

Proposal for 4th Collaborative European Freshwater Sciences Project for Early Career
Freshwater Researchers (“FreshProject”)

Life in plastic, it’s fantastic: unravelling the microalgal community of plastisphere across European lentic systems

PhytoPlastic



Veronica Nava¹ & Julia Gostyńska²

¹ *Department of Earth and Environmental Sciences, University of Milano-Bicocca (Italy)*

² *Department of Hydrobiology, University of Adam Mickiewicz (Poland)*

Keywords: plastic colonization; plastic pollution; phytobenthos; phytoplankton; primary producers.

April 2022

Table of contents

ABSTRACT (ENGLISH)	3
ABSTRACT (ITALIAN)	4
1. STATE OF THE ART	6
2. METHODS AND DEVELOPMENT OF THE PROJECT	7
2.1. METHODS	8
2.2. PROJECT TASKS AND DEVELOPMENT OF THE PROJECT	11
3. IMPACT OF THE PROJECT	14
4. SELECTED REFERENCES	16
5. ANNEX	18
5.1. CURRICULUM VITAE: VERONICA NAVA	18
5.2. CURRICULUM VITAE: JULIA GOSTYŃSKA	21
5.3. SUPPORT LETTERS	23
5.4. LETTERS OF THE EFFS-FEDERATED SOCIETIES	25

Abstract (English)

Among the multiple stressors that affect aquatic ecosystems, plastic pollution is deemed a widespread and pervasive environmental issue. Since the ubiquitous presence of plastic debris in aquatic systems has been established, the focus of research has shifted towards assessing their impact on aquatic organisms and ecosystem functions. The interaction of plastics with aquatic biota starts from low trophic levels; indeed, plastics represent a new habitat for rafting organisms to the point that the term “plastisphere” was coined to define the diverse community growing on the surface of plastic debris. Even if heterotrophic bacteria tend to be the focus of plastisphere research, the presence of microalgae within the epiplastic biofilm has been repeatedly documented. However, further research is needed to explore the microalgae-plastic interactions and several questions remain to be addressed, especially for freshwaters. The present project is aimed at providing insights regarding the microalgal component of the plastisphere, studying the temporal establishment of phytobenthos on different plastic polymers in lakes and reservoirs over a wide geographical scale to better characterize the interaction of plastics with these key organisms. The overarching goal is to understand whether plastic debris can represent a new niche in lentic systems for the microalgal community and determine whether substrate-specific properties or environmental factors prevail in shaping microalgal assemblages on plastic debris. To achieve these objectives, experiments will be conducted in lentic systems with different features. Sheets of polyethylene, polypropylene, and glass (which serve as control being an inert substrate) will be deployed in each system. To assess the temporal and seasonal evolution of the colonization, samples will be collected in succession after 3, 7, 15, and 30 days and the experiment will be replicated in each season. Several physical and chemical parameters will be analyzed alongside (e.g., temperature, dissolved oxygen, nutrient concentration) to understand the relationship with the environmental variables. For each substrate and replicate, we will assess the phytobenthic biomass estimating the chlorophyll a, and the ash-free dry mass. Moreover, microalgae composition will be determined on a subset of samples to understand the community composition colonizing the different substrates. This will represent, at the best of our knowledge, the first coordinated experiment conducted at a large spatial scale to explore the plastisphere and, thereby, a unique dataset will be generated that will allow us to identify the key drivers of the process, leaving aside the site-specific and possibly confounding variables.

Besides, the valuable scientific knowledge that will be acquired, the project will allow a large collaboration among early career researchers in freshwater sciences and will set the basis for further partnerships.

Abstract (Italian)

Tra i molteplici impatti che agiscono sugli ecosistemi acquatici si annovera l'inquinamento da plastica, considerato un problema diffuso e pervasivo. Dal momento che l'onnipresenza dei detriti plastici negli ambienti acquatici è stata ormai accertata, il focus della ricerca si sta gradualmente spostando sulla valutazione del loro impatto sugli organismi e sul funzionamento dei sistemi acquatici. L'interazione della plastica con il biota inizia già dai primi livelli trofici; infatti, i detriti plastici rappresentano un nuovo habitat per gli organismi acquatici al punto che il termine "plastisfera" è stato coniato per definire la variegata comunità che è in grado di svilupparsi sulla loro superficie. Anche se i batteri eterotrofi tendono ad essere al centro della ricerca sulla plastisfera, la presenza di microalghe nel biofilm epiplastico è stata ripetutamente documentata. Tuttavia, sono necessari ulteriori studi per meglio comprendere l'interazione tra microalghe e plastica e vi sono ancora molte domande a cui è necessario rispondere, specialmente per le acque dolci. Il presente progetto vuole contribuire a colmare alcune di queste lacune, tramite lo studio dello sviluppo temporale del fitobenthos su diversi polimeri plastici in vari ambienti lentici distribuiti su un'ampia scala geografica. L'obiettivo principale è comprendere se i substrati plastici rappresentano una nuova nicchia per la comunità microalgale nei sistemi lentici e valutare se sono le proprietà specifiche del substrato o i fattori ambientali a prevalere nel determinare la composizione e quantità delle microalghe epiplastiche. Per raggiungere questi obiettivi, saranno condotti esperimenti in sistemi lentici con caratteristiche differenti. Fogli di polietilene, polipropilene e vetro (usato come controllo essendo un substrato inerte) verranno posizionati in ciascun sistema. Per valutare l'evoluzione temporale e stagionale della colonizzazione, campioni differenti saranno raccolti in successione dopo 3, 7, 15 e 30 giorni e l'esperimento sarà replicato in ogni stagione. Diverse variabili fisiche e chimiche verranno valutate contestualmente. Per ogni substrato e replicato, la biomassa microalgale verrà valutata tramite la misura della clorofilla a e della massa secca al netto delle ceneri. Inoltre, la composizione delle microalghe sarà determinata su un sottoinsieme di

campioni per individuare le specie algali in grado di colonizzare i diversi substrati. Questo rappresenterà il primo esperimento coordinato a studiare la plastisfera su larga scala e ciò permetterà di generare un dataset unico nel suo genere che ci consentirà di identificare i driver chiave del processo, tralasciando le variabili sito-specifiche. Oltre alle rilevanti conoscenze scientifiche che verranno acquisite, il progetto consentirà un'estesa cooperazione tra giovani ricercatori nell'ambito delle scienze acquatiche e getterà le basi per future proficue collaborazioni.

1. State of the art

Among the multiple stressors that affect freshwater ecosystems, plastic pollution has been widely documented as a widespread and pervasive environmental issue and the role of lakes and rivers in global plastic pollution has been increasingly recognized (Horton et al., 2017). Indeed, these systems on one side represent a source of plastic pollution, as they contribute to the transport of plastic debris from land-based sources to coastal and marine environments, on the other they can also represent a sink and can be impaired by the presence of these contaminants (Horton et al., 2017).

