UNIVERSITÀ
POLITECNICA
DELLE MARCHE

Dipartimento
di Scienze
della Vita
e dell'Ambiente
DISVA

PHD COURSE IN LIFE AND ENVIRONMENTAL SCIENCES

Report Form for PhD student annual evaluation (XXXVI and XXXVII cycles)

Name of PhD student: Carola Mazzoli

Title of PhD research: Plastic pollution in rocky coastal areas of high ecological value: development of innovative technologies to assess the impact, removal and recycling of materials

Name of PhD supervisor: Francesco Regoli

Research lab name: Laboratory of Ecotoxicology and Environmental Chemistry

Cycle:
[X] XXXVI
[ ] XXXVII

PhD Curriculum::
[ ] Marine biology and ecology
[ ] Biomolecular Sciences
[X] 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
[ ] Other. Please, indicate: .....

ABSTRACT:

This PhD project aims to investigate the presence and the effects of plastic litter (both macro- and microplastics) in the marine environment with a special focus on rocky coastal areas of high ecological value. The research project includes field sampling activities and laboratory experiments aimed to evaluate: 1) the physicochemical characteristics of microplastics extracted from field matrices; 2) the possible role of plastic items as carrier of inner additives and environmental contaminants; and 3) the adverse effects due to the interaction between marine organisms and compounds released by plastics.

Part 1. Scientific case of the PhD Research

- BACKGROUND

Types and quantities of *marine litter* vary considerably across marine regions due to different hydrodynamics, geomorphologic and anthropic factors, anyway plastics and other artificial polymers are predominant worldwide (Derraik, 2002) and they represent the most common types of marine litter (Fossi *et al.*, 2020). , The Mediterranean basin represents not only a world biodiversity hotspot, but also one of the areas most impacted by marine litter (Fossi et al., 2017; Suaria et al., 2016; UNEP/MAP, 2015; van Sebille et al., 2015). Within the Mediterranean basin, the Adriatic Sea, is predicted as a preferential area of plastics accumulation (Avio *et al.*, 2020, Suaria *et al.* 2014, Fortibuoni *et al.*, 2021). Plastics' resistant to degradation represents the main reason why this material is considered dangerous for the marine environment (Iñiguez et al., 2018). In fact, plastics are estimated to degrade in hundred (Bonanno and Orlando Bonaca, 2018) and even thousand years (Barnes et al., 2009). The plastic fragments generated from the degradation processes (from meso-plastics to micro- and nano-plastics), can pose potential risks to biodiversity, through ingestion among the entire marine food chain, from planktonic communities to large mammals. Moreover, during its permanence in the marine environment plastic can drift, sink or float in the sea, moving for over long distances (Bonanno and Orlando Bonaca, 2018) and acting as a "carrier" not only of alien species but also of chemicals releasing inner additives (Gallo *et al.*, 2018) and/or adsorbing environmental contaminants on its surface, which can be released again in the environment (Teuten, *et al.*, 2009, Endo et al., 2005, Engler 2012).

- SCIENTIFIC AIMS

During this second year of my PhD, I continued to carry out the analysis of WP1: assessing the presence of microplastics in environmental and biological samples collected along the Conero Riviera (Central Adriatic Sea, Italy) and investigating their polymeric nature through Fourier transform infrared spectroscopy (FTIR) technique in attenuated total reflectance (ATR). I further deepened the analysis of WP2, simulating the release of chemicals from plastic items in the marine environment and evaluating their ecotoxicological impact. Finally, within WP3, the potential toxicity of leachates obtained from different recycled plastic materials (that can be used as resources for the production of new objects) was investigated.

- WORKPLAN AND RESEARCH ACTIVITIES

WP 1. Objective. Assessing the presence and the chemical nature of microplastics in abiotic (seawater surface, seawater column and sediment) and biotic samples (mussels, anemones and sea urchins) collected along the Conero Riviera.

Methods.

