Report for NTPC

Name of the project	Arctic warming and methane release controlled by methanogens / methanotrophs - A case study of Arctic Fjords and Lakes.
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Period of field access	Arctic expedition 23-24 1st Batch: May 25th to June 22nd
Details of sampling	Details given below
Details of analysis	Details given below
Results	Details given below

Details of Sampling: Samples were collected from Arctic fjords Kongsfjorden–Krossfjorden syatem that shared common mouth with the adjacent West Spitsbergen Shelf. It is a glacial fjord system, which has some characteristics specific to Arctic fjords. These fjords are influenced by freshwater throughout the year, although only to a slight degree in the long winter season. Six stations from the Kongsfjorden fjord and four stations from Krossfjorden fjord of Ny-Alesund were selected for the present study. Even though sampling from lakes were also proposed for the study, samples could not collect due to the safely issues and the unavailability of a gun man.

Surface and bottom **w**ater and sediment samples were collected from the selected stations. The water samples for methane were collected in glass bottle and capped using a Teflon faced septum and a crimp cap of the appropriate size to fit the bottle. The sample bottles were purged with nitrogen before the sampling. The samples were preserved with 3.5% $HgCl_2$ solution to inhibit methane oxidation.

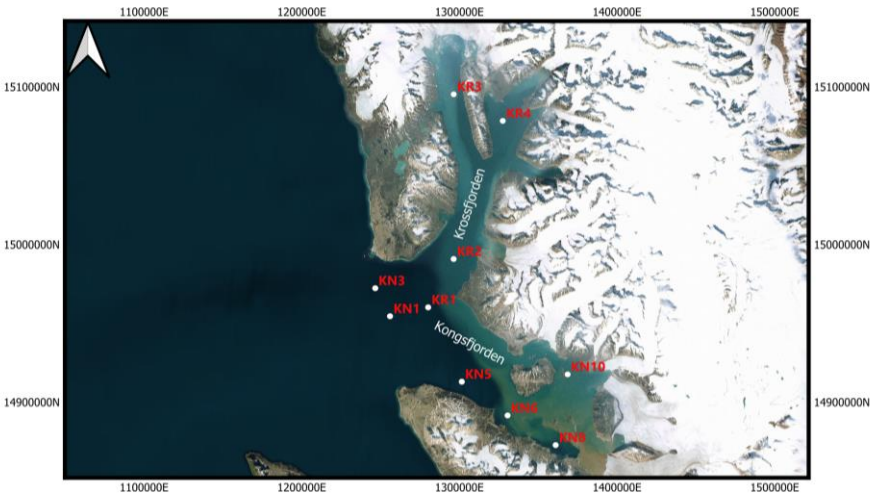


Fig 1: Map showing sampling locations from Kongsfjorden–Krossfjorden system

Water samples for nutrients and sediment samples for fatty acid analysis were also collected and stored in -20 ^{0}C. Surface and bottom water samples for dissolved oxygen were also collected and was analysed using Winkler method. For the identification of methanogens and methanotrophs, one litter of water samples were collected and filtered through 0.22 µm cellulose nitrate filter paper and was stored in -20 ^{0}C.

General Water and Sediment Characteristics

The water samples were analysed following methods suggested by Grasshoff, 1999 and APHA, 1999. All the colorimetric estimations were done using double beam spectrophotometer (Analytik jena Specord 200). pH was measured using a portable pH meter. Salinity, Turbidity, and Temperature were collected from CTD measurements. The Winkler's method was employed for estimation of dissolved oxygen. Nitrite was estimated by colorimetric method after complexation with N-naphthyl ethylene diamine and sulphanilamide. Nitrate was reduced to nitrite in cadmium column and estimated spectrophotometrically. Estimation of ammonia and phosphate were carried out using indophenol blue and molybdenum blue methods respectively (Strickland and Parsons, 1972; Grasshoff, 1999).

