In situ estuarine sediment toxicity assessments: a multi-functional exposure approach

Susana Moreira[1,2], Matilde Moreira-Santos[3], Lúcia Guilhermino[1,2], Rui Ribeiro[3]

[1]CIIMAR, Centro de Investigação Marinha e Ambiental, Lab. de Ecotoxicologia, Rua dos Bragas 289, 4050-123 Porto, Portugal

[2]ICBAS, Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Depart. de Estudos de Populações, Largo Prof. Abel Salazar 2, 4099-003 Porto, Portugal

[3]IMAR, Institute of Marine Research, Departamento de Zoologia, Universidade de Coimbra, Largo Marquês de Pombal, 3004-517 Coimbra, Portugal

Phone: +00-(351)-239855781

E-mail: rui.ribeiro@zoo.uc.pt

Introduction: At present, there is a general consensus on the scarceness of available toxicological tools for ecological risk assessments in estuaries, particularly in what concerns sediment contamination [1]. In opposition to laboratory toxicity testing, in situ assays allow a more realistic exposure, generating more ecologically relevant information for the area under study [2]. In an effort to suit the current needs for toxicology tools specific for estuarine systems and considering that the vast majority of environmental decisions are still based on laboratory toxicity estimates, the main goal of this study was to develop and evaluate procedures specific for in situ estuarine sediment toxicity assessments based on a multi-functional exposure approach.

Methods: Short-term sublethal in situ assays were developed and evaluated using three species with different functions at the ecosystem level: the microalga *Phaeodactylum tricornutum* Bohlin as primary producer, the crustacean *Carcinus maenas* (L.) as consumer, and the polychaete *Hediste (Nereis) diversicolor* Müller as detritivore; either for sediment-overlying water or sediment toxicity assessments. The growth of *P. tricornutum*, immobilized in strontium alginate beads, and the postexposure feeding of both C. *maenas* and *H. diversicolor* were used as short-term individual-level endpoints. The effectiveness of the proposed in situ assays for routine assessments in estuaries was evaluated by deploying them at two reference and five impacted estuaries along the Portuguese coast.

Results and Discussion: In general terms, the assay chambers, with their simple and cost-effective design, and experimental procedures developed for each assay revealed to be suitable for exposing and retrieving the organisms in situ. Particularly, the strontium alginate beads used for microalgae immobilization were found to be appropriate to perform in situ assays, showing no signs of disruption or dissolution after up to eight days of exposure in estuarine sediment-overlying waters, and high organism recoveries were obtained in the in situ assays with *C. maenas* and *H. diversicolor* (from 89 to 100%). Strategies to distinguish a potential toxic effect from confounding factors associated with environmental parameters, by either providing similar conditions across sites or by using methodologies to remove the effect of environmental variables were also developed and successfully applied. This represent an important achievement since, as in any in situ assay but in particular for those developed specifically for highly dynamic systems such as estuaries, the establishment of causal links between exposure and effects may be largely restricted by confounding factors associated with the fluctuating environmental conditions existing in the field. A significant depression in *P. tricornutum* growth (from 24 to 48%), and in *C. maenas* and *H. diversicolor* postexposure feeding (from 16 to 73% and 17 to 90%, respectively) was consistently detected at all the impacted sites relatively to reference sites**,** supporting their responsiveness as sublethal toxicity endpoints. Due to their high effectiveness, the proposed in situ assays were shown to be valuable tools to be routinely employed within the context of risk assessment studies in estuarine areas, for the generation of important ecologically relevant data in a cost- and time-effective way.

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