

# New results on biodegradation of oil and dispersed oil in cold marine environment

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# Impact of dispersants on petroleum hydrocarbon biodegradation and degradation gene abundances in the Baltic Sea

Authors: Ossi Tonteri, Aura Nousiainen, Anna Reunamo, Jari Nuutinen, Jaak Truu and Kirsten S. Jørgensen.

- The effects of dispersant use on the microbial communities at the low temperature in Baltic Sea are unknown.
- Aim was to investigate the impact of dispersant on the petroleum hydrocarbon biodegradation and microbial degradation gene abundance at low temperature
  - Also to compare the differences between Baltic Sea and North Atlantic seawater
- Laboratory scale microcosm experiments at 5 °C for 12 days using naphthenic Northern Sea crude oil and dispersant Finasol 51.
- Seawater for experiments from three locations
  - Gulf of Finland (Tvärminne, coastal area)
  - Gulf of Bothnia (UBS6, open sea)
  - North Atlantic (Narvik, coastal area).

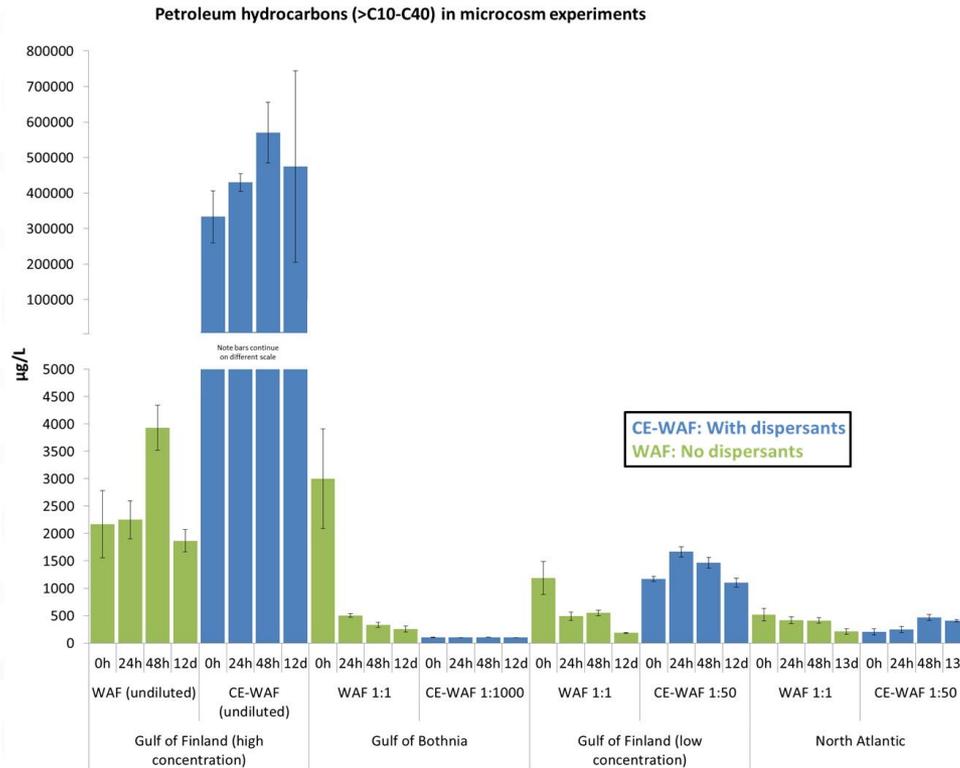


# Microcosm experiment

- Water Accommodated Fractions (WAF) and Chemically Enhanced Water Accommodated Fractions (CE-WAF) of crude oil and dilutions of these were used in the experiments.
- Oil degradation was assessed by analysing the petroleum hydrocarbons with GC-FID
- Microbial biodegradation potential by qPCR targeting alkane and PAH degradation genes



