**TÍTULO DEL PROYECTO (ACRÓNIMO): Evaluación ecotoxico-ómica y multitrófica del riesgo ambiental asociado a los residuos metálicos de productos tecnológicos (E-waste) E-WASTERISK**

***TITLE OF THE PROJECT (ACRONYM):*** Ecotoxico-omic and multitrophic assessment of the environmental risk associated to metallic residues of technological products (E-waste)

E-WASTERISK

**IP 1** (Nombre y apellidos. *Name and surname*)**:** Julián Blasco Moreno

CVA: <https://cvn.fecyt.es/0000-0002-9750-383X>

Publications list: <https://orcid.org/0000-0002-9750-383X>

Summary:

Electrical and electronic products are part of our daily life since interaction with them occurs continuously. This situation has experienced notable growth in the last two decades with an increasingly digitized society, a period in which it is estimated that the increase in the growth of global consumption is being around 2.5 megatonnes (Mt) each year. As a result, the generation of waste from technological products, known by its acronym in English as E-waste (Electronic waste), represents an environmental problem of great concern and global relevance. Furthermore, many of these products contain metallic elements that are potentially toxic and can represent a health problem for ecosystems and ultimately for human beings. The evaluation of the toxicity associated with the contamination of aquatic ecosystems continues to be a scientific challenge since the substances are discharged -in general- in the form of complex mixtures with different modes of action and interaction. Although the risk assessment of pollutants is usually carried out based on information on individual compounds, this may imply a biased view, since the interactions between the components of mixtures may affect the risk. The general objective of this project is to develop an ecotoxicomic and multi-trophic approach to assess the risk of pollution associated with the production of electronic products and their E-waste. To do so, we will use different experimental approaches, including: (i) The selection of the most toxic individual elements present in E-waste and mixtures of associated metallic elements (Li, Hg, Cd, Ni, Co, Al, Mn, and rare earth elements (REEs)) by analyzing their toxicity on marine, pelagic and benthic microalgae. (ii) The molecular analysis of the selected elements/mixtures on the microbiome from waters, sediments and the Scrobicularia plana digestive gland by applying omic approaches (metagenomics and metaproteomics) at environmental exposure conditions. (iii) The implementation of new technologies (MALDI-Imaging) to spatially resolve molecular changes in S. plana due to these pollutants exposure, and the juxtaposition of these changes with histopathological traits of the clam. (iv) The effect of the selected metallic elements/mixtures on the behavioral responses, to move away and recolonize, of the crustacean Palaemon varians. Altogether, the results of this project will inform about the risks associated to the toxicity of E-wastes and will allow us to propose a comprehensive methodology for their evaluation. Our final (ultimate) goal is to generate a repository of valuable information that contributes to improving ecosystem management and implementing the most appropriate strategies to conserve biodiversity.

# ***OBJETIVES***

***General and specific objetives*.**

The general objective is to develop an ecotoxicomic and multi-trophic approach to assess the risk of the pollution associated to the production of electronic products, with the final purpose of contributing to improve ecosystem management and to conserve biodiversity.

The specific objectives are:

1. To design and apply different assays to evaluate the effect, individually and as mixtures, of associated metallic elements (Li, Hg, Cd, Ni, Co, Al, Mn, and rare earths (REEs)) on aquatic organisms belonging to different trophic levels.

2. To analyze the effect of individual elements and of their mixtures on marine, pelagic and benthic microalgae, and to understand the mechanisms involved in their toxicity and detoxification.

3. To analyze the impact of different elements, individually and as mixtures, on the environmental microbiome by applying different omic approaches (metagenomics and metaproteomics), in waters and sediments, and in the microbiome of the clam *Scrobicularia plana*, used as a relevant bioindicator organism.

4. To implement the application of new technologies (MALDI-Imaging) to assess the impact of the individual elements and their mixtures at the molecular level (proteomic/lipidomic) on the different tissues of the biomonitoring species *Scrobicularia plana.* To analyze the subcellular metal distribution in target tissues of the *S. plana.*

5. To examine the effect of individual elements and their mixtures on the behavioral responses, to move away and recolonize, of the crustacean *Palaemon varians*.

6. To model and prioritize the toxicity of individual elements and their mixtures considering metal speciation.

7. To analyze the risks associated to the toxicity of these elements and to propose a comprehensive methodology for their evaluation.

**Elements and mixtures**

Technological products contain toxic metallic elements and REEs in a higher proportion than other types of substances, and they are the objects of this proposal. As previously indicated, Li has been included because it is used in electric vehicle batteries, and consequently its possible release into the environment.

