PHD COURSE IN LIFE AND ENVIRONMENTAL SCIENCES

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

Name of PhD student: Melissa Orsini

Title of PhD research: Presence, effects, and behaviour of MPs in the marine environment

Name of PhD supervisor: Francesco Regoli
Research lab name: Ecotoxicology and environmental chemistry

Cycle:
[ ] XXXVI
[ ] XXXVII

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

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

ABSTRACT (1000 characters, including spaces):

The studies carried out during the second year of the PhD follow and integrate those started the previous year and aim to monitor MPs pollution in the Conero Riviera, an area of high naturalistic, touristic, and economic value located in the Central Adriatic Sea, applying a strategy based on the use of indicator species integrated with the investigation on abiotic compartments.
Local macroalgal species (*Ulva lactuca, Cystoseira compressa, Gongolaria barbata*) were sampled to verify the possible adherence and entanglements of MPs on their fronds, and Insects (Carabidea and Oniscidea spp.) were used for the first time, to conduce a land-based survey of MPs pollution in coastal area revealing ingestion frequencies of about 50%. In addition, experiments under controlled laboratory

conditions were carried out to better understand the role of macro algae in affecting MPs distribution in the water column. Simultaneously, beach cleaning activities were conducted to obtain a more comprehensive characterization of plastic pollution in the Conero Riviera.
Indeed, concerning the introduction of new biobased and biodegradable polymers in the world industry, a mesocosm experiment allowed us to test potential biological effects in Baltic fish and clams exposed to conventional and biobased polymers.

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

BACKGROUND

Microplastics (MPs), commonly defined as synthetic particles <5 mm in diameter (Galgani et al., 2013), are increasingly proving to be ubiquitous in all marine ecosystems and coastal areas. To address their distribution, bioavailability and effects, novel tools and integrated strategies are required.
Since problems with plastic pollution are some of the biggest challenges of our time, biobased plastics have emerged as a promising alternative to synthetic ones. However, the real opportunities and risks of such novel biobased plastic solutions have raised scientific and public awareness.

SCIENTIFIC AIMS

This part of the research project aimed to characterize plastic and microplastics pollution in coastal marine- and land-based areas applying strategies based on i) Development of innovative technologies to counteract the impact of plastics in coastal areas of the Conero Riviera
ii) the introduction of novel bioindicator species to assess the level of impact of MPs in coastal areas
iii) investigation on possible biological effects associated to biobased and biodegradable polymers, given mistrust raised about the harmless nature of these substances.
To achieve these aims, specific field, laboratory and mesocosm investigations were developed.

WORKPLAN AND RESEARCH ACTIVITIES

WP 1. Innovative technologies to counteract the impact of plastics in coastal areas of the Conero Riviera

Objective: The general objective was 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.

Methods

The experimental plan expected a seasonal beach cleaning on a rocky beach of about 200m located along the Conero Coast (*43.602992° 13.5512981°).* The first cleaning was conducted in July 2022 while the second in July 2023.
The sampled marine litter was subdivided into 9 categories including: objects related to fishing and aquaculture activities, bottles, footwear, objects of common use, textile products, pipes and cables fabrics, fragments of rubber and insulating materials, rigid fragments, other.
Each group was weighted and for each category subsamples were chemically analyzed to assess the polymeric nature of the collected plastic items.

Simultaneously with the beach cleaning activity, two overflight and image acquisition surveys conducted by remote controlled drones were performed: one before a cleaning operation and one immediately after. 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).

Results

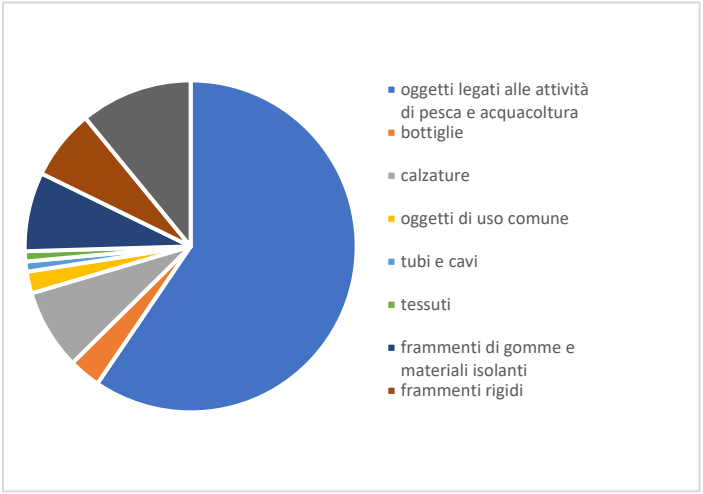
During the beach cleaning activities 22/23 a total of 151.8 kg plastic was sampled for a total number of 3040 items (Table 1).

