W-NH_4rv (6073.00)
Phosphate (ortophosphate)	W-PO_4 (6613.30)
Nitrate and nitrite	W-NO_3NO_2NUG (6591.05)

The concentration series used for toxicity testing was prepared by diluting the stock solution in sterile filtered (0.2 µm) seawater in a logarithmic series with 10 concentrations in the range 100 to 0.5 % of the WAF stock solution. Two controls with clean seawater were added as a negative control. A corresponding concentration series of Ammonium chloride (NH_4Cl) was used as a positive control for ammonia and ammonium toxicity.

The concentrations of nutrients are based on analyses of the stock solutions corresponding to the highest exposure concentrations for both the water accommodated fraction of the fertilizer and the positive reference (NH_4Cl) (Table 1). The other exposure concentrations are calculated from the various dilution factors used for these solutions and the analysis of the stock solution. The fraction of ammonia (NH_3) in the samples was calculated from the total Ammonia + ammonium (as NH_4^+) using recorded temperature, pH and salinity in the exposure solutions as input to a TAN SSAC calculator (Garry Payne, DEP, 2019 - https://floridadep.gov/sites/default/files/TAN%20Criteria%20Calculator5.xlsm).

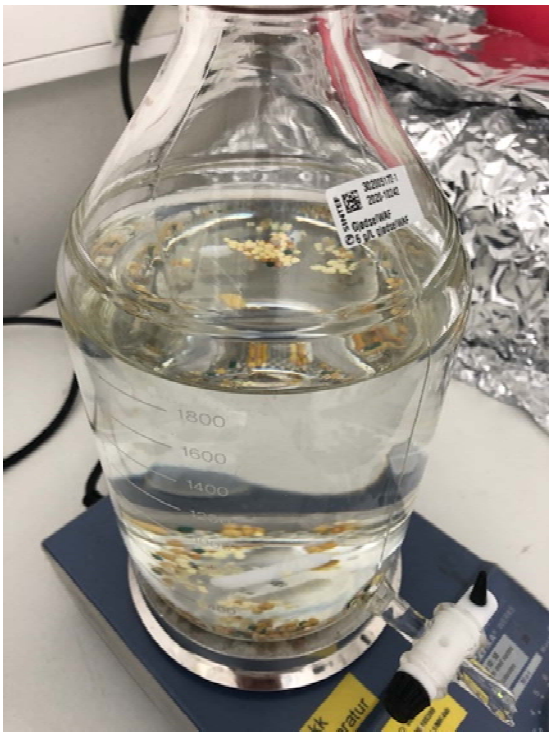

Figure 1. *Equipment for generation of exposure solution. The fertilizer granulate were added to 2 L of seawater (6 g/L) and gently stirred for 20 hours followed by 4 hours of settling before the water phase was collected.*

B.4 Toxicity testing

A toxicity test on early stages of copepods developed by BioTrix and SINTEF previously used for toxicity testing chemicals (Farkas et al. 2020). The test consists of exposure for 72 hours during hatching and the two first nauplii stages (NI and NII) of *C. finmarchicus*.

Testing was performed at 10˚C in 6-well plates with one well plate per concentration tested (6 replicates per concentration). Each well was supplied with 8 ml exposure solution and added approximately 30 fertilized copepod eggs. The copepods were observed every 24 hours for 72 hours whereafter the test was terminated and the final number of hatched eggs and surviving nauplii were recorded. The results presented here are the total mortality (eggs and nauplii) relative to the control groups at the end of exposure.

C Results

C.1 Exposure concentrations

Concentrations of nutrients in the exposure solutions and the highest values recorded in previous sediment column experiments are shown in Table 1. The concentrations in the current WAF from the fertilizer, except for the phosphate, was much higher than those in the surface water in the column experiment (presumably pore water) collected from the columns with 6g/kg fertilizer.

In the concentration series a slight decrease in pH was observed at the two highest WAF concentration (pH 7.76 and 7.99, respectively). For all other groups, the pH was in the range pH 8.05 – 8.12, as expected in sea water. The oxygen saturation was above 95%.