Sampling activities: samples were collected along the Conero Riviera (Ancona coast) in three different sub-areas: "Passetto", "Trave" and "Portonovo", during the autumn and the summer seasons. For what concerns abiotic matrices, sampling of the water surface was performed using a Manta net of 300µm mesh; sampling of water column was performed using a specific pump (KC Denmark's Micro Plastic Particle Pump), while sediments samples were collected using a specific bucket (Van Veen grab). As for the biotic samples, three

different species of invertebrates were collected through snorkelling activity: *Mytilus galloprovincialis* (Mediterranean mussel, filter feeder), *Paracentrotus lividus* (Purple Sea urchin, grazer) and *Anemonia viridis* (Mediterranean snakelocks anemone, planktonivorous/carnivorous), chosen as bioindicator species of different feeding strategies.

Laboratory analysis: The protocol used for the processing of the biological samples is based on a digestion of the whole soft tissues of organisms (5% KOH or 15% H_2O_2 solution depending on the species) followed by a filtration on nitrocellulose filters. For what concerns abiotic matrices: both sediments and sea water samples were processed by colleagues of 'CNR-IAS Genova', with whom UNIVPM collaborates in the framework of some Projects. Extracted particles were firstly microscopically observed through a stereomicroscope and categorized according to both size classes and shapes. Chemical characterization of microplastics was carried out to identify polymer typologies by micro-Fourier Transform Infrared spectroscopy (μFTIR) technique (Avio et al., 2015) in attenuated total reflectance (ATR), using a Spectrum Two spectrometer (PerkinElmer) equipped with the universal ATR accessory and working with the Spectrum 10 software for IR spectra acquisition and analysis.

Results.

Results showed a general high frequency of MPs ingestion within the three species, with minimum values of 30% in mussels, up to 100% in sea urchins and anemones. In particular, *P. lividus* represented the species within the highest number of microplastics per individual, with values included in the ranges 5.8 ± 3.27 (items/organism). No significant seasonal differences (in terms of n° MPs/organism) were highlighted in the three species. As for the different shapes of microplastics, fibers were the most abundant, followed by fragments and films. The most frequently found polymer was polyester (PES), closely connected to textile matrices, followed by polyethylene (PE) and polyamide (PA); most of the microfibers characterized were of natural origin (e.g. cellulose) and then not included as MPs in the final count. Finally, concerning MPs' dimensional classes, the most frequently found were the smallest ones, in the range 20μm-1mm.

WP 2. Objective. WP2 aims to investigate the possible role of plastic as carrier of contaminants in the marine environment, by comparing the chemical characteristics and potential toxicity of leachates obtained from virgin plastic objects and the same objects found beached along the Adriatic coast, through the use of a battery of ecotoxicological assays.

Methods

Description of the sampling area and activity

Plastic litter were sampled along the Conero Riviera in a site not directly accessible by land and therefore not intended for bathing or other anthropogenic activities. For this reason, the hypothesis of a terrestrial contamination of the beach could be excluded validating the hypothesis of beaching phenomenon of wastes from the sea (being port, fishing and mussel farming activities the main inputs of plastic litter).

Samples collection, leachates preparation and laboratory analyses

Plastic litter collected during the sampling was divided into 5 categories (identified as representative): heterogeneous fragments, polystyrene boxes, mussel nets, bottles and Rapido rubber (specific trawl targeting

flat fish). Plastic polymers' identification of single objects selected was performed using FT-IR technique. Finally, virgin plastic items, made of the same polymers of those collected on the beach, were purchased on the market. For leachates' preparation plastic items were firstly manually cut. For chemical analyses, samples were subjected to a first washing in seawater (72h), obtaining the first leachate, and a second sequential acid washing (4h) thus obtaining the second leachate. For both leaching processes plastic water ratio was maintained 100g/L and samples were kept under agitation at room temperature and natural light conditions. Leachates were finally analysed to determine concentrations of polycyclic aromatic hydrocarbons (PAH), aliphatic hydrocarbons (AH), polychlorinated biphenyls (PCBs), flame retardants, pesticides and metals. Leachates for bioassays were prepared using natural filtered sea water and tested at different dilutions using an integrated battery of ecotoxicological bioassays. Different endpoints were evaluated, such as: bioluminescence inhibition of *Aliivibrio fischeri* (Quaderno ISPRA, 4/2021), algal growth inhibition of *Phaeodactylum tricornutum* (UNI EN ISO 10253: 2016E) and embryotoxicity test using *Paracentrotus lividus* (ISPRA notebook, 11/2017). The data obtained from the bioassays were integrated through a "Weight of Evidence" (WOE) approach using "SEDIQUALSOFT" software and obtaining an integrated HQ (Hazard Quotient) of the battery.