The textural characteristics of the sediments were determined by particle size analyser after removing the inorganic carbonates using 10% HCl and organic matter using H_2O_2 (Folk, 1980). CHN-S Analyser was used for the analysis of Total Nitrogen (TN) and Total Sulphur (TS). For the analysis of Total Organic Carbon (TOC), the sample was pre-treated using 1M HCl to remove carbonates. The process was repeated two/three times in order to ensure the complete removal of carbonates, washed with Milli-Q to remove salts and finally freeze-dried and analysed using TOC

analyser. Exchangeable nitrite and nitrate from the sediment was extracted using 2N KCl solution and then was analysed spectrtophotometrically. Concentration of total phosphorus was determined by igniting the dry sediments at 550°C for 2 h in a muffle furnace followed by the extraction of the residue for 16 h with HCl (1 N). Exchangeable phosphate was also extracted with 1N HCl. Then it was analysed spectrtophotometrically.

Extraction of fatty acids: The method described by Harvey (1994) was selected for biomarker analysis. Freeze dried sediment samples was soxhlet extracted for 72 hrs with a mixture of 2:1 dichloromethane (DCM): methanol (MeOH). The extracts was combined and evaporated to dryness using rotary evaporation. The extracted residue was subjected to mild alkaline hydrolysis by refluxing using 6 % methanolic KOH (70 ^{o}C for 6 hours). After the sample was cooled, the neutral lipid was partitioned from the alkaline solution into hexane. The remaining aqueous layer containing the fatty acid salts was acidified to pH 2. Fatty acids in this polar lipid fraction was recovered separately into DCM. It was then converted to fatty acid methyl esters (FAMEs) by treating with 10 ml of 12% BF_3/MeOH (Sigma Aldrich) (70^{o}C for 30 minutes). The FAMEs was subsequently extracted from the reaction solution using DCM. The DCM layer was evaporated to dryness, and the extract was re-dissolved in 1ml methanol for chromatographic analysis.

Microbial DNA analyses: Environmental DNA (eDNA) was extracted in triplicates from sediment using published protocols (e.g. Ghosh and Bhadury 2018, Ghosh et al 2022). For generating high quality metagenome level data based on Nanopore sequencing (a type of HTS), robust amplicon libraries from extracted environmental DNA (eDNA) pool using Ligation sequencing kit (SQK-LSK109; Oxford Nanopore Technologies-ONT, Oxford, UK) and PCR barcoding kit (EXP-PCR096; Oxford Nanopore Technologies, Oxford, UK). The barcode adapter-ligated products will be quantified using Qubit Fluorometer (Thermo Fisher Scientific, USA). Barcoded samples were cleaned up using 1.6X AmPure beads and pooled at equimolar concentrations. For extracted RNA (environmental RNA- eRNA), direct RNA sequencing and cDNA sequencing approaches will be undertaken using ONT sequencing chemistry. Sequencing for metagenomics data generation will be undertaken using a combination of MinION and GridION X5 involving a 48-h sequencing protocol. Raw sequence files in fastq format will be uploaded onto MG-RAST (Meyer et al., 2008). Sequence files will be normalized following quality control. For quality control, artificial duplicate reads will be removed, reads will be trimmed based on quality and length. The MG-RAST pipeline will be used for taxonomic identification and protein prediction using clustering and similarity-based

annotation tools. Taxonomic annotation will be performed against SILVA whereas proteins will be annotated as SEED functions (Overbeek et al., 2005), KO terms, COG classes (Tatusov et al., 2003) and eggNOGs (Jensen et al., 2016). The presence and quantitative abundance of mcrA and pmoA genes linked specifically to CH4 metabolism will be deduced from the metagenome dataset and will be expressed in terms of wet weight of sediment.

Analysis of dissolved methane in water and sediment:

Dissolved Gas Analysis in Water samples will be carried out using a GC Headspace Equilibration Technique. The samples for methane will be collected in glass bottle and capped using a Teflon faced septum and a crimp cap of the appropriate size to fit the bottle. The samples will be preserved with 3.5% $HgCl_2$ solution to inhibit methane oxidation. The sample bottle will purge with nitrogen before the sampling and transported back in the laboratory for analysis. The dissolved methane concentration can be determined after flushing the samples with helium. Equilibrated helium samples can be analysed for methane using gas chromatography.