Table 1 Analyses of exposure solutions

	Fertilizer (mg/L)	NH4Cl (mg/L)	Seawater (mg/L)	Columns 6g fertilizer day 1
Ammonium + Ammonia as NH4+	140 (± 21)	26 (± 3,9)	0,013 (± 0,010)	3,05 (± 0,96)
Phosphate (ortophosphate)	2,55 (± 0,38)		0,067 (± 0,030)	5,83 (± 2,21)
Nitrate and nitrite	100 (± 15)		0,120 (± 0,018)	0,605 (± 0,264)

C.2 Toxicity

The toxicity of the ammonium chloride was similar to that of the fertilizer WAF when related to ammonia + ammonium (Table 2 and Figure 2). The equilibrium distribution between NH_4^+ and NH_3 is determined by temperature, salinity, and pH. Based on measured parameters the fraction of NH_3 relative to NH_4^+ estimated in the exposure solutions were in the range 1 to 2.2%. The toxicity (LC_{10} and LC_{50}) related to ammonium and ammonia are shown in Table 2.

The toxicity (LC_{50}) related to both NH_4^+ and estimated NH_3 was found to be very similar in the two tested solutions (Figure 2) indicating that the ammonia/ammonium complex is the main driver of toxicity observed in the water soluble fraction of the "slow release fertilizer".

Table 2. LC50 related to ammonia (NH_4^+ and NH_3) in the exposure solutions,

	72 hours LC_{10} (mg/L)		72 hours LC_{50} (mg/L)	
	NH_4^+	NH_3	NH_4^+	NH_3
Fertilizer	3,16 (2,52- 3,97)	0,074 (0,059 – 0,093)	9,47 (8,52 - 10,05)	0,210 (0,189 - 0,233)
Ammonium chloride	1,75 (1,21 – 2,53)	0,038 (0,026 – 0,056)	6,74 (5,71 - 7,96)	0,151 (0,179 - 0,128)

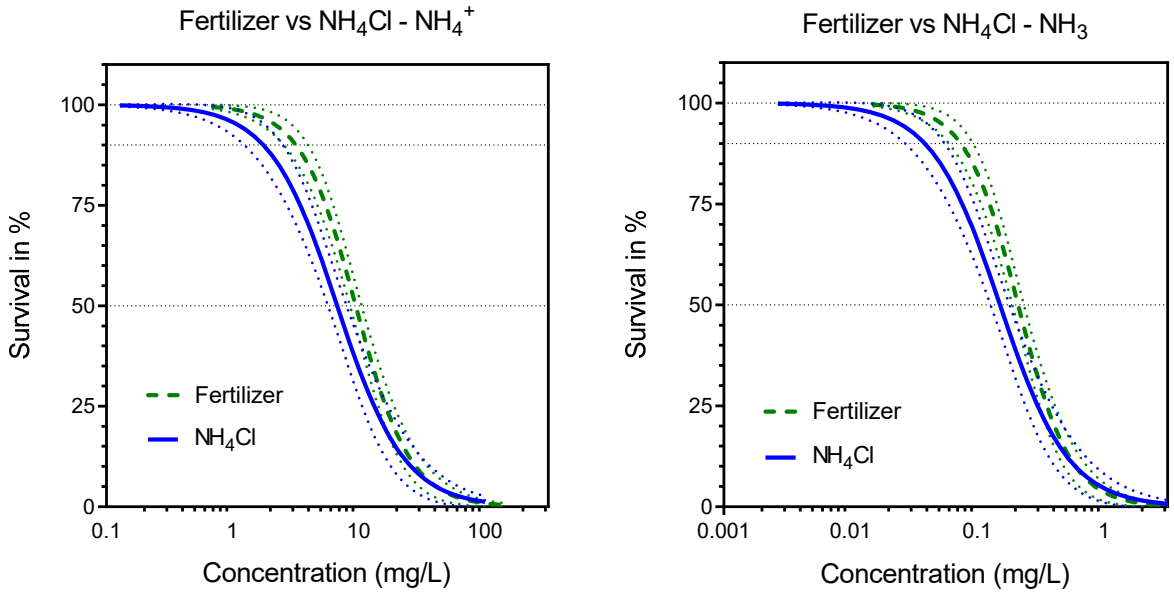

Figure 2. *Comparison of the toxicity of fertilizer WAF and ammonium chloride related to ammonium (NH_4^+) and ammonia (NH_3). Dotted lines indicate the 75% confidence interval.*

