TNA User Report

The completed and signed form below should be returned by email to
eurochamp2020@lisa.u-pec.fr

Project title	Atmospheric deposition impacts on biochemistry and photochemistry in the sea surface layers of oligotrophic systems
Name of the accessed chamber	ISAC
Number of users in the project	2
Project objectives (max 100 words)	The sea surface microlayer as the natural interface region could be assigned as a first sentinel to increasing human impact and climate changes due to fast response in its biological and physico-chemical features. Surface films may undergo photochemical reactions leading to significant abiotic production of volatile organic compounds (VOCs) acting as precursors for the SOA formation. This project is designed to make first steps for a better understanding of the organic matter produced by marine phytoplankton species in relation to the surfactant film formation and photochemical processing at the air-water interface, with the consequences on abiotic VOCs production and atmospheric aerosol formation.
Description of work (max 100 words):	The marine diatom Chaetoceros pseudocurvisetus monoculture was cultivated, analysed and fractionated. Afterwards, different phytoplankton-derived sample fractions were photochemically processed in Quartz cell reactor and fed into the ISAC simulation chamber to identify underlying processes and the organic matter fraction being the main driver for surfactant release and, thus, abiotic photochemical VOCs production. Besides, gas phase and aqueous phase analyses were performed. Afterwards, particle formation and ageing upon oxidation of the produced VOCs was investigated as well.

Principal Investigator's and group's information	
First name	Sanja
Family name	Frka Milosavljević
Nationality	Croatian
Activity domain[1]	Earth Sciences & Environment
Home institution	Ruđer Bošković Institute
Institution legal status[2]	RES
Email	frka@irb.hr
Gender	female
User status[3]	RES
New user	Yes

User 1 Information[4]	
First name	Abra
Family name	Penezić
Nationality	Croatian
Activity domain	Earth Sciences & Environment
Home institution	Ruđer Bošković Institute
Institution legal status	RES
Email	abra@irb.hr
Gender	female
User status	RES
New user	Yes

Trans-National Access (TNA) Scientific Report

The completed and signed form below should be returned by email to eurochamp2020@lisa.u-pec.fr

[1] Physics; Chemistry; Earth Sciences & Environment; Engineering & Technology; Mathematics; Information & Communication Technologies; Material Sciences; Energy; Social sciences; Humanities.
[2] UNI= University and Other Higher Education Organisation;
RES= Public Research Organisation (including international research organisations and private research organisations controlled by public authority);
SME= Small and Medium Enterprise;
PRV= Other Industrial and/or Profit Private Organisation;
OTH= Other type of organization.
[3] UND= Undergraduate; PGR= Post graduate; PDOC= Post-doctoral researcher; RES= Researcher ENG= Engineer; ACA= Academic; TEC= Technician.
[4] Reproduce the table for each user who accessed the infrastructure

Instructions

Please limit the report to max 5 pages, you can include tables and figures. Please make sure to address any comments made by the reviewers at the moment of the project evaluation (if applicable, in this case you were informed beforehand). Please do not alter the layout of the document and keep it in Word version. The report will be made available on the eurochamp.org website. Should any information be confidential or not be made public, please inform us accordingly (in this case it will only be accessible by the European Commission, the EUROCHAMP-2020 project partners, and the reviewers). Please include:

- Introduction and motivation
- Scientific objectives
- Reason for choosing the simulation chamber/ calibration facility
- Method and experimental set-up
- Data description
- Preliminary results and conclusions
- Outcome and future studies
- References

Name of the PI: Sanja Frka Milosavljević
Chamber name and location: ISAC, CNRS, Lyon, France
Campaign name and period: Atmospheric deposition impacts on biochemistry and photochemistry in the sea surface layers of oligotrophic systems; 17/6-29/6/2018