Results:

General wate and sedimentary quality parameters: CTD Data was collected from NCPOR which is part of the project "Long-term monitoring of Kongsfjorden-Krossfjorden for climate change studies". The analysis of dissolved oxygen (DO) and nutrients was carried out for the water samples collected from Arctic fjords. Performed analysis of CHNS, texture, total organic carbon (TOC), total phosphorus (TP), exchangeable nitrite, exchangeable nitrate, and exchangeable phosphate in sediment samples.

pH in the study region ranged from 7.66 to 8.05 (aveg:7.81 ± 0.10) with lower pH in bottom water samples. Concentration of dissolved oxygen (DO) in the study region ranged from 4.31 to 7.76 mg/L (Aveg :6.12±1.01 mg/l), and is found to be almost similar in surface and bottom water samples. Concentration of nitrite and nitrate in the study region ranged from ND to 0.378 µmol/L (Aveg:0.13 ±0.12 µmol/L) and 0.103 to 40.012 µmol/L (Aveg: 4.32 ± 9.69 µmol/L) respectively. Concentration of ammonia varied from ND to 8.289 µmol/L (Aveg:2.10 ± 1.95 µmol/L). Silicate and phosphate in the study region ranged from 1.061 to 53.53 µmol/L (Aveg:6.52 ± 11.40 µmol/L) and 0.35 to 6.433 µmol/L (Aveg:1.88 ± 1.60 µmol/L).