# Results

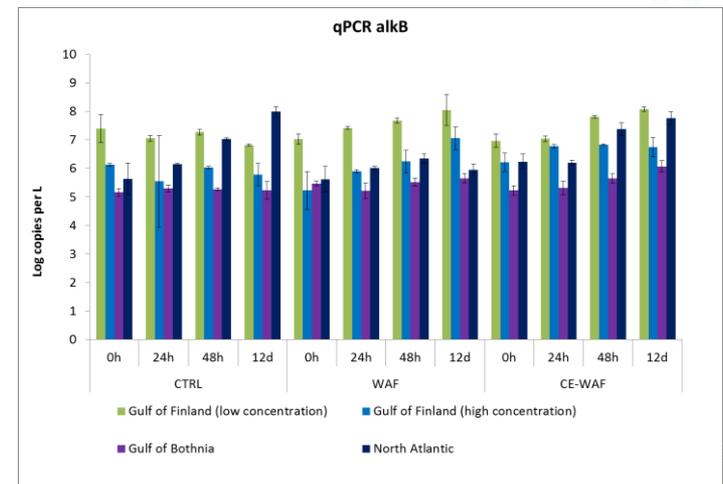
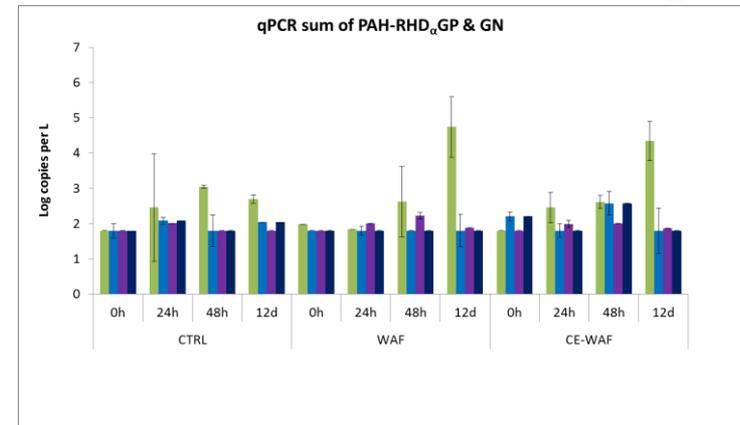


- Highest degradation (92 %) was observed using diluted WAF (no dispersants) with Baltic Sea seawater, degradation with CE-WAF (with dispersants) was 5%
- Baltic Sea microcosms had higher degradation with Gulf of Bothnia and North Atlantic seawater
- Degradation generally lower with dispersant microcosms



# Results

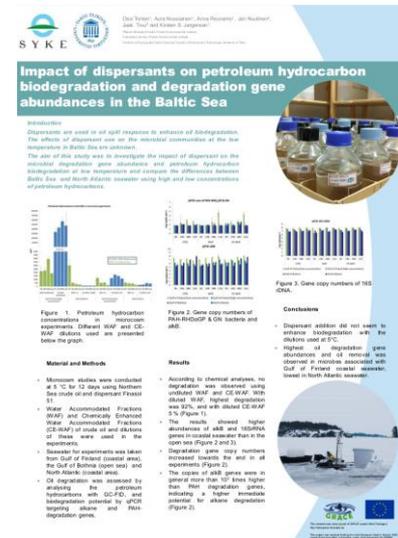
- The results showed higher abundances of alkane degradation (*alkB*) and general (16S rRNA) genes in coastal seawaters compared to open sea seawater
  - Slightly higher in Baltic sea (low concentration) seawater than in North Atlantic
- Degradation gene copy numbers increased towards the end in all experiments
- The copies of *alkB* genes were in general more than  $10^3$  times higher than PAH degradation genes, indicating a higher immediate potential for alkane degradation
- Sequencing data not yet analysed, so no information from microbial communities



# Conclusions

- Oil degradation gene abundances and oil removal was observed to be higher in microbes associated with Gulf of Finland coastal seawater, lower in Gulf of Finland open sea and North Atlantic seawater
- Dispersant addition did not seem to enhance biodegradation in the microcosm experiments with the dilutions used at 5°C
  - Variation in WAF & CE-WAF oil levels between experiments
  - Difficulties finding comparable concentrations with without dispersants

