TNA PROJECT REPORT

1. Project Information

Proposal reference number	JN_CALL_3_10
Project Acronym (ID)	MEPHY
Title of the project	Novel sensing tools to study synergic interaction between trace metals and phytoplankton
Host Research Infrastructure	COSYNA Stationary FerryBox system (COSYNA_SFB)
Starting date - End date	01/04/2019 – 30/06/2019
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2. Project objectives

The MEPHY field campaign aims at, for the first time, synergising state of the art analytical sensing tools to monitor at high resolution a range of trace metals, algal-bacterial species, and macronutrients in impacted and highly dynamic marine coastal areas; and examine if and how they are related. Toward this goal, the specific objectives are:

1: real-time high-resolution quantification of the bioavailable fraction of a range of trace metals and classification of a range of phytoplankton groups using pigment-based taxonomic classification applying two innovative sensing systems recently developed (**www.schema-ocean.eu**). Namely: the TMSM, a compact submersible multichannel Trace Metal Sensing probe incorporating various antifouling gel integrated microelectrode arrays (GIMEs) enabling simultaneous direct detection of the bioavailable fraction of cadmium, lead, copper, zinc, and inorganic arsenic and mercury; the ALPACA, a miniature height-channel Algae Sensing Module enabling discrimination of phytoplankton phyla based on pigment-based taxonomic classification;

2: biosensor assay evaluation of algae toxicity;

3: field and laboratory ancillary measurements of master bio-physicochemical parameters; macronutrients; particulate, total and total dissolved concentrations of the target trace metals as well as Pt (tracer of anthropogenic activity);

4: study of the dynamic behaviour of the bioavailable fraction of a range of trace metals, algal-bacterial species, and macronutrients; and investigation of their main sources and potential interaction by

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coupling all the collected data.

The two-week MEPHY field campaign has been performed at the COSYNA Stationary FerryBox platform (COSYNA_SFB) from May 6th to 16th, 2019. This facility is ideally located for the proposed field activities, namely: in the German Southern North Sea coastal area characterized by frequent Spring and Summer phytoplankton blooms and impacted by anthropogenic sources of trace metals.

3. Main achievements and difficulties encountered

Field activities were successfully achieved according to the work plan. Day 1 was dedicated to inform users on the use of the facilities and FerryBox sensing activities and data file access. Maintenance and calibration of the FerryBox was performed. A sampling pipe, based on Teflon tubes and a Plexiglas sampling chamber built by UNIGE, was installed in parallel to the FerryBox sampling line to avoid contamination problem on on-site sensing of the bioavailable fraction of trace metals and sample collection for ancillary measurements of trace metal analysis as well as metal in phytoplankton. Installation of the user partners sensing and sampling devices; TMSM sensor preparation and calibration; and preliminary tests of the TMSM, ALPAGA and sampling process flow were successfully achieved days 2 to 3. Autonomous operational measurements of the bioavailable fraction of Cu, Pb, Cd, Zn and inorganic As and Hg species (TMSM; time interval 1h); macro-nutrients (COSYMA-SFB sensors; time-interval 1h10); and master bio-physicochemical parameters (COSYNA-SFB instruments 7 sensors; time- interval 10 min) were successfully achieved from days 4 to 11. During the same period, sampling for ancillary measurements of: Pt and speciation of the TMSM target trace metals; (phyto-)plankton community and species (on-site ALPACA and laboratory microscopy analysis); macro-nutrients and organic compounds were performed 3 to 6 times per day prior and after a measurement cycle of 34h (May 13th to 14th) during which sampling was performed every 2h. Laboratory analysis of As(III) and Astot; macronutrients, chlorophyll a and pheopigments; dissolved organic compounds (DOC, FA/HA) for TMSM and FerryBox sensors evaluation/validation and complementary data have been achieved. Phytoplankton morphological investigation by microscopy has been started. Ancillary measurements of the other target trace metals (particulate, total dissolved) and POC are pending.

Within the exception of the absence of the Spring bloom expected due to the April and May months weather conditions (especially lower temperatures that usually observed), no difficulties were encountered. Biosensor assay evaluation of algae toxicity could not be performed due to the very low algae cell density (see section 5).

4. Dissemination of the results

Plan for dissemination of the results are:

- Publication in peer-reviewed papers (1 to 2 reporting exclusively the outcome of the MEPHY field campaign; 1 coupling SCHeMA and MEPHY field campaign data monitored in various dynamic coastal area).
- Oral and Poster presentation in conferences/meetings. Dissemination as part of two meetings are already scheduled: 1) MEPHY Poster at the Final General Assembly of JERICO-NEXT, Brest-France, 3 July 2019. 2) as part of an invited Keynote, for the Experimentation and Instrumentation Workshop: session "From laboratory measurement to routine field

measurement" of the Experimentation and Instrumentation Workshop, Lille-France, 9-11 July 2019.

- First raw data file, and later on validate data file, submitted for open access via the SeaDataNet network and the EMODnet Data Ingestion portal.

5. Technical and Scientific preliminary Outcomes

Main outcomes at the present stage of the MEPHY field monitoring in the Elbe Estuary during the period 7 to 16 May 2019 are summarized below:

TMSM autonomous measurements of the bioavailable fraction of trace metals.