While the benefits of plastics are undeniable, the widespread use of these polymers, namely in discardable forms, ultimately leads to their accumulation in the environment (da Costa et al., 2017). The durability and resistance to degradation of plastics, which render these materials incredibly versatile in several applications, make them difficult or impossible for nature to assimilate (Geyer et al., 2017). Plastic waste is now so ubiquitous in the environment that it has been suggested as a geological indicator of the proposed Anthropocene era (Geyer et al., 2017). The slow breakdown of plastic materials produces successively smaller pieces, called microplastics, facilitating their long-range transport and dispersion in the environment, even being ingested by various organisms (Matsuguma et al., 2017; Nel et al., 2018). Microplastics pose a risk to organisms across the full spectrum of biological organization from cellular to population level. So far, our knowledge about the uptake and biological effects of microplastics comes from laboratory studies that applied simplified exposure regimes (e.g., one polymer and size, spherical shape, high concentrations) often with limited environmental relevance. However, the available data reveals species- and material- related interactions and highlights that microplastics represent a multifaceted stressor (Scherer et al., 2018). The interaction of microplastics with aquatic biota starts from low trophic levels. Indeed, different studies highlighted as plastic particles can interact with primary producers (microalgae) and this has impacts on their respective fates (Yokota et al., 2017). Existing studies on this topic have been mainly focused on the toxic effects of microalgae after exposure to microplastics, reporting effects on growth (Venâncio et al., 2019; Zhao et al., 2019), photosynthetic activity (Mao et al., 2018; Zhang et al., 2017), and morphological changes (Mao et al., 2018). However, the interaction between plastics and microalgae is far more complex with a wide range of consequences. Indeed, plastics constitute suitable substrates for the colonization by

microalgae and represent a new habitat for rafting organisms to the point that the term “plastisphere” was coined by Zettler et al. (2013) to define the diverse community of heterotrophs, autotrophs, predators, and symbionts growing on the surface of plastic debris. Even if heterotrophic bacteria tend to be the focus of plastisphere research, the presence of epiplastic microalgae (i.e., algae growing on plastic surfaces) within the biofilm has been repeatedly documented (cf. Nava and Leoni, 2021). However, further research is needed to explore the microalge-plastic interactions and several questions remain to be addressed. For instance, while it has been shown that the communities differ between biofilms and the ambient environment, there is no consensus on whether biofilms differ between distinct plastic substrates (Rogers et al., 2020), and it remains unclear whether the plastic surface “environment” may exert a strong enough selection to drive species sorting, overcoming other niche-defining factors driven by seasonal and spatial patterns (Nava & Leoni, 2021). The ecological implications of this topic are relevant since this interaction can affect primary productivity and the availability of specific plastic substrates can potentially advantage certain species with consequences for aquatic ecosystem functioning (Zhang et al., 2020). Despite the relevance of this topic, studies addressing the interaction with organisms at the base of aquatic food webs are still extremely limited, especially in freshwater ecosystems (Wang et al., 2019).

2. Methods and development of the project

Given the widespread presence of plastic debris in freshwater systems and the lack of knowledge about the ecological implication of their presence, the present project is aimed at studying the temporal establishment and development of phytobenthos on different plastic polymers over a wide geographical scale to better characterize the interaction of plastics with key organisms of aquatic ecosystems, i.e. microalgae. The overarching goal is to understand whether plastic debris can represent a new niche for the microalgal community in freshwater systems and determine whether substrate-specific properties or environmental factors prevail in shaping microalgal assemblages on plastic debris. In particular, the project aimed at:

- a. evaluate whether different plastic polymers constitute suitable substrates for the development of microalgal communities;

- b. quantify the microalgae biomass developed on microplastics with different polymeric composition and determine whether biomass vary significantly among substrates across a variety of aquatic systems;
- c. identify the microalgae species that are able to develop on different substrates and understand whether plastics exert a strong enough selection to drive species sorting, overcoming other niche-defining factors;
- d. evaluate the temporal and seasonal evolution of the epiplastic community of microalgae in relation to several environmental variables.

Taking advantage of the collaborative nature of the present call, these objectives will be observed over a wide geographical and climatic spectrum of freshwater systems and, therefore, the project will provide new insights and perspectives about the interaction of plastics and microalgae and the implications linked to this process.

2.1. Methods

To achieve the aforementioned objectives, field experiments will be performed to evaluate the colonization by microalgae of plastics with different polymeric composition across a range of environmental conditions in different lentic systems (lakes or reservoirs). The sampling sites will be selected considering their geographical distribution, the trophic levels, and some additional parameters (i.e., mean and maximum depth, surface area, volume, elevation, thermal regime). To explore the impacts of substrate variations on aquatic biofilm communities, three kinds of substrates will be used: one inert (i.e., glass slides) and two plastic polymers (i.e., sheets of polypropylene, PP, and polyethylene, PE). Glass is used because it is widespread and inert in freshwater environments and served as control substrate for biofilm colonization experiments in several studies (Miao et al., 2021; Ogonowski et al., 2018). PP and PE are selected since they are the most common polymers observed in surface waters of freshwater ecosystems (Dusaucy et al., 2021). Plastic sheets will be cut to have the same dimension as the glass slides used (27x47 mm). To allow the deployment of the plastic and glass substrates, an experimental device will be assembled using steel wire. The steel wire will be made into a square shape with a side length of 40 cm and a weight will be placed on the top. Glass slides and plastic films will be tied to the steel wire using a metal wire and three replicates for each substrate will be attached to the device (3 substrates X 3 replicates = 9 samples).

Totally, four devices will be deployed in each site, anchored to a quay or a structure available on the littoral zone of the lentic system at about 10 cm below the surface to apply approximately the same light intensity and ensure that devices are below the water surface even in case of water level fluctuations. To observe the temporal variation of the microalgal community, the devices will be collected in succession after 3, 7, 15, and 30 days. Several physical and chemical parameters will be analyzed in each site on the day of the deployment of the device and during each sampling day (i.e., temperature, oxygen, pH, electrical conductivity); while samples for nutrient concentrations (i.e., total phosphorus, total nitrogen) will be collected only during the first day. Additional meteorological data (i.e., rainfall) will be gathered by each project participant. The experiment will be replicated in Spring, Summer, Autumn, and Winter to observe also seasonal variation in community development.

