

**BORSE DI STUDIO DI DOTTORATO DI RICERCA SU TEMATICHE  
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**Dottorato di Ricerca in  
RESEARCH METHODS IN SCIENCE AND TECHNOLOGY (REMEST)  
XXXVII Ciclo**

***Theme “Synthesis of new fluorescent compounds of biological  
interest: application in the detection of toxic metals in cells of  
marine and terrestrial biomarkers”***



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**SINTESIS AND STUDY OF FLUORESCENT SENSORS OF ENVIRONMENTAL INTEREST**

Tematica GREEN

**RICERCA PROPOSTA**

*breve descrizione della ricerca proposta dal candidato strutturata nel seguente modo:*

- *introduzione della problematica nel contesto scientifico internazionale,*
- *rilevanza del problema,*
- *metodo attraverso il quale il problema verrà affrontato,*
- *obiettivi e risultati attesi,*
- *bibliografia.*

Soil and water pollution represents one of the largest problems that characterized the end of the last century and the beginning of the new millennium. The number of toxic and polluting substances spread into the environment that affect the quality of water and soil with potential environmental risk is constantly increasing, which makes the problem even more pressing.

Europe has moved in this regard with the report "EEA 2020 Signals - Europe towards zero pollution" which examines the different types of pollution and their sources, underlining how pollution is a real problem that must be addressed at a global level.

Soil and water pollution includes the presence of heavy metals which are dangerous because they tend to bioaccumulate. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, relative to the concentration of the chemical in the environment. Compounds accumulate in living things whenever they are absorbed and stored faster than they are broken down (metabolized) or excreted (eliminated). Heavy metals can enter a water network from industrial and consumer waste, or even from acid rain that hits the ground and releases heavy metals into streams, lakes, rivers and groundwater. Allergies are not uncommon, and repeated long-term contact with certain metals or their compounds can cause numerous



short and long-term problems. Heavy metals are important in many respects to humans, especially in the manufacture of some important human products, such as accumulators (Pb), mercury arc (Hg) lamps and thermometers, kitchen utensils, and for holding stuff (Al) and a wide range of other products<sup>6,7</sup>. Nevertheless, the biotoxic effects, if improperly disposed of or stored, could potentially be life-threatening, therefore, they cannot be neglected<sup>1</sup>. Although heavy metal poisoning can be clinically diagnosed and medically treated, the best option is to prevent heavy metal pollution and subsequent human poisoning<sup>2</sup>.

Science is focusing on studying new tools that can assess the presence of pollutants in both water and soil and the interaction mechanism with living organisms. One approach is to study species able to bio-accumulate toxic substances, and in particular of heavy metals, found in soils and waters contaminated by spills of waste substances<sup>3</sup>. These organisms accumulate the substances present in the environment around them within specific cellular compartments. Two of these species, namely *Armadillidium vulgare* (crustacea, isopoda)<sup>4</sup> and *Mytilus galloprovincialis*,<sup>5</sup> have resulted in recent studies indicating the state of soil pollution and the presence of heavy metals, as regards the former (also named Saint Anthony piglet), and the state of water, as regards the latter.



Fig. 1 *Armadillidium Vulgare*

([https://it.wikipedia.org/wiki/Armadillidium\\_vulgare](https://it.wikipedia.org/wiki/Armadillidium_vulgare))



Fig. 2 *Mytilus Galloprovincialis*

([https://www.researchgate.net/figure/Three-perspectives-of-Mytilus-galloprovincialis-a-shell-appearance-b-side-view-of\\_fig4\\_263550724](https://www.researchgate.net/figure/Three-perspectives-of-Mytilus-galloprovincialis-a-shell-appearance-b-side-view-of_fig4_263550724))

My PhD project concerns the development of fluorescent probes capable of signaling the presence of toxic metals such as Cr, Mn, Cu, Cd, Hg and Pb in a physiological environment that can be used to map the distribution of polluting species within the cells of the organisms mentioned above. Fluorescent probes of this type consist of a coordinating unit containing donor groups such as amines, imines, sulphides, phenols,

carboxylates and carbonyls, connected through an aliphatic spacer to a fluorophore, which must vary its optical properties as a result of the coordination of the analyte.

