PHD COURSE IN LIFE AND ENVIRONMENTAL SCIENCES

Report Form for PhD student evaluation (XXXVII cycle, PON)

Name of PhD student: Melissa Orsini
Title of PhD research: Presence, behaviour, and effects of microplastic and microfiber in marine environment

Name of PhD supervisor: Prof. Francesco Regoli
Research lab name: Ecotoxicology and Environmental Chemistry

Mesi in IMPRESA (Italia): not done yet
Mesi in IMPRESA (Estero, se previsti): not esxpected

PhD Curriculum:
[X] Marine biology and ecology
[ ] Biomolecular Sciences
[ ] Civil and environmental protection

DISVA instrumentation labs/infrastructure eventually involved in the project:
[X] Actea Mobile Laboratory
[ ] Advanced Instrumentation lab
[ ] Aquarium
[ ] MassSpec lab
[ ] MaSBiC
[ ] Simulation/informatics lab
[X] Other. Please, indicate: FTIR room

ABSTRACT (1000 ch):

The general aim of this research is to study the presence and typology of macroplastics, microplastics (MPs) and microfibers (MFs) in different matrices (water, beach, seabed, and biota) focusing on the Adriatic region. Specifically, the research focuses on:

- Characterization of MPs and MFs in *Mytilus galloprovincialis* of the Adriatic Coast
- Application of a multi-matrices approach for monitoring macroplastics, MPs and MFs in the Conero area
- Investigation on how mussel beds and macroalgal forests might influence the fate of MPs in the marine environment
- Development of innovative technologies (drones equipped of artificial intelligence) to counteract the impact of plastics in coastal areas of the Conero Riviera

Preliminary results show that the MPs-MFs ingestion frequency in mussels of the Central- south Adriatic reaches 50%, while almost 87% was reached in sea urchins and anemones, with fibres being in all organisms the dominant shape. Conversely, in water matrices, fragments were the most abundant part.

Part 1. Scientific case of the PhD Research (2 to 3 pages)

BACKGROUND

Studies on the spatial distribution of marine litter have identified the Adriatic region as a preferential area for plastic accumulation within the Mediterranean Sea, especially due to transboundary effects caused by sea currents (Palatinus et al., 2019). Microplastics, commonly defined as particles <5 mm in diameter (Galgani et al., 2013), are increasingly proving to be ubiquitous in all water systems, microplastic transportation pathways are characterized by complex dynamics due to processes such as movement mechanisms (windage and sinking velocities) as well as changes in physical and chemical characteristics (loss of structural integrity, fragmentation and aggregation (L.Andrady, 2017) as well as interactions with biota (Chiara Schmid, 2021).
Since the assessment of plastic ingestion in marine species has become a research priority in the context of the Marine Strategy Framework Directive, MSFD, 2008/56/EC, Descriptor 10, Indicator 10.1.3 (Bellas et al., 2016) the investigation of plastic particles in marine biota and habitats is one of the key points of our research.

SCIENTIFIC AIMS

The research project is focused on assessing the fate of plastics in the marine environment integrating analyses on different environmental matrices: quantification and characterization of macro and microplastics will be carried out on beach, seabed sediment, water, organism, and algae collected along the Adriatic Sea with special focus on the Conero Riviera (central Adriatic Sea). Since macroalgal forests and mussels could mediate the vertical distribution of MPs, through ingestion and biodeposition, a key point of the research is to determine their capacity in retaining these particles. In addition, the research aims to develop an innovative model for analysing the risk of plastic pollution in the sea, to apply advanced and innovative technologies for the identification, recovery, and reuse of plastics and to enhance sustainable development approaches.

WORKPLAN AND RESEARCH ACTIVITIES

WP1. Characterization of MPs and MFs in *Mytilus galloprovincialis* of the Adriatic Coast

Objective: The objective of this workplan is to detect and assess the presence of MPs and MFs in mussels chosen for their properties as ideal bioindicator and for their commercial value. This will allow to provide valuable information also on MPs and MFs as contaminants in seafood products and on the possible risk for human health related to consumption of edible bivalves.

Methods

A total of 155 specimens of *Mytilus galloprovincialis* were collected during the summer season 2021 along the Central Southern Adriatic coast in 4 areas: Conero (n= 35), Vasto (n=20), Bari (n=60) and Brindisi (n=40). In Vasto, mussels were obtained from a farm unlike the other sites where they were taken from natural beds through snorkeling activity. Mussels were carried under controlled conditions in the laboratory, the entire soft tissue was removed from the shells and stored at -20°C until required for analysis.
The processing of the sample and the extraction of microplastics was performed following (Pittura et al., 2022). The polymeric identification of extracted items was carried out through µFTIR spectroscopy (Bour et al., 2018).