D Discussion

The objective of the current tests was to investigate if the fertilizer could be contributing to the observed toxicity of fertilized sediment collected from experiments with oil degradation in the SINTEF sediment column system (Nordtug et al. 2019). The composition of the nutrients in the current test-solution was different from that of the samples collected from the column experiment in that the nitrate/nitrite concentration relative to both the phosphate and the ammonium/ammonia was significantly elevated in the fertilizer test relative to the column samples (Table 1). In addition, the relative concentration of phosphate was much lower in the current test.

The results show a close similarity between the toxicity of the pure fertilizer WAF and ammonium chloride when the toxicity is related to ammonia/ammonium concentrations. Other candidates to contribute to toxicity in the fertilizer WAF are phosphate and nitrate/nitrite. Phosphates are not regarded to be toxic and may protect against toxicity by for instance reducing the uptake of toxic metals (Karadjova et al., 2008). If the toxicity of this component was the driver for toxicity in the fertilizer it is expected that the LC_{50} in the WAF of the fertilizer would be much lower than for the ammonium chloride. The toxicity of nitrite to shrimp post-larvae is reported to be approximate 20 times less than for ammonia. Consequently, the correlation between the LC_{50}-values and the ammonium/ammonia concentration in the fertilizer WAF and the ammonium chloride suggest that this is the likely driver for toxicity in the WAF from the fertilizer. The ratio between ammonium and ammonia changes with temperature, salinity and pH and the ammonia is the component of most concern with LC_{50} values for crustaceans reported around 1 mg/L (Alcaraz et al., 1999). The current results indicate that the *Calanus* early stages are slightly more sensitive than the "average" crustacean.

Tests previously conducted with oiled sediment did not produce sufficient amount of exposure solution to allow for analyses. We are therefore not able to directly compare the results from the two tests. However, by comparing to the analyses from the column experiment (Table 1) the ammonium/ammonia on day 1 in the columns added 6 g/kg sediment is about one third of the recorded LC_{50}-values and in the same range as the

estimated LC_{10}-values in the current test. It is therefore plausible that the ammonia added by the fertiliser can contribute to the observed toxicity in the testing of the sediments from the column experiment.

Early stages of *C. finmarchicus* have previously been shown to be rather sensitive to contaminants, and thus expected to be more sensitive than "the average" sediment organisms. Based on the current results, however, we recommend that the amounts of the Plantagen slow-release fertilizer used on shoreline sediments should be maximum 3 mg/kg sediment.

E Conclusion

The toxicity (LC_{50}) related to both NH_4^+ and estimated NH_3 was found to be very similar in the two tested solutions (Figure 2) indicating that the ammonia/ammonium complex is the main driver of toxicity observed in the water-soluble fraction of the "slow release" fertilizer.

The concentrations of ammonium/ammonia in samples from the previously conducted degradation experiments are all below the LC_{50}-values recorded in the toxicity test. The concentrations in the pore water of the columns added 6 g fertilizer/kg sediment are in a range that may contribute to mortality in early stages of *C. finmarchicus*. We thus recommend that the amount of the current fertilizer (Plantagen slow release) used on shoreline sediment should be limited to 3 g/kg sediment.

F Literature

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Nordtug, T., Brakstad, O.G., McFarlin, K., Whyte, L.G., Greer, C.W. (2019). Biodegradation of marine fuels in beach sediments at low temperature – A laboratory study. SINTEF report OC2019 F-133 (restricted).