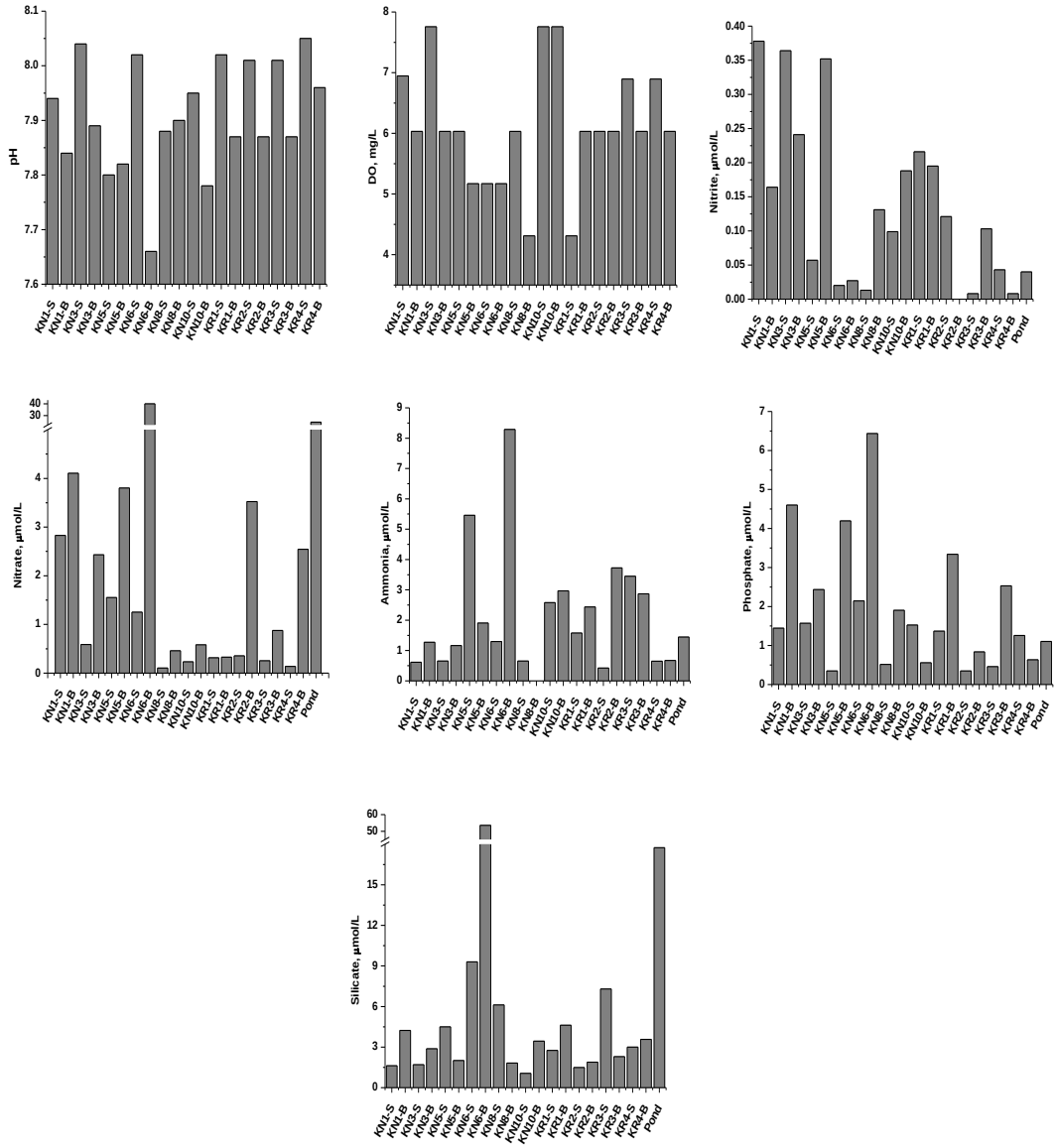


Fig2: Variation of water quality analysis from the study region

Total organic carbon (TOC) content in the arctic sediment ranged from 0.06 to 1.64 % (Aveg:0.66±0.61). TN was found to be ranged from 0.01 to 0.6 (Aveg:0.14±0.17) %. TOC/TN ratio ranged from 2.05 to 19 in the study region with values in Kongsfjorden ranging from 2.05 to 14.50 and in Krossfjorden from 2.33 to 19. Moisture content ranged from 24.78 to 40.80 % (Aveg:35.08±5.69 %) increasing from the glacier front to outer stations. Particle analysis showed lower percentage of sand and higher percentage of silt followed by clay in Krossfjorden. In the mouth of Kongsfjorden sand percentage was very low. Higher sand percentage was found in the mid of the Kongsfjorden (KN5:20.39 % and KN6:16.77 %). The silty fraction dominated in the fjords, ranging from 74.93 to 79.65% and 65.7 to 85.71% in

Krossfjorden and Kongsfjorden, respectively. The clay class component ranged from 20 to 25% and 12 to 18% in Krossfjorden and Kongsfjorden, respectively.