The metallic composition of E-waste varies widely depending on the investigated product (Chen *et al.,* 2021). The elements that have been selected correspond to the so-called “heavy metals”: Cu, Cd, Pb and Zn. In the case of rare earths, the lack of knowledge about these compounds, from the regulatory and environmental point of views, has been related to their limited industrial use. However, its presence in technological products urges the need to improve the knowledge that we have about them. The REEs used in technological products correspond to 15 elements of the lanthanide group: La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Y and Sc. All these compounds present high reactivity due to their electronic configuration. Among the most used ones in technological applications are La, Ce, Gd and Nd. In addition, Li will be included in the proposal among the elements to be tested for the reasons previously mentioned. However, other metals as Ni, Co, Al and Mn are present in the Li-battery and will be also included to assess the joint effect of leachable metals from Li-batteries.

The assays with each of the pelagic microalgae species will be carried out in a first phase by exposing them to the elements individually in a wide range of concentrations, to calculate the ECx (the effect concentration at which x% effect) of growth inhibition. The algal species showing the highest sensitivity will be selected for the second phase, where binary, ternary and quaternary mixtures of the REEs and other metals will be tested at EC10 concentration. We will proceed to identify the mixtures that show a greater toxicity to later make combinations between a heavy metal, a mixture of REEs and Li. This approximation will make possible to identify the mixtures that exert the greatest toxicity, which will be the object of study with the other experimental designs (*S. plana*/microbiome, molecular imaging; *P. varians*/non-forced assay). In addition, assays will be carried out with the mixtures that show the greatest toxicity at concentrations similar to those that occur in leachates from E-waste storage discharges, 5 μg/L (Brewer et al., 2022). To study the biological effects of real Li batteries, exposures will be made to the metallic component of their cathode (which in addition to Li contains Ni, Co, Al and Mn). Real samples from commonly used batteries will be provided by the FQM-175 group.

**Selection of organisms**

Microalgae

Microalgae are an essential trophic level in marine ecosystems, which respond sensitively when exposed to classic and emerging pollutants, and their mixtures. Algae have also shown differential responses when exposed individually or in mixtures to these pollutants.

Selected species are: *Phaeodactylum tricornutum* (Bacillariophyta) (CCMM 07/0402), commonly used in ecotoxicological tests (ISO 1995). Two other pelagic species are: *Nannochloropsis gaditana* (Eustigmatophyta) (CCMM 04/0201) and *Pleurochrysis cf. roscoffensis* (Prymnesiophyta) (CCMM 05/0701). In the case of the experiments to be carried out with a benthic microalga, a very sensitive species will be used: *Cylindrotheca closterium* (Bacilariophyta) (CCMM 07/0301). These species are part of the microalgae collection (CCMM) that is kept at ICMAN, and therefore they are continuously available.

Crustaceans

The crustacean *Palaemon varians* will be used (Leach 1814) (marine decapod). This species is found in areas of marshes, salt flats and pipes. The organisms will be captured in the Guadalquivir River, in the coastal marsh areas around the Bay of Cádiz or in marine culture facilities. The research group has experience in collecting this species, its maintenance under laboratory conditions and its use in bioassays (Araujo *et al*., 2019).

Mollusks

The bivalve mollusk *S. plana* (da Costa, 1778), whose habitat is the intertidal zone of soft, clayey, or muddy bottoms, with abundant organic matter, will be selected. It is an endobenthic bivalve widely distributed on the Atlantic and Mediterranean coasts. It has a key role in the structure and functioning of estuarine and coastal ecosystems and is an important link in the trophic webs of both systems. It also plays a critical role in the biogeochemical cycles of both nutrients and pollutants because of its bioturbation activity. This species is used both as a bioindicator in field studies (Sole *et al.* 2009) and in laboratory experimental designs to test the potential ecotoxicological risk of metals, metal nanoparticles, pharmaceutical compounds, microplastics, etc. (among others: Buffet *et al.*, 2013; Freitas *et al.*, 2016; Mouneyrac *et al.*, 2014; Ribeiro *et al.*, 2017). Our recent publication of the *S. plana* transcriptome has vastly widened the application of molecular approaches in this organism (Amil-Ruiz *et al*., 2021).

