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Microbes as indicators of Hg contaminated sediments

Studies in the Gulf of Bothnia

Agneta Andersson, Juanjo Rodriguez, Elizabeth Sands, Sonia Brugel, Erik Björn, Sofi Jonsson, Sonja Gindorf, Li Zhao

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SWEDISH ENVIRONMENTAL
PROTECTION AGENCY

The Swedish Environmental Protection Agency
Phone: + 46 (0)10-698 10 00
E-mail: registrator@naturvardsverket.se
Address: Naturvårdsverket, SE-106 48 Stockholm, Sweden
Internet: www.naturvardsverket.se

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Preface

This report *Microbes as indicators of Hg contaminated sediments – studies in the Gulf of Bothnia* presents the results of one of the six funded projects within the call Contaminated Sediments from 2020. The research results from this call aim to provide the knowledge base needed for measures and policy instruments regarding risks with and spread of contaminated sediments and hazardous substances from contaminated sediments.

The project has been financed with the environmental research grants from the Swedish Environmental Protection Agency (SEPA) to support the knowledge needs of SEPA and the Swedish Agency for Marine and Water Management.

This report is written by Agneta Andersson (Umeå University), Juanjo Rodriguez (Umeå University), Elizabeth Sands (Umeå University), Sonia Brugel (Umeå University), Erik Björn (Umeå University), Sofi Jonsson (Stockholm University), Sonja Gindorf (Stockholm University), and Li Zhao (Umeå University).

The authors are responsible for the content of the report.

Stockholm, March 2025

Marie Uhrwing
Department head, Sustainability Department

Förord

Denna rapport med titeln: *Microbes as indicators of Hg contaminated sediments – studies in the Gulf of Bothnia* presenterar resultaten av ett av sex beviljade projekt inom utlysningen *Förorenade Sediment* från 2020. Forskningsresultaten från denna utlysning syftar till att ta fram kunskapsunderlag som behövs för åtgärder och styrmedel avseende risker med och spridning av förorenade sediment och farliga ämnen från förorenade sediment.

Projektet har finansierats med medel från Naturvårdsverkets miljöforskningsanslag till stöd för Naturvårdsverkets och Havs- och vattenmyndighetens kunskapsbehov.

Denna rapport är författad av Agneta Andersson (Umeå universitet), Juanjo Rodriguez (Umeå universitet), Elizabeth Sands (Umeå universitet), Sonia Brugel (Umeå universitet), Erik Björn (Umeå universitet), Sofi Jonsson (Stockholms universitet), Sonja Gindorf (Stockholms universitet), och Li Zhao (Umeå universitet).

Författarna ansvarar för rapportens innehåll.

Stockholm, mars 2025

Marie Uhrwing
Avdelningschef för Hållbarhetsavdelningen

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Summary

Chemical pollution resulting from human activities is a significant global concern for environmental authorities. Pollutants released into aquatic ecosystems often accumulate at levels far exceeding natural background concentrations, leading to harmful effects on local organisms and the overall functioning of these ecosystems. Bioindication plays a critical role in the implementation of environmental legislation, particularly within the European Union, for assessing the quality of various natural environments. Microbes are found in a wide array of habitats, which would make them particularly valuable as rapid and sensitive indicators of environmental disturbances caused by pollutants, for example mercury.

The overall aim of this project was to elucidate if bacteria can be used as indicators of contaminated sediments. We focused on mercury, which is a common pollutant in Gulf of Bothnia fiber banks. The project was carried out by developing a portable molecular sequencing laboratory (Omibox), performing microcosm experiments mimicking climate change scenarios and by executing field studies in pollution gradients to identify possible bacterial indicators for mercury pollution.

The Omibox portable laboratory was successfully developed, enabling fast examination of natural microbial communities. The Omibox is compact and does not need much space. Microbial DNA and RNA were analyzed directly after sampling, and after 2 months storage in –20 °C, –80 °C and at room temperature. The results showed highest yield at immediate analysis and lowest at storage in room temperature. The recommendation is therefore to analyze the samples directly after sampling, and if that is not possible to keep samples frozen until analysis.

In microcosm experiments we showed that terrestrial dissolved organic matter (tDOM) induces MeHg formation, via increased bacterial activity. Heterotrophic bacteria use the fresh tDOM as a substrate, which increase the bacterial production and the MeHg production. Since climate change leads to elevated precipitation in northern areas, the inflow of tDOM will increase in the northern Baltic Sea as well as the formation of MeHg.

The link between bacterial community composition and mercury pollution was studied in three fiber bank gradients in the Gulf of Bothnia; Bureå (Bothnian Bay), Köpmanholmen and Sundsvall (Bothnian Sea). The bacterial community composition was determined from 16S sequencing and compared with the level of Hg pollution. We found that some groups of bacteria were enriched at the Hg polluted sites. These were, for example, bacteria of the phylum Campylobacterota (Class Campylobacteria) and the phylum Desulfobacterota (Class Desulfobaccia). The results were relatively similar in response to total Hg and MeHg. At the unpolluted "control" sites, the phylum Proteobacteria was more prominent. Experiment also showed that bacterial communities from contaminated areas were more tolerant to Hg addition than those from un-contaminated areas. Our results imply that high relative abundances of the phyla Campylobacterota and Desulfobacterota could be used as indicators of mercury pollution. We continue this project by analysing the gene composition, especially mercury methylation genes (*hgcAB*) and tolerance genes (*mer*) and their link to taxonomic groups.

In conclusion, the developed portable molecular laboratory, the Omibox, was found to be a useful and compact full laboratory unit, which can be used onboard ships and allows for fast analysis of natural microbial communities. Increased tDOM loads would enhance MeHg formation due to increased bacterial activity. Finally, certain bacterial taxa (Campylobacterota and Desulfobacterota) were observed to be enriched in polluted fiber-rich sediments, suggesting that these groups can be used as indicators of Hg pollution. Taken together, the project contributes with a framework on bacterial community structure and function as a response to Hg pollution in sediments, which can be useful in ecosystem monitoring.

Sammanfattning

Kemiska föroreningar orsakar problem världen över. Miljögifter som hamnat i akvatiska miljöer ackumuleras ofta till nivåer som har skadliga effekter på organismerna och hela ekosystemen. Inom EU-lagstiftningen används biomarkörer för att analysera statusen på naturliga miljöer. Mikroorganismer skulle kunna vara väl lämpade som bioindikator, eftersom de i stort sett finns överallt på jorden och snabbt svarar på miljöförändringar. Målsättningen med detta projekt var att klarlägga om bakterier kan användas som indikator för kontaminerade sediment. Vi fokuserade på kvicksilver, som är ett vanligt förekommande miljögift i fiberrika sediment i Bottniska viken. Inom projektet utvecklades ett portabelt molekylärt sekvenseringslaboratorium (Omibox), experiment utfördes för att testa effekter av terrestra organiska ämnen samt mikroorganismers toleransnivåer för kvicksilverbelastning. Därutöver utfördes fältstudier i gradienter av kvicksilverbelastade områden i Bottniska viken för att hitta möjliga indikatorer i bakteriesamhället.

Omibox-laboratoriet utvecklades enligt plan och fältprover testades för olika behandlingar. Mikrobiellt DNA och RNA analyserades direkt efter provtagning, samt efter 2 månader i –20 °C, –80 °C och rumstemperatur. Resultaten visar att högst utbyte erhålls vid direktanalys, och sämst vid 1 månads lagring i rumstemperatur. Rekommendationen är därför att fältprover analyseras direkt efter provtagning, och om det inte är möjligt att de förvaras frysta till analys.

I mikrokosmexperiment kunde vi visa att terrestert organiskt material (tDOM) inducerar bildning av metylkvicksilver. Bildningen visade sig vara kopplad till bakteriell aktivitet, bakterieproduktionen. Heterotrofa bakterier använde det färska terrestra organiska materialet som substrat, varvid bakterieproduktionen och bildningen av MeHg ökade. Eftersom klimatförändringar leder till ökad nederbörd i nordliga områden, förväntas också inflödet av tDOM och bildningen av MeHg att öka i norra Östersjön.

Kopplingen mellan bakteriesamhällets sammansättning och kvicksilverföreningar undersöktes i tre fiberbanksgradienter i Bottniska viken; Bureå (Bottenviken), Köpmanholmen och Sundsvall (Bottenhavet). Bakteriesamhällets sammansättning analyserades genom s.k. 16S sekvensering och resultaten jämfördes med graden av Hg förorening. Resultaten visar att vissa bakteriertaxa var anrikade i Hg förorenade områden; exempelvis Campylobacterota och Desulfobacterota. Vid de oförorenade kontrollstationerna dominerade däremot Proteobacteria. Experiment visade också att sediment från kontaminerade sediment var mycket mer toleranta mot kvicksilvertillsats än de från rena områden. Campylobacterota och Desulfobacterota skulle därför kunna användas som indikator på Hg förorenade sediment. Studien kompletteras med analys av Hg metyleringsgener *hgcAB* samt resistensgener (*mer*).

Sammanfattningsvis, visade projektet på att det är en stor fördel att analysera fältprover direkt efter provtagning. Detta kan exempelvis göras med Omibox-laboratoriet som kan användas ombord på fartyg. Med det pågående klimatinducerade ökade inflödet av tDOM, kan man förvänta sig ökad produktion av MeHg i kontaminerade områden i norra Östersjön. Bakterier inom de taxonomiska grupperna Campylobacterota och Desulfobacterota skulle kunna användas som indikatorer på Hg kontaminerade sediment, även om vissa pusselbitar återstår att analyseras. Våra experiment indikerar dessutom att dessa grupper kan vara tämligen toleranta mot kvicksilverföroreningar. Sammantaget har det här projektet tagit fram ett ramverk för hur bakteriesamhällets struktur och funktion påverkas av Hg förorening, vilket borde vara användbart i miljöövervakningen.

Terms and abbreviations

THg	Total mercury
MeHg	Methylmercury
tDOM	Terrestrial dissolved organic matter
Omics	A term describing the field of large-scale data in biology. Includes for example genomics and transcriptomics
Bacterial production	Bacterial biomass production. Can be measured by the “leucine” or the “thymidine” method.
FDOM	Fluorescent dissolved organic matter
Fiber bank	Fiber rich sediment originating from paper industry
Beta-diversity	Change in species composition across habitats and ecosystems
16S amplicon sequencing	Sequencing of the 16S gene in prokaryotes (bacteria)
Shotgun sequencing	Sequencing of metagenomes, i.e. random genes of a microbial community.
ANCOM-BC	Analysis of compositions of microbiomes with bias correction. Here used to analyse enrichment of specific taxa in relation to Hg level.
Sulfate reducing bacteria	Anaerobic microorganisms that can use sulfate as terminal electron acceptor. Common bacterial group in anoxic sediments. Carries genes coding for MeHg formation.
Omibox	A portable molecular and sequencing laboratory, developed within the project. DNA and RNA sequencing is performed by two MinION sequencers (Oxford Nanopore Technologies).

1. Introduction

Sediment bacteria have an important ecosystem function as degraders of sinking plankton (e.g. de Moraes et al. 2014). Industrial pollution can, however, change the bacterial community composition and function or even knock out their activity. Thus, bacterial composition and activity can potentially be useful as bioindicators of environmental pollution.

In ecology and environmental management, a bioindicator refers to any biological entity or metric that can be employed to evaluate environmental conditions by observing deviations from its previous state or established threshold values (Sperlea et al. 2022). Previous research has suggested the use of microbes as bioindicators of environmental pollution, highlighting their potential as rapid and sensitive indicators of environmental disturbances caused by contaminants (Caruso 2013). Due to the diverse chemical structures and properties of hazardous substances, different microbial taxa appear to be equipped with particular metabolic machineries to metabolise different types of compounds (Toyama et al. 2009, Podar et al. 2015, Yan et al. 2022). This means that the presence or dominance of certain microbial taxa could serve as an indicator of high concentrations and/or the bioavailability of these compounds.

At the same time, the specific microbial taxa that become dominant in response to pollutants can also be influenced by biogeographical and physicochemical factors, such as redox conditions, organic carbon content, and nutrient levels, as well as sediment type (Wang et al. 2018, Shi et al. 2021, Jia et al. 2023). Consequently, different microbial communities may be expected in different geographic regions. Despite these variations, natural microbial communities often exhibit a high degree of functional redundancy (Chen et al. 2022), where various members of the community contribute to maintaining certain ecosystem functions, regardless of the exact taxonomic composition. Given this functional redundancy, biologically derived indicators like gene expression levels or enzymatic activity can be used to assess the status of specific ecosystem functions (Morris et al. 2020). For instance, genes involved in particular metabolic processes, such as the mercury methylation genes *hgcAB* (Podar et al. 2015) and the Hg tolerance genes *mer* (Jeffrey et al. 1996).

Climate change in northern regions and fate of environmental pollutants

Huge amounts of terrestrial dissolved organic matter (tDOM) is already today transported from land to the Baltic Sea (Gustafsson et al. 2014), and this transport is expected to increase in the future due to climate change (Andersson et al. 2015, Meier et al. 2022). In aquatic environments, tDOM plays a crucial role in determining the fate and distribution of pollutants (Iubel et al. 2021). In particular, increased concentrations of tDOM have been linked to the enhanced formation and bioaccumulation of methylmercury (MeHg) in various aquatic systems, including sediments and waters (He et al. 2019, Poste et al. 2019, Rodriguez et al. 2022, Rodriguez 2023). The way DOM interacts with mercury in water largely depends on both the amount and type of DOM present (Seelen et al. 2023). Different components within DOM can strongly

attach to mercury, which affects how much mercury is available to organisms and how much gets converted into the more toxic form of Hg (MeHg).

On one hand, when mercury binds with a type of DOM that is easily used by microorganisms (let's call this labile DOM or LDOM), it forms aggregates that can be readily taken up by these organisms. This uptake leads to more mercury entering the cells, where it can be converted into MeHg. A good example of LDOM is the organic matter produced by phytoplankton, often referred to as exudates (Koefoed Björnsen 1988). These exudates break down easily or are directly absorbed by microbes, leading to higher rates of MeHg production. On the other hand, when mercury binds with more resistant types of DOM (we can call this refractory DOM or RDOM), it forms aggregates that are not easily degraded. As a result, less mercury is taken up by organisms, and less MeHg is produced.

This interaction between DOM and mercury is a slow process and involves a lot of competition between different molecules in the DOM pool for binding with mercury (Rodriguez et al. 2022, Rodriguez 2023). This has important implications, especially in the context of climate change, as the amount and type of DOM in boreal aquatic systems are expected to increase. Therefore, understanding the chemical makeup of DOM is crucial when studying how mercury becomes available in these environments and how much MeHg is produced and accumulated in the food web. This is particularly important because the organic matter in aquatic systems is a complex mix of materials from both land (allochthonous) and within the water body itself (autochthonous), making it more diverse in composition than in terrestrial environments. Therefore, to better understand how climate change might affect mercury bioaccumulation in aquatic food webs – especially given the anticipated rise in terrestrial organic matter in northern aquatic systems – it is essential to investigate the interaction dynamics between mercury species, organic matter, and the microbial communities that inhabit these environments.