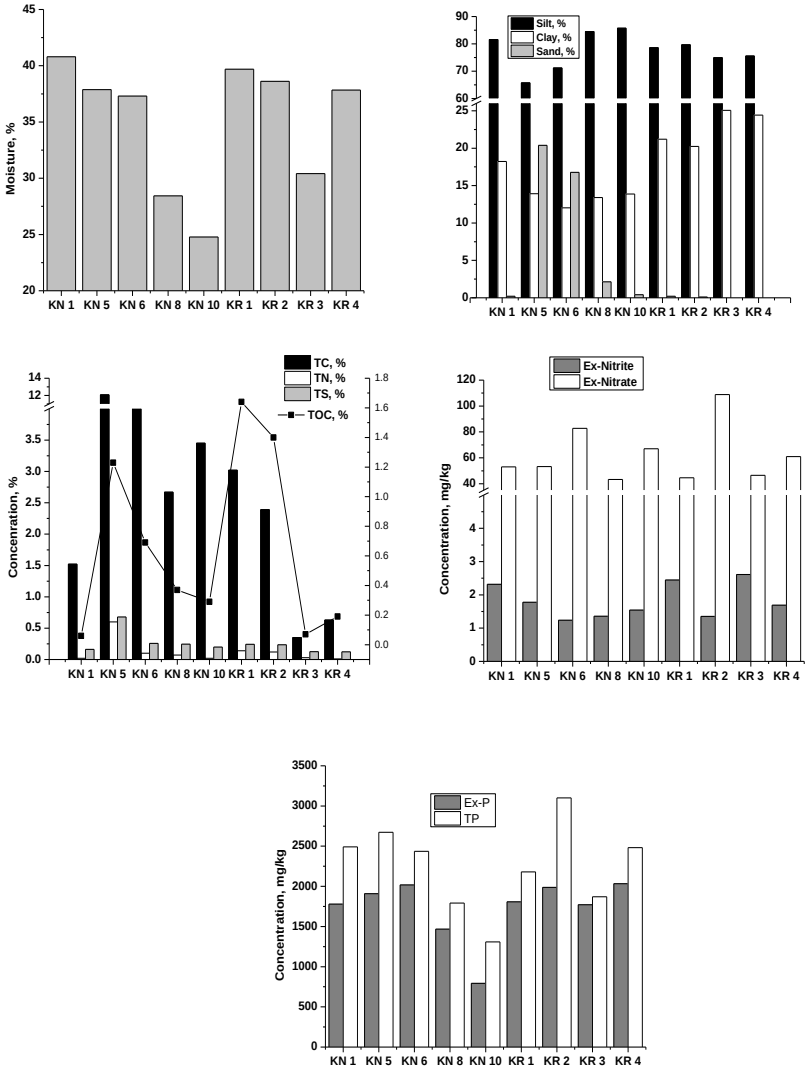


Fig 3: Variation of sedimentary parameters in the study region

Exchangeable nitrite ranged from 1.24 to 2.61 mg/kg (Aveg:1.81±0.52 mg/kg) with the highest concentration reported from the middle of the Krossfjorden. Its concentration in Kongsfjorden, concentrations decreased from outer to inner parts of the fjords. Exchangeable nitrate ranged from 43.36 to 108.80 mg/kg with highest concentration was observed in the inner Krossfjorden. In Kongsfjorden, highest concentration was reported from middle of fjord. TP and exchangeable phosphorous ranged from 1309.38 to 3101.49 mg/kg (Aveg: 1729.21± 391.80 mg/kg) and 793.17 to 2032.55 mg/kg (Aveg: 2259.27± 535.34 mg/kg) respectively. Highest concentration of exchangeable nitrite was reported from the middle of the Krossfjorden (KR2). For the sediment samples taken from Kongsfjorden it was found to be decreases from outer to

inner parts of fjords. In Kongsfjorden highest concentration of Exchangeable nitrate was reported from mid of the fjord (KR6). Highest exchangeable phosphorous was observed from the mouth of the Krossfjorden (KR1). In the Kongsfjorden, exchangeable P and TP exhibited highest concentration in mid of the fjord of and lowest concentration was reported from in the western arm of the fjord (Lilliehöökfjorden-KR3).

Analysis of fatty acids extracted from sediment: Quantification of fatty acids was achieved by calibration of FAMEs standards supplied by Sigma Aldrich (Supelco 37 Component FAME Mix, 18919-1AMP, Bacterial Acid Methyl Esters BAMEs-47080-U). Sample FAMEs extracted from the arctic fjords were also injected in the same condition that of standards and their concentrations were determined from the calibration plot.

