Ph.D. SCHOLARSHIPS ON THE THEMATIC AREAS OF INNOVATION AND GREEN

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Ph.D in RESEARCH METHODS IN SCIENCE AND TECHNOLOGY - Ciclo XXXVII

Bound Topic of Research "Sviluppo di biotecnologie basate sui foraminiferi per la mitigazione dell'impatto antropico e il ripristino degli ecosistemi marini "

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Development of foraminiferal-based biotechnologies for the mitigation of the anthropogenic impact and restoration of marine ecosystem	
THEME: ☐INNOVAZIONE ☒ GREEN	
PROJECT PROPOSAL	**Introduction of the problem in the international scientific context** Coastal marine environments provide a plethora of ecosystem services with immense social benefits that are today threat by anthropogenic activities (Townsed et al., 2018). The release of carbon dioxide (CO_2) has led to decrease in ocean pH and carbonate saturation state. At local scale, high amounts of organic (e.g., hydrocarbons) and inorganic (i.e., trace elements) pollutants are discharged in marine ecosystem and are potentially hazardous to aquatic life. The marine environments are also affected by emerging pollutants (i.e., engineered nanomaterials, microplastics). Most of them are classified as harmful by the EU (Dulio et al., 2018).

Nowadays, strategies and solutions to mitigate and tackle marine pollution have become an issue of global focus. The recent scientific and technological advancements have enabled the implementation of marine biotechnology, where marine organisms represent resources for different sectors of society including bioremediation. Some studies have, for instances, focused on the effectiveness of storing CO_2 in biologic reservoirs (i.e., Caldeira and Wickett, 2005), while others have explored the potential application of innovative biotechnological approaches for the bioremediation of marine polluted areas from hydrocarbons, metals and plastic among others (Townsed et al., 2018).

Benthic foraminifera (BF) are single celled micro-organisms. The majority of BF species secretes a calcium carbonate test (i.e., shell). Previous studies have documented how calcifying organisms could benefit from electricity density for increasing their growth. This biotechnological application has been already tested on corals (Huang et al., 2021) and oysters (Karissa et al., 2012), but not on BF yet. BF may represent a way for storing the extra amounts of CO_2 in oceans. On the other hand, agglutinated BF species have the ability to collect small grains from the sediment for their tests' constructions (Armynot du Chatelet et al., 2013). The agglutinated BF could then be used to remove toxic microparticles (i.e., plastic) from

	the sea sediment, reducing the pollution level and creating a new and sustainable form of bioremediation in impacted marine areas. The exploitation of BF as an emerging biotechnology may open indeed new horizons in the development of strategies for restoring the aquatic ecosystems at both local and global scale. **Significance of the matter** The exploitation of marine resources (e.g., biotechnological) represents a new frontier of international scientific, industrial, and economic interaction among countries. The impacts of human activities have reached a critical level that is destined to worsen due to the current climate changes. The proposal of this PhD project meets perfectly the objectives of European Union actions and policy including those established within the Horizon 2020 and the Marine Strategy Framework Directive. Additionally, it represents the first effort to evaluate the potential application of BF for reducing the anthropogenic impacts. The challenge of bioremediation and mitigation has a great socio-economic potential impact due to the growing awareness that ecological issues affect also the human health. The key role of the presents research is also supported at EU and international level by the Blue Economy of the European Union Blue Growth and Maritime Spatial Planning Directive.

Methodology

The implementation of the project is carried under laboratory-controlled conditions. The first part of the PhD project is devoted to assess the viability and physiological response of larger benthic foraminifera (LBF) to different electrical current stimulations. The viability will be evaluated through fluorescent probes (i.e., CTG or CHB; Frontalini et al., 2019), whereas the physiological response through protein and enzymes assays (Betti et al., 2021). These steps ensure the definition of the appropriate electrical density range and will be followed by a time-course experiment to check the growth rates of LBF (i.e., *Amphistegina*) by optical microscopy. The second part of the project involves the agglutinated foraminferal species (i.e., *Eggerelloides scaber*) that will be exposed to different concentrations, types and dimensions of microplastics and used to check, at first, their physiological response and retention capability. The former is evaluated by fluorescent probes (i.e., Nile Red, Acridine Orange and Cell-Rox), whereas the latter by using fluorescent microplastic with a confocal microscope (Ciacci et al., 2019).

Objectives and expected findings

The main aim of the PhD is to develop new foraminiferal-based biotechnologies for a) storing CO_2 in oceans and buffering the effect of ocean acidification; and b) removal of microplastic from the sediment. The stimulation by electric current density is expected to improve the LBF calcifying capability, creating a new potential method of CO_2 sequestration with huge implications at global scale. The development of agglutinated foraminiferal substrate is expected to retain microplastic and opens a revolutionary strategy for bioremediating marine sediment. The potentiality of biotechnological advances might support a green use of benthic foraminifera to tackle human-induced impacts and, in the future, find even *in situ* applications.

Bibliography

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Briefly highlight the coherence characters between the project, the SNSI, and the PNR with reference also to the capacity to foster innovation and interchange between the world of research and the productive world in the field innovation, digital and enabling technologies, as well as the potential scientific, economical and social repercussion.	The present PhD proposal perfectly follows the National Smart Specialization Strategy (NSSS) that defines long-term investment priorities for meeting innovative products and services and, more importantly, the development of key enabling technologies (KETs) including biotechnologies, one of

	four KETs. The application of marine resources (e.g., biotechnological) to mitigate the anthropogenic impact plays a key role for addressing several social challenges. In fact, the KETs are expected to stimulate competitiveness in terms of global competition and, at the same time, to develop solutions to tackle challenges such climate changes and for a sustainable development. In this way, biotechnological applications like the ones here proposed involving benthic foraminifera might support European industries in terms of innovation, scientific collaboration, technological and innovation advances, all under a green perspective. The present project therefore perfectly falls within "*Smart and sustainable industry, energy and environment*" thematic area and, more specifically, within two Development trajectories "*Water and waste treatment systems and technologies*" and partly with "*Systems and technologies for reclaiming contaminated sites and decommissioning nuclear plants*" of NSSS. The former fore-

	sees the potential development of sustainable technologies, and in the context of the present PhD, to pursuit biotechnological advances for environmental bioremediation. Additionally, the PhD research has been thought and developed to meet the National Research Program (PNR) defining national research policies and the lines of intervention in the field of research to overcome the weaknesses of the national research system. The line of research of the present PhD proposal falls within "*5.6 Food products, bioeconomics, natural resources, agriculture and environment*" as large areas of research and innovation and related areas of intervention and specifically within two areas of intervention, namely "*5.6.1 Green Technologies: Articulation 3. Prevention of soil and water contamination*" and partly "*5.6.5 Knowledge of technological innovation and sustainable management of marine ecosystems: Articulation 1. Knowledge of marine ecosystems and the coastal strip*". The development of foraminiferal-based biotechnology for the mitigation of human-induced

	impact represents an innovative exploitation of marine resources. This approach is also contemplated with the Green New Deal and incorporated within governmental strategies (i.e., European Green Deal), that aim at re-shaping the European Union in a modern, resource-efficient and competitive economy that will not generate net greenhouse gas emissions by 2050 even using green tech-nologies (i.e., biotechnologies) such as ben-thic foraminifera.