Relevance of in situ multi-omic studies environmental quality and risk assessment

In recent years, the advent of high-throughput sequencing technologies has transformed environmental microbiology, shifting the focus towards the study of complex microbial communities in their natural habitats (Tedersoo et al. 2021). Metagenomic and metatranscriptomic analyses have become essential tools for exploring these communities and understanding their functional roles (Aplakidou et al. 2024).

Traditionally, environmental samples for omic studies, such as DNA/RNA sequencing, required transport of samples to fully equipped laboratories for analysis. However, recent studies have demonstrated the feasibility and advantages of performing on-site sequencing immediately after sample collection, particularly in remote or challenging environments (Goordial et al. 2017). This approach offers several benefits, including real-time data acquisition, a lower risk of sample contamination, and the ability to adjust research methods based on immediate findings. This is particularly useful in environments where traditional lab-based sequencing would be impractical or time-consuming.

While laboratory-controlled experiments are valuable, the intricate interactions between microbial communities and their environments make in situ field experiments essential. Such experiments, which combine metagenomics and metatranscriptomics, help unravel the biochemical processes active in specific

environments at particular times. In contrast, ex situ incubation can disrupt natural conditions, potentially altering microbial community composition or causing molecular degradation. With the purpose of improving these aspects, we developed OmiBox, a fully portable laboratory for in situ multiomic analyses of environmental samples. Integrated into a rolling portable hardcase, it contains all the equipment and power supply required to process environmental samples, including DNA/RNA extraction, library preparation and nanopore sequencing (Oxford Nanopore Technologies). This innovative approach represents a significant advancement in the application of in situ multi-omic analyses. By processing and analyzing samples immediately after collection in the field, this methodology enhances our ability to study microbial communities in real time, within their natural environment. This project marks the first time that in-field multi-omic experiments have been conducted in remote habitats, providing insights into the phylogenetic and functional aspects of microbial life under natural conditions.

In order to develop a fast and reliable method to assess the environmental status and potential toxicity of pollutants in waters, sediments, or soils, it is also vital to identify the microbial taxa, functional genes, and metabolic pathways characteristic of natural communities in the habitat being evaluated. These biological indicators should also be capable of responding to varying pollutant loads under different physicochemical conditions, such as nutrient and organic carbon concentrations. The decreasing costs and increasing miniaturization of omic technologies present an opportunity to develop and implement rapid, sensitive methods for assessing the environmental health of contaminated areas using natural microbial communities.

Although previous studies have demonstrated microbial community responses to environmental pollutant loads, to our knowledge this project represents the first attempt to comprehensively describe the response of microbial communities performing in their natural environment using comparative functional and taxonomic analyses simultaneously. In the project we performed laboratory experiments studying tDOM-induced MeHg formation, executed field surveys in fiber bank gradients, tested Hg resistance in contaminated and clean sediments and developed the Omibox for near in situ analysis. We expected specific bacterial taxa to be enriched in Hg contaminated sediments and that these have developed tolerance to Hg pollution. The knowledge and methodologies developed through this research could be integrated and standardized for applications in environmental monitoring, management, and risk assessment.

2. Methods

2.1 Development of portable lab – Omibox

Manufacture and workflow development

OmiBox was initially designed and subsequently manufactured with technical support from the Department of Physics at Umeå University. Various components and equipment were either purchased, custom-made, or modified as needed. These elements were then assembled, followed by several rounds of preliminary testing to ensure functionality.

A list of the different components of OmiBox is provided below and shown in Figure 1:

- **MinION Mk1C:** The MinION Mk1C is a portable, real-time DNA and RNA sequencing device developed by Oxford Nanopore Technologies. It integrates real-time sequencing, a powerful GPU-based computing capability, and a high-resolution screen in a single portable device, enabling users to sequence and analyse genomic material in the field or in remote locations. The device is highly versatile, supporting various applications such as metagenomics and metatranscriptomics, and is particularly suited for environments where space and resources are limited.
- **Bento Lab:** The Bento Lab is a compact, portable molecular biology workstation designed for DNA analysis in the field or in small labs. It integrates key equipment, including a PCR thermocycler, centrifuge, gel electrophoresis chamber, and blue light transilluminator, allowing for sample preparation, amplification, and visualization in one device. Bento Lab is user-friendly, making it suitable for both professionals and educational purposes, enabling DNA extraction and analysis outside of traditional laboratory settings.
- **Invitrogen Qubit 4 Fluorometer:** The Invitrogen Qubit 4 is a next generation portable size fluorometer designed to accurately measure DNA, RNA, and protein quantity, integrity and quality.
- **MonoLyser:** The MonoLyser is a compact, portable bead mill homogenizer designed for efficient sample disruption and lysis. It is used to prepare biological samples, such as cells and tissues, for DNA, RNA, or protein extraction. The device is lightweight and battery-powered, making it ideal for field use or in laboratories with limited space. Its high-speed bead milling process ensures consistent and rapid sample homogenization, suitable for various research and diagnostic applications.
- **SoloLAB Pop-Up Benchtop Isolator:** The soloLAB™ Pop-Up Benchtop Isolator is a compact, portable containment unit designed for performing sensitive biological or chemical procedures in a controlled environment. It features a transparent, flexible enclosure that creates a sterile or contained workspace. The isolator was customized with two pass-through ports to maintain isolation of the inner environment while allowing wide and unrestricted hand movements.

Additionally, a custom-made ventilation system was installed to provide a continuous inflow of filtered air, ensuring sterile conditions inside the isolator. This setup is ideal for fieldwork or laboratory environments requiring contamination control.

- **Goal Zero Yeti 400 Portable Power Station and Goal Zero 200 solar panels:** The Goal Zero Yeti 400 is a power station with a capacity of 396 Wh and 33Ah (12V). It is suitable to run lights, phones, laptops, and small appliances in off-grid situations such as research expeditions, and can be charged using Goal Zero solar panels.
- **IFISH IceHotel 6-p:** The IFISH IceHotel 6-p is an insulated, portable tent designed to accommodate up to six people (200 cm high and with 7.3 m² floor space). It features a durable, weather-resistant exterior with insulated walls to provide warmth and protection in extreme cold conditions.

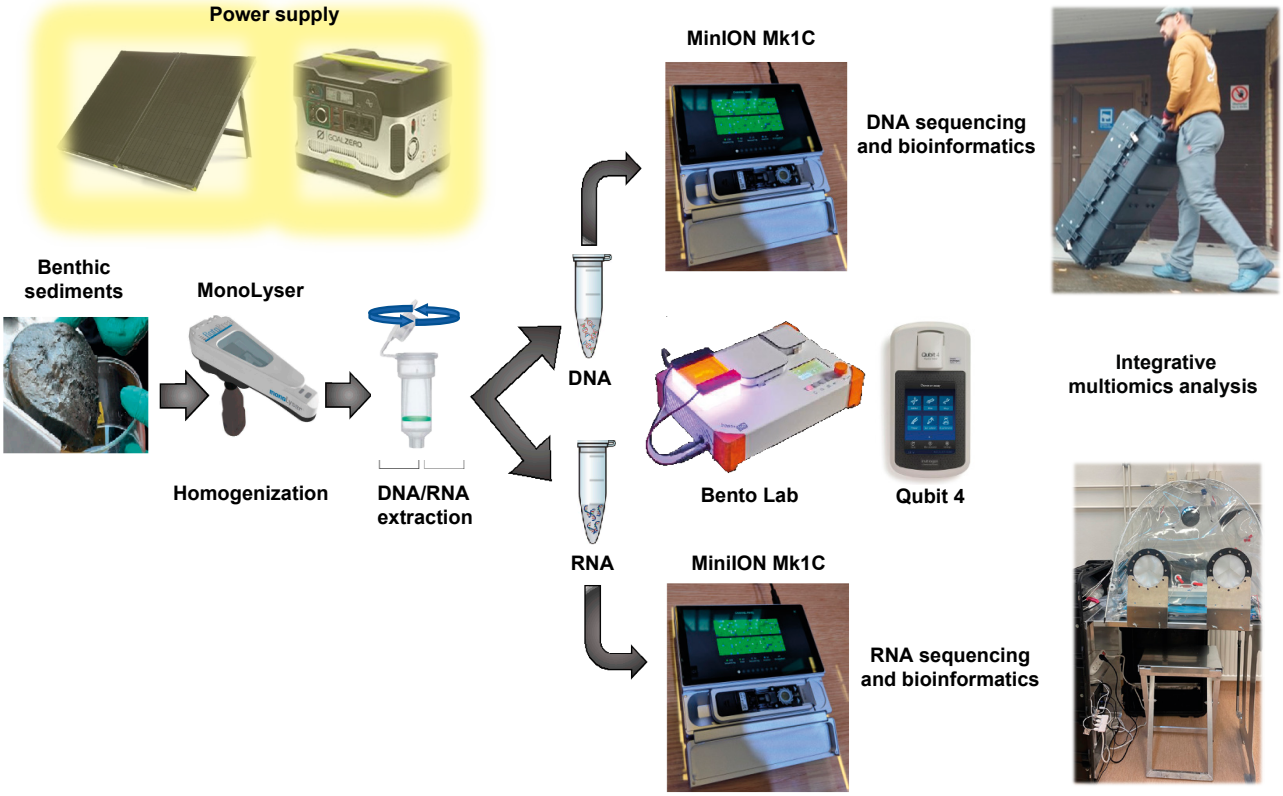


Figure 1. Sample processing workflow and equipment integrated in OmiBox.

Figure 2 provides a detailed overview of the DNA/RNA extraction, library preparation, and sequencing workflow. DNA/RNA co-extraction was carried out using the Zymo-BIOMICS DNA/RNA Miniprep Kit, with centrifugation conditions adapted to the Bento Lab's specifications. The workflow was further customized for DNase I treatments to ensure complete DNA removal. During RNA library preparation, bacterial rRNA was depleted using the NEBNext® rRNA Depletion Kit (Bacteria), enriching for mRNA. Polyadenylation of RNA and cDNA barcoding were performed, followed by sequencing on the MinION Mk1C. Both metatranscriptomic and 16S amplicon sequencing were carried out in parallel using two MinION Mk1C units.

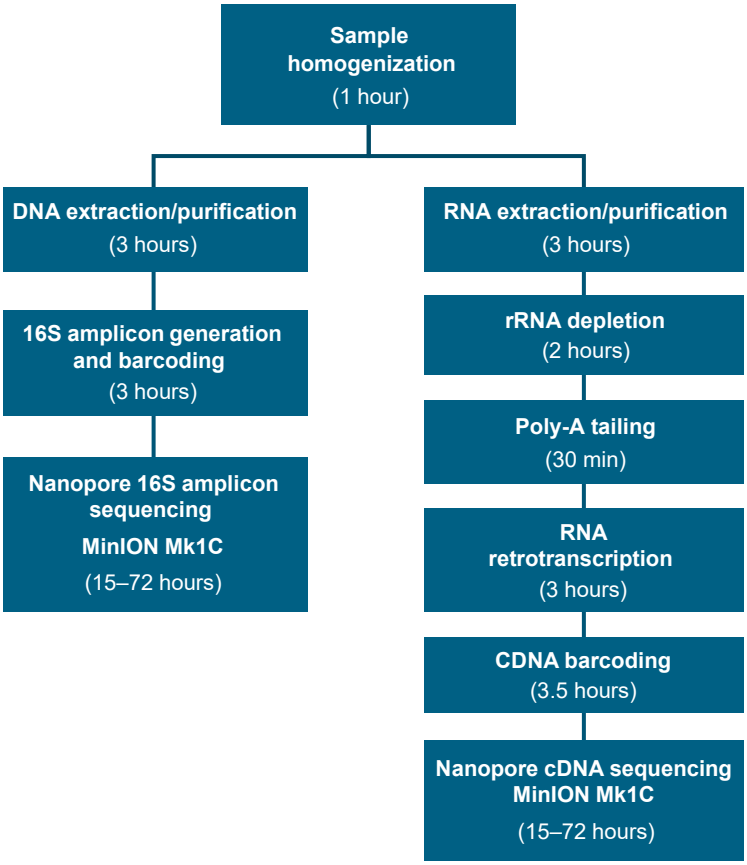


Figure 2. Workflow including the different steps throughout DNA/RNA extraction, 16S rRNA and cDNA library preparation and Nanopore sequencing. Estimated processing times for each step are shown in brackets.

Initial tests

OmiBox was first field-tested on March 24, 2022, near the Umeå Marine Sciences Centre (UMF). The system was deployed inside the IFISH IceHotel 6-p tent to protect against harsh weather. Onboard a hovercraft, sediment cores were collected from the Bothnian Sea through the frozen surface layer (Figure 3). The sediment cores were processed immediately in the field laboratory. A total of six sediment samples (~300 mg) underwent DNA/RNA co-extraction and 16S amplicon sequencing. All equipment was powered by solar panels connected to the Goal Zero Yeti 400 power station.

In September 2022, OmiBox was tested onboard the research vessel KBV181 (Figure 4). Benthic sediment cores were collected from a transect with increasing mercury concentrations (Köpmanholmen area, 63° 10.36’ N, 18° 35.72’ E), and nine samples were processed for DNA/RNA co-extraction and 16S amplicon sequencing onboard.

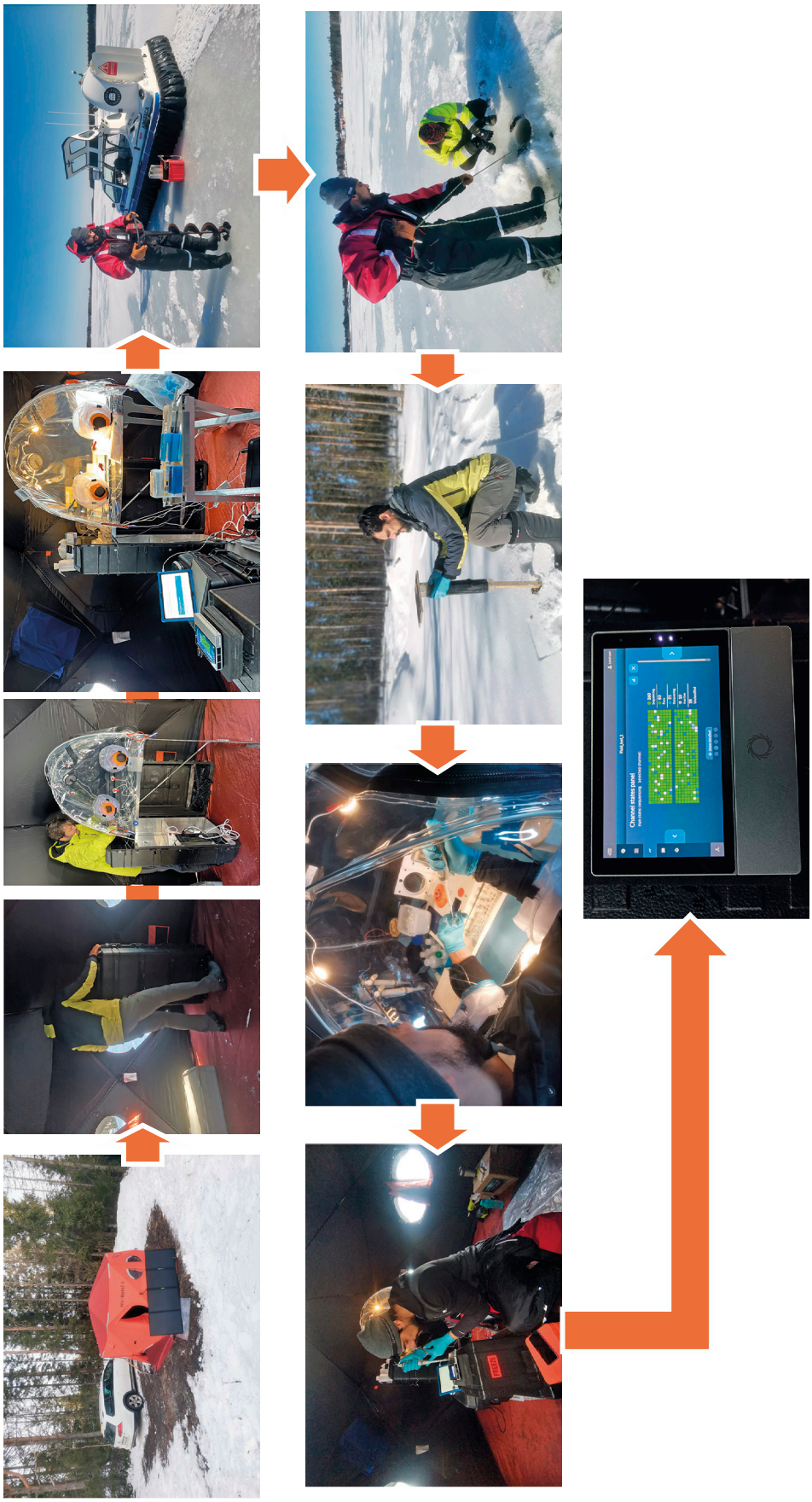


Figure 3. Field experiment carried out to test OmiBox performance in the field under cold conditions, from OmiBox deployment to sample collection, processing and sequencing.