• Introduction and motivation

Once deposited through dry and wet processes, atmospheric aerosols provide the aqueous ecosystems with an external source of nutrients, such as iron, phosphorus, and nitrogen.[1] This, in turn, influences the quality and quantity of organic matter (OM) produced by the phytoplankton within the photic zone, changes carbon uptake and indirectly affects the climate. The contribution of atmospheric deposition (AD) can be especially important and efficient in oligotrophic environments which represent 60% of the global ocean. Such external AD inputs should become even more important in a future scenario with increased aerosol emissions and a shallower ocean mixed layer depth, owing to increased temperatures, over which aerosols may leach out nutrients. Being the top millimetre of the sea surface, the SML represents the natural interface region of the major environmental importance; it could be assigned as a first sentinel to increasing human impact and climate changes due to fast response in its biological and physico-chemical features.[2] The SML has a gel-like nature with dissolved polymeric carbohydrates and amino acids, as well as lipids present along with gel particles, being mainly of autochthonous origin, produced by the phytoplankton community. The OM within the SML possesses different degree of surfactant activity comprising the pool of the surface active substances (or surfactants; SAS) which form films at the air-water interface.[3] Surface films influence the air-sea gas exchange,[4] and when irradiated, may undergo unique photochemical reactions leading to significant abiotic production of unsaturated and functionalized volatile organic compounds (VOCs) acting as precursors for the secondary organic aerosol (SOA) formation.[5] Such VOCs emissions could be especially important in oligotrophic regions where abiotic photochemistry might be of the same importance as the biological VOCs production. Thus, changes of marine surfactant films due to photochemistry could give rise to new processes, affecting chemistry in the marine boundary layer and having a global impact on the air-sea exchange processes necessary to understand oceanic feedbacks on the atmosphere on a global level.[6] Photochemical processes at the air-water interface of authentic SML samples[5] as well as biofilm-containing solutions comprising mixed population of different microorganisms (i.e., bacteria, fungi, algae)[7] have been studied. Up to our knowledge there are no studies on the contribution of biogenic OM produced by particular marine phytoplankton species, especially that which is produced under environmental stress. Namely, it is apparent nowadays that ocean biogeochemistry is facing increasing pressures from anthropogenic forcing which have the potential to alter the biological, chemical and physical characteristics of the oceans on local and global scales in the foreseeable future.

• Scientific objectives

This project is designed to make first steps for a better understanding of the OM produced by marine phytoplankton species in relation to the surfactant film formation and photochemical processing at the air-water interface, with the consequences on abiotic VOCs production and atmospheric aerosol formation, and thus, the climate in general. Project specific objectives may be summarized as follows:

1. to better understand the mechanism of phytoplankton-derived OM photochemical processing at the air-water interface;
2. to identify the most important constituents of phytoplankton-derived OM responsible for abiotic VOCs production;
3. to assess the interfacial photochemistry and the particle formation potential of the VOCs produced by marine phytoplankton surfactant films in the foreseeable future.

• Reason for choosing the simulation chamber/ calibration facility

Our project goals could only be achieved by combining our expertise with the unique opportunities to study interfacial photochemical processes at the ISAC facility, including the most advanced analytical equipment to investigate both gas and liquid phases.

• Methods and Experimental set-up

Phytoplankton monoculture growth, fractionation and analyses

- Prior to the experiments at CNRS, ISAC, the marine diatom Chaetoceros pseudocurvisetus monoculture was cultivated to reach stationary growth phase at Ruđer Bošković Institute (RBI, Zagreb, Croatia). Batch culture of C. pseudocurvisetus was maintained within sterile tissue culture flasks containing low-nitrogen F/2 medium (NO_3 20 µmol L^{-1})[2] being relevant for N-depleted oligotrophic waters. This sample presents a case prior to atmospheric deposition influences, and marks a first step towards assessment of the atmospheric deposition. All cultures were grown on a 12/12 light/dark cycle under illumination of 4500 lx and 20 °C. The cell number concentration and the growth phases were determined every second day by cell counting with Fuchs-Rosenthal Chamber hemocytometer under Olympus BX51-P polarizing microscope. The growth was terminated at onset of stationary growth phase (eighth day) when the enhanced dissolved OM release by phytoplankton cells to the external medium is expected.
- The samples were taken from the incubation bottles immediately after culturing to obtain authentic phytoplankton solution (APS) containing jointly particulate phytoplankton cells and dissolved organic exudates (i.e. nonfiltered fraction). Dissolved phytoplankton solution (DPS) was obtained after filtration of APS through 0.7 µm Whatman GF/F filters pre-burned at 450 °C for 5 h (i.e. filtered fraction). Since dissolved phytoplankton solution contains a mixture of organic material, including polysaccharides, proteins and lipids, we further performed the extraction of lipid material from DPS by standard liquid-liquid extraction method. Organic lipid extracts were evaporated to dryness in dark vials. All phytoplankton sample fractions have been frozen immediately after processing at -20 °C until experiments at CNRS, ISAC.
- Released phytoplankton organic matter was analysed from sample aliquots at RBI to obtain initial data necessary for further sample photochemical processing:

- Dissolved and particulate organic carbon (DOC and POC); a model TOC–V_{CPH} (Shimadzu) carbon analyzer with a platinum silica catalyst and a non–dispersive infrared detector for CO_2 measurements was used for DOC measurements and calibrated with potassium hydrogen phthalate. POC was analyzed by a solid sample module SSM–5000A connected to a TOC–V_{CPH} calibrated with glucose.
- Dissolved and particulate surfactant analysis; the phase sensitive ac voltammetry (*out-of-phase* mode, frequency 77 Hz, and amplitude 10 mV) of sample aliquots were performed by tree-electrode system with µAutolab-type II (Eco Chemie B. V., The Netherlands), GPES 4.6 software (Eco Chemie B. V., The Netherlands).
- Dissolved and particulate lipid analysis; after particulate and dissolved lipid extractions lipid analysis include quantification of 18 polar and unpolar lipid classes. The lipid class analysis was performed using a thin-layer chromatograph-flame ionization detector (TLC-FID) Iatroscan MK-VI (Iatron, Japan), with a hydrogen flow of 160 mL/min and an air flow of 2000 mL/min. Lipid classes were separated on Chromarods SIII and quantified with an external calibration using a mixture of standard lipids.

Photochemical processing at the air-water interface

Different phytoplankton-derived sample fractions were photochemically processed in a Quartz cell reactor and fed into the ISAC simulation chamber to identify underlying processes and the OM fraction being the main driver for surfactant release and, thus, abiotic photochemical VOCs production. Within, gas phase and aqueous phase analysis were performed simultaneously. Afterwards, particle formation and ageing upon oxidation of the produced VOCs was investigated as well.

Photochemical chamber

Pytoplankton culture samples APS and DPS (7-9 mL) were introduced into a cylindrical Quartz cell reactor, thermostated by a water bath (20 °C). The experiment with the lipid surface film was conducted by spreading the hexane lipid extract aliquots on top of salt water bulk (0.55M NaCl) filling the ca. half of the cell reactor. A Xenon lamp (150 W; LOT QuantumDesign, France) placed at a distance of 13 cm from the reactor was used to mimic solar irradiation on the Earth's surface. A quartz water filter of 5 cm was mounted in front of the lamp to remove infra-red irradiation, whereas short wavelengths (λ < 290 nm) were eliminated by a Pyrex filter positioned directly in front of the reactor. A flow of 200–300 mL of compressed, filtered and purified air was pushed continuously through the reactor. Typically, after introduction of the samples and acquisition of a stable background signal, the suspensions were irradiated for 1 h. Experiments were stopped as soon as signals reached background levels again, after switching off the light. Illumination experiments were followed by the gas phase and aqueous phase analyses as described below.

Gas Phase analysis

A PTR-ToF-MS (SRI-PTR-ToF-MS 8000, Ionicon Analytik GmbH, Innsbruck, Austria) was used to quantify the emitted VOCs using H_3O^+ as source reagent ion Air was sampled at a constant flow of 100 mL min^{-1} at an inlet temperature of 60 °C. Typically, a drift voltage of 600 V, a drift temperature of 60 °C and a drift pressure of 2.25 mbar were used, resulting in an E/N-ratio of about 135 Td. VOCs concentrations were calculated according to Cappellin et al.[8] Fluxes of VOCs will be calculated according to Ciuraru et al.[5]

