Employment of marine protozoa to assess the quality of coastal and estuarine sediments.

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Introduction: Contamination of the aquatic environments by pollutants has become a serious problem in the recent years. Furthermore, it has been observed that the pollutant concentration in sediments and sediment's interstitial water can be more than 10-100 times higher than the one present in the overlying water column. The interest in the toxicity of pollutants occurrence in these matrices and their biological effects, has increased during the last decades. On this context, it is generally agreed that, in the environmental biomonitoring, biomarkers can provide information for pointing out those situations requiring a close investigation at an early stage [1]. From this point of view, it is of increasing interest to identify a set of organisms displaying sensitive responses to sediment and pore water from coastal environments under anthropic pressure. Thus, the possibility of utilizing *E. crassus*, an interstitial single-cell marine protozoa, was examined by applying a battery of tests including cell viability and growth as typical ecotoxicological high-level endpoints and utilizing biological sublethal parameters more sensitive such as lysosomal membrane stability and endocytotic rate, which are typical stress biomarkers in eukaryotic cells [2]. The sensitivity of *E. crassus* assay has been evaluated in a concentration range of two heavy metals (Cu and Hg), an organic pollutant – the benzo(a)pyrene – and their mixtures. In addition, considering the emerging issue of climate change impacts the effects of different levels of temperature and pH is considered.

Methods: The *cell viability* was assessed adapting the method described by Dondero et al. (2006). The alteration on the mean *replication rate* was performed according to Trielli *et al.*, [6]. *Endocytotic rate* was assessed as described by quantifying cellular intake of fluorescent bioparticles E. coli (Dondero et al. 2006). *Lysosomal membrane* stability was estimated with the retention time of Neutral Red (NRRT) using a modified of method described by Dondero et al. (2006).

Results: The results of the *E. crassus* cell viability and replication rate test are reported as compared to the control value (only artificial seawater): treated protozoa showed a decreasing trend in sub-samples exposed to increasing chemicals concentration (10−80%). At the same time, a significant reduction of lysosomal membrane stability (up to 70%) and endocytosis rate was observed (from 10 to 90%). Data for both sublethal and physiological parameters showed significant differences between the values measured even after the exposure of organisms to nano/micro molar concentration of heavy metals. The endocytosis rate data showed that there was a particularly strong decrease of organisms predation capability treated with Hg. The results obtained by the organisms exposed to binary mixtures showed different type of response: additive and/or synergic toxic effects on protozoa. The cumulative potential effects of different levels of T° and pH will be also evaluated.

Discussion: Results of laboratory studies indicated that estimated sub-lethal index of stress presented significant differences between reference and treated animals even after exposure to nano/micro molar trace elements concentrations. The endocytotic rate data showed a particularly strong decrease of predation capability in organisms treated with Hg. Sub-lethal tests, like endocytosis rates and lysosomal membrane stability, were able to detect biological effects of n/μM concentrations of toxicants. Furthermore, a significant synergic effect was observed on lysosomal membrane stability and replication rate of ciliate protozoa exposed to binary mixtures of selected toxicants. These results, confirming the suitability of using *E. crassus* as sentinel organism for monitoring sediment toxicity will be also discussed in relation to the effects of environmental parameters variations like temperature and pH[7].

References:

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