Biodiversity restoration and conservation of inland water ecosystems for environmental and human well-being

BioReset

BiodivRestore-406

2020 - 2021 Joint Call

Joint COFUND Call on “Conservation and restoration of degraded ecosystems and their biodiversity, including a focus on aquatic systems”

Deliverable 3.X

Diatom Sampling Protocol

Lead Beneficiary	Work package	Delivery month
CIIMAR	**3**	**18**

1. Executive Summary

Diatoms are unicellular microalgae with a porous siliceous cell wall. They can be found in practically all aquatic environments and are well-recognised as bioindicators of ecological water quality. They respond to abiotic stress in short time scales, showing differentiated responses to a variety of aquatic contaminants. Diatoms are the main components of biofilms. These are aggregates of various microorganisms (diatoms and other microalgae, protozoa, fungi and bacteria) embedded in a polymeric matrix. The biofilms are visible macroscopically as greenish to brownish layers colonising different substrates (rocks, pebbles, blocks, shells, clay...).
Diatom sampling is the basis for the evaluation of the ecological water quality with taxonomic quality indices as recommended in the Water Framework Directive. This is a suitable and widely used method, but it is cumbersome and expensive, requiring sample preparation and identification knowledge and experience, among others (Pinto et al., 2021). Various methods have thus been considered for its suitable replacement, though the proposals available are also expensive and require sample preparation significant technical resources and expertise (Pinto et al., 2021). **BioReset** proposes the development of an innovative approach based on Raman Spectroscopy, which does not require sample preparation, can be employed with a minimum number of measurements done by non-specialised personnel, with results analysed using pre-established chemometrics routines. For this development, the first relevant step was to produce a diatom sampling protocol that all **BioReset** partners, and others, non-specialised on diatoms or ecology, could use to obtain suitable diatom samples from their target aquatic systems and send for analysis. This deliverable reports on the methods and procedures for suitable sample collection providing quality samples for both the classical diatom analysis and the innovative diatom Raman spectroscopy.

2. Task description

The work was developed within the scope of WP3 (Evaluation of ecosystem conservation and restoration: diatom biofilms), coordinated by CIIMAR, and relates to the samplings necessary to develop its tasks, including the maintenance of diatom biofilms in the laboratory and all the field assessments considered in the partners target ecosystems (Portugal, Spain, Norway, Sweden). Diatom sampling for the purposes described should be done preferentially in locations with low shadowing and coarse substrates (rocks, pebbles, blocks, shells) close to the water surface. In the absence of coarse biofilm substrates, samples can be collected from fine substrates (sand, lime or clay). As a last resource, they can also be collected from water vegetation. The method and protocol presented herein are based on the methods described in Oliva-Teles et al. (2022) and Pinto et al. (2022).

3. WP3 – Team members involved in the work development

The following CIIMAR team members contributed to the development of the work and the protocol presented herein:

Name	Organization	Role
Laura Guimarães	CIIMAR	Institutional leader and WP coordinator
António Paulo Carvalho	CIIMAR	Task coordinator
Luís Oliva-Teles	CIIMAR	Task coordinator
Raquel Pinto	CIIMAR	PhD student allocated to the project (FCT grant)
Marco Veludo	CIIMAR	Technician hired through the project

4. Developed activities

Concerning this deliverable, the rational for the selection of the sampling sites was as follows. The sampling took place in ecosystems associated with the wastewater treatment plants (WWTP) involved in the project. The specific sampling sites within the ecosystem should be chosen to reflect a potential contamination gradient, allowing to characterise the input of the WWTP into the system contamination and a possible recovery along the downstream river or system flow, reflecting better conditions. An example is the selection of a site before the WWTP effluent release into the ecosystem, a site imediately after the point of effluent release (a meter at most) and two to three more sampling sites after this one up to 5 to 10 km in distance from the contamination source. Depending on the objective of the assessment, in cases of interventions for ecosystem recovery or restoration, the sites should be established to allow sample collection before and after the restoration or mitigation intervention to allow for suitable comparison, as well as the

necessary long-term follow-up needed to maintain the good ecological status, once this has been reached. The protocol was developed for the sampling of CIIMAR, based on the procedures available in the literature and adapted for the objectives of **BioReset**. The specific protocol to adopt is presented in section 5.