Aqueous Phase analysis

- Liquid-phase sample extracts (after liquid-liquid extraction with dichloromethane, re-dissolved in acetonitrile:water, 1:1, v:v) were analysed by reversed phase ultra-high performance liquid chromatography coupled to an Orbitrap high resolution mass spectrometer (Q Exactive, Thermo Scientific, Bremen, Germany) using heated electrospray ionization (HESI). For the LC separation, a Dionex Ultimate 3000 ultraperformance liquid chromatograph was equipped with a HSS T3 Acquity UPLC column (1.8 µm, 5 2.1 × 100 mm). Acidified water (eluent A; 0.1%, v/v, formic acid) and acidified acetonitrile (eluent B; 0.1%, v/v, formic acid) were used as mobile phase. An HESI voltage of −3.0 kV was applied for negative ionization mode measurements. The sheath gas flow rate was set to 42 arbitrary units (a.u.) and the auxiliary gas flow rate to 25 a.u. A capillary temperature of 350 °C and a heater temperature of 250 °C were used. All measurement were performed using the highest possible mass resolution (R=1.4·107 at 10 m/z 200). Data were processed and evaluated by XCalibur 2.2 (Thermo, USA). Further data analysis will be performed using the non-target screening approach of the MZmine 2.21 software package (http://mzmine.github.io).
- APS and DPA sample aliquots were frozen immediately after irradiation experiments at -20 °C for further DOC and surfactant analyses (described above) at RBI, Zagreb.
- In order to investigate the influence of the sunlight irradiation on the OM complexing capacity, the APS and DPA sample aliquots were collected before and after irradiation experiments for the speciation anaysis of selected metal species (zinc, cadmium, copper and lead). The samples were filtrated through celulose acetate filters with pore size of 0.45 µm, and acidified to pH 2 with nitric acid and will be analised at RBI, Zagreb by differential pulse anodic stripping voltammetry.
- APS and DPA sample aliquots were taken before and after irradiation experiments for UV-Vis analysis performed at CNRS by UV/Vis spectrophotometer (Agilent Technology), 10 mm pathlength cell.

Multiphase atmospheric simulation chamber

Particle formation upon oxidation of the produced VOCs was investigated using a 2 m^3 chamber made of FEP film (fluorinated ethylene propylene). The chamber was surrounded by 12 UV-Vis lamps (OSRAM lamps, Eversun L80W/79-R) to mimic solar irradiation. When all chamber background levels were established, the overnight filling the chamber with VOCs produced after sample irradiation in Quartz cell was conducted. Gas phase concentration of VOCs was monitored with a PTR-ToF-MS using H_3O^+ as source reagent ion. Afterwards, the

ozone was injected in the dark, reaching a maximum of 300 ppb_V. The ozone was generated by UV photolysis of oxygen (Stable Ozone Generator 1, Ultra-Violet Products Ltd., USA). The resulting O_3 concentrations in the chamber were continuously measured (49i, Thermo Scientific, USA). A Particle number concentrations and corresponding size distributions were measured using an ultrafine condensation particle counter (UCPC, 3776, TSI, USA) and a scanning mobility particle sizer (SMPS, 3936, TSI, USA). After the particle concentrations reached an approximate steady state concentration, the UV-Vis lamps were turned on for 1 h to further study SOA ageing.

• Data description

The experiments at CNRS, Lyon were operated for 10 days, typically with two irradiation setup per day. As such, we conducted 15-20 irradiation experiments in Quartz cell, including blank experiments performed on empty cell as well as on the cell filled with Milli-Q water. Also, the temperature impact on VOCs production was studied. In parallel to illumination experiments, gas phase and aqueous phase analyses were performed. In addition, we performed 3 long-time experiments using the multiphase simulation chamber to study particle formation and growth.