For the collection of the samples, nylon brushes will be used to scratch the whole surface (both the layers) of the plastic sheets and glass slides (Rimet et al., 2020). The surfaces will be scratched in a previously cleaned tray and the materials obtained will be collected in a centrifuge tube and make up to 50 mL volume using distilled water. Then, the samples will be transferred to the laboratory in a closed bag using freezer packs to keep samples chilled and in darkness. For the subsequent analysis, each sample will be equally divided into subsamples for the determination of chlorophyll a, and ash-free dry mass (AFDM). Specifically, chlorophyll a will be determined by spectrophotometric techniques according to APHA (1998), after being filtered using glass microfiber filters (Whatman GF/C filters), and extracted using 90% acetone. To avoid interference of phaeopigments that have the same absorption peak as for chlorophyll a, an acidification step as a second part of the analysis will be performed (Biggs and Kilroy, 2000). AFDM will be determined by filtering the subsample through pre-ashed Whatman GF/C filters, then drying for 24 h at 105°C, weighing, ashing in a muffle furnace for 4 h at 550°C, and weighing again (Biggs and Kilroy, 2000). Chlorophyll a concentration and AFDM will be converted to biomass per unit area. An "Autotrophic Index" (AI) will be calculated as the ratio of AFDM to chlorophyll a (Biggs and Kilroy, 2000). If the biomass developed would not be enough to perform the biomass determination, the three replicates will be merged.

In addition to biomass measurement, phytobenthic organisms will be identified. To this end, samples will be collected at the beginning and the end of the experiment (i.e., after 3 and 30 days since the deployment of the device), and the subsample of the three replicates of each substrate will be merged. Totally for each site and each season, six samples will be collected for

microalgae identification and counting (Fig. 1). Subsamples for species identification will be preserved in Lugol's iodine in a cool, dark place and counted within one month. The counting and identification will be performed using an inverted microscope (sedimentation chamber) or a normal compound microscope (Palmer-Maloney chamber), based on the availability of the equipment of the teams (Biggs and Kilroy, 2000). To help in microalgae identification a workshop will be organized before the starting of the project (see Section 2.2.) and electronic keys for the identification will be provided to each member. Moreover, identification performed by each project member will be verified by an expert taxonomist, by sending pictures of the algae identified or samples that need to be checked. Algae density will be evaluated, and biomass will be estimated based on geometric approximation.

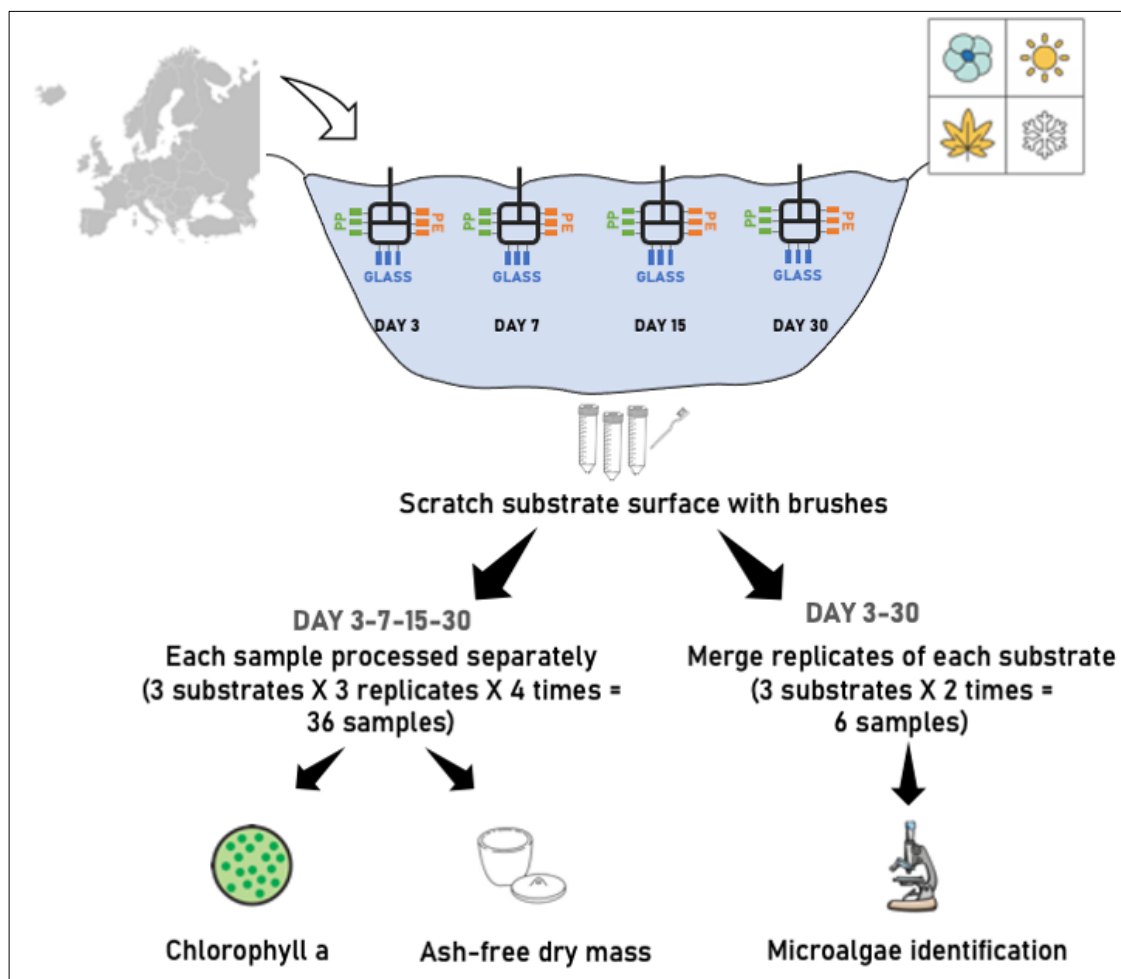


Figure 1. Schematic representation of the sampling design.

Data will be then analyzed to evaluate the main drivers of the microalgae-plastic interaction. Diversity indices will be calculated to evaluate the alpha diversity (i.e., the number of taxa or number of functional characteristics within a location, cf. Rolls et al., 2018). Moreover, to

measure the association between species and the different polymers colonized, the composite index called "IndVal" (indicator value) by Dufrêne and Legendre (1997), will be calculated. Besides, beta diversity will be also evaluated using appropriate multivariate analysis. Since functional diversity may be more sensitive to anthropogenic stress than taxonomic diversity, results will be also discussed in this respect. Statistical differences among the different substrates will be also tested and multivariate analyses will be employed to evaluate the influence of the different environmental factors. Given the large-scale of the study and the high number of data that we will gather, machine learning algorithm might be applied to verify our hypothesis. Statistical analyses will be performed using the software R.

2.2. Project tasks and development of the project

The project will be developed in two years, starting from May 2022, and it will be divided into four project tasks: the pre-experimental phase; the experimental and laboratory phase; data analysis and publications; and communication and dissemination (Fig. 2).

