EVALUATION OF GENOTOXICITY OF SEDIMENTS FROM THE SADO-RIVER ESTUARY USING SOLVENT EXTRACTIONS OF DIFERENT POLARITIES

A. M. Vicente[1]; J. Sacadura[1]; M. Pinto[1]; H. Louro[1]; P. M. Costa[2]; S. Caeiro[2,3]; J. Lavinha[1]; M. J. Silva[1]

[1]Department of Genetics, National Health Institute Dr. Ricardo Jorge, I.P., Portugal; [2]IMAR, Department of Science and Environmental Engineering, Faculty of Science and Technology of the New University of Lisbon, Portugal; [3]IMAR, Department of Exact and Technological Sciences of the Portuguese Distance Learning University, Portugal.

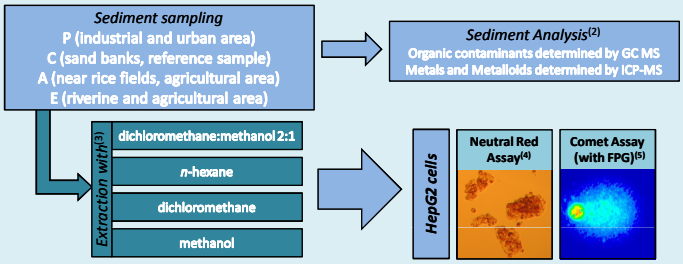
INTRODUCTION

The river Sado Estuary (SW Portugal) is affected by various sources of pollution, such as heavy-industry, urbanism, mining, agriculture and maritime traffic. Mostly classified as a natural reserve, it also remains a privileged site for fishing activities performed by the local population. Previous studies revealed sizable amounts of contaminants in the estuary sediments, namely metals, pesticides and polycyclic aromatic hydrocarbons(1). These compounds can be accumulated in the edible parts of estuarine species with commercial value or local agricultural products and enter the human food chain, posing a health problem, especially for the local community.

OBJECTIVES

The present study aims to assess the cytotoxic and genotoxic potential of Sado Estuary sediments following a fractioning method, in order to elucidate whether their toxicity can be attributed to a particular group of contaminants, or is rather the result of the complex interaction of contaminants.

METHODS



Positive and solvent controls were used in every assay.

CONTAMINANTS IN SEDIMENTS

➢ Sediment sample P was especially contaminated with moderate levels of PAHs (particularly acenaphthylene, acenaphthene, fluoranthene, pyrene, and dibenzo[a,h]anthracene) and metals (particularly As, Cu, Cr, Ni, Zn and Pb).

➢ Sediment samples E and A were especially contaminated with moderatelevels of metals (particularly As, Cr, Ni, Cu, Zn and Pb)

➢ Sample C, consisting of a sandy sediment, from an area with high oceanic influence, showed low levels of contaminants.

Data obtained from (2).

RESULTS - CYTOTOXICITY

Sample	Extraction Method			
	DCM:methanol (2:1)	DCM	*n*-hexane	methanol
C	-	-	-	-
P	+++	-	-	-
A	+++	-	-	-
E	+++	-	++	-

+++, POSITIVE - Significant dose-dependent decrease, > 2 significant doses.
++, POSITIVE - Significant dose-dependent decrease, high dose significant (≤ 2).
+, POSITIVE - No significant dose-dependent increase, ≥ 2 significant doses.
(+), EQUIVOCAL - No significant dose-dependent increase, 1 significant dose.
-, NEGATIVE.

➢ The highest cytotoxicity was observed for extract $P_{DCM/met}$ from 100 mg SEQ/ml ($p < 0.05$).

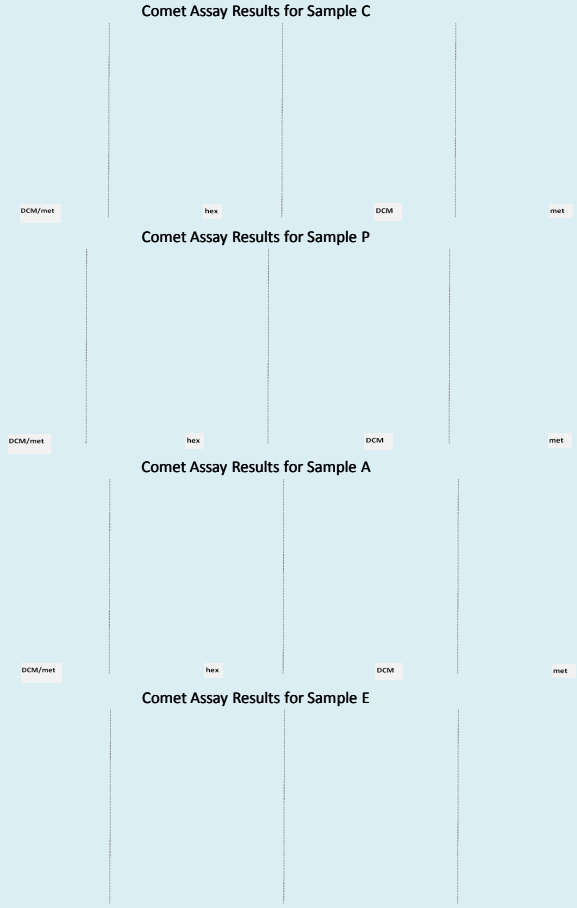
➢ Extracts $E_{DCM/met}$, $A_{DCM/met}$ and E_{hex} similarly reduced cell viability up to approximately 60% with statistical significance from 150 and 175 mg SEQ/ml ($p < 0.05$), respectively.