• Preliminary results and conclusions

Background experiments were firstly performed on an empty reactor and on deionized water. The experiments on APS (i.e., non filtered authentic sample containing particulate phytoplankton dead cells and dissolved OM exudates) and DPS (i.e., filtered APS containing only dissolved phytoplankton exudates) samples arising from diatom Chaetoceros pseudocurvisetus cultivation were conducted after all chamber gas phase background levels were established in the different ionization modes of the PTR-ToF-MS. The APS and DPS solutions were filled ca. half of a small Quartz cell and irradiated by means of a Xenon lamp continuously flushed with purified air. Fig. 1a shows typical VOCs signals followed by PTR-ToF-MS measurements upon sample irradiation as a function of time for APS samples. All VOCs signals followed were found to increase as soon as the sample was irradiated. It should be noted that for all samples VOCs fluxes decreased immediately after switching off the light, reaching background levels typically after 30–60 minutes, and clearly linking VOCs production to irradiation. We tested also the change in VOCs production after addition of photosensitizer humic acid (30 mg L^{-1}) within the APS sample bulk but no further increase in VOCs signal was observed. Further, we observed similar increase in VOCs signal intensities for the irradiation experiments on DPS samples. This is an indication that dissolved surface active constituents released from diatom C. pseudocurvisetus cells are possibly the main driver for abiotic photochemical VOCs production observed. This was in agreement with the recent findings on biofilms, being the population of different microorganisms (i.e., fungi, algae, archaea).[7] However, our results point even further that dissolved surfactants, originating solely from marine phytoplankton cell lysis could lead to significant abiotic production of VOCs upon sea surface irradiation. Preliminary VOCs identification indicated production of saturated and unsaturated VOCs, including isoprene.

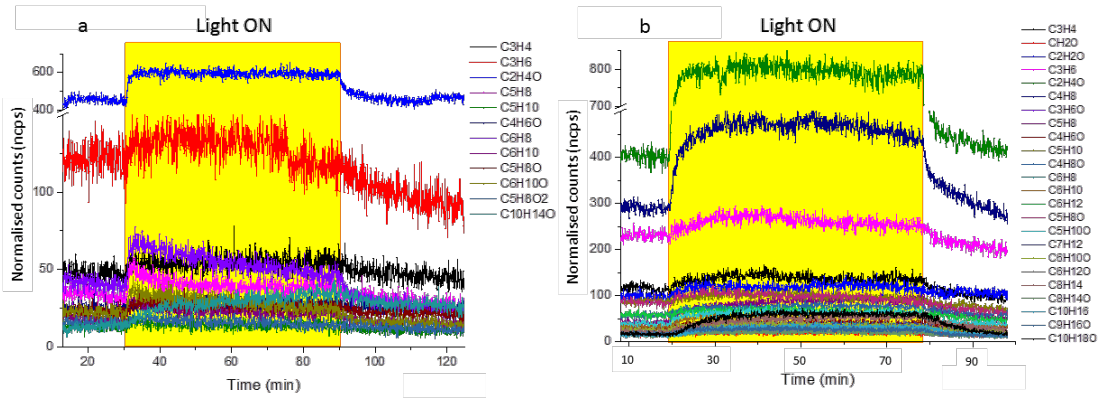

Figure 1. Identified VOCs signal increase as a function of time identified by SRI-PTR-ToF-MS upon: (a) APS sample irradiation and (b) irradiation of biogenic lipid films.

To gain more insight to the phytoplankton dissolved surface-active constituents being the main contributors to observed VOCs production, the irradiation experiments on phytoplankton dissolved lipid material were perform after spreading the hexane lipid extract aliquots on top of salt water bulk. Fig. 1b shows typical VOCs signals upon biogenic lipid film irradiation at the air-water interface as a function of time. We observed significant increase in number of identified saturated and unsaturated VOCs as well as in signal intensities when biogenic lipids were concentrated on the air-water interface in comparison to APS and DPS experiments. Lipid material is present in

marine OM pool in a very low degree but due to the extremely high surface affinity their ubiquitous presence at the air-water interface is the result of their competitive adsorption and segregation from other macromolecular constituents.[3] Thus, phytoplankton-derived lipid fraction in particular due to photochemical processing at the air-water interface could be a significant contributor to the abiotic VOCs production globally.