**Research team**

Dr. Julián Blasco. Member 1. PhD in Chemistry. Research Professor at the ICMAN-CSIC and PI1 of this proposal. He has a strong expertise in marine chemistry en ecotoxicology. He has experience in the use of analytical techniques for metal trace analysis and biomarker responses to environmental contamination (legacy and emergent pollutants). He has published more than 220 articles in international journals (mainly in Q1 journals. He has edited from books and he is Associate editor of journals Science Total Environment, Heliyon and Environmental Toxicology and Chemistry and member of editorial board of several journals

Dr. José Alhama. Member 3 PhD in Biology Sciences, Assistant Professor at the Department of Biochemistry and Molecular Biology of the UCO. Furthermore, he has been already accredited to Full Professor and will be defending the position next month. He will be dedicated full time to this project. He has strong expertise in the study of biomarkers sensitive to pollutants in different organisms, using conventional and -omic techniques, to study the biological effects of environmental stress. He brings also strong expertise in redox proteomics, and image-related massive approaches. He will be involved in all activities of the project, being responsible for the proteomic analyses. He has published 44 scientific papers in SCI journals including articles in Environmental Pollution, Science of the Total Environment, Microbial Biotechnology, Process Safety and Environmental Protection, Journal of Environmental Management, etc. He is also the coordinator of the BIO-187 group.

Dr. Ignacio Moreno. Member 4. Degree in Biology (specialty in Botany), PhD Marine Sciences, ‘Científico Titular’ at the ICMAN from 2008, Director of the Ecology and Coastal Management Department from March 2013 to June de 2019. Curator of the Culture Collection of Marine Microalgae. Wide experience in microalgal toxicity tests and the design of pioneer bioassays (for whole sediments, for immobilized microalgae, avoidance experiments). Use of flow cytometry. 89 publications: 60 SCI (45 of them Q1), 16 no-SCI and 13 book chapters. *h* index Scopus = 26; Total cites Scopus = 2320; *h* index Google Scholar = 29 (22 from 2018); *i*10 index Google Scholar = 56 (33 from 2018); Total cites Google Scholar = 3270 (1785 from 2018).

**Work-team**

Dr. Rosa Freitas. PhD. In Biology from University of Aveiro (2005). She is specialized in Ecology of Coastal Ecosystem and the effect of global changes in transition systems, her research is focused on the impacto f climate change and pollution in marine invertebrates with ecological and economic relevance. She is Auxiliary Professor in University of Aveiro and she has published 193 articles in international scientific journals including Environmental pollution, Aquatic Toxicology, Environmental Toxicology and Chemistry, etc.

Dr. Pedro M Costa. PhD. in Environmental Science from NOVA University of Lisbon (2010). Biologist specialized in Environmental Toxicology, dedicated especially to genotoxicology, toxicopathology and molecular toxicology. His main technical expertise molecular biology, bioinformatics, genotoxicology and, especially, histopathology. He founded and leads the SeaTox Lab@UCIBIO, based in the Department of Life Sciences of the School of Science and Technology of NOVA University of Lisbon, where he is an Auxiliary Professor. He has published 120 scientific papers in SCI journals including articles in Marine Drugs, Marine Environmental Research, Aquatic Toxicology, Scientific reports, Environmental Research, etc

Catia Gonçalves. MSc. in Environmental Engineering from NOVA University of Lisbon She has participated in both national and international research projects about the ecotoxicological effects of microplastics in marine organisms. In 2018, she joined the SeaTox Lab team where she has expanded her knowledge on toxicology, molecular biology and exploring new histochemical techniques. She is finishing her PhD on exploring new functions and biotechnological applications of cephalotoxins. She has published eight SCI research papers in journals as Marine Drugs, Frontiers in Marine Science, Histochemistry and Cell Biology, Environmental Pollution or Marine Environmental Research.

Dr. Almudena Benítez. PhD in Chemistry, Post-doct at the Department of Inorganic Chemistry and Chemical Engineering of the UCO. She will be dedicated part-time to this project. She is expertise in the study of graphene-based materials for the development of lithium-sulfur batteries. She will be involved in activities related to the selection of mixtures for the exposure assays, selection of residues from real lithium-based batteries and in the analyses of the exposure results. She has published 24 scientific papers in SCI journals including articles in One Earth, Sustainable Energy & Fuels, Journal of Cleaner Production, Journal of Energy Chemistry, Science of the Total Environment, etc.

Ana Patricia Rodrigo PhD. in Biotechnology. Expertise in chromatography (HPLC), toxicity assays, proteomics, histological techniques and light (bright field and fluorescence) and electron microscopy. She has also worked in the physiology of the production of bioactive compounds, the chemical characterization of substances and the assessment of their potential as biocides, cytotoxic agents, anti-proliferative properties and other issues of upmost relevance for drug discovery and application in biomedical research. She has published 13 SCI research papers in journals as Marine Drugs, Scientific Reports, Environmental Research or Frontiers in Marine Science.

Marina Barbudo. MSc. in Biotechnology from the Universidad de Córdoba. She has participated in both national and regional research projects about the pollution effects in marine organisms. She joined BIO187 team in 2021 where she has expanded her knowledge on omic techniques and its application in ecotoxicology studies. She is starting her PhD. and hopes to get a PhD. grant from the UCO. She has several international congress communications and two research MS under preparation.