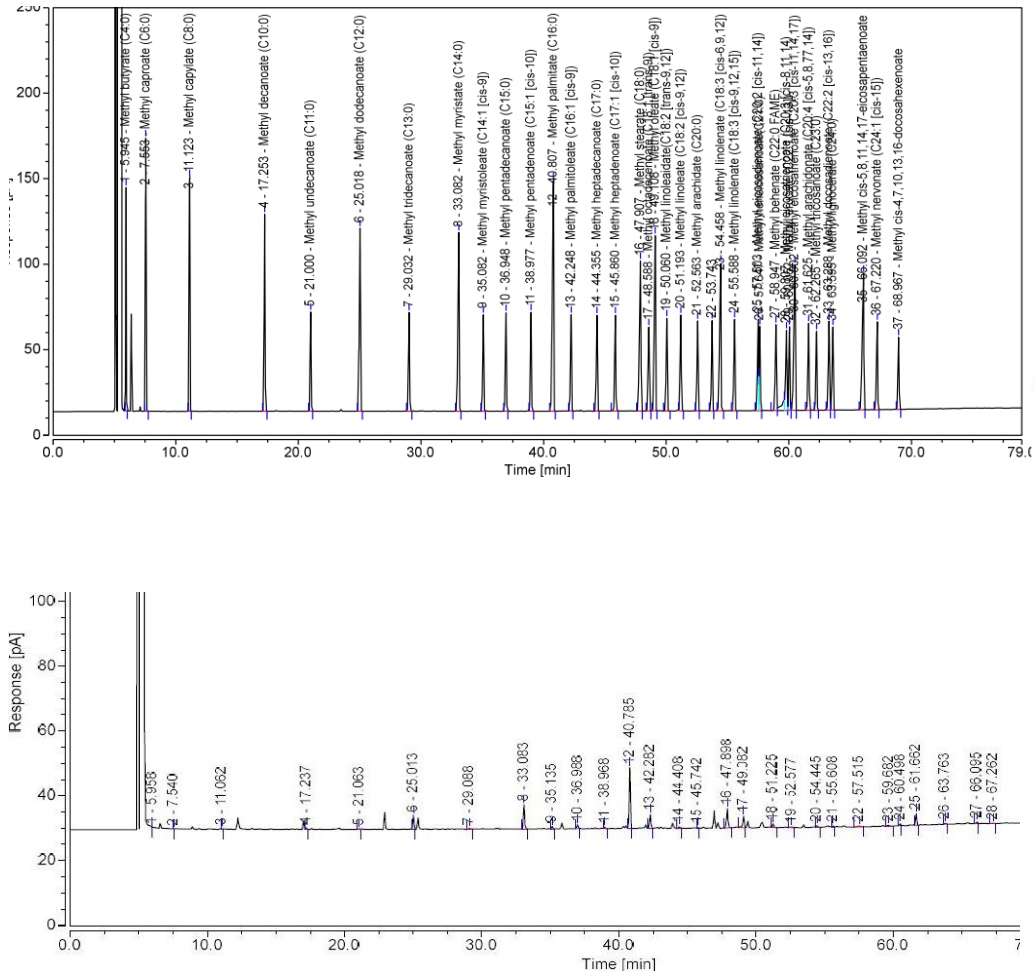


Fig 4: Fatty acid profiling of standard and sample