In the first phase, which will last around 5 months, the project will be advertised to reach out to potentially interested early-career researchers (ECRs). At this stage, a virtual meeting will be arranged to better inform all potential candidates about the project objectives, the equipment needed and what is required to be actively involved in the project. If some ECRs are potentially interested in collaborating on the project but lacking the necessary equipment, this will be recorded and possible solutions will be found (i.e., samples can be sent to the laboratory of the universities of the PIs and processed there), but also alternative ways for collaborating will be proposed (i.e., contribution to data elaboration and analysis, paper writing). After this, the teams will be assembled, and a preparatory workshop will be organized. During the workshop, the sampling devices will be constructed, the sampling protocol will be circulated and discussed and specific lessons about phytobenthos identification will be provided in collaboration with experts in the field. Prof. Beata Messayasz has been already contacted and she agrees to provide help in the taxonomic identification during the workshop phase and throughout the whole project. In particular, during the workshop, each participant will be encouraged to bring phytobenthic samples collected from their sampling site to provide guidance on species that are likely to be dominant in the water bodies where they will work. Electronic keys with pictures of the algal species will be created and made available to all the project participants. The

workshop will represent an opportunity for the members of the project to expand their knowledge about phytobenthos, to have a throughout understanding of the field procedures that should be performed and at the same time it will represent an opportunity to establish a solid network among the participants. Considering the possible limitation linked to the current situation, a hybrid format will be arranged in order to maximize the participation of the members.

After this preparatory phase, the first sampling round will be performed in October 2022. Biomass measurements will be carried out directly after the sampling activities, while phytobenthos identification will be accomplished in the subsequent month. If problems are encountered during the identification stage, samples can be sent to PIs' laboratory that will verify the samples in collaboration with Prof. Messyasz. Data about in-field parameters, phytobenthos biomass and phytobenthos identification will be uploaded in a shared folder, which will be created and will serve as centralized location to store project files and data. The data will be provided by each team by filling in a standardized datasheet, in this way the data will be already arranged in an appropriate format for subsequent elaboration. A meeting will be organized after the first sampling campaign to understand possible problems that occurred during the sampling session and solutions will be provided to overcome difficulties. The following sampling sessions will occur in January, April, and July 2023. After the ending of the sampling campaigns, all the data will be available and ready for subsequent analysis. A sub-team interested in data analysis will be assembled and it will start working immediately on data cleaning and analysis, collecting the inputs and ideas from the whole team. To facilitate this collaborative work, tools such as git repositories (e.g., GitHub) will be used. Once the data analysis will be performed, the writing phase will start and at least two publications will be prepared: the first one will discuss biomass measurements on different substrates across the lentic systems studied, the second will focus on the different microalgal species and diversity indices. The aim is to publish the papers in high-impact and open-access journals. Additional publications will be also evaluated and active proposals and participation from the project members will be encouraged. All the data obtained will be then published in digital repositories which respect the FAIR Data Principles (Findability, Accessibility, Interoperability, and Reusability), to allow future research to be performed and thus increase the impact of the project.

Throughout the whole project, particular attention will be paid to scientific communication and outreach initiatives. To this extent, we will create a project website, together with social media channels (i.e., Twitter, Instagram, ResearchGate), and we will take care to constantly update them. This will allow increasing the visibility of the project and, reflexively, of all the ECRs involved. SEFS conferences will be attended to present the results, in addition to other national and international conferences. In particular, we will encourage each project member to personally present the project results during conferences or other initiatives. Within the communication strategies, we will also contribute to creating a strong community among the participants in the project. To do this, dedicated meetings to get better acquainted with each other and to share personal research activities will be arranged both virtually and in-person during scientific conferences.

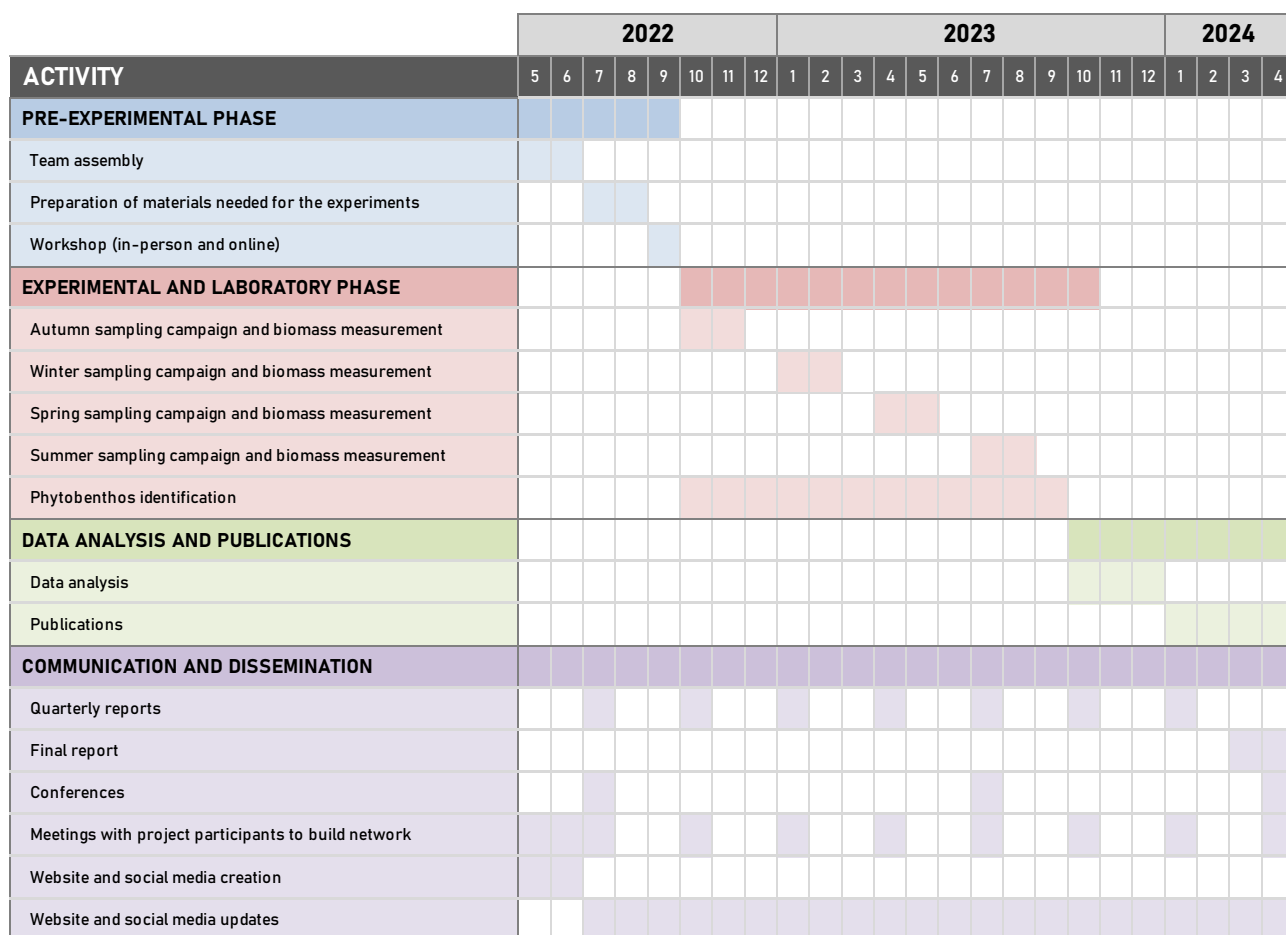


Figure 2. Gantt chart of the proposed project with the identified project tasks.