➢ Sediment sample C was not cytotoxic, as well as all DCM and methanol and *n*-hexane extracts (except E_{hex}).

➢ Significant dose response curve correlations (Spearman's *R*) for sediment extracts DCM/met ($p < 0.05$), ranked as: and P > E > A > C.

➢ Only extract concentrations yielding ≥ 50% cell viability were used in the genotoxicity assays.

RESULTS - GENOTOXICITY



* Statistical significant difference over the respective solvent control. Concentration 0 mg SEQ/ml refers to DMSO 2% v/v.

➢ Extract $P_{DCM/met}$, $E_{DCM/met}$ and E_{met} raised significantly the level of DNA damage, without FPG treatment, only at the highest concentration of 200 mg SEQ/ml ($p < 0.001$; p =0.006 and p = 0.041, respectively).

➢ Extracts $A_{DCM/met}$,$E_{DCM/met}$ and E_{met} exhibited similar patterns, inducing DNA damage, with FPG treatment, at concentrations 100 and 200 mg SEQ/ml (p = 0.001 and < 0.001; p = 0.006 and < 0.001; p = 0.036 and 0.002; respectively)

➢ Similarly, extracts $P_{DCM/met}$ and P_{met} induced DNA damage from concentrations 25 (p = 0.032 and 0.006 respectively) up to the highest tested concentration (100 and 200 mg SEQ/ml, with $p < 0.001$ and p = 0.001, respectively), whereas for extract P_{hex} only the highest tested concentration revealed DNA damage, with FPG treatment (p = 0.031).

➢ Extraction with *n*-hexane, for sediment samples E and A, failed to induce genotoxicity.

➢ Overall, all extracts from sample C, as well as all DCM extractions, failed to induce significant DNA damage in HepG2 cells..

CONCLUSIONS

➢ All sediment samples differ significantly, producing different patterns of cytotoxic and genotoxic effects in HepG2 cells, which is in accordance with sediment contamination analysis.

➢ We suggest that the presence of metals, PAHs and other organic contaminants are responsible for the observed effects, either by inducing genotoxic effects alone or as co-mutagens in a mixture.

➢ DCM and *n*-hexane (non-polar solvents) should be able to extract many organic compounds, mainly PAHs, which is compatible with the low levels of cytotoxicity. Nevertheless only extract P_{hex} revealed genotoxicity, which could reflect that the levels of PAHs present do not induce detectable genotoxic effects at the tested concentration range.

➢ Genotoxicity (particularly oxidative DNA damage) was observed with the methanol extraction (P_{met}, E_{met}) which, along with the contamination data, could suggest that these extracts might contain predominantly metals.

➢ Data indicates that the mixture of DCM:methanol ($P_{DCM/met}$, $E_{DCM/met}$ and $A_{DCM/met}$) might be the most appropriate solvent extraction to determine the overall effects of a complex environmental sample.

➢ The fractioning with solvents of different polarities was expected to allow to establish an association between a set of contaminants and its particular biological effects. However, possible interactions between contaminants might be responsible for the detected effects in DCM/met extracts, that were lost after fractioning.

➢ The use of a human cell line is a suitable model to survey the responses and effects of exposure to environmental pollutants and my be used to estimate the hazard to human health.

(1) Caeiro, S., Costa, M.H., DelValls, A., Repolho, T., Gonçalves, M., Mosca, A., Coimbra, A.P., Ramos, T.B., Painho, M., 2009. Ecological risk assessment of sediment management areas: application to Sado Estuary, Portugal. Ecotoxicology 18, 1165–1175.
(2) Carreira, S., Costa, P.M., Martins, M., Lobo, J., Costa, M.H., Caeiro, S., 2013. Ecotoxicological heterogeneity in transitional coastal habitats assessed through the integration of biomarkers and sediment-contamination profiles: a case study using a commercial clam. Arch Environ Contam Toxicol 64, 97-109.
(3) Srut, M., Traven, L., Stambuk, A., Kralj, S., Žaja, R., Mičović, V., Klobučar, G.I., 2011. Genotoxicity of marine sediments in the fish hepatoma cell line PLHC-1 as assessed by the Comet assay. Toxicol In Vitro 25, 308-314.
(4) Repetto, G., del Peso, A., Zurita, J.L., 2008. Neutral red uptake assay for the estimation of cell viability/cytotoxicity. Nat Protoc 3, 1125-1131.
(5) Dusinska, M., Collins, A., 1996. Detection of oxidised purines and UV-induced photoproducts in DNA of single cells, by inclusion of lesion-specific enzymes in the comet assay. Atla-Alternatives to Laboratory Animals 24, 405-411.

Acknowledgements
This work was supported by the Foundation for Science and Technology (ref. PTDC/SAU-ESA/100107/2008)