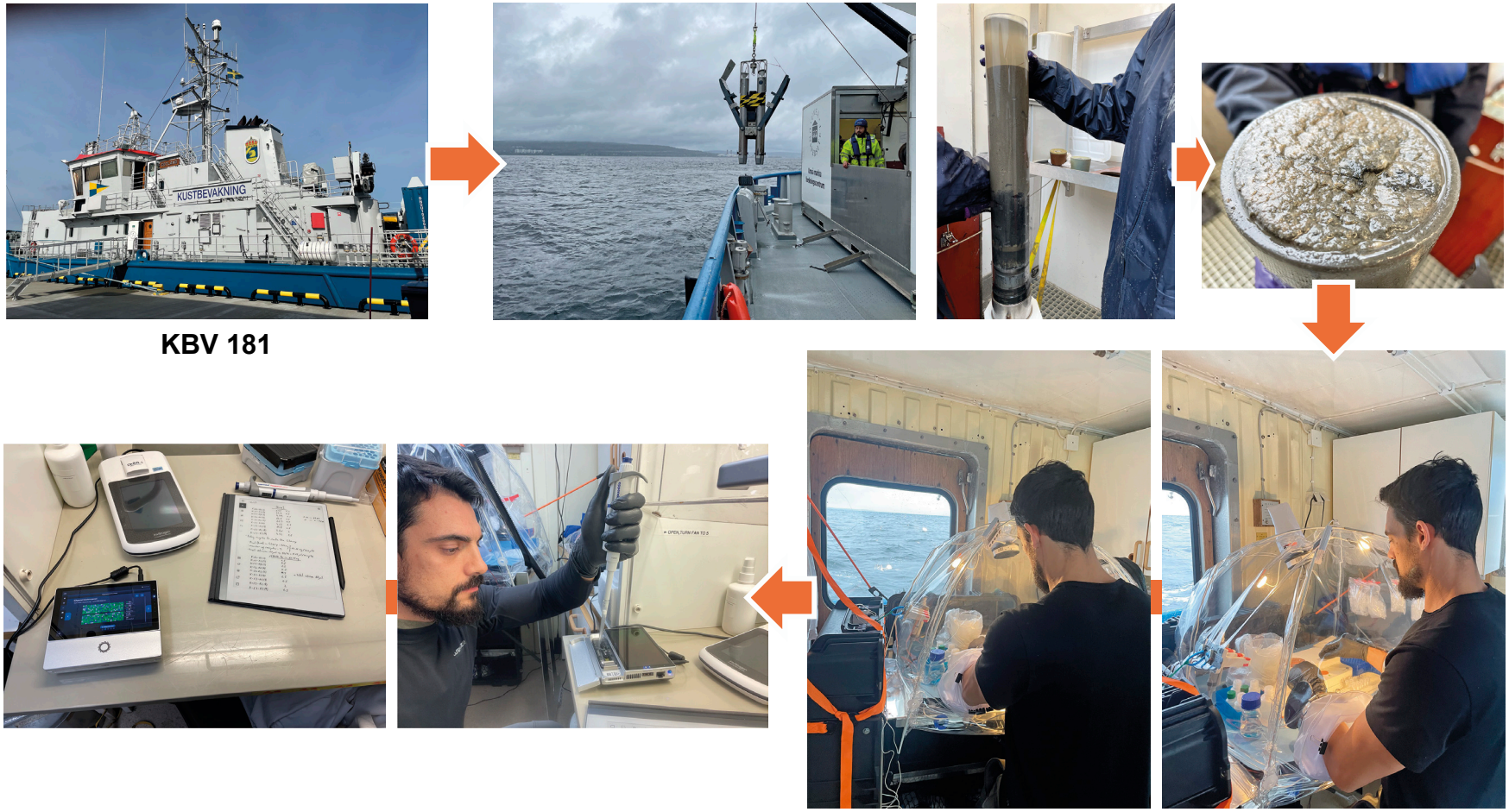


Figure 4. Field experiment onboard the KBV181 research vessel. Collection of benthic sediments, sample processing and sequencing are shown sequentially.

Feasibility of in situ sample processing and multiomics

To test the practicability of *in situ* processing of samples, benthic sediments were collected on April 13th 2024 from a coastal area next to UMF facilities (Öre estuary) using a bottom grab dredge sampler. The sediments were homogenized, and samples were either immediately preserved or processed right after collection, making a total of four treatments: 1) Situ (samples processed immediately after collection); 2) –80 °C (preservation at –80 °C); 3) –20 °C (preservation at –20 °C); 4) RT (preservation at room temperature in 600 µl of Shield Buffer (Zymo Research). Five replicates were set up for each treatment.

Samples were transported to UMF facilities within 10 minutes. OmiBox was deployed in advance so that the processing of samples from in situ treatment started immediately. DNA/RNA co-extraction, 16S amplicon sequencing, and cDNA sequencing were performed as described above (Figure 2). After 60 days of storage, samples from the treatments –80 °C, –20 °C, and RT were processed using exactly the same workflow as previously for the in situ treatment. 16S amplicons and cDNA were simultaneously sequenced in parallel over 48 hours using the two Mk1C devices integrated in OmiBox.

Quality control was carried out using the Qubit fluorometer (DNA/RNA concentrations) included in OmiBox and an Agilent 2100 Bioanalyzer (RNA Integrity Number, RIN).

2.2 Microcosm study of the effect of tDOM on MeHg formation

A microcosm experiment was performed to study the production of MeHg in oxygenated coastal waters and the influence of tDOM inputs (Rodriguez et al. 2022). Terrestrial DOM was added according to a climate change scenario for the northern Baltic region, projecting that the inputs of terrestrial matter to coastal areas in the Baltic Sea will increase 30–100 % within the next 100 years (Hägg et al. 2010). The study focused on MeHg formation mediated by enhanced bacterial production, which in turn was induced by tDOM addition.

Coastal water from the Gulf of Bothnia was incubated in a laboratory at Umeå Marine Sciences Centre at 15 °C and light intensity of ~30 µE m^{-2} s^{-1}. Air was gently pumped into the aquaria to ensure aeration of the seawater. Four treatments were applied 1) ambient conditions (Control), i.e., natural DOC concentration and no Hg addition; 2) addition of Hg (Hg^{+}); 3) addition of DOC corresponding to ~40 % increase from natural levels, as well as addition of Hg (DOC_{40}-Hg^{+}); 4) addition of DOC corresponding to ~70 % increase from natural levels, plus addition of Hg (DOC_{70}-Hg^{+}). The increase in DOC concentration was achieved by the addition of a tDOM extract derived from humic rich soil material collected locally at the Öre river bank, as described by Rodriguez et al. (2022). Hg(II) was added as $HgCl_2$ to a final concentration of ~250 pM Hg(II) to treatment 2–4. Samples for analysis of DOC, nutrients and bacterial production were collected over 5 days.

DOC and nutrients were analysed using standard methods and bacterial production was measured using the [^{3}H-methyl]-thymidine incorporation method, as described in Rodriguez et al. (2022). Total Hg was measured using the US EPA method 1631E combined with isotope dilution analysis using cold-vapor inductively

coupled plasma mass spectrometry (CV-ICPMS), as described in Rodriguez et al. (2022). MeHg was measured by thermal desorption-gas chromatography combined with ICPMS (TDGC-ICPMS 6890 Agilent GC, and 7700 ICPMS) (Rodriguez et al. 2022).

2.3 Field study of sediment microbial communities in Hg gradients

A field survey was carried out in the Gulf of Bothnia in September 2022 onboard the ship KBV181 (Figure 5). Three fiber bank gradients were sampled: one in the Bothnian Bay (Bureå) and two in the Bothnian Sea (Köpmanholmen and Sundsvall (Table A1A–B) (Norrlin et al. 2016). We also sampled unpolluted control stations in the Bothnian Bay (A5) and in the Bothnian Sea (C3 and C14).

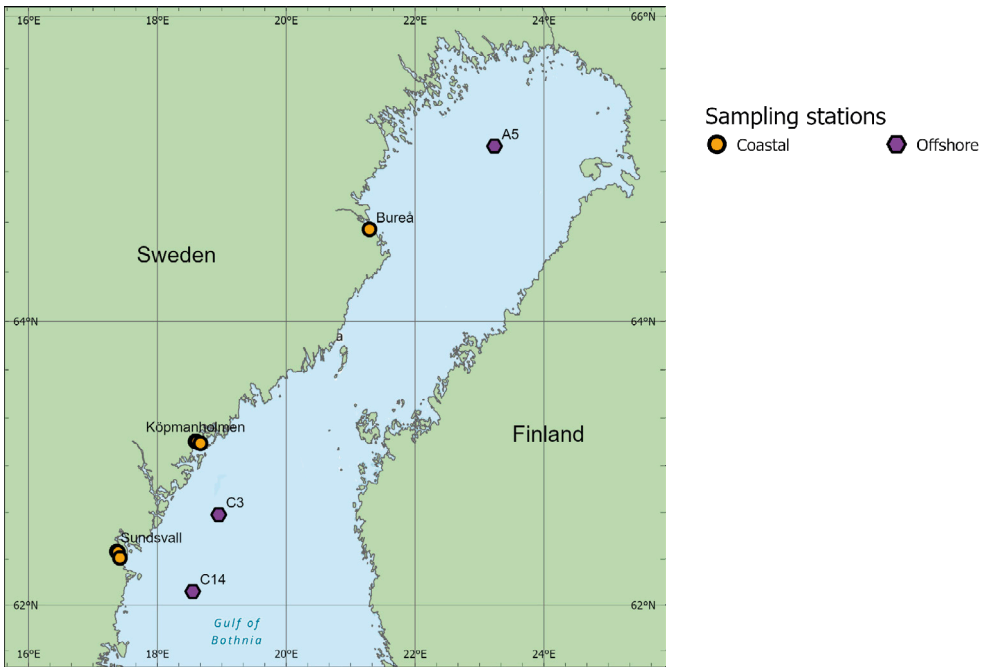


Figure 5. Map of the sample sites.

Sediment cores were collected using a Gemini sampler connected to a crane. Sediments from the upper 5 cm were then collected from the cores with a spoon. 50 ml was collected in Falcon tubes for MeHg and total Hg analysis, and 2 ml Eppendorf tubes for microbial analyses. The Eppendorf tubes were placed on dry ice immediately after collection and then in liquid N_2 once all samples from the site were collected. Chemical analyses (C, N and P) were performed on both solid sediments (freeze-dried and homogenized) and pore water (sediment pore water extracted and filtered on 0.2 µm).

Hg and MeHg analysis in water

Total Hg was determined following the US EPA 1631E method and using isotope dilution analysis. Samples were preserved by acidification to 0.1 M HCl immediately after collection and a ^{200}Hg isotope enriched standard (Oak Ridge National Laboratory, batch 185091) was added prior to BrCl digestion. The measurements were done by an on-line cold vapor generation system (HGX-200, Cetac) with $SnCl_2$ reduction connected to an Agilent 8900 inductively coupled plasma mass spectrometer. Methylmercury was determined as previously described (Lambertsson & Björn 2004; Munson et al. 2014). Samples were acidified to 0.1 M HCl immediately after collection. Prior to analysis, a Me^{200}Hg isotope enriched standard (synthesized in-house from ^{200}HgO, Oak Ridge National Laboratory, batch 185091) was added. MeHg was ethylated by sodium tetraethylborate followed by purge and trap onto Tenax adsorbent tubes. Thermal desorption-gas chromatography combined with inductively coupled plasma mass spectrometry (TDGC-ICPMS, 6890 Agilent GC and 7700 ICPMS) were employed for the measurement of ethylated mercury species.

FDOM analysis

Absorbance and Fluorescence measurements were carried out using a HORIBA AquaLog UV-800 fluorometer. For analysis, the samples were transferred into three-fold rinsed 10 mm quartz cuvettes. In a first step, the absorbance at 254 nm was measured for each sample. Waters with absorbance254 values between 0.03 and 0.3 RU were analyzed undiluted, whereas waters with higher values were diluted. This procedure minimizes oversaturation and inner-filter effects. Fluorescence and absorbance were then measured simultaneously in the range of 200 to 800 nm at increments of 5 nm and integration time of 1 second. Fluorescence and absorbance data were further used to compute a PARAFAC model in the dreem toolbox Version 0.6.4 for Matlab (version 2023a) (Murphy et al. 2013). The same toolbox was used to identify fluorescence peaks and indices.

DNA extraction, sequencing and bioinformatics

Sediment samples were stored at −80 °C prior to extraction. DNA was extracted using ZymoBIOMICS DNA/RNA Miniprep Kit (Ver 2.0.0), eluted in 50 µl elution buffer, and quantified using a Qubit fluorometer HS kit. DNA concentrations extracted ranged from 17 to 43 ng/µl.

Amplification of 16S gene was performed by PCR using the 16S Nanopore Barcoding Kit 24 V14 (SQK-16S114.24), 10 ng of DNA template, and the standard kit protocol. The kit amplifies full length 16S using the primers 27F 5′-AGAGTTTGATCMTGGCTCAG and 1492R 5′-CGGTTACCTTGTTACGACTT. Amplicons were tested using Qubit which detected a mean concentration of 9 ng/µl and fragments were assessed using agarose gel electrophoresis. Samples were pooled together in equimolar quantities for a total 50 fmol per library for sequencing on Nanopore MinION Mk1C (Oxford Nanopore Technologies plc.). The library was split over two flow cells, type FLO-MIN114, which generated a mean of 250 000 reads per sample after trimming for quality and length.