3. Impact of the project

The proposed project (*PhytoPlastic*) provides an opportunity to develop a large-scale study about the interaction of plastics with fundamental components at the base of aquatic food webs, i.e. microalgae, and thus understanding the key drivers of the process, while overlooking the site-specific and possibly confounding factors. After the widespread presence of plastic debris in aquatic environments was proven, researchers turned their attention to assessing their impact on ecosystem functioning and food webs. The study of plastisphere is becoming increasingly important, particularly because of the ecological implications of this process. The role of biogeography or substrate properties in shaping the plastisphere community is a topic of debate and the proposed project can contribute to fill some of the current gaps in this research field. Indeed, only by applying a large-scale analysis is possible to understand which is the role of the substrate in the colonization compared to other relevant environmental factors. This will represent the first coordinated project to look into the plastisphere and it will considerably improve understanding in the subject, particularly because the project will be undertaken in freshwater systems, where data is extremely scarce.

Besides, the data obtained can be useful not only in the context of plastic research but also for wider-ranging ecological research questions. Indeed, data about community composition and biomass from a variety of different systems can provide insight into their phytobenthic community, thus contributing to extending the knowledge about this key component of the aquatic community. All the data obtained will be published in open databases, and this will allow future studies to be set based on the knowledge acquired.

Since the topic of plastic pollution is of great interest to society, this project will also represent an opportunity to set different science outreach activities, which will provide opportunities to increase public support and awareness of science, while also stimulating the ECRs involved in the project, enhancing their creativity and motivation and extending their communication capabilities.

One strength of the project is the limited equipment needed for its achievement. This will allow large participation from many ECRs, with different backgrounds. Moreover, the majority of the analysis (i.e., biomass estimates: chlorophyll a and AFDM) will be performed in a limited amount of time, ensuring to obtaining the first dataset in a fast and consistent way and ensuring the success of the project. Moreover, the experience of PIs in studies concerning microalgae and

microplastics (see CVs in Annexes) provides optimal bases for a successful accomplishment of the project and the proposed publications. In addition to this, the collaborations with expert in the field have been already settled and this will provide guidance and fundamental supports for the validity of the data obtained.

The organization of the workshop at the initial stage and the different engagement strategies that will be put in place throughout the whole project will help in building a solid network of freshwater scientists. Indeed, it is especially important for ECRs to expand their network, and this will be highly facilitated during the project and considered one of the most important objectives that need to be achieved. Contribution and feedback will be often required from the project members to improve the project outcome and impact during its development and make this an outstanding learning experience for both the PIs and all the participants.

Through a large-scale collaboration, the proposed project will enable to gain scientific knowledge on a highly relevant topic, understanding the implications of the presence of plastics in freshwater environments, and it will contribute to the creation of a solid network of freshwater scientists, which will foster future collaborations (Fig. 3).

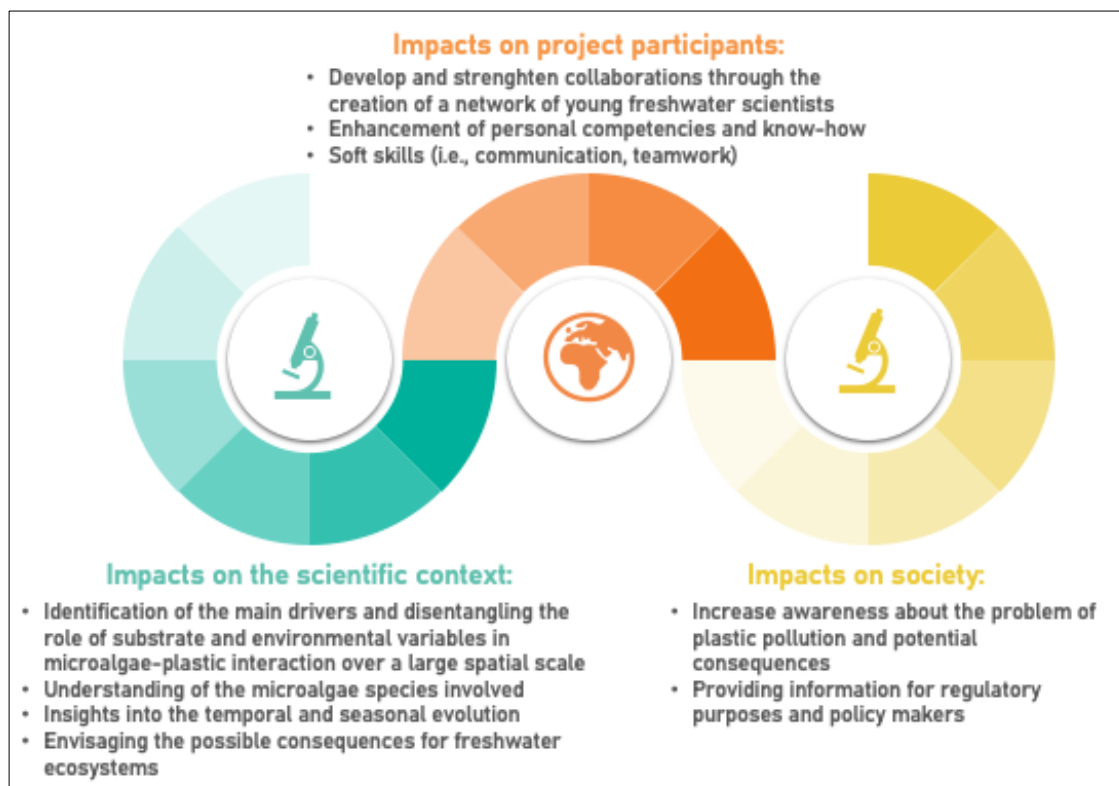


Figure 3. Summary of the potential impacts of the proposed project.