The Elbe Estuary is a major European estuary impacted by anthropogenic sources of trace metals. The TMSM records allowed producing the first data for the Elbe Estuary on the concentrations of a range of trace metals under their forms potentially available for bio-uptake. Bioavailability is of primary concern when considering if a metal behaves as nutrient or toxicant. While the global regulatory environmental quality standards (EQS) for metals in water bodies are still mainly based on total (dissolved) concentrations, the revised WFD Priority Substances Directive (2013/39/EU) highlights the need of measurement of the bioavailability of hazardous trace metals either indirectly by modelling of their speciation or directly by applying specific measurement methodology. Significant temporal variations in the bioavailable fraction of all the trace metals targeted were observed. The temporal variation of the As(III), Hg(II), and Pb(II) available for bio-uptake were found to be mainly anti-cyclic to the temporal variation of the salinity, suggesting a conservative behavior (i.e. dilution of the metal bioavailable species carried out by the Elbe River by mixing with marine water). This is supported by the concentration vs salinity distributions of these metals. The temporal variations observed for the bioavailable fraction of Cu(II), Cd(II), Zn(II), and possibly As(V), are more complex suggesting that other processes, in addition to dilution, control the fate of the potentially more toxic fraction of these metals. It is expected that coupling the TMSM data with those of particulate, total and total dissolved metal concentrations, measured by ICP-MS in the collected samples, will deepen information on these processes.

ALPACA direct detection of algae.

During MEPHY field campaign, we evaluated the presence of relevant phytoplankton groups combined with an approximation of the microalgae cell density directly after the sample collection. The samples were pumped through the detection unit using a peristaltic pump at flow rate of 1.5 mL/min. Pump velocity and data-acquisition rate ensure that each cell event is recognized at each measurement channel with at least three measurement points. This strategy is crucial to enabling and improving the individual signal analyses of mixed algal samples. As blank and baseline correction, we processed sterile seawater (by filtration 0.22µm). At the end of each measurement 70% ethanol was processed to wash the entire fluidic system and avoid biofouling. During the entire monitoring activity, first we evaluated sterile seawater (for at least 5 min) and immediately after, without stopping the measurement, the properly mixed marine sample (for at least 10 min). In general, the sample fluorescence pattern changed very slightly compared to the blank (sterile seawater) indicating the low amount of algal cells present in the collected samples. For identification of algae groups, a multivariate pattern recognition algorithm combined with internal calibration and standardization strategies were applied. In same sample, the system has indicated that Miozoa constituted the major phylum of the sample, followed by Rhodophyta and Haptophyta. In order to validate the results, each sample was taken in duplicate and preserved with

Lugol's (2 to 10 %) for morphological investigations by microscopy. This analysis showed evidence of 0-10 cells in 50 ml in the marine samples, in which dinoflagellates (belonging to Miozoa phylum) and diatoms constituted the major groups.

COSYNA FerryBox autonomous measurements of macronutrients.

The temporal variation of the macronutrients (nitrate, nitrite, phosphate, silicate) monitored at high-resolution by the FerryBox were anti-cyclic to the variation of salinity with a strong correlation ($R2 \geq 0.7$) for the concentration vs salinity distributions indicating the conservative behavior of the four macronutrients (nitrate, nitrite, phosphate, silicate) monitored at high-resolution by the FerryBox. This can be expected during a period characterized by low biological activities as revealed by the ALPACA results.

Synergic interaction between trace metal speciation and phytoplankton.

The results suggest that the biological activity may influence dissolved arsenic speciation. Indeed, As(III) bioavailable concentrations as well as the As(III)/Astot ratio peak up with increasing effective light penetration and increase of pheopigments. This would support the hypothesis that the reason behind the relatively high concentrations of As(III) in oxygen saturated water can be found in reduction of As(V) to As(III) by microorganism (e.g., phytoplankton and cyanobacteria) activity. Phytoplankton and bacteria uptake arsenate because of its chemical similarity to phosphate, after which it can be reduced to As(III), methylated, and excreted, mostly as As(III) and/or DMAs(V). Influence or not of the biological activities on the dissolved speciation of the other metals must be still evaluated.

TMSM and FerryBox evaluation/validation.

As(III) quantification. As(III) concentrations and temporal trends measured on-field by the TMSM and in laboratory by FIAAS were similar. As the TMSM measure specifically the bioavailable species while FIASS measurements reflect the total dissolved concentrations, this result revealed that As(III) is mainly under the bioavailable (more toxic) form. Moreover, it validates the AuNP-GIME performance for direct autonomous field detection and quantification of As(III).

Nutrients. Results obtained in laboratory for nutrient concentrations monitored in collected samples during the 34h measurement cycle were compared with field autonomous measurements from the FerryBox. For the nitrates, data are well inter-compared and give similar results. For the phosphates, qualitative variations seem similar but values measured in the collected samples are much lower than those reported by the FerryBox. For nitrite, variations are very different both qualitatively and quantitatively and concentration values obtained from laboratory based technique are much lower.

Chlorophyll-a. No link in the temporal variations were observed between the in situ and lab chlorophyll a data, and laboratory values are much higher than those from the Ferrybox.

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