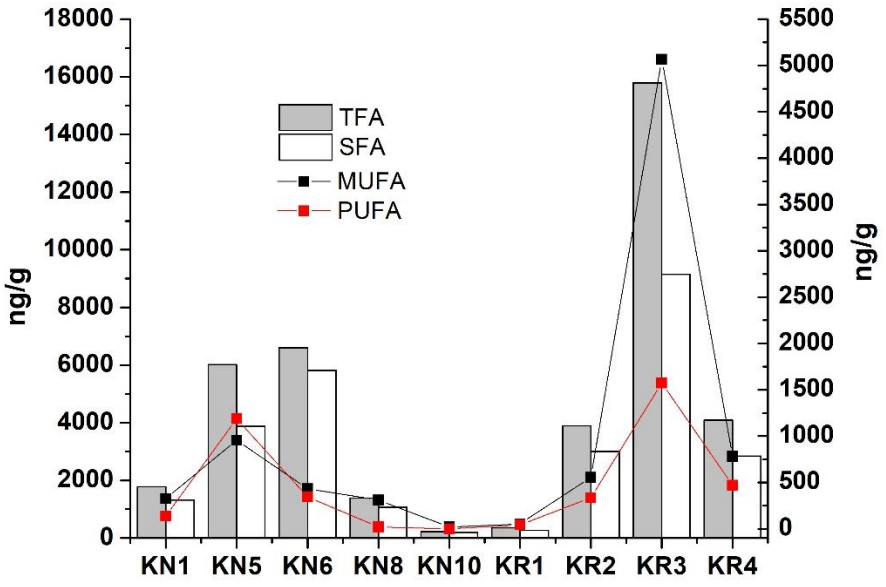
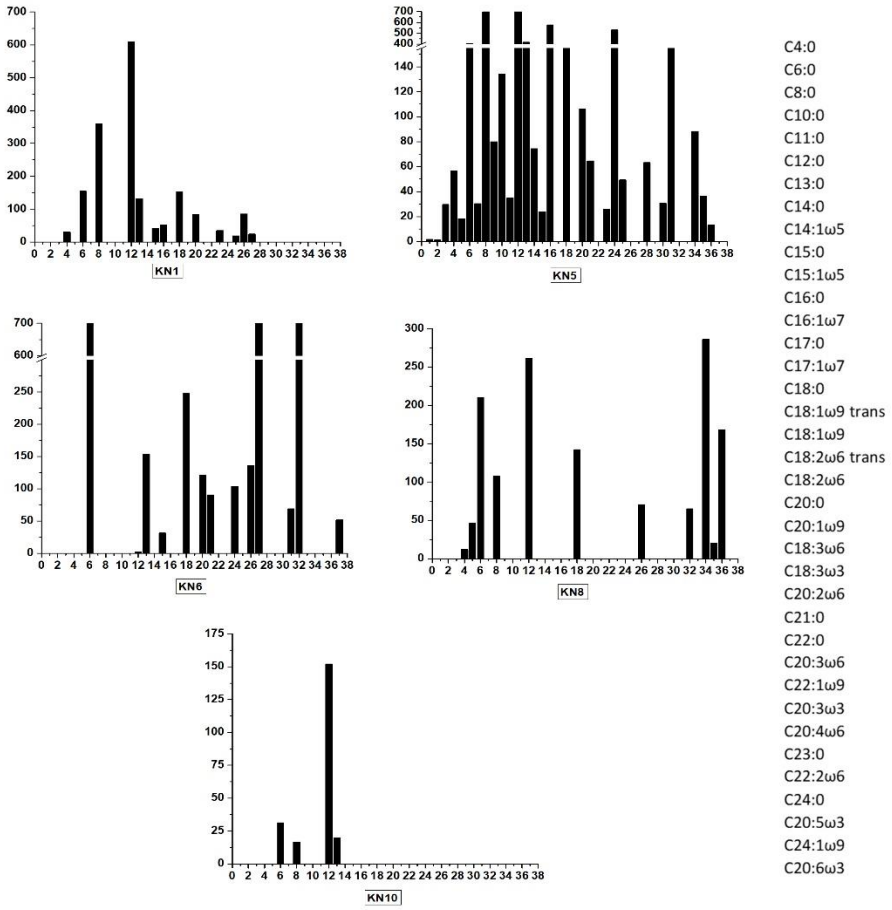