4. Selected references

- APHA, 1998. Standard Methods for the Examination of Water and Wastewater. Am. Public Heal. Assoc. Water Work. Assoc. Environ. Fed. 552.
- Biggs, B.J.F., Kilroy, C., 2000. Stream Periphyton Monitoring Manual. NIWA, Nation Institute of Water and Atmospheric Research, Christchurch, New Zealand.
- da Costa, J.P., Duarte, A.C., Rocha-Santos, T.A.P., 2017. Microplastics – Occurrence, Fate and Behaviour in the Environment, in: Rocha-Santos, T.A.P., Duarte, A.C. (Eds.), Characterization and Analysis of Microplastics. Elsevier, pp. 1–24. <https://doi.org/10.1016/bs.coac.2016.10.004>
- Dufrêne, M., Legendre, P., 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecol. Monogr.* 67, 345–366. [https://doi.org/10.1890/0012-9615\(1997\)067\[0345:SAAIST\]2.0.CO;2](https://doi.org/10.1890/0012-9615(1997)067[0345:SAAIST]2.0.CO;2)
- Dusaucy, J., Gateuille, D., Perrette, Y., Naffrechoux, E., 2021. Microplastic pollution of worldwide lakes. *Environ. Pollut.* 284, 117075. <https://doi.org/10.1016/j.envpol.2021.117075>
- Geyer, R., Jambeck, J.R., Law, K.L., 2017. Production, use, and fate of all plastics ever made. *Sci. Adv.* 3, e1700782. <https://doi.org/10.1126/sciadv.1700782>
- Horton, A.A., Walton, A., Spurgeon, D.J., Lahive, E., Svendsen, C., 2017. Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities. *Sci. Total Environ.* 586, 127–141. <https://doi.org/10.1016/j.scitotenv.2017.01.190>
- Mao, Y., Ai, H., Chen, Y., Zhang, Z., Zeng, P., Kang, L., Li, W., Gu, W., He, Q., Li, H., 2018. Phytoplankton response to polystyrene microplastics: Perspective from an entire growth period. *Chemosphere* 208, 59–68. <https://doi.org/10.1016/j.chemosphere.2018.05.170>
- Matsuguma, Y., Takada, H., Kumata, H., Kanke, H., 2017. Microplastics in Sediment Cores from Asia and Africa as Indicators of Temporal Trends in Plastic Pollution. *Arch. Environ. Contam. Toxicol.* 73, 230–239. <https://doi.org/10.1007/s00244-017-0414-9>
- Miao, L., Yu, Y., Adyel, T.M., Wang, C., Liu, Z., Liu, S., Huang, L., You, G., Meng, M., Qu, H., Hou, J., 2021. Distinct microbial metabolic activities of biofilms colonizing microplastics in three freshwater ecosystems. *J. Hazard. Mater.* 403, 123577. <https://doi.org/https://doi.org/10.1016/j.jhazmat.2020.123577>
- Nava, V., Leoni, B., 2021. A critical review of interactions between microplastics, microalgae and aquatic ecosystem function. *Water Res.* 188, 116476. <https://doi.org/10.1016/j.watres.2020.116476>
- Nel, H.A., Dalu, T., Wasserman, R.J., 2018. Sinks and sources: Assessing microplastic abundance in river sediment and deposit feeders in an Austral temperate urban river system. *Sci. Total Environ.* 612, 950–956. <https://doi.org/10.1016/j.scitotenv.2017.08.298>
- Ogonowski, M., Motiej, A., Ininbergs, K., Hell, E., Gerdes, Z., Udekwu, K.I., Bacsik, Z., Gorokhova, E., 2018. Evidence for selective bacterial community structuring on microplastics. *Environ. Microbiol.* 20, 2796–2808. <https://doi.org/10.1111/1462-2920.14120>
- Rimet, F., Vautier, M., Kurmayer, R., Salmaso, N., Capelli, C., Bouchez, A., Hufnagl, P., Domaizon, I., 2020. River biofilms sampling for both downstream DNA analysis and microscopic

counts. <https://doi.org/dx.doi.org/10.17504/protocols.io.ben6jdhe>

- Rolls, R.J., Heino, J., Ryder, D.S., Chessman, B.C., Growns, I.O., Thompson, R.M., Gido, K.B., 2018. Scaling biodiversity responses to hydrological regimes. *Biol. Rev.* 93, 971–995. <https://doi.org/10.1111/brv.12381>
- Scherer, C., Weber, A., Lambert, S., Wagner, M., 2018. Interactions of microplastics with freshwater biota, in: Wagner, M., Lambert, S. (Eds.), *Freshwater Microplastics: Emerging Environmental Contaminants?* Springer International Publishing, Cham, pp. 153–180. https://doi.org/10.1007/978-3-319-61615-5_8
- Venâncio, C., Ferreira, I., Martins, M.A., Soares, A.M.V.M., Lopes, I., Oliveira, M., 2019. The effects of nanoplastics on marine plankton: A case study with polymethylmethacrylate. *Ecotoxicol. Environ. Saf.* 184, 109632. <https://doi.org/10.1016/j.ecoenv.2019.109632>
- Wang, W., Gao, H., Jin, S., Li, R., Na, G., 2019. The ecotoxicological effects of microplastics on aquatic food web, from primary producer to human: A review. *Ecotoxicol. Environ. Saf.* 173, 110–117. <https://doi.org/10.1016/j.ecoenv.2019.01.113>
- Yokota, K., Waterfield, H., Hastings, C., Davidson, E., Kwietniewski, E., Wells, B., 2017. Finding the missing piece of the aquatic plastic pollution puzzle: Interaction between primary producers and microplastics. *Limnol. Oceanogr. Lett.* 2, 91–104. <https://doi.org/10.1002/lol2.10040>
- Zettler, E.R., Mincer, T.J., Amral-Zetter, L.A., 2013. Life in the “plastisphere”: microbial communities on plastic marine debris. *Environ. Sci. Technol.* 47, 7137–7146. <https://doi.org/10.1021/es401288x>
- Zhang, C., Chen, X., Wang, J., Tan, L., 2017. Toxic effects of microplastic on marine microalgae *Skeletonema costatum*: Interactions between microplastic and algae. *Environ. Pollut.* 220, 1282–1288. <https://doi.org/10.1016/j.envpol.2016.11.005>
- Zhao, T., Tan, L., Huang, W., Wang, J., 2019. The interactions between micro polyvinyl chloride (mPVC) and marine dinoflagellate *Karenia mikimotoi*: The inhibition of growth, chlorophyll and photosynthetic efficiency. *Environ. Pollut.* 247, 883–889. <https://doi.org/10.1016/j.envpol.2019.01.114>

5. Annex

5.1. Curriculum vitae: Veronica Nava

PERSONAL DETAILS

<i>Address</i>	Piazza della Scienza 1, 20126, Milano (MI), Italy
<i>Date of birth</i>	24/10/1993
<i>Nationality</i>	Italian
<i>E-mail</i>	veronica.nava@unimib.it

EDUCATION

11/2018 – 01/2022	<i>Ph.D. in Environmental Sciences</i> University of Milano-Bicocca (Italy) Thesis: "Microplastics in freshwater systems: characterization, quantification and interaction with aquatic organisms" Honors: cum laude, certification of Doctor Europaeus
11/2015 – 07/2017	<i>M.Sc. in Environmental Sciences</i> University of Milano-Bicocca (Italy) Thesis: "Interspecific relationships and ecological requirements of two potentially harmful Cyanobacteria in Lake Iseo" Honors: 110/110 cum laude
10/2012 – 07/2015	<i>B.Sc. in Environmental Sciences</i> University of Milano-Bicocca (Italy) Thesis: "Assessment of anthropogenic pressures on Brembo River basin: a comparison between biological methods" Honors: 107/110