Poster nr. 6



# In situ electrokinetic treatment pilot test of petroleum hydrocarbon contaminated marine sediment

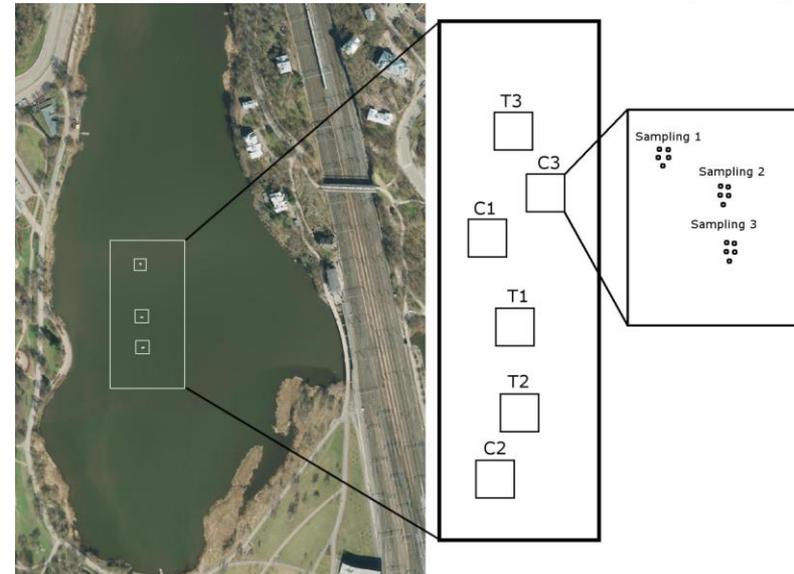
Authors: Ossi Tonteri, Miikka Tunturi, Emil Vahtera, Laura Hoikkala, Kaarina Lukkari, Miira Jääskeläinen and Kirsten S. Jørgensen

- In situ pilot-scale testing of electrokinetic remediation of oil polluted sediments conducted by Lamor and SYKE
- WP2.3 "Report on enhanced oil remediation in marine sediments using electrokinetic treatment"
- Experiment and sampling was conducted in co-operation with Erased project (City of Helsinki, SYKE and Ekoharden Technologies) in Töölönlahti Bay, Helsinki



# Experiment location and equipment

- According to previous studies sediment has been polluted with petroleum hydrocarbons ranging between 2000-3000 mg/kg dw
- Three experiment areas (= 3 platforms) with identical setup and equipment
- Remediation equipment (Ekogrid) placed on floatable platforms
  - Each platform with electrical control box and 16 electrodes arranged in rectangular shape around the platform