Downstream analysis

For the downstream analyses, MinKNOW software version 24.06.14 with Dorado 7.4.13 (c) 2024 Oxford Nanopore Technologies PLC was used to basecall reads during sequencing in 450 bps HAC mode (high accuracy), min read length 200 bp. 16S reads were demultiplexed and barcode indexes trimmed using MinKNOW.

Reads were trimmed according to expected lengths using Chopper v0.6.0 (De Coster and Rademakers, 2023). Min length 200, max 2000 for 16S gave a mean quality of Q15.7.

QIIME2 (version qiime2-amplicon-2024.5) was used to perform closed reference clustering by VSearch at 97 % identity to form OTUs. The same databases were used for clustering and for taxonomic assignment. Low abundance features of less than ten occurrences across all samples were removed. Taxonomy for the 16S reads was assigned using the Greengenes database version 2022.10 (McDonald et al. 2023) which identified 715 named OTUs.

Shotgun analysis

The extracted DNA was used to prepare standard shotgun metagenomics sequencing libraries using NEBNext® Ultra™ II DNA Library Prep Kit (Cat No. E7645). Sequencing was performed on a NovaSeq 6000 S4 flowcell with paired-end 150 bp reads, generating at least 10 G of raw data per sample (Novogene, Cambridge, UK). Sequence read quality was assessed with FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Adapter sequences and low-quality bases were removed using fastp with default parameters (Chen et al. 2018). Clean reads from all the samples will be merged and co-assembled using MEGAHIT (Li et al. 2015). The resulting contigs will be used to predict functional genes by prodigal (Hyatt et al. 2010). The *hgcA* and *hgcB* genes, indicators of Hg methylation (Podar et al. 2015), and the Hg tolerance mer genes (Jeffrey et al. 1996), will be selected for further analysis.

Statistical analyses

Analysis of dissimilarity of bacterial composition between contaminated and clean sites

Bray-Curtis plots were performed to analyze the dissimilarity of bacterial communities at contaminated and clean sites. The analysis was based on a distance matrix, where distances are calculated within and among groups (Birtel et al. 2015). All samples in the clean category were compared pairwise to one another and values calculated, then all samples in the contaminated category were compared to each sample in the clean category. Bray-Curtis takes into account presence and absence of species as well as their abundances. The values are distances calculated using the Bray-Curtis dissimilarity index; the higher the value, the more different the samples are.

Analysis of possible indicator taxa

To find possible indicator taxa related to Hg pollution, multivariate analysis was performed. To test for significant differential abundances of taxa alongside total mercury concentrations measured at each site, ANCOM-BC (analysis of composition with bias correction which uses linear regression) was used (Lin and Peddada, 2020). Mercury concentrations (DW) were grouped for ANCOM-BC analysis as follows: low < 0.1 ug g^{-1}, medium < 1 ug g^{-1}, and high ≥ 1 ug g^{-1}. Methylmercury concentrations (DW) were grouped as follows: low < 1 ug g^{-1}, medium 1–5 ug g^{-1}, high ≥ 5 ug g^{-1}.

2.4 Test of bacterial tolerance to Hg addition in contaminated and clean sediments

The aim of this sub-project was to elucidate at what Hg pollution load the benthic ecosystem is at risk of losing its important ecosystem function to degrade sinking plankton. A Hg addition experiment was carried out with natural sediments from polluted and clean areas. Yeast extract was used as a model substance for sinking plankton. Yeast has a similar cellular composition to eukaryotic phytoplankton. Heterotrophic bacterial production was monitored over a three-day period, as a signal for ecosystem degradation of sinking plankton. Bacterial production was measured using the leucine method (Kirchman et al. 1985, Van and Riemann 1993), as the thymidine method has been shown to be less suitable for anaerobic bacteria (Winding 1992). Additionally, the bacterial community composition is presently being analyzed.

Field sampling

Sediments from two contaminated sites (Köpmanholmen and Obbola (63°41'33.14"N, 20°19'48.32"E) and two unpolluted sites (Öre and Gavik) were collected at 20 m depth during autumn 2024. Sediment cores were transported to the department of Ecology and Environmental Science (EMG), Umeå University, where the experiments were carried out.

Laboratory experiment

The upper 5 cm sediment was collected and diluted in seawater to obtain a slurry. The slurry samples were incubated in Erlenmeyer flasks and kept homogenized and oxygenated by magnetic stirring. Experiments were carried out in a temperature-controlled room.

A fixed amount of yeast extract was added to all samples, 1 mg C l^{-1}. Metallic Hg was added in a gradient following: 0.1, 0.5, 1.5, 5, 15, 50, 150, 400 and 800 µg Hg g^{-1} (wet sediment) at unpolluted sites and 1.5, 5, 15, 50, 100, 200, 400, 800 and 1 500 µg Hg g^{-1} (wet sediment) at contaminated sites. Samples were incubated in darkness at 10 °C for three days.

At the beginning and end of the experiment, samples for Hg analysis, DNA sequencing and bacterial production were collected. Hg analysis will be performed at the Erik Björn lab (https://www.umu.se/forskning/grupper/erik-bjorn-lab/). Bacterial production was analyzed using the "leucine method" (Kirchman et al. 1985), which has been shown useful for benthic samples. These samples were analyzed in sealed capsules in a scintillator at Umeå Marine Sciences Centre. Bacterial DNA was sequenced using an Oxford Nanopore MinION sequencer (Mikheyev and Tin 2014), which is available at the EcoChange laboratory, EMG, UmU.

Used material were treated according to regulations. A permit to perform the experiment has been approved by Kemikalieinspektionen (Diarienr. 5.2-H24-06327).

3. Results

3.1 Portable laboratory-Omibox

Initial tests

During the March 2022 field test, OmiBox operated entirely off-grid using solar energy from the Goal Zero Nomad 200 panels. Once the samples were collected and transported to the field camp, a total of approximately 6 hours were required to complete the workflow from DNA/RNA extraction and purification to 16S amplicon sequencing. cDNA library preparation had to be suspended due to cold conditions towards the end of daylight, which caused the freezing of some of the reagents required during the workflow. The 16S amplicon sequencing by the MinION Mk1C proceeded over approximately 24 hours, which generated circa 11 million reads in total (i.e., circa 1.8 million reads per sample) with an average fragment size of 1.52 kb.

During the field test onboard the KBV181 research vessel, OmiBox was deployed inside a lab container and operated on-grid by connecting the power station to the vessel's power supply. DNA/RNA co-extraction and 16S library preparation were completed within 24 hours, where the workflow had to be interrupted and subsequently resumed two times due to adverse weather conditions (intense shaking caused by waves). 16S amplicon sequencing by MinION Mk1C was run for about 24 hours, obtaining a total of circa 13 million reads (around 1.4 million reads per sample) with an average fragment size of 1.51 kb. Data from both field experiments are currently being analysed.

Feasibility of in situ sample processing and multiomics

Both DNA and RNA were successfully extracted, purified, and sequenced within 24 hours after sample collection. Figure 2 shows approximate times from each of the steps during 16S amplicon and cDNA library preparation. As expected, cDNA library preparation was significantly the longest phase, taking circa 40 % of the entire workflow. 16S amplicon and cDNA sequencings were run in parallel by the two MinION Mk1C units for approximately 36 hours, which included around 1.5 hours of sequencing time on the road while transporting the equipment back to EMG facilities at Umeå University.

Total genomic DNA concentration after purification ranged between 36.2 and 64.56 ng/µl (in 100 µl elution volume) with no significant differences between in the samples processed in situ and samples stored at –80 °C and –20 °C. However, samples preserved at room temperature using Shield buffer showed significantly lower DNA concentrations, ranging between 2.6 to 9.6 ng/µl. Similarly, total RNA concentrations after purification were not significantly different among in situ, –80 °C, and –20 °C samples, ranging between 21.2 to 35.4 ng/µl (in 16 µl elution volume), while RNA concentrations in samples preserved at room temperate were significantly lower (8.72–19 ng/µl).

After quality control, samples extracted in situ showed the highest RNA Integrity Number (RIN) values (8.42 ± 0.47), followed by samples stored at –80 °C and –20 °C (7.83 ± 0.24; no significant differences). The samples stored at room temperature showed a high degree of degradation as indicated by the electropherogram profiles and low RIN values (2.40 ± 0.16) (Figure 6). Furthermore, cDNA concentrations after RNA polyadenylation and retrotranscription were generally higher in samples processed in situ (2.27 ± 0.36 ng/µl) compared to –80 °C and –20 °C samples (0.256 ± 0.06). Samples stored at room temperature showed significantly lower cDNA concentrations (0.19 ± 0.06).

Differences in taxonomic composition and gene expression patterns are being currently analyzed.

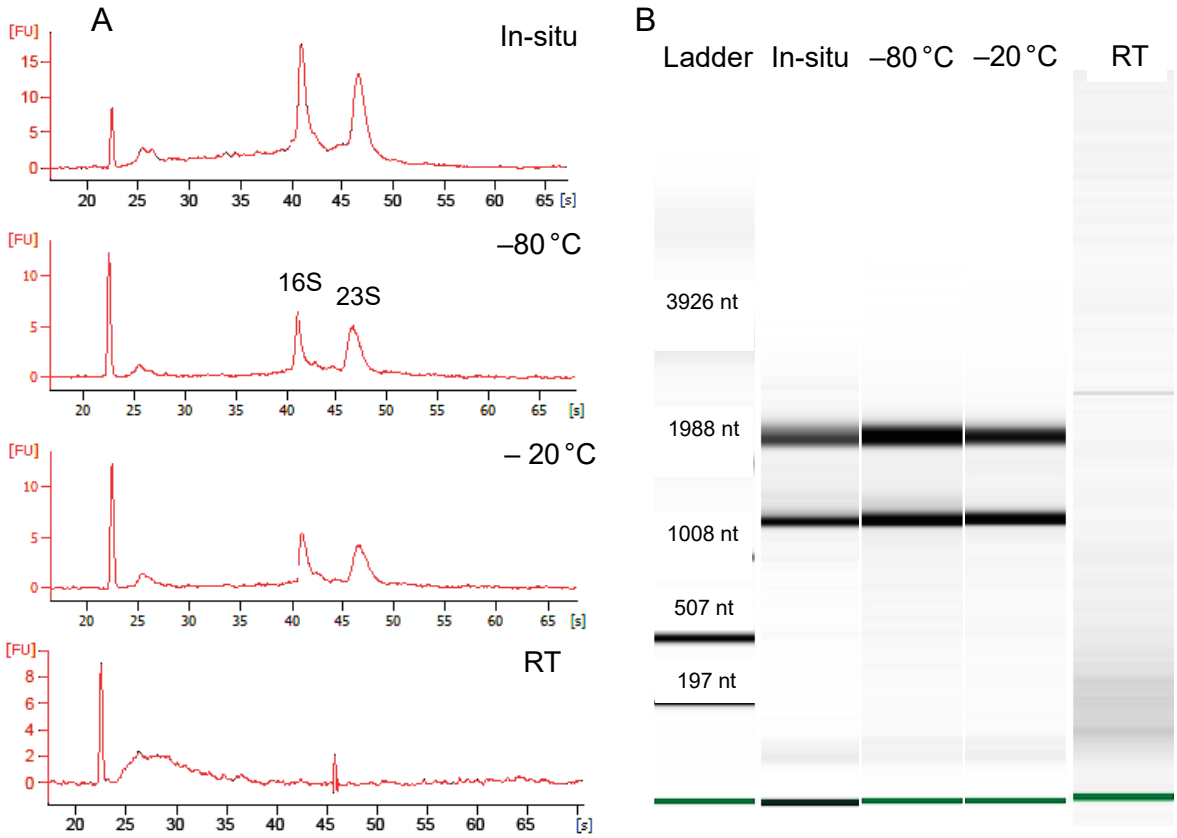


Figure 6. Electropherogram (A) and gel electrophoresis from Agilent 2100 Bioanalyser showing integrity of total RNA extracted from samples processed in situ and samples stored at –80 °C, –20 °C, and at room temperature (RT) in Shield buffer. Peaks in the electropherograms refers to the ladder, 16S rRNA, and 23S rRNA respectively. In the gel electrophoresis, the size (nucleotides) of the bands from the ladder are shown.

3.2 Production of MeHg in oxygenated coastal water – influence of tDOM

The experimental results showed that heterotrophic bacterial production increased with tDOM addition (Figure. 7 A and B), and that MeHg was quickly formed in treatments where $HgCl_2$ was added (Figure 8). Moreover, MeHg formation was coupled to the magnitude of bacterial production, which in turn was regulated by the tDOM addition (Figure 9). The enhanced Hg methylation activity was not linked to any substantial changes in bacterial community composition or gene pool, but instead it was influenced by the bacterial overall activity.

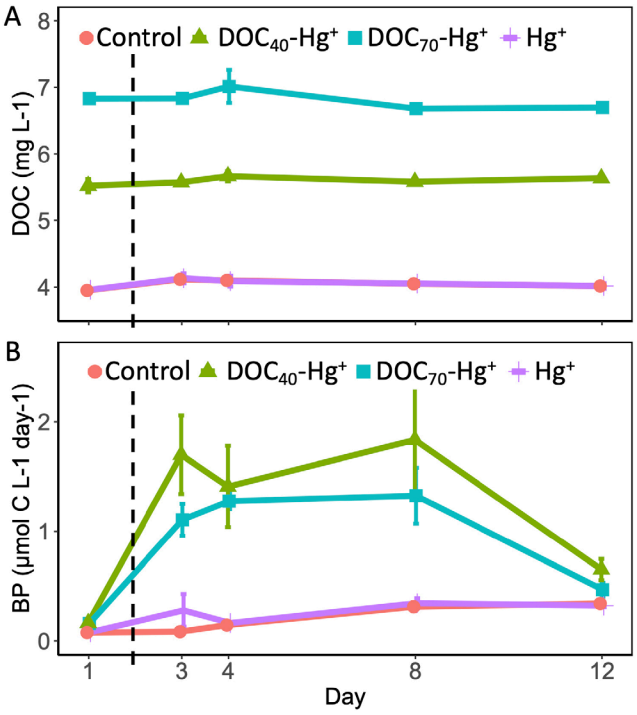


Figure 7. Concentration of dissolved organic carbon (A) and bacterial production (B) in the different treatments throughout the experiment. The dashed line indicates the addition of Hg(II). Error bars represent the standard error ($n = 3$). From Rodriguez et al. 2022.

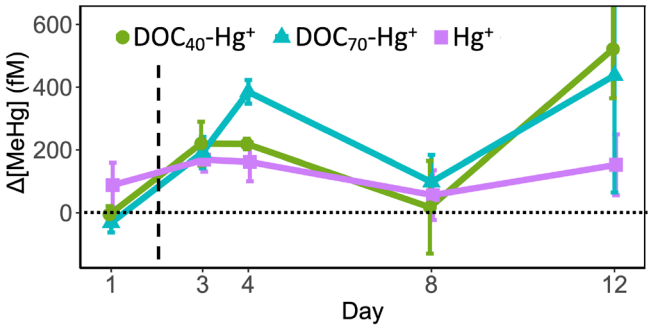


Figure 8. Increment of methylmercury (ΔMeHg) concentration over the unexposed control throughout the experiments in the treatments with addition of Hg(II). $\Delta[MeHg] = Value_{(treatment)} - Value_{(Control)}$. The dashed line indicates the addition of Hg(II). Error bars represent the standard error ($n = 3$). From Rodriguez et al. 2022.