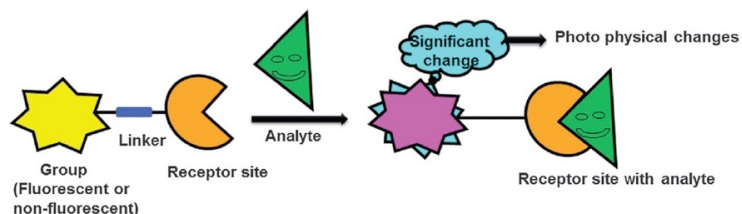


Fig. 3 General scheme of the recognition of the analyte by the probe <sup>8</sup>

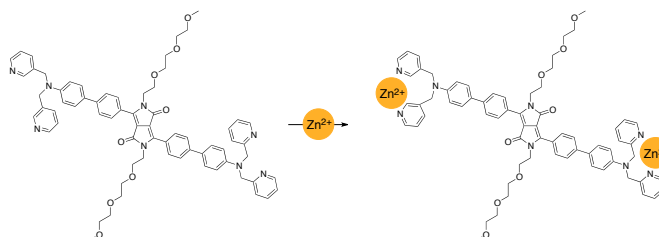


Fig. 4 Example of a probe for the recognition of  $Zn^{2+}$  cations

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The first part of the project involves the design and synthesis of a series of probes and their photochemical characterization.

Subsequently the probes are going to be tested on cellular preparations to evaluate their behavior in the biological environment.

Once the effectiveness of the probes and the cellular compartment where certain metals accumulate have been ascertained, control and treatment experiments (incubation of



probes together with polluted substrates, due to the accumulation of metals) are going to be performed both to evaluate the sensitivity of the probes in the presence of pollutants as well as to assess whether the values present in the soil are comparable and proportional to those found in the analyzed species.

The aim of the project is to set up a protocol that puts in place probes that evaluate the presence of polluting metals in water and soils. We expect positive and statistically significant results with an increase in the presence of metals in the treated Saint Anthony piglets compared to controls since the selected species are born accumulators of substances present in the surrounding environment and the probes allow to selectively detect specific components in complex biological systems, such as tissues and cells, both fixed and in culture. The aim is also to broaden the range of biological samples to which the probes can be applied. For this reason, the synthesis of the probes plays a fundamental role in this project.

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*Evidenziare sinteticamente i caratteri di coerenza tra il progetto, la SNSI ed il PNR con riferimento anche alla capacità di favorire l'innovazione e l'interscambio tra mondo della ricerca e mondo produttivo nei settori dell'innovazione, del digitale e delle tecnologie abilitanti, nonché le potenziali*

People's Health is the main focus and ultimate goal of the project; our work would represent an important element to offer different products and services that speed-up the analytic response time, provide a better service to waste treatment implants, industrial areas and polluted sea coasts.

The months in the Company are thought to possibly promote and provide Reagents for Pollution Diagnostics and Research, with high brilliance and clarity of image, stability and sensitivity. These features are the characteristics needed in the different synthesised fluorescent probes.

Furthermore I will propose to test firstly these technical aspects in "in vitro" models (different cell lines, artificially



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polluted), whereas in a second step of the project I will move on bioindicators of toxic polluted areas. This is the procedure normally used to test and eventually validate a fluorescent compound employed in biological fields.

In fact, before any future and desirable commercialization: the probes need to demonstrate not only their usefulness, the lack of an important toxicity and their easy application.

In this way, several Companies will be interested in optimization, commercialization of the probes, whereas waste treatment implants, industrial areas and polluted sea coast centers can equip themselves with a flow cytometer and/or a fluorescence microscope to perform their own controls