PROFESSIONAL APPOINTMENTS

03/2022 - ongoing	<i>Postdoctoral researcher</i> University of Milano-Bicocca (Italy) Project: "Effects of plastics and anthropogenic impacts on freshwater food-webs and ecosystem functioning"
11/2021 – 02/2022	<i>Research assistant – Ecology and Management of Freshwater Ecosystems</i> University of Milano-Bicocca (Italy)
09/2017 – 10/2018	<i>Research assistant – Ecology and Management of Freshwater Ecosystems</i> University of Milano-Bicocca (Italy)
03/2015 – 05/2015	<i>Internship O.U. Water Quality Monitoring</i> Environmental Protection Agency (ARPA – Bergamo)

PUBLICATIONS

1. **Nava** et al. (2022). Microalgae colonization of different microplastic polymers in experimental mesocosms across an environmental gradient. *Glob. Chang. Bio.* 28(4), 1402–1413.
2. Lučić, Mikac, Vdović, Bačić, **Nava** et al. (2022). Spatial and temporal variability and sources of dissolved trace elements in the Sava River (Slovenia, Croatia). *Environ. Sci. Pollut. Res.* (Online Early).
3. **Nava**, Frezzotti, Leoni (2021). Raman analysis for the analysis of microplastics in aquatic systems. *Appl. Spectrosc.* 75(11), 1341-1357.
4. **Nava**, Leoni (2021). A critical review of interactions between microplastics, microalgae and aquatic ecosystem function. *Water Res.* 188, 116476.
5. **Nava**, Leoni (2021). Comparison of Different Procedures for Separating Microplastics from Sediments. *Water* 13(20), 2854.
6. Leoni, Zanotti, **Nava** et al. (2021). Freshwater system of coral inhabited island: availability and vulnerability (Magodhoo Island of Faafu Atoll - Maldives). *Sci. Total Environ.* 785, 147313.
7. Leoni, Patelli, **Nava**, Tolotti (2021). Cladocera paleocommunity to disentangle the impact of anthropogenic and climatic stressors on a deep subalpine lake ecosystem (Lake Iseo, Italy). *Aquat. Ecol.* 55, 607–621.
8. Rotiroti, ..., **Nava** et al. (2020). Overlapping redox zones control arsenic pollution in Pleistocene multi-layer aquifers, the Po Plain (Italy). *Sci. Total Environ.* 758, 143646.
9. Jenny, ..., **Nava** et al. (2020). Scientists' Warning to Humanity: Rapid degradation of the world's large lakes. *J. Great Lakes Res.* 46(4), 686-702.
10. **Nava** et al. (2019). Chloride balance in freshwater system of a highly anthropized subalpine area: load and source quantification through a watershed approach. *Water Resour. Res.* 56(1), e2019WR026024.
11. **Nava** et al. (2019). An R package for estimating river compound load using different methods. *Environ. Model. Soft.* 117, 100-108.
12. Leoni, Spreafico, Patelli, Soler, Garibaldi, **Nava** (2019). Long-term studies for evaluating the impacts of natural and anthropic stressors on limnological features and the ecosystem quality of Lake Iseo. *Adv. Oceanogr. Limnol.* 10(2)
13. Stefania, Rotiroti, Buerge, Zanotti, **Nava** et al. (2019). Identification of groundwater pollution sources in a landfill site using artificial sweeteners, multivariate analysis and transport modeling. *Waste Manage.* 95, 116-128.
14. Rotiroti, Bonomi, Sacchi, McArthur, Stefania, Zanotti, Taviani, Patelli, **Nava** et al. (2019). The effects of irrigation on groundwater quality and quantity in a human-modified hydro-system: the Oglio River basin, Po Plain, northern Italy. *Sci. Total Environ.* 672, 342-356.
15. Rotiroti, Zanotti, Fumagalli, Taviani, Stefania, Patelli, **Nava** et al. (2018). Multivariate statistical analysis supporting the hydrochemical characterization of groundwater and surface water: a case study in northern Italy. *Rendiconti Online Soc. Geol. Ital.* 47, 90-96.
16. Leoni, **Nava**, Patelli (2018). Relationships among climate variability, Cladocera phenology and the pelagic food web in deep lakes in different trophic states. *Mar. Freshwater Res.* 69(10), 1534- 1543.
17. Leoni, Patelli, Soler, **Nava** (2018). Ammonium transformation in 14 lakes along a trophic gradient. *Water* 10, 265.
18. **Nava** et al. (2017). Interspecific relationship and ecological requirements of two potentially harmful Cyanobacteria in deep south alpine lake (L. Iseo, I.). *Water* 9, 993.

AWARDS AND GRANTS

1. Winner of the student travel award for the Virtual Meeting – ASLO (2021).
2. Winner of the travel award for the GLEON 2019 – All Hands’ Meeting, Huntsville, Ontario, Canada provided by “Cary Institute of Ecosystems Studies”, New York, USA (2019).
3. Winner of the Transnational Access (TA) grant from AQUACOSM (funded by the European Commission EU H2020-INFRAIA-project No 731065) for the project “MPhyto: Influence of microplastics on primary productivity” (2019).
4. Winner of the “Wetzel SIL Congress Travel Award” for the participation at the 34th Congress of the International Society of Limnology – SIL, Nanjing, China (2018).
5. Winner of the fellowship for the participation at “XIV Incontro dei Dottorandi e Giovani Ricercatori in Ecologia e Scienze dei Sistemi Acquatici”, Genova, Italy (2018).

RESEARCH PROJECTS

- GALACTIC - GlobAl LAke miCroplasTICs, Global Lake Ecological Observatory Network: Principal Investigator
- Lake, stream and groundwater modeling to manage water quality in the system of lake Iseo-Oglio river, Cariplo: Participants
- Europonds, European Federation of Freshwater Sciences: Participants
- DOMSeasons, Global Lake Ecological Observatory Network: Participants
- Day 'N' NightZ, Global Lake Ecological Observatory Network: Participants
- Urban algae - Ecological Status and the Perception of Ecosystem Services of Urban Ponds, European Federation of Freshwater Sciences: Participants

OTHER ACTIVITIES

- Didactic seminars for MSc course 'Ecology and Management of Inland Waters' -Dep. Earth and Environmental Sciences, University of Milano-Bicocca
- Participation in the professional mobility program Pioneers into Practice - Climate-KIC, 2019
- Webmaster for the International Society of Limnology - SIL (www.limnology.org)
- Co-leader 'Communication Committee' for the International Society of Limnology (SIL)
- Member of 'Communication Committee' for the GSA-GLEON (Global Lake Ecological Observatory Network)
- Co-chair at the XXXIV and XXXV congresses of the International Society of Limnology (SIL)
- Supervisor of 8 MSc and 9 BSc theses in freshwater sciences
- Counseling activities and didactic tutoring at the University of Milano-Bicocca
- Reviewer for the following scientific journals: Environmental Science & Technology, Environmental Pollution, Environmental Chemistry Letters, Journal of Limnology
- Member of the following scientific society: International Society of Limnology (SIL), Association for the Sciences of Limnology and Oceanography (ASLO), Global Lake Ecological Observatory Network (GLEON), Società Italiana di Ecologia (SIte), Associazione Italiana di Oceanologia e Limnologia (AIOL)