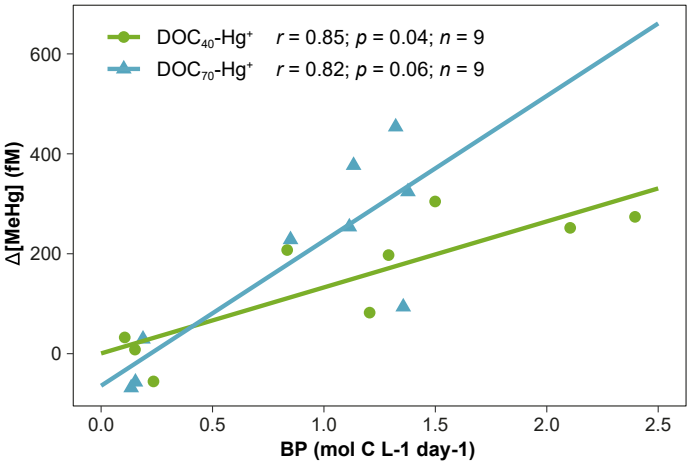


Figure 9. Pearson correlation between MeHg concentration (normalized against control; Δ[MeHg]) and bacterial production (BP) over the first 4 days of the experiment in the DOM and Hg treatments. Pearson correlation coefficients (*r*), *p*-values (*p*) and number of observations (*n*) are shown. Modified after Rodriguez et al. 2022.

3.3 Sediment chemistry and bacterial taxonomic composition in Hg pollution gradients

CNP concentrations in sediments

The pore water CNP concentrations showed variation, but there was no general up- or down-going trend between fiber banks and the unpolluted sites, and no general geographical difference (Figure A1). The pore water content of the sediment was on average 74 %, the DOC concentration ~100 mg l^{-1}, TDN 1 570 ugl^{-1} and TDP 160 ugl^{-1}. The pore water molar C:N ratio was 76, C:P 1 640 and N:P 20. The carbon and nitrogen content of the particulate sediment also showed variations, but as for the pore water there was no general differences between fiber banks and unpolluted sites (Figure A2). It may be noted that for one of the fiber bank sites, Sundsvall 1, the particulate carbon content was extremely high, maybe due to sampling in a locally fiber-rich area. Average molar C/N ratio was higher in particulate sediment than in pore water, but the opposite condition prevailed for the C/P and the N/P ratios (Table 1).

Table 1. Average CNP stoichiometry of sediment pore water and particulate sediment. For pore water DOC, TDN and TDP were used. For dry particulate sediment, data are total C, N and P.

Average	C/N (mol/mole)	C/P (mol/mole)	N/P (mol/mole)
Pore water	17	9 286	452
Sediment	90	1 931	23

Qualitative variations of organic matter in pore water

In difference to CNP concentrations, the FDOM analysis showed qualitative geographical differences of the organic matter in the sediment pore water. The PARAFAC analysis identified three different components: C1, which has previously been described as protein-like (Stedmon and Markager 2005, Yamashita et al. 2010, Harjung et al. 2019), C2 representing humic substances of terrestrial origin (Shutova et al. 2014, Tanaka et al. 2014) and C3 comprising humic substances of microbial origin (Li et al. 2015, Brogi et al. 2019). We cannot quantify the concentrations of these components, instead we calculated their relative contribution to the total fluorescence signal in %. Based on this, samples showed a geographical and site-specific variation: The relative contribution of the protein-like component was higher in the Bothnian Sea than in the Bothnian Bay (Figure 10). Within the Bothnian Bay, the protein component was higher at the control station (A5) than in the Bureå fiber bank. The terrestrial humic substance component was higher in the Bothnian Bay than in the Bothnian Sea. Within the Bothnian Bay, the highest fraction of the terrestrial humic FDOM was observed in the Bureå fiber bank. The fraction of microbial humic substances was in general higher in the Bothnian Bay than in the Bothnian Sea.

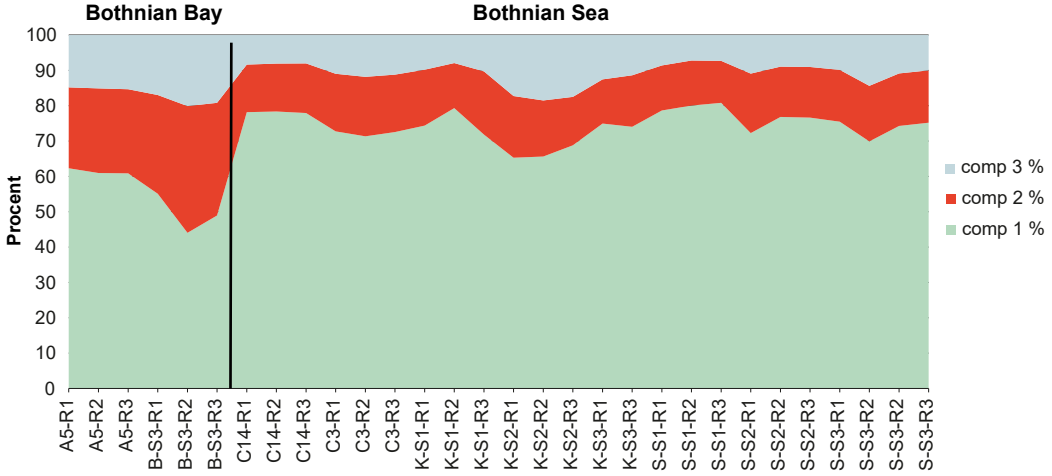


Figure 10. Results of FDOM analysis at different sites. Replicates are technical replicates. C1 = protein component, C2 = humic substances or terrestrial origin, C3 = humic substances of microbial origin.

Total mercury and methylmercury in fiber banks and at control sites

The total mercury and methyl mercury concentrations were much higher in the Bureå, Köpmanholmen and Sundsvall fiber banks than at the control sites (A5, C3 and C14) (Figure 11A–C). The MeHg concentrations were 20–320 times higher in the fiber banks than at the control sites, and the total Hg concentrations were 10–80 times higher at the control sites. THg and MeHg did not show the same spatial pattern. Highest THg levels were found in Bureå and Köpmanholmen 1–2, while highest MeHg concentrations were observed at Sundsvall site 2 and Köpmanholmen site 2 (Figure 11A–B, Table A2). The Hg methylation level was highest in Köpmanholmen and Sundsvall, reaching 5–8 % (Figure 11C).

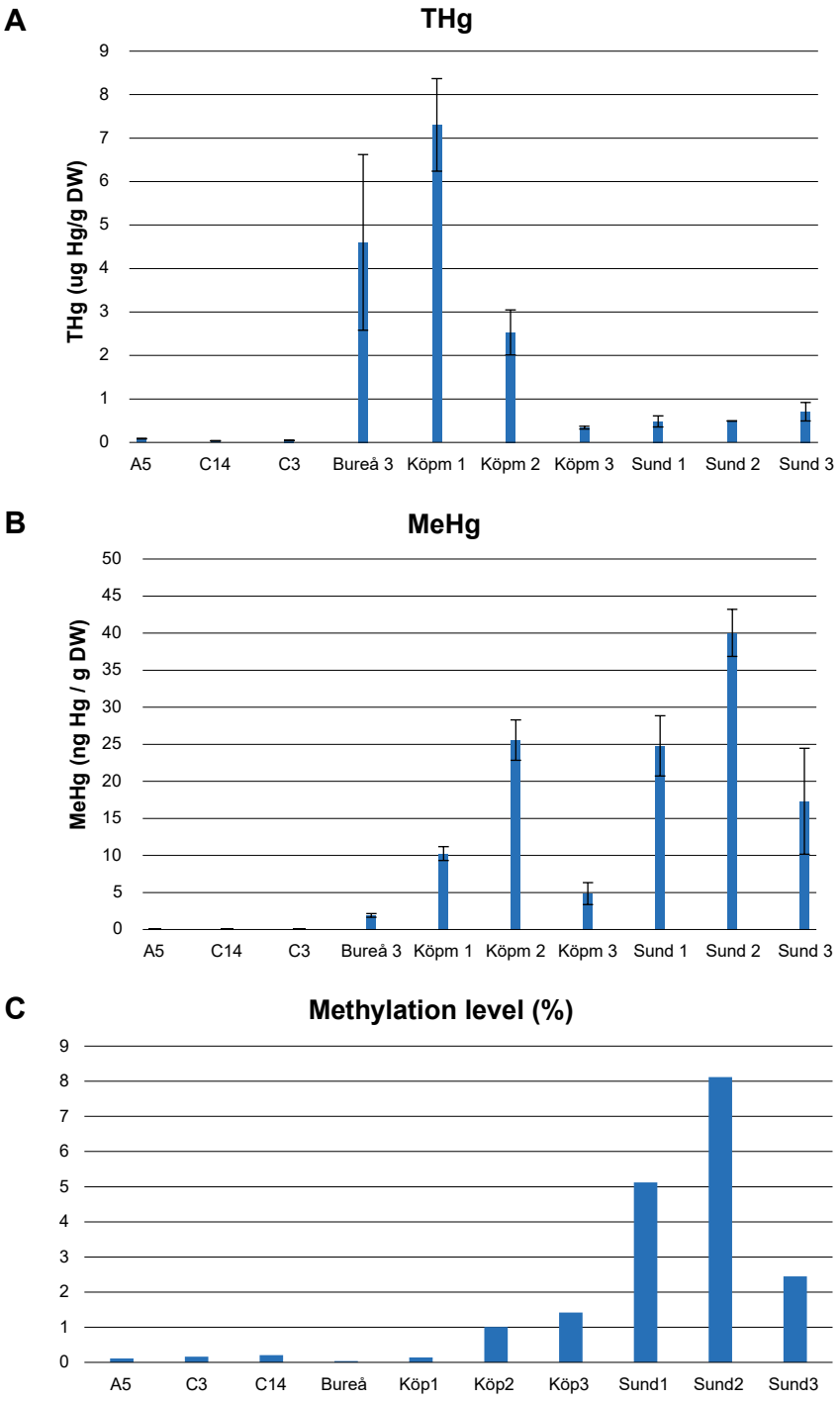


Figure 11 A–C. Average total Hg (A) and MeHg (B) concentrations and methylation level (C) at different sampling sites. Error bars denote standard error of 3 technical replicates.

Bacterial taxonomic compositions in fiber banks and at control sites

The bacterial community compositions at contaminated and unpolluted "clean" sites were compared, using Bray-Curtis dissimilarity analysis. The results showed that community composition differed between polluted and clean sites (Figure 12). The box plot indicates that the bacterial communities at the clean sites have some spread, but the bacterial communities at the contaminated sites show larger distance to the clean sediments.

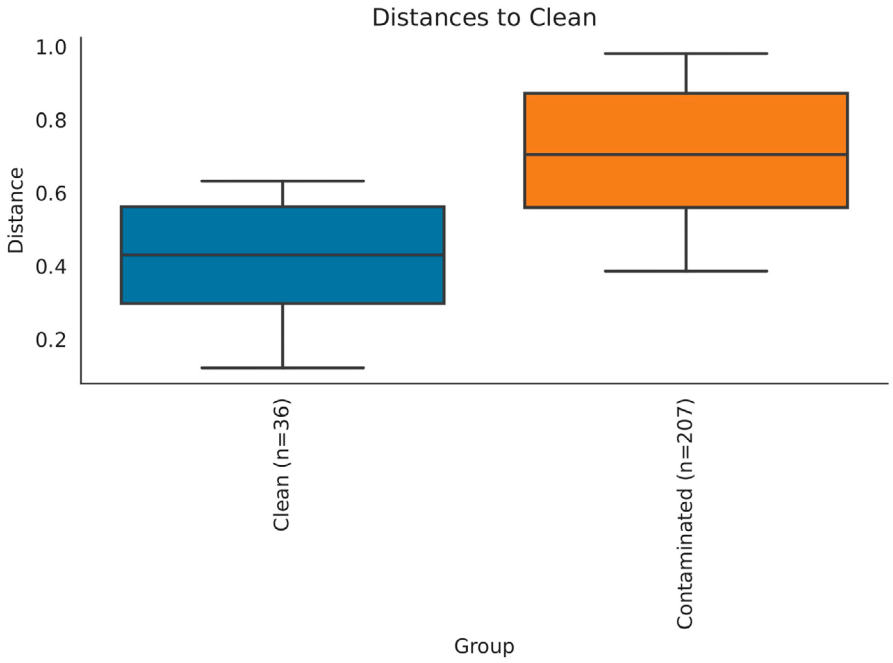


Figure 12. Plots of the dissimilarity between bacterial community composition at clean and Hg contaminated sediments, Bray-Curtis dissimilarity (e.g. Birtel et al. 2015).

Looking more in-depth into the bacterial community composition, we found occurrence of many different phyla, for example Cyanobacteria, Proteobacteria, Desulfobacteriota and Campylobacterota. We ordered the bacterial groups along total Hg and MeHg pollution gradients; high, moderate and low pollution (Figure 13–14). From that we could envisage taxonomic gradients along the pollution gradients. However, to further analyze if such a relationship exists, we performed ANCOM-BC analysis, and found that certain bacterial groups were enriched at high and moderately high total Hg (THg) and MeHg levels (Figure 15–16). These groups were the phylum Campylobacterota and a number of Desulfobacterota groups. In general, these groups showed enrichments both by THg and MeHg.