Fig 5: Fatty acid indices for the samples collected from the arctic fjords



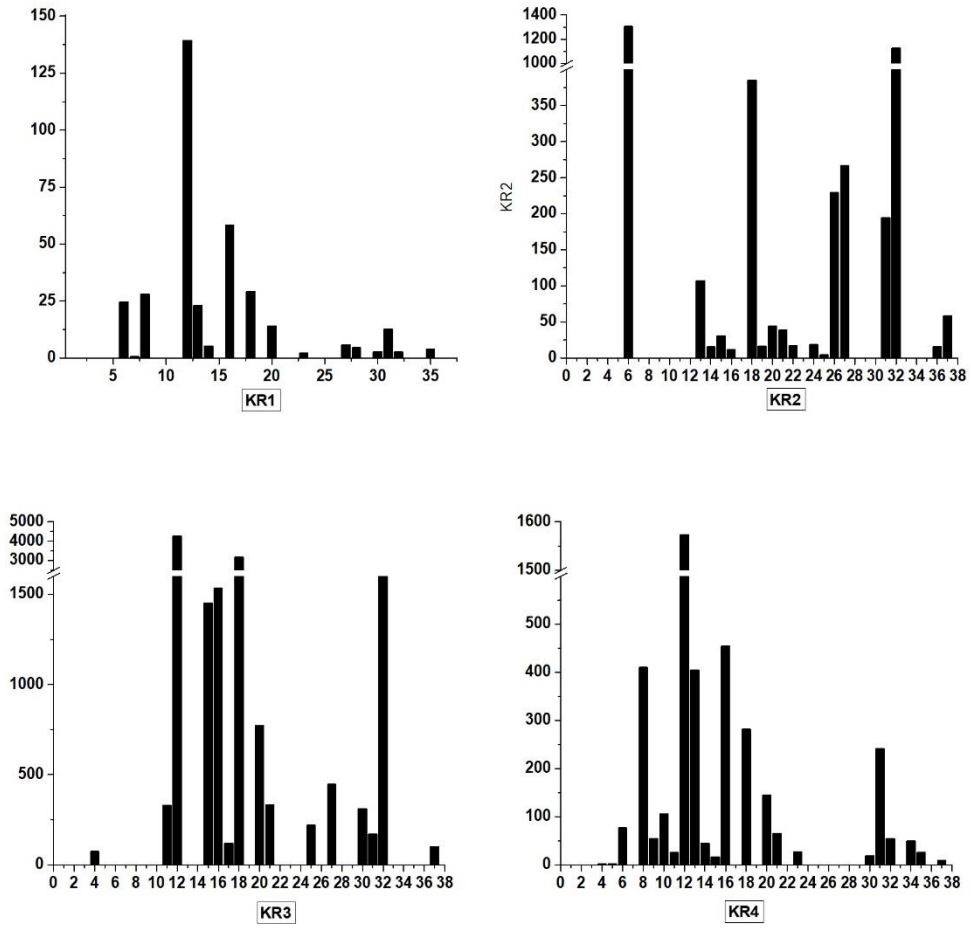


Fig 6: Concentration of different fatty acids in the arctic fjords

TFA (Total Fatty acids) was calculated as sum of all fatty acids and was ranged from 219.64 to 15790 (4526.28±4800.06) ng/g. SFA (Saturated straight chain Fatty Acids) contributed 55.08 to 90.98 % to the total fatty acid pool and its concentration was varied from 199.84 to 9149.10 (3057.47 ± 2925.13) ng/g . TFA and SFA followed the same trend in the entire study region. In Kongsfjorden the concentration was found to be increasing from the outer to middle of the fjord and then it is found to be decreasing towards the inner fjord. Innermost station near glacier in Kongsfjorden recorded lowest concentration in the entire study region. MUFA (Mono Unsaturated Fatty Acids) varied from 19.80 to 5068.04 (944.75 ± 1576.33) ng/g and it was contributed 6.56 to 32.09 % to the TFA pool. Contribution of Poly Unsaturated Fatty Acids (PUFA) to the total fatty acid pool ranged from ND to 19.73 % and its concentration ranged from ND to 1573.02 (456.31 ± 556.99) ng/g. Lilliehöökfjorden, the western arm of the fjord recorded the highest concentration of TFA and SFA in the Krossfjorden and it is very low in the outer fjord. Concentration of MUFA and PUFA followed the similar concentration except at the Lilliehöökfjorden, which showed very high concentration of MUFA

compared to PUFA. Among individual fatty acids C16 is the dominant fatty acids in the most of samples. In KN6 and KR2, dominance of C12 fatty acid was observed which could be originated from bacterial source. Only in KN8, C24 fatty acid was dominant fatty acid.