Beach cleaning	2022	2023
items	1424	1611
Kg	71,3	80,5
items/mq	6	7

Table 1 Sampled plastic items during the campaign 2022/23

About 52% of the collected plastic was associated to fishing activity including fishing devices, nets, and polystyrene boxes. While the categories footwear, fragments of rubber and insulating materials were almost equally represented in both samplings with an average of 10% each (Figure 1 a, b). Rubber was present with a frequency of 30% while polyester covered 20% of the polymer abundancy in 2022 and 40% in 2023. The great contribute of rubber is due to the presence of a specific fishing device that is used for bottom trawling along the central Adriatic Sea, this environmentally unsustainable fishing causes the loss of thousands of rubber filaments that strand on the Adriatic beaches and contribute to the mass stranded marine litter. All in all, the individual categories are represented in both samplings with very similar frequencies, as well, the polymeric composition remains constant.

a.) 2022



b.) 2023

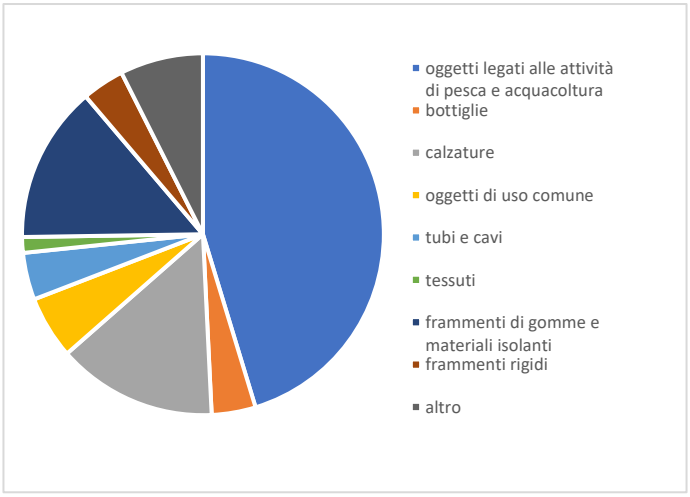


Figure 1a, b Frequencies of the collected plastic litter expressed in categories.

WP2. Use of novel bioindicator species

Objective: The general objective of this part of the project was to introduce new bioindicator species (aside from traditional ones as *Mytilus galloprovincialis*) in order to estimate the distribution and the bioavailability of plastic pollutants in marine and shrubland ecosystems. Macroalgal forests were used to study their capacity to work as sinks for MPs particles, accordingly to that, experiments under controlled laboratory conditions were carried out to better understand the role of these species in affecting MPs distribution in the water column, focussing on how algal surface complexity might influence surface

adhesiveness. Secondly, insects from the order Carabidea and Oniscidea were used for the first time as bioindicator for microplastic ingestion in coastal areas.

Methods

Three algal species with different morphologies and of high ecological relevance were collected seasonally: *Cystoseira compressa, Gongolaria barbata* and *Ulva lactuca.*
For each species the apical fronds were cut of and analyzed after MPs under a stereomicroscope.

To conduct an experiment in controlled laboratory conditions, *Cystoseira compressa* and *Ulva lactuca* were chosen for MPs exposure.
For both specimens 3 replicates were sampled and exposed to known concentration of MPs in 5L beacher provided with an aerator. A rinsing phase followed to assess how many particles remain attached after the flushing. The water in which the algae were exposed was filtered to assess their releasing capacity, while algae were observed under a stereomicroscope to count the trapped particles.