5. Diatom sampling protocol

5.1. Materials and equipments

- Medium to hard toothbrush
- Plastic pipettes (1-3 mL) with the tip cut off
- Small sampling tray
- Sampling flasks (120 mL; Figure 1 *left*) (three to four per sampling site) and a dark glass bottle (60 mL, Figure 1 *right*) with a few drops of fixation solution, *i.e.* Lugol (0.33%) or buffered formaldehyde solution (4%) (one per sampling site)
- Multiparametric measurement device for the measurement of water pH, dissolved oxygen and conductivity/salinity
- Camera and GPS
- Thermic box with ice accumulators to store non-fixed samples
- Common labelling and protection materials (e.g. markers, tracing paper, labels, pencil, notebook, waterproof boots, latex/nitrile gloves...)

Figure 1. Examples of sampling flasks for diatom collection and fixation (120 mL, *left*; 60 mL, *right*).

5.2. Procedures

Data on pH, dissolved oxygen, conductivity and salinity should be collected in each sampling site, even if with simpler methods. GPS coordinates and photos should also be taken. At the end of a sampling, the materials can be cleaned with local water, before use in the next site. Three plastic flasks are required for the application of the diatom Raman spectroscopy method. The glass flask (with lugol or formaldehyde) is required to preserve the diatoms and prepare them for the taxonomic identification. While the diatom Raman spectroscopy method is under implementation in a new lab it is advisable to compare the results with the established method in use to assess water quality with diatom autoecological indices (Water Framework Directive), for callibration. If no expertise in taxonomic identification is available in the local lab, CIIMAR can be contacted to provide sample identification. Within BioReset, all taxonomic identifications and assessments of samples collected, in Portugal and the other partner countries, were done by CIIMAR.

The sample fixation can be done directly in the field (the glass flasks are prefilled with the fixation solution and taken to the sampling site) or on arrival at the lab. Each sampling site should be selected to cover a total area of about 100 cm^2 of biofilm colonisation. The colonised area is visible through the water as the greenish and brownish layer at the surface of the substrate (Figure 2). Substrates without visible signs of biofilm colonisation are not suitable for sampling. After collection, the samples should be conditioned in the thermic box. All samples need to be labelled with the date of sampling, denomination of the watercourse, city, substrate sampled and project. Three alternative methods for diatom collection are provided below, to be used according to the characteristics of the target ecosystem.

Sampling coarse substrates

The substrates and water are collected and processed in the margin.

1. Select a few rocks, blocks or pebbles and put them in the plastic tray filled with ~500 mL of local water.
2. Scrap each substrate, one by one, with a toothbrush and dissolve the biofilm in the tray water (Figure 2).
3. Repeat the process until the tray water becomes opaque and brownish.
4. Pour the content of the tray into the sampling flasks.

Figure 2. Example of a coarse substrate with evidence of biofilm colonisation (*left*) and sampling (*right*).

Sampling fine substrates

Areas with fine substrate (sand, lime, clay) with signs of biofilm colonisation can also be sampled.

1. Select the substrates with the most amount of biofilm within the sampling area.
2. With the plastic pipette collect as much biofilm as possible into the sampling flasks.

Sampling water vegetation

If none of the methods above can be employed and there is an obligation to sample the target ecosystem, the following method can be used. The biofilm installed in the aquatic vegetation is sampled by washing and squeezing.

1. Collect about 500 mL of local water into a plastic tray.
2. Collect several submerged water vegetation and place it in the tray water.
3. Clean the plants/algae with tray water and squeeze them with your hands.
4. Pour into the flasks the suspension obtained.

BioReset samples

The samples should be sent away for analysis in cold conditions, for suitable preservation. An express mail service should be used and samples should be sent in thermic boxes filled with dry ice. Besides CIIMAR samplings in Portugal, this collection method was employed by BioReset colleagues in Spain, Norway and Sweden. Samples from these countries were sent to CIIMAR for analysis. Unfortunately, unexpected Customs problems, with unpredicted retention of samples, led to loss of the Norway samples. Samples from Spain and Sweden were of high quality and provided excellent results.

6. Associated indicators

The indicators which led to the development of this protocol and made use of it are indicated below.

