Evaluation of Exposure to Microplastics and Nanoplastics Associated with the Consumption of Clam *Chamelea Gallina*

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This PhD thesis research project aims to develop analytical protocols for the determination of microplastics (MPs) and nanoplastics (NPs) in the clam Chamelea Gallina, through two instrumental techniques, the Raman spectroscopy and Pyrolysis coupled with gas chromatography-mass spectrometry (Py-GC/MS), that provide morphological and chemical information. The data obtained will allow to carry out a preliminary evaluation of the human exposure to the MPs/NPs taken through the diet, deriving from the consumption of this mollusc.

Valutazione sull’esposizione a microplastiche e nanoplastiche associata al consumo di vongola *Chamelea Gallina*

Questo progetto di tesi di dottorato mira a mettere a punto protocolli per la determinazione di microplastiche (MP) e nanoplastiche (NP) nella vongola *Chamelea Gallina*, mediante due tecniche strumentali, la spettroscopia Raman e la Pirolisi accoppiata a gas cromatografia-spettrometria di massa (Py-GC/MS) che forniscono informazioni di tipo morfologico e chimico. I dati ottenuti permetteranno di effettuare una valutazione preliminare dell’esposizione umana alle MP/NP assunte attraverso la dieta, derivante dal consumo di questo mollusco.

# **1. State-of-the-Art**

Microplastics (MPs) are polymer particles with dimensions between 0.1 µm and 5 mm, ubiquitously dispersed in the environment and present in various forms including fibers and fragments. Their further fragmentation leads to the formation of nanoplastics (NPs) with dimensions up to 1 nm. The presence of these plastic particles has aroused considerable interest in recent decades due to their toxicity detected in marine organisms, and their potential role as a vehicle for other pollutants, thus increasing the exposure and facilitating the entry of substances toxic to humans, being at the top of the food chain.

The bivalve molluscs, given their nature of filter feeding, ingest many substances, including the MPs/NPs, which accumulate between tissues and internal organs based on the size. This feature allows them to be used as indicators of the healthiness of the marine environment and therefore possible to think of their use as bioindicators for plastics contamination (Ding J et al, 2021). Some protocols for the analysis of the MPs content in mussels that use different analytical approaches are available in literature (Pinto da Costa J et al, 2019).

These protocols involve the use of non-destructive techniques such as Raman spectroscopy, capable of characterizing the MPs by shape and size, as well as identifying them by comparing the spectrum obtained with characteristic reference spectra, optimizing a series of parameters such as the laser power, the number of scans and the exposure time.

Another technique that is recently taking hold for the analysis of MPs/NPs is pyrolysis coupled to gas chromatography-mass spectrometry (Py-GC/MS), a structural investigation technique that involves thermal degradation with temperatures above 500 °C and characterization of microplastics and any additives through pyrolysis products (Ishimura T et al, 2021).

Furthermore, the choice of analytical technique becomes fundamental in this case according to the information to be obtained; on the one hand, in fact, spectroscopic techniques make it possible to obtain information on the size and shape of the microplastics, while thermodegradation techniques allow to exploit lower sensitivities by analysing a minimum amount of sample, losing the morphological information.

*Chamelea Gallina* is a bivalve mollusc that feeds by filtering the surrounding waters and grows on the seabed. it is highly appreciated both in Italy and in the rest of Europe and the use of this food, consumed completely without removing internal organs, can constitute a source of contamination from microplastics. Although molluscs are among the foods most taken into consideration when it comes to contamination in the marine environment, there is still little data available on the number of MPs present, necessary to evaluate the risk of exposure associated with the consumption of the clam *Chamelea Gallina* (Gedik K, Gozler AM, 2022).

The proposed research project aims to fill the lack of analytical methods for the extraction, identification, and quantification of MPs/NPs in *Chamelea Gallina* clams. The use of different instrumental techniques will allow to obtain combined information on physical characteristics (color and size of the particles) and chemical characteristics (mass of the particles, or of the relative thermodegradation products). Finally, the application of validated protocols on real samples will provide data on MPs/NPs contamination levels, filling the lack of data in the literature and allowing a first assessment of exposure to these contaminants with their intake.

# **2. PhD Thesis Objectives and Milestones**

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Sampling planning\* of the *Chamelea Gallina* clams in Abruzzo, Molise and Marche region** considering different parameters as collection areas and fishing block periods, and the consumption of the product (A1.1). The number of samples must be sufficient to be representative and must take place over 12 months (A1.2).

A2) **Analytical protocol\*\* for the determination of the MPs/NPs with Raman spectroscopy** which will include the optimization of the microplastics extraction protocol from the matrix (A2.1), the instrumental method for the identification of polymers by varying key parameters (laser power, number scans and exposure time) (A2.2) and the analysis of the samples of clams (A2.3).

A3) **Analytical protocol for the determination of the MPs/NPs with Py-GC/MS** which will be conducted using the extraction method used for spectroscopy analysis, adapted for the preparation of the sample to pyrolysis (A3.1). The parameters to be optimized will concern the pyrolysis process and the identification of microplastics through the characteristic pyrolysis products and with certain values of *m/z*, as well as the acquisition parameters in mass spectrometry (A3.2). After the validation of the analytical protocol, the analysis of the samples of clams will be made (A3.3).

A4) **Definition of the levels of contamination of the *Chamelea Gallina* clams of MPs/NPs** by developing the data obtained by the two complementary analytical techniques (A4.1) and preliminary evaluation on the risk of exposure to MPs/NPS associated with the consumption of *Chamelea Gallina* clams (A4.2).

A5) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

\*The Sampling planning (A1.1) will end in June 2023, while the collection of samples will begin in July 2023 (first year of the PhD thesis project) and will finish in June 2024.

\*\* The extraction protocol (A2.1) and instrumental optimization (A2.2) will be conducted during the first year of the PhD thesis project.

***Table 1***Gantt diagram for this PhD thesis project.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Activity Months | | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** |
| A1) | ***Sampling of Chamelea Gallina*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) Sampling planning |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 2) Collection of samples |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A2) | ***Optimization of the Raman spectroscopy analytical protocol*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) Extraction protocol |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 2) Instrumental optimization |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 3) Samples analysis |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A3) | ***Optimization of the Py-GC/MS analytical protocol*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) Extraction protocol re-optimization |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 2) Instrumental optimization |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 3) Samples analysis |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A4) | ***Definition of contamination levels*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) Data elaboration |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 2) Risk evaluation |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A5) | ***Thesis and Papers preparation*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

# **3. Selected References**

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