Since the highest intensities of VOCs signals were observed during the irradiation experiments on lipid films, further particle formation and grow experiments were performed after overnight filling the chamber with VOCs produced during irradiation of lipid films. As illustrated in Fig. 2, new particle formation occurred in the chamber directly afterwards the ozone was injected in the dark and reaching a maximum of 300 ppb_V, indicating the formation and nucleation of low-volatile oxidation products. Nucleation of SOA particles up to a maximum concentration of ca. 200 cm^{-3} was observed after dark ozonolysis. Particle size-distribution maximum shifted over time to a larger particle sizes, reaching the maximum values of ca. 20 nm. After the particle concentrations reached an approximate steady state levels, UV lights were turned on to further study the effects of SOA ageing. As can be seen from Fig. 2, an immediate particle growth followed, demonstrating the oxidation of yet unreacted VOCs and the formation of further low volatile compounds, condensing on already existing particles.

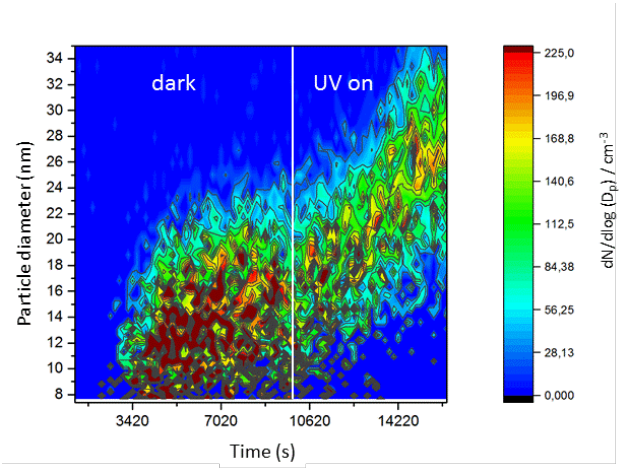

Figure 2. Particle number and size distribution after ozone injection (reaching ca. 300 ppb_V) as a function of time for a chamber experiment. Phytoplankton lipid films were irradiated prior to ozone injection for 24h hours to induce sufficient VOCs production for filling up the chamber. After 300 min of dark ozonolysis, the UV lights were turned on.

• Outcome and future studies

All of the shown data are preliminary and require further analysis. However, we have already shown that the photochemical processing of OM produced by marine diatom Chaetoceros pseudocurvisetus results in abiotic VOCs production and further aerosol particle formation and growth. Most probably the biogenic lipid material is prominent phytoplankton-derived OM fraction responsible for the observed VOCs production. Future analysis will comprise overal data on VOCs fluxes, HPLC-HRMS measurements, DOC, surfactants, lipid classes, metal speciation and UV-Vis properties of biogenic samples studied. Based on the promising results obtained from this project, the joined COGITO project proposal on above issue has been submitted in June 2018 by Christian George and Sanja Frka Milosavljević.

• References

1. Kanakidou, M., Duce, R. A., Prospero, J. M., Baker, A. R., Benitez-Nelson, C., et al. Atmospheric fluxes of organic N and P to the global ocean, Global Biogeochem. Cycles, 26, GB3026, 2012.
2. Cunliff, M., Engel, A., Frka, S., Gašparović, B., Guitart, C., et al. Sea surface microlayers: a unified physicochemical and biological perspective of the air-ocean interface. Progr. Oceanogr. 109, 104–116, 2013.
3. Frka, S., Pogorzelski, S., Kozarac, Z., Ćosović, B. Physicochemical signatures of natural sea films from Middle Adriatic stations. J. Phys. Chem. A. 116 (25), 6552–6559, 2012.
4. Engel, A.; Bange, H. W.; Cunliffe, M.: Burrows, S. M.; Friedrichs, G.; et al. Frontiers in Marine Science 2017, 4, 165, 1–14.
5. Ciuraru, R.; Fine, L..; van Pinxteren, M.; D'Anna, B.; Herrmann, H.; George, C. Environ Sci Technol 2015, 49,

13199–13205.
6. Bruggemann, M.; Hayeck, N.; George, C. Nature Communications 2018, 9.
7. Bruggemann, M.; Hayeck, N.; Bonnineau, C., Pesce, S.; Alpert, P.; et al. Faraday Discussions 2017, 200, 59–74.
8. L. Cappellin, T. Karl, M. Probst, O. Ismailova, P. M. Winkler, C. Soukoulis, E. Aprea, T. D. Märk, F. Gasperi and F. Biasioli, Environ. Sci. Technol., 2012, 46, 2283–90.