Insects from the order Carbidea and Oniscidea were sampled along 3 selected sites of the Conero coast using specific hidden traps. Organisms were collected, weighted, measured, and then dissected to isolate the gastrointestinal tract (GIT) for the analysis of MPs. Pools of 5 GIT were processed and analyzed after MPs.

Results

In the field:
All algal species trapped MPs with levels varying depending on the sampling period, reaching the highest concentration of 3.8 MP/g w.w (*Gongolaria barbata, April).*
Fibers were the dominant shape (98%), and polyester was the most frequent polymer. Most MPs fell in the 1-3 mm size class.

In the lab:
Cystoseira and Ulva retain 5.2% and 5.9% MPs respectively.
In Cystoseira only 0.8% of the MP particles was trapped by the algae after the rinsing phase while 1.68% in Ulva.
Concerning the MP/g of dry tissue, 0.8 MPs/g were counted on *Cystoseira* fronds while 22 MPs/g on Ulva leaves. The average size class retained by Ulva is 378µm and 502µm in *Cystoseira*.

About insects, results reported a high MPS ingestion frequency, suggesting insects as valid bioindicators. 57% of Carabids and 36% of Onisco resulted positive to MPs ingestion.
Considering only those insects positive to MPs ingestion, the mean value of ingested particles was of 1.04 in Carabids and 0.34 in Onisco.
In both species, fragments were the dominant shape and natural fibers were widely more diffused than synthetic ones.

WP3. Biological effects in *Gasterosteus aculeatus* and *Macoma balthica* exposed to conventional and biobased polymers a mesocosm experiment.

Objective: The aim of the mesocosm experiment was to figure out if biobased polymers are more or less harmful than conventional plastics. To test this, the balthic clam *Macoma baltca* and the local fish

Gasterosteus aculeatus have been exposed to well-known concentrations of different micronized polymers: PHBv, PLA, and PP, for a period over 4 weeks.

Methods

Approximately 10 organisms of *Gasterosteus aculeatus* and *Macoma balthica* were taken and dissected before the start of the experiment, to have reference values. A second and a last dissection were carried out at the middle and at the end of the experiment to observe if biological effects are modulated during the exposure time.

For the clams, the whole tissue was dissected, while for fish, muscle tissue, gills, eyes, liver, and brain were dissected separately and stored at -80.

The selected species have been previously used as bioindicators of water and sediment quality for their ability to bioaccumulate contaminants and their sensitivity in biological responses.

Analyzes were focused on a panel of biological responses at biochemical and cellular level (including oxidative metabolism, biotransformation pathway and neurotoxicity) to assess potential effects induced by microplastics from either conventional or biobased polymers.

Results

Concerning neurotoxic effects, preliminary results showed a certain modulation of acetylcholinesterase activity both in fish and in clams, with a general induction of this enzyme in fish. In clams no different induction between synthetic- and biobased-polymers were evident.

Expected results will give an indication on the modulation of biotransformation (CYP450), and antioxidant systems potentially affected by organic additives and trace metals leached from MPs.

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. Raman- FTIR Spectroscopy workshop (24h)

List of periods spent abroad

1. 10 days in Sweden for a mesocosm experiment part of the Project JPI Oceans
2. 4 days at the NCR of Genoa to define a standardized protocol for the extraction of MPs from marine sediments.

List of conferences/workshops attended and of contributions eventually presented

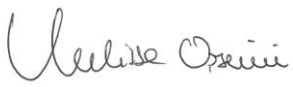
1. final project meeting Life blue Lakes, Rome 26-27th September 2023
2. RESPONSE project meeting 26-28 april 2023, poster exhibition “Effects of microplastics on ecological functioning
3. Artropodi utilizzati come bioindicatori di microplastiche in ambiente terrestre: il caso dei carabidi (Coleoptera:Carabidae), XVII Congresso Nazionale Italiano di Entomologia (CNIE), 12 -16 june 2023, Palermo

Part 3. PhD student information on publications

in preparation, provisory title: “Biological effects in *Gasterosteus aculeatus* and *Macoma balthica* exposed to conventional and biobased polymers.”

08/11/23

Student signature



Supervisor signature