Results

A mean of 1.4±0.4 MPs/individual were found in Adriatic mussels with no significant difference in the abundance observed among the different selected areas. On the contrary, the percentage of organisms which ingested at least one MP (frequency of ingestion) varied from a 30%, recorded in a specific area of the Conero called Trave, to 75% observed in mussels taken in Vasto. Of the 111 extracted and characterized MPs, 76% were in the shape of fibres, 56% were made of polyester and the size class of 1-3mm was in general the most abundant. In addition, a high frequency of fibres of natural origin (86%) was found in mussel tissues confirming these materials as possible new emerging contaminants that requires attention beside synthetic microfibers.

WP2. Application of a multi-matrices approach for monitoring MPs and MFs in the Conero area

Objective: Applying a monitoring strategy for microplastics pollution in the Conero Riviera that integrates information resulting from analyses of both biotic (species with different feeding strategies) and abiotic (surface water, water column and seabed sediments) compartments.

Methods
Samples of *Parancetrotus lividus* (sea urchin, grazer) and of *Anemonia viridis* (anemones, predator) has been collected through snorkeling activity. For each species n=30 organisms have been analyzed for MPs content, processing their entire soft tissues. Surface water samples (n=5 coastal transect) were sampled using a Manta net of 300 µm mesh size trawled by boat on the water surface at 1-2 Knots for 10/20 minutes. The material concentrated in the final collector was collected and transferred to sample containers, that were carried under controlled conditions in the laboratory and stored in refrigerators at -20°C until required for analysis.
Water column samples were taken using an innovative device named Microplastic particle pump. This pump is equipped with 50 µm,100 µm and 300 µm steel meshes and was used to filter 500L of water at two different depths, 1m and 5m to obtain information about the distributions of MPs along the entire water column.
In conclusion, samples of sediment were taken along a near-offshore transect (100, 500, 2000 and 4000 m from the coast) in 3 specific sites: Passetto, Trave and Portonovo using a Van veen grab sampler. Only the surface sediment (first 5 cm) was taken, carefully transferred into 500ml glass jars, carried under controlled conditions in the laboratory and stored in refrigerators at -20°C until required for analysis. Sediment samples were processed as reported by (Frias et al., 2018) using a Sediment-Microplastic Isolation (SMI) unit that was assembled as reported by Coppock et al. (2017).

The filters obtained from the processing of biota, water and sediment samples were analysed for their microplastic content under a stereomicroscope: isolated items were classified and characterized in terms of shape, size and polymer typologies as described in WP1 section.

Results
About 87% of both *P. lividus* and *A. viridis* analysed species, resulted positive to MPs ingestion. However, a higher intake was calculated for grazers (sea urchins) than for predators (anemones), showing on average 3.3 ± 1.99 and 1.7 ±0.37 items/organism, respectively. In both species fibers were dominating, but with a higher frequency in sea urchins (70%) while in anemones the shape distribution was more uniform (44% were fibres, 35% fragments and 23% films)
Considering results from surface water samples, collected by using the Manta net (330 µm), MPs abundance mean values found in the Conero area are of 0.26 ± 0,27 items/m^3 (103,495 ± 106,674items/km^2). Fragments dominated, 1-3 mm was the dimensional class most represented and polypropylene, and polyethylene were the polymers most frequently found.
The presence of microplastics in sediments of the Conero area was confirmed in 10 of the 15 analyzed samples with a mean abundance of 13 (± 14) items/kg d.w., with different intensities among sampling locations (Trave less affected by MPs than Passetto and Portonovo) and moving offshore from the coast (the highest MPs concentration at 2000 m from the coast). Overall, on a total of 20 isolated items, fragments resulted to be the dominant shape represented by 67%, followed by fibres 20% and films 13%. The most representative size class was 0.3-1 mm (76%), followed by the range of 0.1-0.3 mm (17%). Polyethylene and polyester were the most represented polymers.
Water column samples are still to be analysed

WP3. How do mussel beds and macroalgal forests influence the fate of MPs in the marine environment
Objective: The objective of this WP is to analyze the capacity of macroalgal forests to work as sinks for MPs particles, since different macroalgal surface structures may influence the MPs retention capacity. Secondly, we will analyze how filtering mussel beds can affect the fate of MPs in the marine environment.

Methods
Three algae species with different morphologies and of high ecological relevance were collected seasonally: *Cystoseira compressa, Gongolaria barbata* and *Ulva lactuca.*
At the same time sediment samples were taken under the macroalgal populations and outside the algal forest to assess if the presence of macroalgae influences the MPs abundance and typology in the sediment.
Additionally, mussels were sampled from a submerged rock, to assess the benthic pelagic coupling. Since mussels could mediate the vertical distribution of MPs through ingestion and biodeposition, sediment samples were taken under the mussel population and outside to assess if their presence influences the MPs typology and abundance in the seabed.

Results
Results are still in progress

WP4. Development of innovative technologies to counteract the impact of plastics in coastal areas of the Conero Riviera
Objective
The general objective of the project is to develop an innovative model for the management of plastic pollution in the sea, particularly focused on the rocky coasts of the Conero, based on advanced technological solutions for the identification, recovery, and reuse of these materials. The goal is to validate an integrated approach that unites the world of research, the industrial sector and civil society in the fight against plastic pollution, environmental recovery and towards models based on circular economy and sustainability. Monitoring and research must be focused on quantification and characterization of the types of plastic affecting the area, and sites at which it accumulates.