Results.

Obtained results confirmed the initial hypothesis of plastic acting as a vector of environmental contaminants. Leachates obtained from beached plastics showed higher concentrations of metals (µg/g), PAHs (ng/g) and AHs (µg/g) than virgin plastic ones. On the contrary, the ecotoxicological bioassays revealed, a higher toxicity for virgin plastic leachates than for the beached ones. Being the virgin 'rubber of Rapido' the most toxic sample. The elaboration of results within the WOE model, revealed that all categories of beached plastic leachates expressed a Moderate HQ, at highest concentrations, except for polystyrene. While for virgin plastic leachates only the 3 categories of 'polystyrene', 'bottles' and 'Rapido rubber' had significant levels of HQ, also obtaining Severe HQ levels for 'Rapido rubber'.

WP 3. Objective

The study aims to explore the potential toxicity of leachates obtained from different recycled plastic materials, considered secondary raw materials that can be used as resources to produce new objects.

Methods

Samples preparation and laboratory analyses: four different types of plastic fragments: white rubber "LV1"; white rubber "LV2"; tennis ball "PT"; shoes "SC" were characterized using FT-IR technique to determine their polymeric nature and were used for the realization of plastic leachates. Leachates were prepared following the method previously described for WP2 and tested at different dilutions using the same battery of ecotoxicological bioassays (*A. fischeri, P. tricornutum P. lividus)*. The results obtained from the bioassays were finally integrated through the "Weight of Evidence" (WOE) Model using "SEDIQUALSOFT".

Results

For *A. fischeri* the EC10 and LOEC values were respectively included in the ranges 0.2-0.9 g/L and 0.1-0.5 g/L, while for *P. tricornutum* the values were higher: 0.7-4.4 g/L EC10 and 0.8-20 g/L LOEC. The *P. lividus*

embryotoxicity test was the most sensitive with EC10 values ranging from 0.0006-0.014 g/L and LOEC 0.1-3.2 g/L. The sample with the highest toxic effect was PT. The results of the processing of WOE model showed Moderate and Major HQ levels at concentration of 1 g/L for all samples and even at a lower concentration for PT and SC (0.5 g /L).

WP 4. Objective

Bibliographic research activity aimed at obtaining data relating to abundance, polymeric nature, shape and interaction with the biota of microplastics in different compartments of marine environment (water, sediment and beach). The literature search was conducted to provide data for the development of a Weight Of Evidence (WOE) Model for risk assessment of microplastics in the marine environment.

- REFERENCES

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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. Getting Started with R: Environmental Computing, Giuseppe d'Errico (1 cfu)
2. "Current threats to research ethics and how to cope with them", Marco Seeber, Department of Political Science and Management, University of Agder, Norway, 9 June 2022.

Other Activities

1. Disciplinary Tutor for Applied Biology Laboratory 2021/2022
2. Educational and laboratory activities related to PLS with high school students (: Istituto Istruzione Superiore "VOLTERRA - ELIA", Ancona; Liceo scientifico "Galileo Galilei", Ancona; Istituto Istruzione Superiore Corinaldesi- Padovano, Senigallia; Istituto Tecnico Tecnologico Nautico "Leone Acciaiuoli", Ortona).

List of conferences/workshops attended and of contributions eventually presented

1. 21st International Symposium on **P**ollutant **R**esponses **I**n **M**arine **O**rganisms May 22-25, 2022 Gothenburg, Sweden. Contribution: Poster Presentation #P60 "Leachates from Virgin and Beached Plastic Items: Assessment of Chemical Composition and Ecotoxicity to Marine Organisms" C. Mazzoli*, D. Fattorini, M. Di Carlo, G. d'Errico, F. Iezzi, L. Pittura, A. Nardi, S. Gorbi, F. Regoli. Receiver of Best Student Poster Presentation Award.