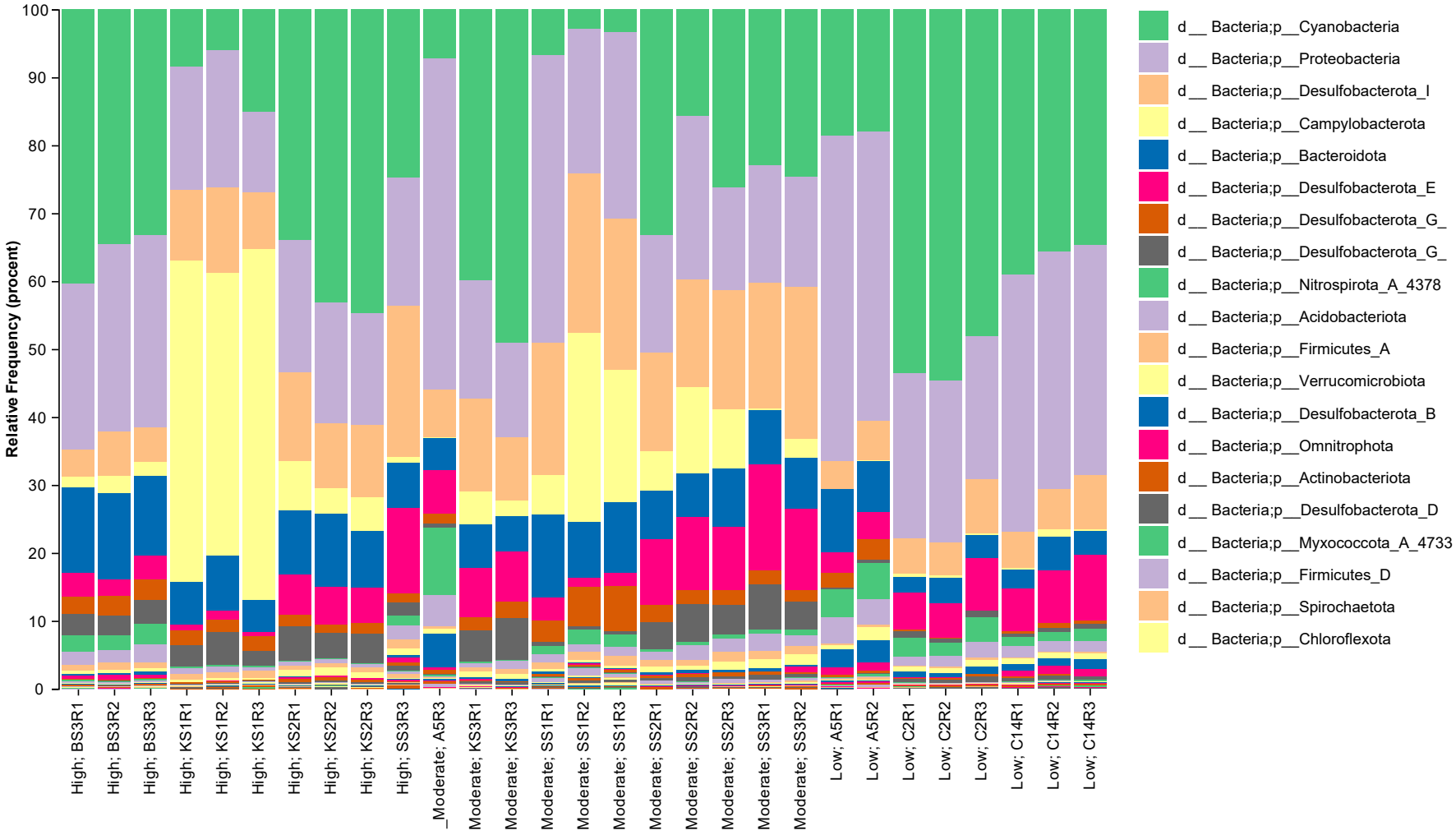


Figure 13. Bacterial taxonomic composition at the phylum level at high, moderate and low total Hg contamination level. Different technical replicates are also included.

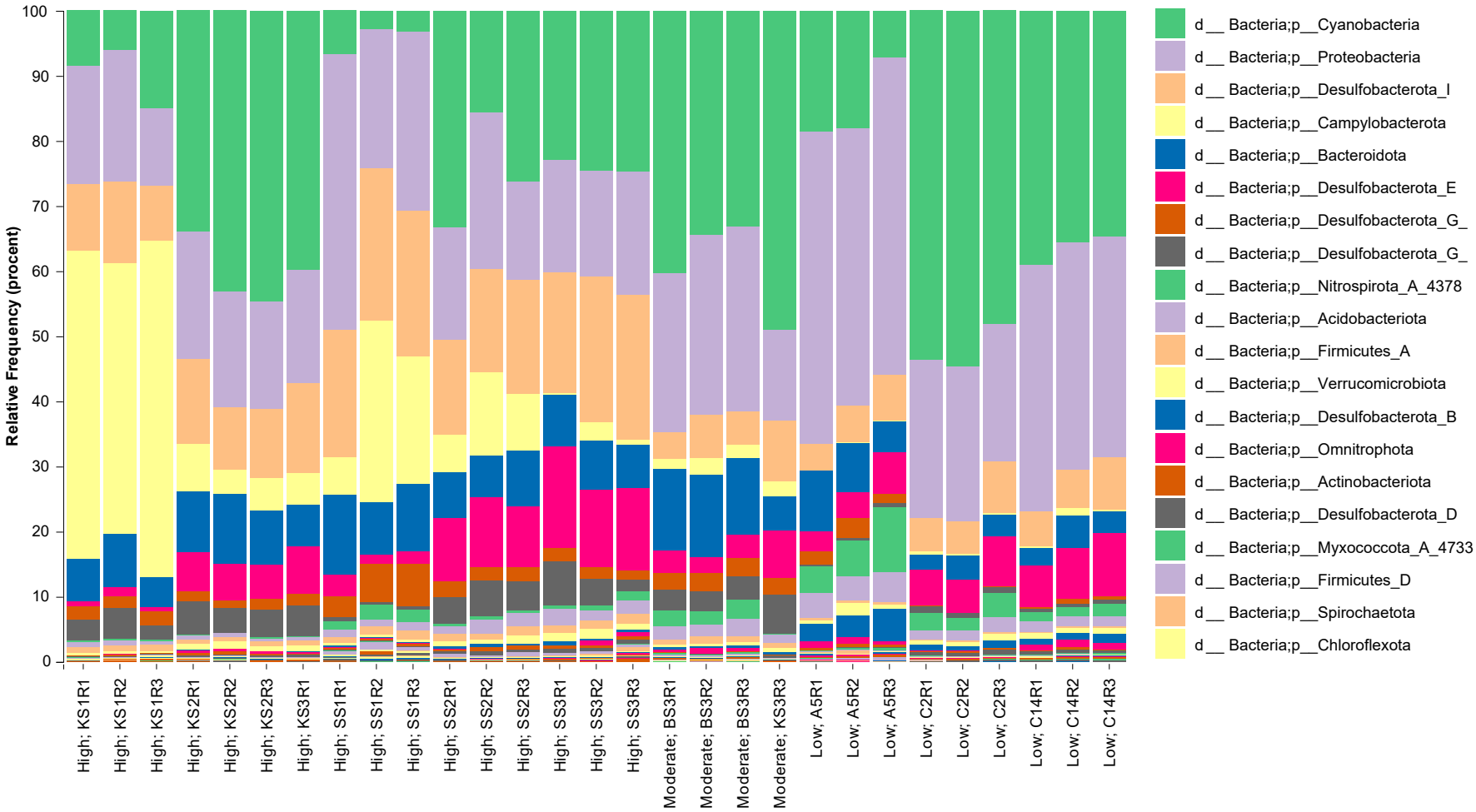


Figure 14. Bacterial taxonomic composition at the phylum level at high, moderate and low MeHg contamination level. Different technical replicates are also included.

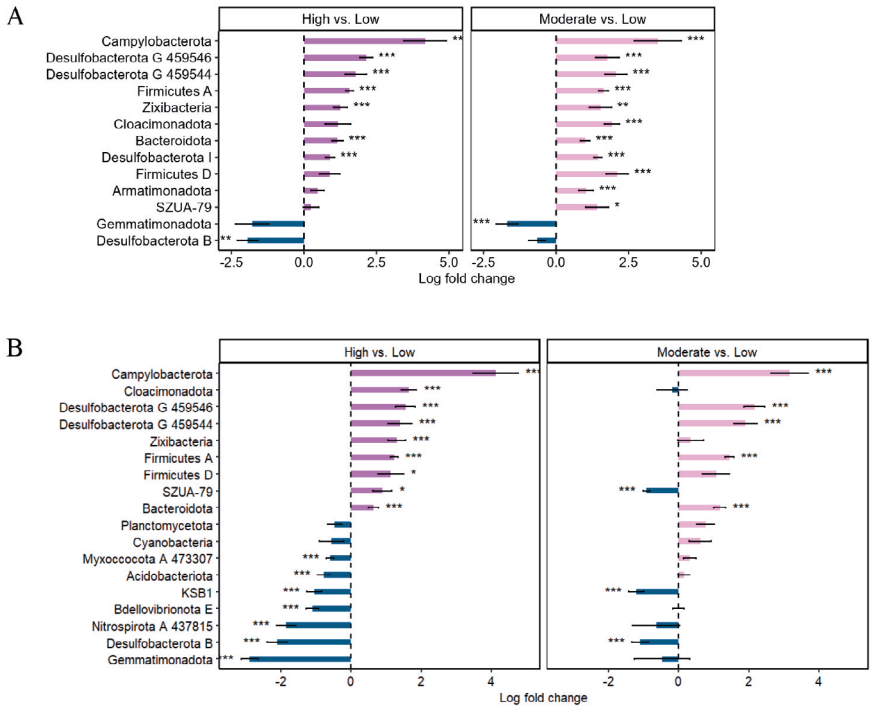


Figure 15. Bacterial phyla enriched by THg (A) and MeHg (B). Taxa compared from high to low THG and MeHg concentrations, and from moderate to low concentrations.

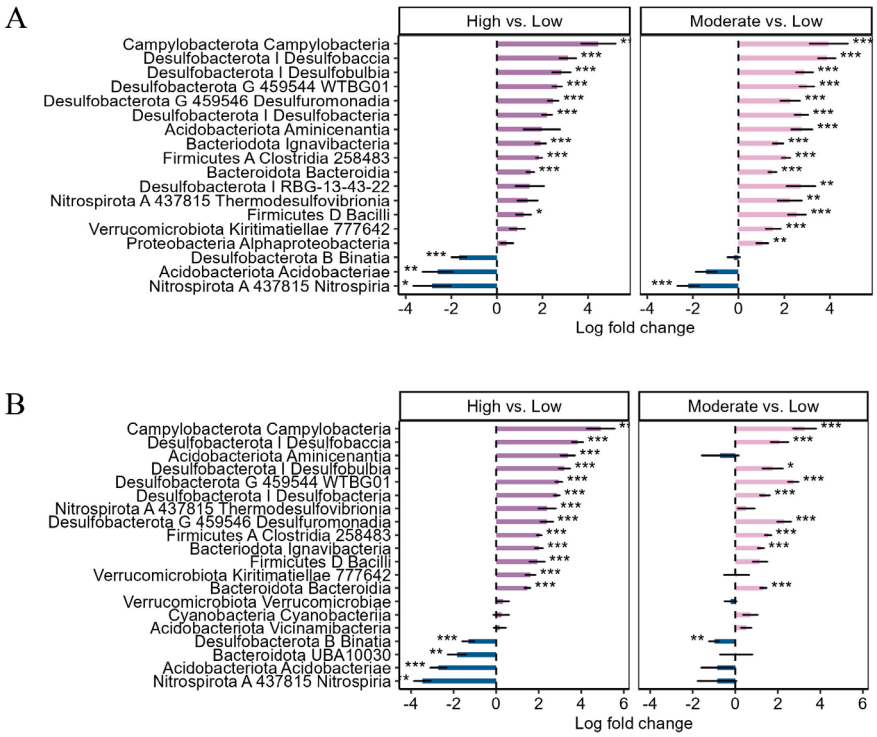
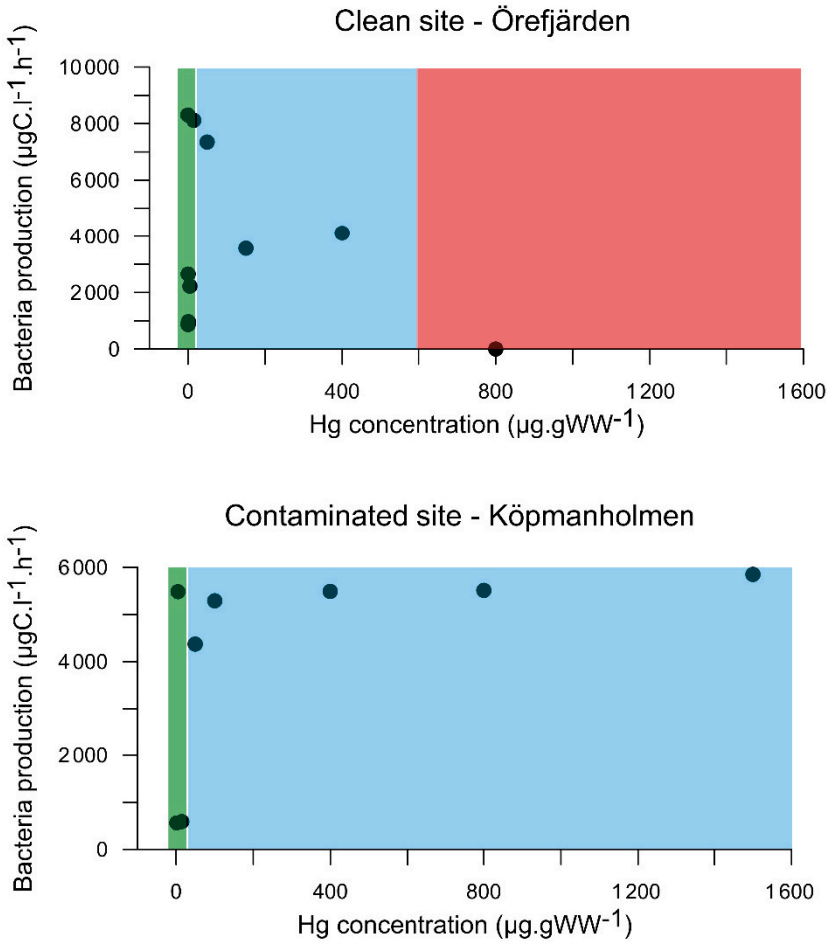


Figure 16. Bacterial classes enriched by THg (A) and MeHg (B). Taxa compared from high to low THG and MeHg concentrations, and from moderate to low concentrations.

3.4 Bacterial tolerance to Hg addition

The bacterial community at the clean site, Örefjärden, was much more sensitive to Hg addition than the community at the contaminated site, Köpmanholmen (Figur 17). At Örefjärden, the bacterial production was totally inhibited at 800 ug.gWW-1 Hg, while at Köpmanholmen additions of 1 500 ug.gWW-1 Hg did not cause any reduction of the bacterial production. Here, the bacterial production was at its maximum at the highest Hg addition, indicating that the bacterial community was resistant to Hg. The green area in the figure 16 describes conditions where bacteria were growing at fast rate and where they were not affected by Hg addition. The blue area describes the condition where bacteria were growing slowly due to Hg addition, and the red area where the bacterial growth was totally inhibited. Taken together, bacteria at the clean site were sensitive to Hg addition, while bacteria from the contaminated site was tolerant to high Hg additions.



Figur 17. Hg addition experiment. Bacteria production for Örefjärden (clean) Köpmanholmen (contaminated) and sediments. Green area: fast growth – no Hg effect, blue area: slow growth – moderate Hg effect, red area: no growth – toxic Hg effect.

4. Discussion

4.1 Omibox – a usable tool for analyzing microbial communities in field

There is high need to be able to perform in situ or near in situ field sequencing of microbial communities (Goordial et al. 2017). The field tests conducted with OmiBox demonstrated its ability to rapidly generate high-quality data on microbial community composition and gene expression from natural samples, with results available within 48 hours post-sample collection. The platform's autonomous operation, including its ability to function entirely off-grid, was validated under challenging environmental conditions. However, the test conducted in cold environments highlighted the necessity for an integrated heating system to maintain internal temperatures and ensure an optimal performance of the different chemical reagents used, independent of external temperatures.

The test conducted aboard the research vessel KBV181 presents promising potential for the deployment of OmiBox or similar platforms in routine environmental monitoring programs. Other biological parameters, such as bacterial production and primary production, are already implemented in monitoring activities currently ongoing (Umeå Marine Sciences Centre, Umeå University, [accessed October 10, 2024]), which also required in situ sample processing onboard the research vessel KBV181.