LANGUAGE

Italian – Native speaker

English – Professional (IELTS test held on 2018: C1)

5.2. Curriculum vitae: Julia Gostyńska

PERSONAL DETAILS

<i>Address</i>	Dąbrowskiego 1/26, 62 - 300, Września, Poland
<i>Date of birth</i>	03/02/1997
<i>Nationality</i>	Polish
<i>E-mail</i>	julgos@amu.edu.pl

EDUCATION

10/2021 – currently	<i>Ph.D. student in Doctoral School of Natural Sciences</i> University of Adam Mickiewicz (Poland) Thesis: "The role of polyphenolic substances in shaping the competition mechanism at the level of algae - pleustophytes - cyanobacteria in the subsurface layer of water reservoirs"
10/2019 – 07/2021	<i>M.Sc. in Faculty of Biology</i> University of Adam Mickiewicz (Poland) Thesis: "Distribution patterns of the dioecious charophytes species (<i>Chara tomentosa</i> L.) in the depth gradient of the lake"
10/2016 – 07/2019	<i>B.Sc. in Faculty of Biology</i> University of Adam Mickiewicz (Poland) Thesis: "The spatial structure and relationships between species of aquatic vegetation in a trophically stable, but subject to anthropogenic pressure, shallow-water lake (Jarosławieckie Lake, Greater Poland National Park)"

SCIENTIFIC ACTIVITIES

21 – 24.11.2021	" <i>Physico-chemical factors shaping small pleustophytes communities</i> ", Julia Gostyńska, Radosław Pankiewicz, Bogusława Łęska, Zuzanna Piotrowicz, Łukasz Tabisz, Beata Messyasz, XLV International Scientific and Technical Seminar "Chemistry for Agriculture", Poland (poster)
07.06.2021	" <i>Distribution patterns of the dioecious charophytes species (Chara tomentosa L.) in the depth gradient of the lake</i> ", 7th Polish - Kazakh Meeting "Relationship between chemistry and biology", Adam Mickiewicz University (oral presentation)

OTHER ACTIVITIES

2019 - currently	Activity in the Organizing Committee of the Celebration of the 100th Anniversary of Natural Sciences Club of Adam Mickiewicz University, member of the editorial team of the Natural Sciences Club historical book
------------------	--

2016 – 2021 2019 – 2021	Member of Natural Sciences Club of Adam Mickiewicz University: Representative of the Tropical Biology Section
12/2020	Participant of courses organized by the University of Adam Mickiewicz: - "Surface water quality and its assessment using the bioindication method", date of obtaining the certificate: 04.12.2020 - "Digital natural environment course for applications in spatial planning", date of obtaining the certificate: 5.12.2020
14.06. – 02.07.2019	Participant of the Tropical Biology course at Kibale National Park in Uganda
2018/2019	Participant in project: "Higher competences - greater chance on the labor market. Program for the development of competences of students of the Faculty of Biology of the University of Adam Mickiewicz in Poznań ", under which I obtained the certificate: - Workshops and certified training educating the professional competences of an ecologist diver, 25.02 - 25.08.2019 - Communication in specialized English, 11.12.2018 – 01.21.2019 - Effective interpersonal and professional communication (including FRIS® styles of thinking and acting), 17.11.2018 – 15.12.2019

LANGUAGE

Polish – Native speaker

English – Upper – Intermediate

5.3. Support letters



UNIVERSITÀ DEGLI STUDI
DI MILANO - BICOCCA



DIPARTIMENTO DI SCIENZE DELL'AMBIENTE E
DELLA TERRA

01 April 2022

Re: Veronica Nava – Letter of support

To whom it may concern,

Herewith, I express my full approval for the research proposal of Dr. Veronica Nava to the “FreshProject” call. I also confirm that she will be hosted at the University of Milano-Bicocca during the proposed project.

Sincerely,

A handwritten signature in cursive script that reads 'Barbara Leoni'.

Barbara Leoni

Associate professor at University of Milano-Bicocca

barbara.leoni@unimib.it



01 April 2022

Re: Julia Gostyńska – Letter of support

To whom it may concern,

Herewith, I express my full approval for the research proposal of MSc Julia Gostyńska to the "FreshProject" call. I also confirm that she will be hosted at the Adam Mickiewicz University Poznań, Faculty of Biology during the proposed project.

Sincerely,

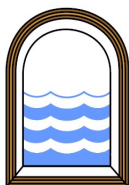
DEAN
Faculty of Biology
Beata Messyasz
Beata Messyasz, PhD, DSc

Beata Messyasz

Associate professor at Adam Mickiewicz University Poznań

messyasz@amu.edu.pl

5.4. Letters of the EFFS-federated societies



Associazione Italiana di Oceanologia e Limnologia A.I.O.L.

CONSIGLIO DI PRESIDENZA

Presidente

Nico Salmaso
S. Michele all'Adige (TN)

Padova, 06 aprile 2022

Vice Presidente

Domenico D'Alelio
Napoli

ATTESTATO DI SOCIO AIOL

Segretaria

Monica Tolotti
S. Michele all'Adige (TN)

L'Associazione Italiana di Oceanologia e Limnologia, nella persona del Presidente, Dott. Nico Salmaso, conferma l'adesione in qualità di Socio di

Consiglieri

Caterina Bergami
Bologna

VERONICA NAVA

Silvia Bianchelli
Ancona

Alessandro Cau
Cagliari

Il presente attestato ha validità per il corrente anno, 2022.

Mauro Celussi
Trieste

Diego Copetti
Brugherio (Monza-Brianza)

Aldo Marchetto
Verbania

Alessandra Pugnetti
Venezia

Tesoriere

Tiziana Tonsi
Verbania

Il Presidente

WEB: www.aiol.info

Presidenza e Segreteria: c/o Fondazione Mach-Istituto Agrario di S. Michele all'Adige, Via E. Mach, 1 - I-38010, S. Michele all'Adige (Trento), Italy
Tesoreria: c/o CNR-Istituto di Ricerca sulle Acque (IRSA), Largo Tonolli, 50, 28922 Verbania-Pallanza (VB)
E-mail Presidenza: nico.salmaso@fmach.it - E-mail Segreteria: segreteria.aiol@gmail.it - E-mail Tesoreria: tiziana.tonsi@irsa.cnr.it



Barcelona, 5 April 2022

To whom it may concern.

I, Romina Álvarez Troncoso, secretary of the Iberian Association of Limnology – AIL, hereby

CERTIFY

that **Julia Gostyńska** is a member of this Association since 2022 (member ID nº 2021019). This status implies automatic membership to the Iberian Society of Ecology – SIBECOL, as AIL is affiliated to SIBECOL since its creation.

Yours sincerely,

Dr. Romina Álvarez Troncoso,
Secretary of the Iberian Association of Limnology