2. 21st International Symposium on **P**ollutant **R**esponses **I**n **M**arine **O**rganisms May 22-25, 2022 Gothenburg, Sweden. Contribution: Oral Presentation #O67 "Cellular Disturbance and Effects on Thermal Stress Response in Mussels Exposed to Synthetic and Natural Microfibers" A. Nardi*, L. Pittura, C. Mazzoli, F. Mongera, G. d'Errico, M. Benedetti, S. Gorbi, F. De Falco, M. Cocca, M. Avella, F. Regoli.

3. X_a Edizione delle Giornate di Studio "Ricerca e Applicazione di Metodologie Ecotossicologiche, September 28-30, 2022 Lido di Camaiore, Lucca (Italy). Poster Presentation "Preliminary Investigations on the Toxicity of Recycled Plastic Materials and Potential Risks for the Marine Environment" C. Mazzoli *, G. d'Errico, F. Iezzi, M. Orsini, L. Painting, M. Benedetti, S. Gorbi, F. Regoli.

4. X_a Edizione delle Giornate di Studio "Ricerca e Applicazione di Metodologie Ecotossicologiche, September 28-30, 2022 Lido di Camaiore, Lucca (Italy). Oral Presentation "Comparison in the toxicity of virgin plastic objects compared to those beached along coastal environments" C. Mazzoli*, G. d'Errico, F. Iezzi, D. Fattorini, M. Di Carlo, L. Pittura, A. Nardi, M. Benedetti, S. Gorbi, F. Regoli.

Part 3. PhD student information on publications

List of publications on international journals

J1. Pittura L., Nardi A., Cocca M., De Falco F., d'Errico G., Mazzoli C., Mongera F., Benedetti M., Gorbi S., Avella M., Regoli F. "Cellular disturbance and thermal stress response in mussels exposed to synthetic and natural microfibers" Frontiers in Marine Science, 9, 2296-7745(2022) DOI: 10.3389/fmars.2022.981365

J2. G. Liberatori, C. Mazzoli, F. Ferraro, L. Sturba, M. L. Vannuccini, D. Baroni, P. A. Behnisch, M. Puccini, S. Vitolo, I. Corsi. "Aryl hydrocarbon reporter gene bioassay for screening polyhalogenated dibenzo-p-dioxins/furans and dioxin-like polychlorinated biphenyls in hydrochar and sewage sludge" Journal of Hazardous Materials, 428, 0304-3894, (2022) DOI: 10.1016/j.jhazmat.2022.128256

J3. C. Mazzoli*, D. Fattorini, M. Di Carlo, G. d'Errico, F. Iezzi, L. Pittura, A. Nardi, S. Gorbi, F. Regoli. "Leachates from Virgin and Beached Plastic Items: Assessment of Chemical Composition and Ecotoxicity to Marine Organisms" *(in preparation)*

J4. C. Mazzoli *, G. d'Errico, F. Iezzi, M. Orsini, L. Painting, M. Benedetti, S. Gorbi, F. Regoli. "Preliminary Investigations on the Toxicity of Recycled Plastic Materials and Potential Risks for the Marine Environment" *(in preparation)*

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

B1. Book Chapter: Title *Microplastics and Nanoplastics*. Authors: Lucia Pittura, Stefania Gorbi, Carola Mazzoli, Alessandro Nardi, Maura Benedetti, Francesco Regoli. In *Marine Analytical Chemistry*, Julián Blasco and Antonio Tovar-Sánchez (Eds). ISBN: 9783031144851. Publisher: Springer International Publishing. Publication date: 26 November 2022

B2. Report "Plastic Litter in the Adriatic Basin" https://www.greenpeace.org/static/planet4-italy-stateless/2021/06/98c3eab4-report-plastic-litter-in-the-adriatic-basin-2021.pdf

B3. Report "Insieme per il Mar Mediterraneo, come ottenere energia pulita dai rifiuti di plastica recuperati dal mare"; https://marevivo.it/wp-content/uploads/2021/10/REPORT_INSIEME-PER-IL-MAR-MEDITERRANEO.pdf

12/10/2022

Student signature

Supervisor signature