Although data analysis is still in progress, the multiomic experiment conducted at the Umeå Marine Sciences Centre (UMF) revealed that RNA extracted immediately after sample collection exhibited superior quality compared to samples preserved under various conditions. While snap freezing at –80 °C or lower is typically considered adequate for preserving environmental samples (Pavlovska et al. 2017, Veilleux et al. 2021), in this study, RNA extracted in situ showed higher integrity than that from samples stored at –80 °C. Further analysis will determine whether these differences in RNA quality are reflected in the gene expression profiles. Our working hypothesis is that in situ extraction provides a more accurate representation of the actual taxonomic composition and gene expression of microbial communities.

4.2 MeHg formation in coastal waters linked to tDOM and bacterial production

Our microcosm experiment showed that Hg methylation occurs in oxygenated brackish coastal waters, a finding that very few studies have shown before. The methylation level (% MeHg of total Hg) was up to ~0.2 % (up to 500 fM MeHg for an addition of 250 pM inorganic Hg) which is comparable to the low end of methylation levels observed in sediments (Figure 11C, Table A2). It is, however, expected that the maximum Hg methylation capacity is lower in water due to the relatively low abundances of microorganisms in seawater compared to that in sediments. Further, our results indicate that methylation is a fast process that can take place before Hg-DOM equilibrium has been reached, and is coupled to high bacterial biochemical activity induced by increased tDOM concentration.

We did not find any coupling to specific genes, such as the gene pair *hgcAB*, but this may be due to the commonness of methylation processes in all living cells (Sanchez-Romero et al. 2015). Methylation is a universal biochemical process where methyl groups are added to a variety of molecular targets, including lipids, proteins and DNA (Menezo et al. 2020). It is for example involved in DNA repair, protein function and gene expression.

Our findings have ecological implications in a global change perspective, where increased terrestrial runoff in the northern hemisphere is expected to exacerbate the entrance of tDOM and environmental pollutants (including Hg) into coastal waters. Terrestrial DOM will nurture heterotrophic bacteria, which may lead to increased levels of MeHg, which biomagnifies in the pelagic and benthic food webs (Jonsson et al. 2017, 2022).

4.3 Sediment Hg pollution gradients show chemical and taxonomic variations

Geographical differences of organic carbon in sediments

We observed qualitative differences in Gulf of Bothnia sediments that can be explained by ecological variations in the Bothnian Sea and Bothnian Bay basins. The yearly primary production is ~5-fold higher in the Bothnian Sea than in the Bothnian Bay (Andersson et al. 1996), which may explain the higher proportion of protein-like compounds in the Bothnian Sea sediments. In contrast, the proportion of terrestrial and microbial derived humic substances was higher in the Bothnian Bay sediments. This may be explained by the 2-fold higher humic substance concentrations in the Bothnian Bay and the generally higher bacterial production in that basin (Paczkowska et al. 2016, monitoring data unpublished). Bacterial production is especially high along the coasts where river water meets the saline water (Figueroa et al. 2016, Andersson et al. 2018). The fraction of terrestrial and microbial humic substances in the FDOM pool tended to be higher at the coast, where fiber banks were located. On the other hand, we did not observe any geographical differences of the CNP concentrations in the pore water and in the particulate sediment. In general, the C/P and N/P ratios were higher in the pore water than in the solid sediment, maybe indicating that P is exploited by bacteria from the sediment pore water. The fiber banks did not distinguish from the unpolluted sites, except a few exceptions. Altogether, this indicates that the whole region is very rich in organic carbon but that qualitative differences occur, which is related to variations in ecosystem productivity and terrestrial influence.

High mercury levels in fiber-banks

Earlier studies have shown fiber-rich sediments in the Gulf of Bothnia hold high concentrations of Hg and persistent organic pollutants (Apler et al. 2014, Norrlin et al. 2016, Dahlberg et al. 2020, 2021). Our study is in line with earlier studies. The measured total Hg and MeHg levels were comparable or higher than observations in earlier studies (Apler et al. 2014, Norrlin et al. 2016). Apler et al. (2014) reported THg values of 0.3–2.84 mg/kg dry weight. The highest value (2.84) in that study was assessed to be much deviating from the background levels. In our study we

observed values of 4–30 ug THg /g dry weight (which would be equal to mg/kg dry weight) in the Sundsvall sampling area, which must be considered to be very high levels. An earlier study reported THg concentrations to be 0.34–1.1 ug THg /g dry weight in Köpmanholmen and 1–4 ug THg /g dry weight in Bureå (Norrlin et al. 2016). We measured 4–20 ug THg /g dry weight in Köpmanholmen and 2.5 ug THg /g dry weight in Köpmanholmen. Norrlin et al. (2016) reported that the Hg methylation level varied between 0.03 and 1.6 %, and in our study the level varied from 0.12–8 %. Interestingly, the THg and MeHg did not peak at the same sites. The THg peaked in Bureå, and Köpmanholmen 1 and 2, while the MeHg peaked at the Sundsvall 1–3 sites and showed high values also at Köpmanholmen 2. The methylation level was highest at the Sundsvall 1 and 2 sites, reaching levels of 5–8 %. We can conclude that THg and MeHg levels were enhanced at all studied fiber-rich areas in the Gulf of Bothnia (this study and other studies), but variation between the studies most likely depend on small scale local variations. We assume that bacteria were most active where the MeHg levels and the methylation levels were highest, namely at the Sundsvall 1–2 sites.

Potential bacterial indicator groups

The bacterial class showing the largest enrichment related to MeHg and THg was Campylobacteria, which in literature is described as being chemolithoautotrophic (e.g. Wang et al. 2022, 2024), which is using inorganic reduced compounds as a source of energy, for example hydrogen (H_2), hydrogen sulfide (H_2S), and reduced metals. Fiber rich sediments are very rich in organic carbon, as for example in our study the pore water DOC concentrations were about 30-fold higher than in seawater (sediment pore water ~120 mg/l and seawater ~4 mg/l). The studied fiber banks should be rich in sulfur and, as demonstrated, also rich in mercury. It was notable that the enrichment of this group was seen consistently from low to high mercury and form low to moderate concentrations. However, we cannot be sure that it is the presence of mercury that is the driver for occurrence of this group, rather it is the combination of multiple environmental factors that is the causative factor.

Other enriched bacterial classes were, for example, Desulfobaccia and Clostridia, which are part of core communities in subterranean estuaries where genes for denitrification, sulfate reduction, and methanogenesis are amplified (Adyasari et al. 2024). Also, Desulfobulbia, Desulfobacteria and Desulfuromonadia are well-known from marine sediments and likely they are sulfate reducers (Yue et al. 2023). This is interesting as sulfate reducing bacteria have been identified as the causative organisms in methylmercury formation. Next, we will search for occurrence of the methylmercury genes *hgcA* and *hgcB* genes in our metagenome data, as they are indicators of Hg methylation (Podar et al. 2015). It would be especially interesting to see if these genes are enriched at the Sundsvall 1–3 sites, as the methylation degree showed highest values at these sites (Figure 11C). However, this could also be a consequence of low demethylation rates, as previously noted in Sundsvall sediments (Erik Björn pers. com.). The observed MeHg concentrations reflect the net MeHg accumulation resulting from both enhanced methylation and reduced demethylation.

4.4 Tolerance adaption and risk assessment

The Hg addition experiment shows that the bacterial community at the contaminated site had developed tolerance to high Hg concentrations. Either by changing the species composition to resistant taxa or by developing tolerance. We are presently analyzing the taxonomic composition in sediments. The results of the test are interesting from an evolutionary perspective, and they are in line with an earlier study (Jeffrey et al. 1996), reporting expression of Hg tolerance genes, *mer*, in Hg contaminated sediments. Our results therefore point at generality, i.e. tolerance will be selected for or evolve in Hg contaminated areas. However, it also means that the ecosystem function of sediment bacteria as degraders of sinking phytoplankton will not be erased in Hg contaminated areas. The ecosystem function of sediment bacteria as degraders of sinking phytoplankton can be maintained even in Hg contaminated areas. Thus, from a risk assessment point of view, the ecosystem function as plankton degraders is likely to remain in highly contaminated areas. Nevertheless, there are other risks in Hg contaminated areas, such as biomagnification of MeHg in the marine food web (Jonsson et al. 2022).

4.5 Conclusions

The project shows that fast analysis of microbial communities is very important in order to get accurate taxonomic results. Sampling in natural environments often require long transport times, which can result in freeze-thawing of collected samples and thus degenerated samples. The portable molecular and sequencing laboratory, the Omibox, would be a highly usable tool to achieve good quality taxonomic or "omics" results. The Omibox will be usable in remote field sampling sites as well as in simple standard laboratories.

We focused on Hg, a pollutant that is of great concern in the Baltic Sea as well as in Swedish lakes. MeHg formation is executed by bacteria, and once Hg is transformed into that form it can be biomagnified in the food web. The results show that MeHg formation can occur already in pelagic oxygenated waters, and is mediated by tDOM. Since climate change causes increased precipitation and thus increased inflows of tDOM to the northern Baltic Sea, there is a risk of elevated MeHg formation in the ecosystem.

Fiber-rich sediments are a legacy of the paper industry along the Gulf of Bothnia coast. In the past Hg was used in the paper production process, which mean that the fiber-rich sediments are polluted with Hg as well as other toxic compounds. Our studies showed that specific taxa of bacteria are enriched in fiber-rich sediments in the study area. These are chemolithoautotrophic bacteria (Campylobacteria), which are using H_2, H_2S and reduced metals as energy source, and different types of sulfate-reducing bacteria (e.g. Desulfobaccia). This is interesting and makes sense, as sulfate-reducing bacteria have been recognized as major contributors to MeHg production (Achá et al. 2012). Furthermore, our Hg addition experiment showed that bacterial communities from contaminated sites exhibited a clear tolerance to high Hg concentrations, suggesting that these taxa are not only enriched in such environments but may also be functionally adapted to Hg exposure. In the ongoing research we search for MeHg coding genes, the gene pair *hgcAB* and for Hg tolerance genes (*mer*) in the metagenomic data. We see the possibility that both the bacterial taxonomic composition and the gene composition can be used as indicators of Hg polluted sediments.

5. Recommendations

Based on the results of the project, we recommend incorporating microbial analyses into the monitoring of Hg-contaminated sediments, especially in contexts where chemical measurements alone may not capture ecological risk or ongoing microbial activity.

1. **Analysis of bacterial community composition using 16S amplicon sequencing**
   This method is particularly useful for detecting enrichment of key microbial groups, such as sulfate-reducing bacteria and Campylobacteria, associated with MeHg formation in sediments. It is valuable in sediments where MeHg concentrations are low but methylation potential may exist, or in sites undergoing remediation, where microbial shifts can serve as early indicators of changing risk. Suitable statistical tools (e.g. Bray-Curtis, ANCOM-BC) should be applied to identify significant taxonomic patterns related to Hg contamination levels.

2. **Monitoring of bacterial gene composition using metagenomics**
   Focus on detecting genes associated with mercury methylation (*hgcAB*) and Hg tolerance (*mer)*. Shotgun sequencing and metagenome analysis provide insight into the functional capacity of microbial communities, which is essential in legacy fiber-rich sediments, climate-sensitive areas with increasing tDOM input, or areas at risk of future MeHg production. These functional markers help evaluate the potential for ongoing or future mercury transformation processes, even in the absence of current high MeHg levels.

3. **Fast analysis of collected samples using portable technologies, such as the OmiBox**
   OmiBox enables rapid on-site analysis of microbial community composition and gene expression, reducing degradation risks from storage or transport. This is particularly valuable in remote or logistically challenging environments where monitoring activities usually take place, and when real-time results would be rapidly integrated into monitoring or management decisions.

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Appendices

Table A1a. Sediment samples collected during the field survey, September 2022. Table presents pore water (p.w.) content, DOC, TDN and TDP.

Site	Station	Repl.	Sample	Lat.	Long.	Depth (m)	Pore w (%)	DOC (mg.l-1)	TDN (µg.l-1)	TDP (µg.l-1)
Offshore	A5	1	A5-R1	65.1667	23.2333		75.3	77.21	2 057	149
Offshore	A5	2	A5-R2	65.1667	23.2333		74.7	74.25	1 910	152
Offshore	A5	3	A5-R3	65.1667	23.2333		74.2	75.07	2 118	174
Offshore	C14	1	C14-R1	62.0998	18.5485		63.3	99.23	1 453	136
Offshore	C14	2	C14-R2	62.0998	18.5485		67.8	102.04	2 126	181
Offshore	C14	3	C14-R3	62.0998	18.5485		68.0	100.79	1 805	162
Offshore	C3	1	C3-R1	62.6528	18.9523		75.9	96.18	2 489	210
Offshore	C3	2	C3-R2	62.6528	18.9523		76.5	97.15	2 914	131
Offshore	C3	3	C3-R3	62.6528	18.9523		76.8	88.62	1 934	165
Bureå	3	1	B-S3-R1	64.6201	21.2909	17	81.4	109.85	1 331	66
Bureå	3	2	B-S3-R2	64.6201	21.2909	17	81.1	98.56	987	92
Bureå	3	3	B-S3-R3	64.6201	21.2909	17	81.6	88.71	1 113	88
Köpmanholmen	1	1	K-S1-R1	63.1703	18.5905	76	56.7	129.88	1 157	154
Köpmanholmen	1	2	K-S1-R2	63.1703	18.5905	76	55.0	163.31	2 111	243
Köpmanholmen	1	3	K-S1-R3	63.1703	18.5905	76	62.7	125.30	727	97
Köpmanholmen	2	1	K-S2-R1	63.1679	18.6171	65	77.6	107.41	1 209	182
Köpmanholmen	2	2	K-S2-R2	63.1679	18.6171	65	76.7	112.79	1 422	178
Köpmanholmen	2	3	K-S2-R3	63.1679	18.6171	65	77.5	135.06	1 124	83
Köpmanholmen	3	1	K-S3-R1	63.1559	18.6673	34	76.1	148.96	867	57
Köpmanholmen	3	2	K-S3-R2	63.1559	18.6673	34	80.2	103.42	719	63
Köpmanholmen	3	3	K-S3-R3	63.1559	18.6673	34	80.4	167.85	779	188
Sundsvall	1	1	S-S1-R1	62.3894	17.3718	35	82.5	73.00	2 854	398
Sundsvall	1	2	S-S1-R2	62.3894	17.3718	35	85.9	56.78	2 090	390
Sundsvall	1	3	S-S1-R3	62.3894	17.3718	35	85.9	79.88	2 413	305
Sundsvall	2	1	S-S2-R1	62.3839	17.3867	36	74.7	88.50	1 008	107
Sundsvall	2	2	S-S2-R2	62.3839	17.3867	36	75.6	95.91	965	222
Sundsvall	2	3	S-S2-R3	62.3839	17.3867	36	75.5	121.12	1 203	64
Sundsvall	3	1	S-S3-R1	62.3414	17.4191	7	71.0	120.12	2 296	82
Sundsvall	3	2	S-S3-R2	62.3414	17.4191	7	69.4	64.95	606	163
Sundsvall	3	3	S-S3-R3	62.3414	17.4191	7	70.9	65.73	1 425	166

Table A1b. Sediment samples collected during the field survey, September 2022. Table presents pore water fluorescent DOM components (FDOM): protein-like, humic-like of terrestrial origin and humic-like of microbial origin.