Appendix 1

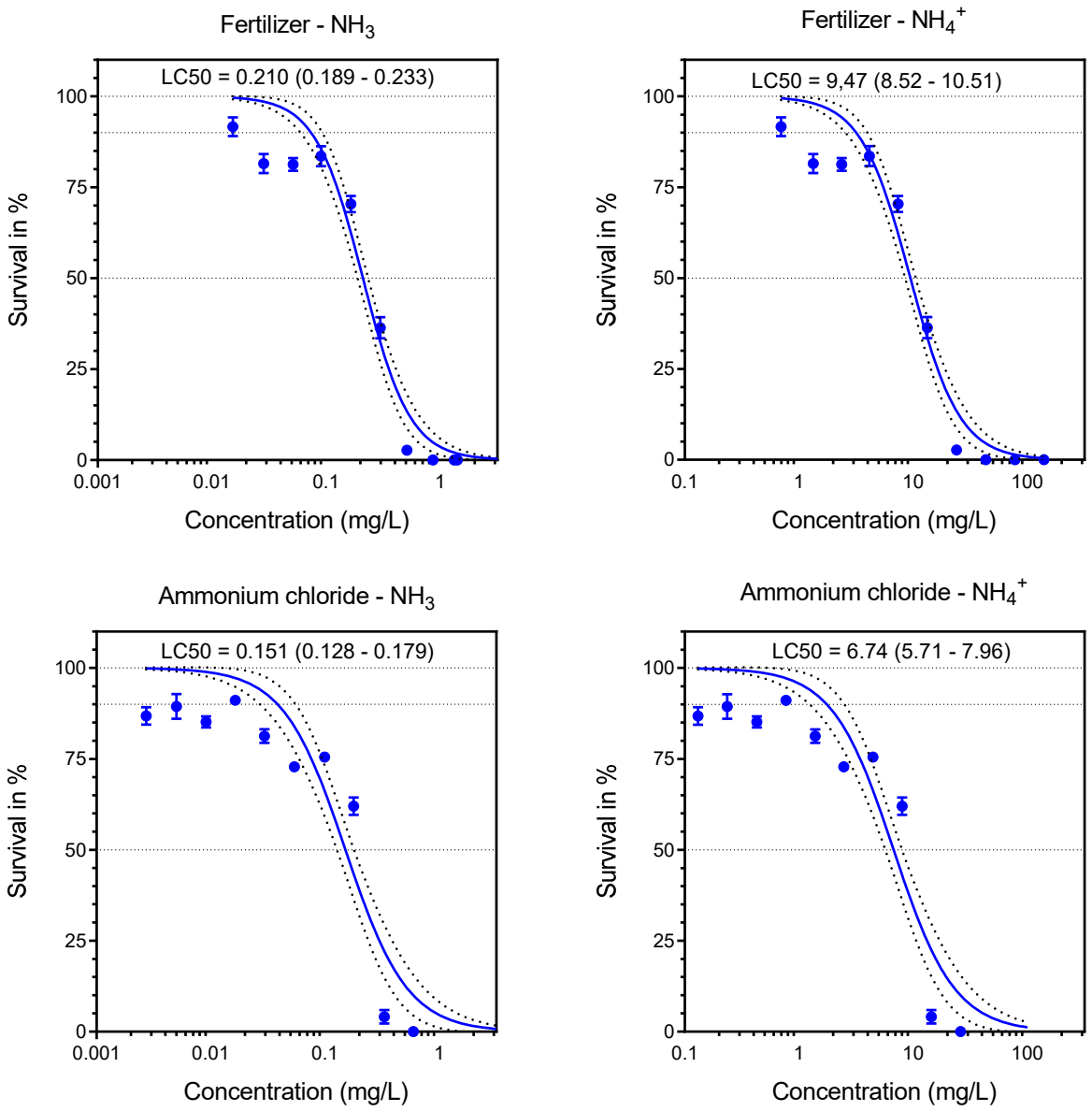

Figure A1. *Survival of Calanus finmarchicus early stages compared to control groups related to ammonium (NH_4^+) and ammonia (NH_3) after 72 hours exposure to fertilizer WAF and ammonium chloride. Dotted lines indicate 95 confidence intervals. Vertical bars indicate standard error based on 6 replicates.*