Publications

1. Pinto R, Vilarinho R, Carvalho AP, Moreira JA, Guimarães L, Oliva-Teles L. 2022. Novel Approach to Freshwater Diatom Profiling and Identification Using Raman Spectroscopy and Chemometric Analysis. Water 14:2116. DOI: 10.3390/w14132116
2. Oliva-Teles L, Pinto R, Vilarinho R, Carvalho AP, Moreira JA, Guimarães L. 2022. Environmental diagnosis with Raman Spectroscopy applied to diatoms. Biosensors and Bioelectronics 198:113800. DOI: 10.1016/j.bios.2021.113800

Communications

1. Sousa, M., Pinto, R., Carvalho, A.P., Guimarães, L., Oliva-Teles, L. Diatom taxonomic identification in the Lima River Estuary. Blue Think Conference 2023. Poster presentation | September 13, 2023 | Matosinhos, Portugal
2. Pinto, R., Vilarinho, R., Carvalho, A.P., Agostinho, J., Guimarães, L., Oliva-Teles, L. Raman spectroscopy applied to diatoms as an alternative to conventional water quality assessment. Blue Think Conference 2023, Poster presentation | September 13, 2023 | Matosinhos, Portugal
3. Pinto, R., Vilarinho, R., Carvalho, A.P., Agostinho, J., Guimarães, L., Oliva-Teles, L. Potential of Raman Spectroscopy for Environmental diagnosis using diatoms. Ciência 2023 - Encontro com a Ciência e a Tecnologia - Ciência e Oceano para Além do Horizonte. Poster presentation | July 5-7, 2023 | Aveiro, Portugal
4. Sousa, M., Pinto, R., Carvalho, A.P., Guimarães, L., Oliva-Teles, L. Diversity of Saltmarsh Diatom Communities in the Lima River Estuary. Ciência 2023 - Encontro com a Ciência e a Tecnologia - Ciência e Oceano para Além do Horizonte. Poster presentation | July 5-7, 2023 | Aveiro, Portugal
5. Sousa, M., Pinto, R., Carvalho, A.P., Guimarães, L., Oliva-Teles, L. Community Composition of saltmarsh diatoms in the Lima River Estuary. OCEAN3R Final Workshop and meeting. Poster presentation | June 27, 2023 | Matosinhos, Portugal
6. Pinto, R., Vilarinho, R., Carvalho, A.P., Agostinho, J., Guimarães, L., Oliva-Teles, L. Water Quality assessment using Raman Spectroscopy applied to Diatoms. OCEAN3R Final Workshop and meeting. Poster presentation | June 27, 2023 | Matosinhos, Portugal

PhD thesis

1. Raquel Pinto. Ecosystem assessment and monitoring using diatom Raman spectroscopy. PhD in Biology, University of Porto (Portugal) | 2023 (ongoing), Fundação para a Ciência e a Tecnologia (FCT) (Portugal) grant 2022.09984.BD

Master dissertation

1. Marco Sousa (2022) Characterisation of saltmarsh diatoms in the Lima River Estuary: Community composition and Raman Spectroscopy applied for environmental diagnosis. Master in Toxicology and Environmental Contamination. Institute of Biomedical Sciences Abel Salazar (ICBAS), University of Porto.

References

Pinto, R., Vilarinho, R., Carvalho, A.P., Moreira, J.A., Guimarães, L.*, Oliva-Teles, L. Novel Approach to Freshwater Diatom Profiling and Identification Using Raman Spectroscopy and Chemometric Analysis (2022) Water, 14 (13): 2116, doi: 10.3390/w14132116

Oliva-Teles, L., Pinto, R., Vilarinho, R., Carvalho, A.P., Moreira, J.A., Guimarães, L.* Environmental diagnosis with Raman Spectroscopy applied to diatoms (2022) Biosensors and Bioelectronics 198: 113800, doi: 10.1016/j.bios.2021.113800

Pinto, R., Vilarinho, R., Carvalho, A.P., Moreira, J.A., Guimarães, L.*, Oliva-Teles, L. Raman spectroscopy applied to diatoms (microalgae, Bacillariophyta): Prospective use in the environmental diagnosis of freshwater ecosystems (2021) Water Research 198: 117102, doi: 10.1016/j.watres.2021.117102