Methods
The experimental plan expects a seasonal beach cleaning on a rocky beach of about 200m located along the Conero Coast (*43.602992° 13.5512981°).*
Simultaneously with the beach cleaning activity, three overflight and image acquisition surveys conducted by remote controlled drones were performed: one before a cleaning operation, one immediately after and a third one month after the cleaning operation. The images resulting from this phase were used to train a model with deep learning techniques in order to automatically detect areas within an image that contain plastic fragments (detection by bounding box).
The first beach cleaning and drone overflight was performed mid-June 2022.
A total of 83kg plastic waste was collected along the selected area. Depending on the level of impact, 3 subareas were identified: low, medium, and high impact. Items were cataloged after their polymeric identification and stored for their recovery and reuse with the support of dedicated companies.
To have an integrated model of data, surface water, water column and seabed samples were collected as well to see if periodical beach cleaning activities influences the MP abundancy in the surrounded environment.

Results
Data obtained from the beach cleaning activity will be useful to understand if periodical beach clean ups might have any correlation with the plastic concentrations analyzed in the environmental matrices.
Moreover, final results will get an integrated model of data to see whether microplastics accumulate in sediments as their final destination or if they are well distributed along the water column.
A total of 83kg plastic was sampled for a total number of 1825 items of which 64% was associated to fishing activity including fishing devices, nets, and polystyrene boxes. Polystyrene and rubber were the two more frequent characterized polymers.
Analysis of surface water, water column and seabed samples are still in progress, but preliminary results show a decreasing concentration of MPs particles going from the water surface to the bottom.

REFERENCES

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Juan, B., José, M.-A., Ariana, M.-C., Besada, V., & Martínez-Gómez, C. (2016). Ingestion of microplastics by demersal fish from the Spanish Atlantic and Mediterranean coasts. Marine pollution bulletin 55-60.

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Pittura, L., Tavoloni, T., Ventura, L., Stramenga, A., d'Errico, G., Vaglio, G. L., Regoli, F., Piersanti, A., Gorbi, S. (2022). Microplastics and Brominated Flame Retardants in freshwater fishes from Italian lakes: implication for human health. Frontiers in Water, 89.

Palatinus, A., Kovač Viršek, M., Robič, U., Grego, M., Bajt, O., Šiljić, J., Suaria ,G., Liubartseva, S., Coppini, G., Peterlin, M. (2019). Marine litter in the Croatian part of the middle Adriatic Sea: Simultaneous assessment of floating and seabed macro and micro litter abundance and composition. Marine Pollution Bulletin 427-439.

Schmid, C.; Cozzarini, L.; Zambello, E. (2021). A critical review on marine litter in the Adriatic Sea: Focus on plastic pollution. Environmental pollution

Part 2. PhD student information on the overall year activity (courses/seminars/schools, mobility periods, participation to conferences)

List of attended courses/seminars/schools

1. Technology transfer and innovation (course)

2. Design of research: European projects (course)

3. Analisi di regressione mediante Microsoft Excel (course)
4. Getting Started with R: Environmental Computing (course)
5. “Cambiamenti climatici, comunità e Sindaci resilienti: contributi e riflessioni con il mondo universitario (seminar)

6. "The resolution revolution in Cryo-electron-microscopy, in Structural Biology and in Life Sciences", Martino Bolognesi, Department of Biosciences, University of Milan (seminar)

List of periods spent abroad

1. Not expected

List of conferences/workshops attended and of contributions eventually presented

1. participation at the PhD week 6-10 July at the Università Politecnica delle Marche with poster exhibition

2. contribution to poster entiteled
   - **“Indagini preliminari sulla tossicità di materiale di plastica riciclata e potenziali rischi per l’ambiente marino** “
     Carola Mazzoli, Giuseppe d’Errico, Federica Iezzi, **Melissa Orsini**, Lucia Pittura, Maura Benedetti, Stefania Gorbi, Francesco Regoli

Exposed in lido di Camaiore 28-30/09 during the study days of ecotoxicology

- “**First insights into leachate toxicity of field collected plastics towards marine zooplankton**“

Chiara Gambardella, Roberta Miroglio, Elisa Costa, Roberta Minetti, Veronica Piazza, Laura Castellano, Natalia Perez, Lucia Pittura, **Melissa Orsini**, Veronica Viviani, Bénédicte Morin, Jérôme Cachot, Marco Faimali, Francesco Regoli, Francesca Garaventa
At the International congress MICRO 2022 14-18/11, Lanzarote, SP 3.

3. Scientific and didactic support for PLS activity and support during workshops for first and second grade schools.

Part 3. PhD student information on publications

Not yet published (in preparation)

List of publications on international journals

Not yet

List of publications on conference proceedings

Not yet

List of other publications (books, book chapters, patents)

Not yet

Date 15/11/2022

Student Signature