Victoria Muñoz. She is responsible of the communication department of the ICMAN-CSIC. She has got experience in the preparation of scientific events and dissemination and divulgation activities.

Luis David Salvatierra. He is PhD student (FPU Junta Andalucía) the ICMAN-CSIC and he is working on the effect of the pollution on the avoidance and recolonization responses. He has published 4 articles in intenational journals (Science of the Total Environment, Chemosphere

Chiara Trombini. She is PhD in Marine Sciences. She is working as technician in the ICMAN-CSIC, currently. She has got experience in the use of HPLC-MS, HPLC-FL/UV-VIS and extraction and purification techniques and collection and preparation samples for microbiome analysis and molecular techniques (PCR, DNA and RNA isolation). She has published 20 articles in international scientific journals (Chemosphere, Aquatic Toxicology, Science of the Total Environment, Ecotoxicology and Environmental Safety, etc.)

María del Carmen Agulló. She is lab technician graduate with wide experience in the use of sampling treatment for metal analysis and quantification using ICP-OES and ICP-MS. She is responsible of these equipment within the platform PIAMEM of the ICMAN-CSIC for trace metal and nanoparticle analysis.

Antonio Moreno. He is a member of the Field Operations Unit, with extensive experience in field sampling of water, sediment and organisms. He is also a qualified boat skipper and professional diver.

# ***TRAINING CAPACITY***

***Training program planned in the context of the requested project***

A very relevant aspect is that advanced scientific knowledge on how pollution can affect aquatic ecosystems, generated in this project, can facilitate the training of personnel that can be incorporated during the execution of the project in powerful and state-of-the-art techniques and methodologies, thus facilitating their employment.

The PhD student will be enrolled in the PhD program “Biociencias y Ciencias Agroalimentarias” (Biosciences and Agro-Food Sciences) run by the University of Córdoba. The thesis will be in the research field “Biología Molecular de los Mecanismos de Respuesta a Estrés” (Molecular Biology of Stress Response Mechanisms), which is focused on the application and development of molecular techniques for the analysis of biological responses to environmental stress, e.g., pollution exposure. The PI2 (Carmen Michán) and one member of the research teams (José Alhama) are member and responsible, respectively, of this Ph.D. program.

The training activities of the Ph.D. program are: (1) A mandatory course of legislation related to the doctorate grade. (2) Participation in different local, national and international specific congresses, e.g., “Congreso Científico de Investigadores en Formación eidA3-ceiA3, UCO”, “Jornadas de Divulgación de la Investigación en Biología Molecular, Celular, Genética y Biotecnología”, “Congreso de la Sociedad Española de Toxicología AETOX”, SETAC Europe anual meeting, etc. (3) Specific courses selected by those proposed in the doctorate program distributed over a 3-year period, e.g., related with specific techniques, science communication, information management, bioinformatics, etc. Due to the objectives of the project, the Ph.D. student will be trained in analytical skills and high throughput techniques related with the measurement of legacy and emergent pollutants in different environmental compartments. For the use and application of analytical techniques ICP-MS, sp-ICP-MS, HPLC-MS will be trained in the ICMAN-CSIC, and for the molecular and imaging techniques plus the corresponding bioinformatic analysis in the UCO by the research team members. It should be mentioned that the Genomic, Proteomic and Bioinformatic units of the Central Services of the UCO (SCAI-UCO) and the Mass Spectrometry and Molecular Imaging unit of the IMIBIC (UCO-FIBICO-SAS) will participate in this project and have expressed their support (see supplementary material). Regarding to the courses offer of the Ph.D. program, this is renovated each year and the selection of the courses will consider his/her skills in relation to the tasks project.

In the frame of training activities -in high level international research groups- to complement his/her formation, one or two stages for a period of three months have been considered: Host #1, Associate Laboratory i4HB - Institute for Health and Bioeconomy, NOVA School of Science and Technology, NOVA University Lisbon, under the supervision of Prof. Pedro M. Costa. His research group have experience in the use of (tissue/organ/whole organism) spatially-resolved techniques to analyze the responses to the stressors. The stage of the Ph.D. student in this research group will contribute to improve his/her knowledge of different techniques for imaging analysis and the interpretation of the resulting histopathological changes. This professor and two members of this group will contribute directly to this project as members of the working team. Host #2, Maastricht MultiModal Molecular Imaging (M4I) institute, Maastricht University, under the supervision of Dr Tiffany Porta and Dr. Berta Cillero-Pastor. This group is leader in the MSI imaging fields and provides the opportunity to gain insights on different aspects, including instrumental development, sample preparation and quantification.