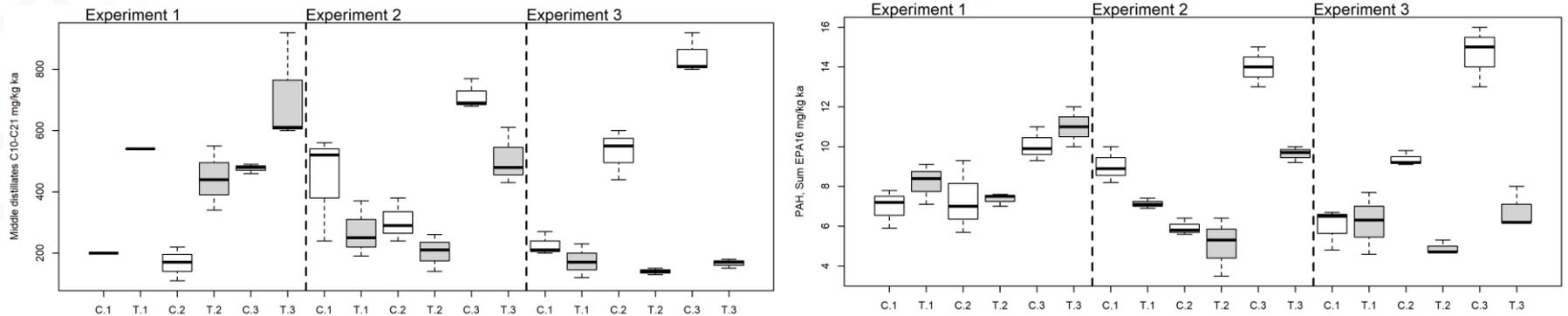


# Experiment

- Experiment started in August 2017 and ended August 2018
  - Overall treatment time ca. 10-11 months per plot
  - (Original plan was to have longer treatment time of ca. 16-18 months, but was shortened due to funding problems in Erased project)
- Sediment sampling was conducted in the treatment and control plots to evaluate effects of the treatment
  - Chemical analysis (C<sub>10</sub>-C<sub>40</sub> hydrocarbons, PAH, THC)
  - Microbial analysis (DAPI, qPCR, sequencing)
  - (Bioassay testing to evaluate remediation success on toxicity level was conducted in WP3/WP2.4)



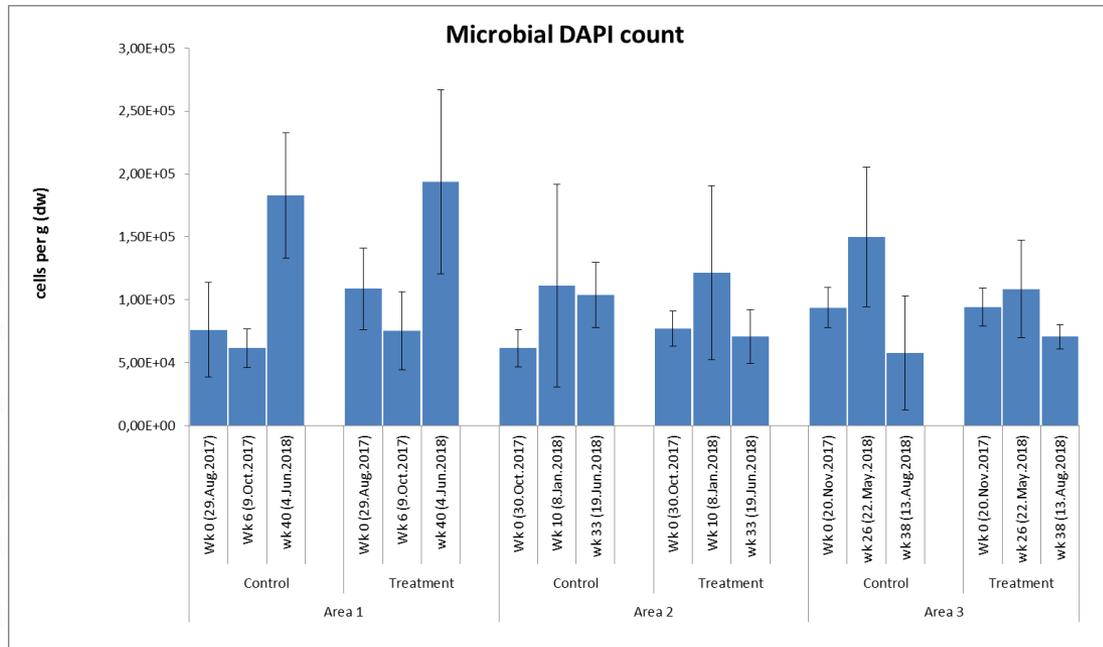
# Results



- High variations in hydrocarbon and PAH concentrations between and within experiment areas
- Some treatment plots showed decrease in hydrocarbon concentrations in sampling round 2, but concentrations increased in all control and treatment plots in sampling round 3
  - New source of oil?
- Likelihood Ratio Tests of the Linear Mixed Effects model indicated some reduction in  $C_{10}$ - $C_{20}$  and PAH concentrations in some treatment plots, but variation is too high to make clear conclusions



# Results



- Generally low microbe levels, below  $10^5$  cells per g (dw)
- No clear increase in treatment samples vs control
  - High variation, difficult to count
- qPCR and sequencing not yet finished

# Conclusions

- Based on the results it is difficult to make clear conclusions on the effects of the treatment, mainly due to large variation in the sediment between and within the test plots
- Several reasons that could have affected the remediation:
  - Too short treatment time
  - The effect of cold winter season in the remediation process
  - Some indication that remediation equipment was not working optimally on all experiment areas

Poster nr. 5