Site	Station	Repl.	Sample	Comp1%_protein	Comp2%_humic_terr	Comp3%_humic_microbe
Offshore	A5	1	A5-R1	62.29	22.87	14.84
Offshore	A5	2	A5-R2	60.96	23.96	15.09
Offshore	A5	3	A5-R3	60.84	23.82	15.35
Offshore	C14	1	C14-R1	78.15	13.49	8.37
Offshore	C14	2	C14-R2	78.35	13.57	8.08
Offshore	C14	3	C14-R3	77.94	14.03	8.02
Offshore	C3	1	C3-R1	72.73	16.30	10.97
Offshore	C3	2	C3-R2	71.33	16.84	11.83
Offshore	C3	3	C3-R3	72.52	16.29	11.19
Bureå	3	1	B-S3-R1	55.08	27.92	17.00
Bureå	3	2	B-S3-R2	44.02	35.85	20.13
Bureå	3	3	B-S3-R3	48.93	31.87	19.19
Köpmanholmen	1	1	K-S1-R1	74.38	15.78	9.84
Köpmanholmen	1	2	K-S1-R2	79.33	12.72	7.96
Köpmanholmen	1	3	K-S1-R3	71.81	17.89	10.30
Köpmanholmen	2	1	K-S2-R1	65.26	17.45	17.29
Köpmanholmen	2	2	K-S2-R2	65.62	15.85	18.54
Köpmanholmen	2	3	K-S2-R3	68.80	13.70	17.50
Köpmanholmen	3	1	K-S3-R1	74.96	12.49	12.55
Köpmanholmen	3	2	K-S3-R2	74.27	14.88	10.85
Köpmanholmen	3	3	K-S3-R3	74.01	14.57	11.42
Sundsvall	1	1	S-S1-R1	78.64	12.74	8.63
Sundsvall	1	2	S-S1-R2	80.06	12.74	7.20
Sundsvall	1	3	S-S1-R3	80.83	11.85	7.32
Sundsvall	2	1	S-S2-R1	72.27	16.79	10.94
Sundsvall	2	2	S-S2-R2	76.83	14.22	8.95
Sundsvall	2	3	S-S2-R3	76.60	14.35	9.05
Sundsvall	3	1	S-S3-R1	75.45	14.66	9.89
Sundsvall	3	2	S-S3-R2	69.90	15.71	14.39
Sundsvall	3	3	S-S3-R3	75.18	14.79	10.02

Table A2. Sediment samples collected during the field survey, September 2022. Table presents sediment content of total carbon (TC), total nitrogen (TN), MeHg and total Hg, presented per wet weight and per dry weight.

Site	Station	Repl.	Sample	TC (gC.gDW-1)	TC (gC.gWW-1)	TN (gN.gDW-1)	TN (gN.gWW-1)	MeHg (ng Hg/g DW)	MeHg (ng Hg/g WW)	THg (µg Hg/g DW)	THg (µg Hg/g WW)
Offshore	A5	1	A5-R1	3.17	2.39	0.27	0.20	0.07	0.05	0.08	0.06
Offshore	A5	2	A5-R2	3.13	2.34	0.27	0.20	0.10	0.07	0.08	0.06
Offshore	A5	3	A5-R3	3.05	2.26	0.26	0.19	0.12	0.09	0.10	0.08
Offshore	C14	1	C14-R1	2.31	1.46	0.25	0.16	0.09	0.06	0.03	0.02
Offshore	C14	2	C14-R2	2.24	1.52	0.25	0.17	0.06	0.04	0.04	0.03
Offshore	C14	3	C14-R3	2.19	1.49	0.24	0.16	0.10	0.07	0.04	0.03
Offshore	C3	1	C3-R1	2.96	2.25	0.29	0.22	0.08	0.06	0.05	0.04
Offshore	C3	2	C3-R2	2.97	2.27	0.29	0.22	0.10	0.08	0.05	0.04
Offshore	C3	3	C3-R3	2.86	2.20	0.29	0.22	0.06	0.05	0.05	0.04
Bureå	3	1	B-S3-R1	3.98	3.24	0.35	0.29	1.76	1.43	2.85	2.32
Bureå	3	2	B-S3-R2	4.06	3.29	0.34	0.28	1.59	1.29	2.32	1.88
Bureå	3	3	B-S3-R3	4.14	3.38	0.34	0.28	2.41	1.96	8.63	7.05
Köpmanholmen	1	1	K-S1-R1	2.86	1.62	0.21	0.12	10.85	6.15	7.35	4.17
Köpmanholmen	1	2	K-S1-R2	2.47	1.36	0.20	0.11	8.41	4.63	5.44	2.99
Köpmanholmen	1	3	K-S1-R3	3.20	2.01	0.22	0.14	11.47	7.19	9.12	5.72
Köpmanholmen	2	1	K-S2-R1	3.95	3.07	0.36	0.28	30.92	24.00	3.56	2.76
Köpmanholmen	2	2	K-S2-R2	3.78	2.90	0.37	0.28	23.67	18.15	2.12	1.62
Köpmanholmen	2	3	K-S2-R3	3.80	2.95	0.38	0.29	22.07	17.12	1.91	1.48
Köpmanholmen	3	1	K-S3-R1	3.51	2.67	0.38	0.29	6.06	4.61	0.35	0.27
Köpmanholmen	3	2	K-S3-R2	3.61	2.89	0.38	0.30	6.60	5.29	0.39	0.32
Köpmanholmen	3	3	K-S3-R3	3.71	2.98	0.39	0.31	1.92	1.54	0.28	0.22
Sundsvall	1	1	S-S1-R1	14.04	11.58	0.46	0.38	32.86	27.11	0.73	0.61
Sundsvall	1	2	S-S1-R2	19.58	16.83	0.48	0.41	19.87	17.07	0.41	0.35
Sundsvall	1	3	S-S1-R3	19.36	16.62	0.49	0.42	21.61	18.56	0.31	0.26
Sundsvall	2	1	S-S2-R1	4.72	3.53	0.31	0.23	46.02	34.38	0.50	0.37
Sundsvall	2	2	S-S2-R2	4.76	3.60	0.30	0.23	38.89	29.41	0.48	0.36
Sundsvall	2	3	S-S2-R3	4.57	3.45	0.31	0.23	35.21	26.58	0.50	0.38
Sundsvall	3	1	S-S3-R1	3.48	2.47	0.26	0.18	9.88	7.02	0.51	0.36
Sundsvall	3	2	S-S3-R2	3.08	2.14	0.24	0.17	10.42	7.24	0.48	0.33

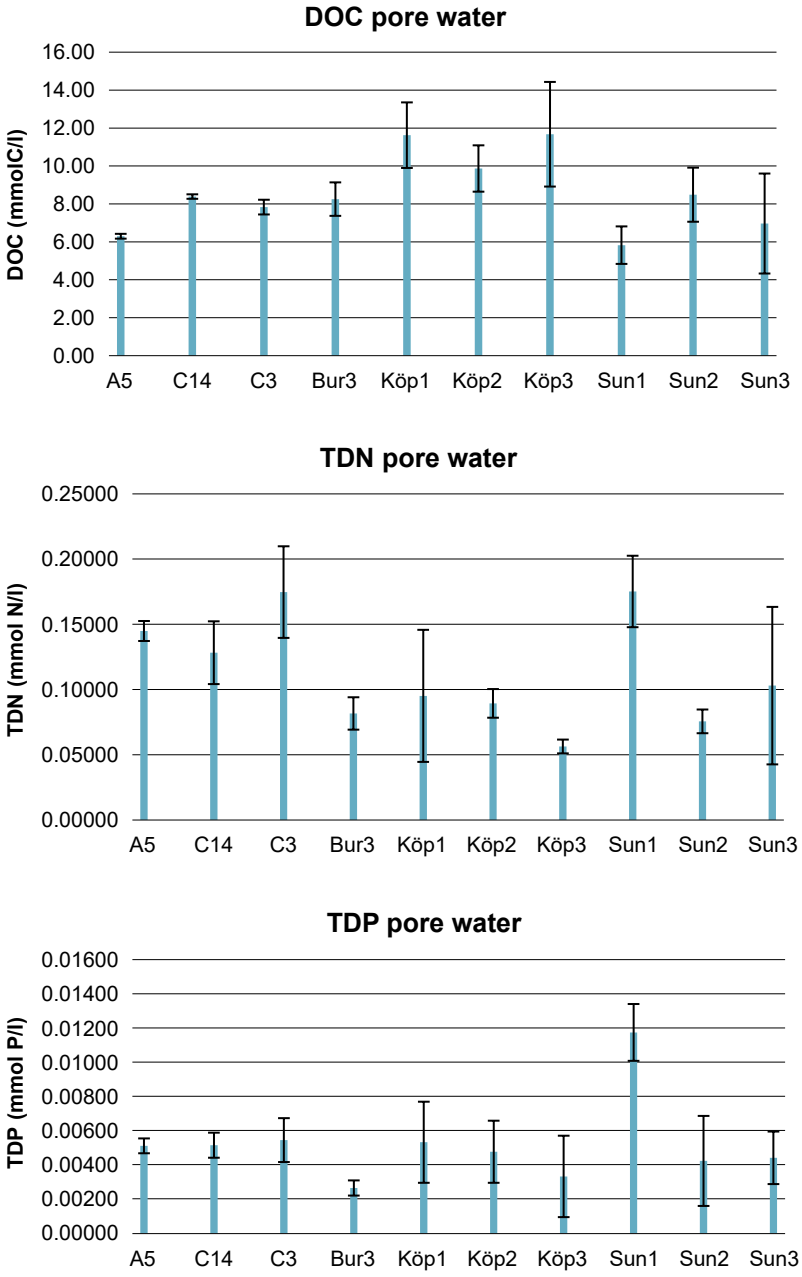

Figure A1. Average DOC, TDN and TDP concentrations in sediment pore water at the different sampling sites. Error bars denote standard deviation.

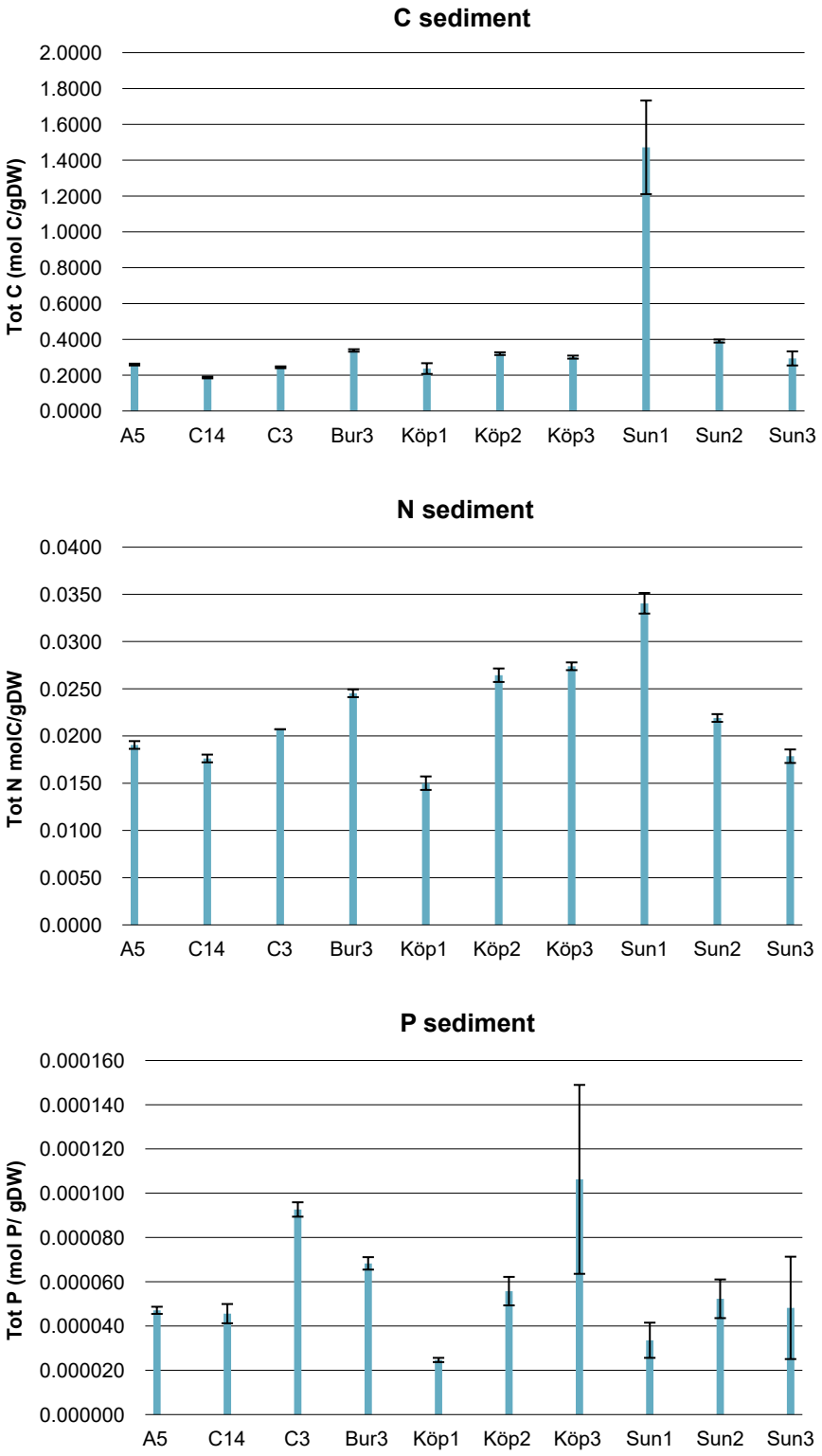

Figure A2. Average total C, N and P in dry sediment at the different sampling sites. Error bars denote standard deviation.

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Microbes as indicators of Hg contaminated sediments

Studies in the Gulf of Bothnia

This report presents results from a project investigating the use of microbes as indicators of mercury (Hg) pollution in sediments. Microbes respond rapidly to environmental change, making them excellent bioindicators. However, to ensure reliable results, it is essential to analyze microbial communities soon after sampling to prevent degradation or loss of activity.

To address this, a portable molecular sequencing laboratory, OmiBox, was developed, enabling near in situ analysis of microbial taxonomic composition and gene expression. Additionally, microbial community composition was studied in Hg-contaminated fiber banks in the Gulf of Bothnia. The results revealed that certain taxonomic groups, such as bacteria from the phyla Campylobacterota and Desulfobacterota, were enriched in fiber-rich, Hg-polluted sediments.

The study also included tolerance experiments comparing bacteria from clean and contaminated sediments in response to Hg addition. Bacteria from polluted sites exhibited significantly greater tolerance, suggesting evolved resistance and retained microbial functionality. Nonetheless, Hg-contaminated sediments pose ecological risks, as methylmercury (MeHg) can bioaccumulate and magnify through the food web.

This project contributes a framework for understanding how bacterial community structure and function respond to Hg pollution in sediments, offering valuable tools for environmental monitoring and assessment.

NATUR VÅRDS VERKET
SWEDISH ENVIRONMENTAL PROTECTION AGENCY

Naturvårdsverket, SE-106 48 Stockholm. E-mail: registrator@naturvardsverket.se, www.swedishepa.se
Visiting address Stockholm: Virkesvägen 2. Visiting address Östersund: Forskarens väg 5, hus Ub.