

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

A. M. Vicente<sup>1</sup>; J. Sacadura<sup>1</sup>; M. Pinto<sup>1</sup>; H. Louro<sup>1</sup>; P. M. Costa<sup>2</sup>; S. Caeiro<sup>2,3</sup>; J. Lavinha<sup>1</sup>; M. J. Silva<sup>1</sup>

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

## INTRODUCTION

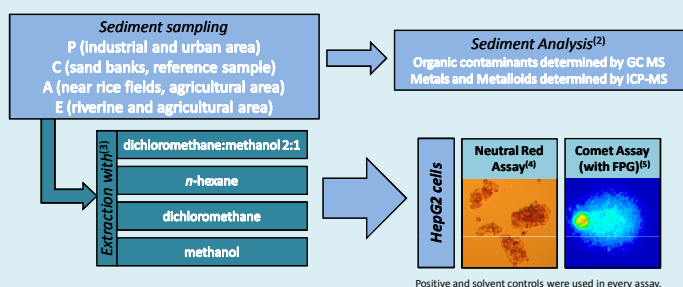
The river Sado Estuary (SW Portugal) is affected by various sources of pollution, such as heavy-industry, urbanism, mining, agriculture and maritime traffic. Mostly classified as a natural reserve, it also remains a privileged site for fishing activities performed by the local population. Previous studies revealed sizable amounts of contaminants in the estuary sediments, namely metals, pesticides and polycyclic aromatic hydrocarbons<sup>(1)</sup>. 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



## 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 moderate levels 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).

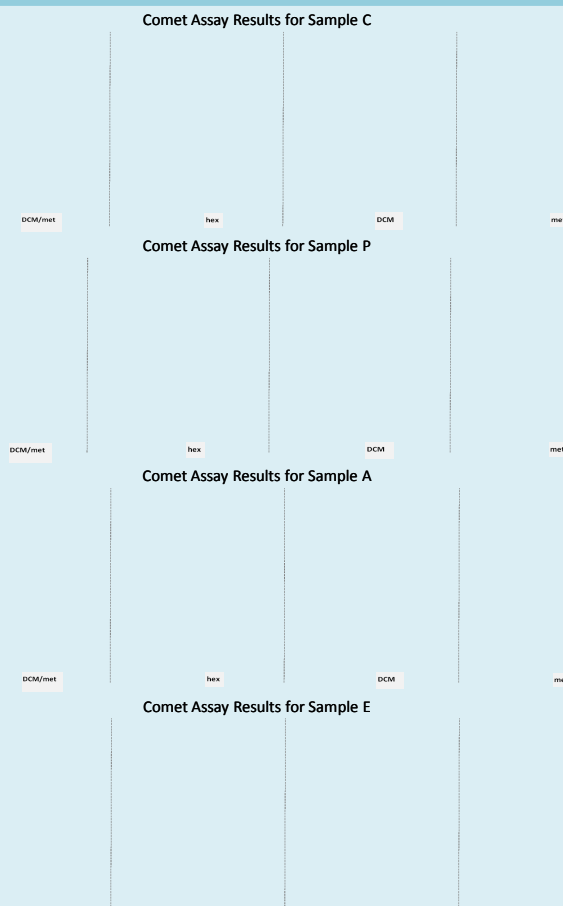
## RESULTS - CYTOTOXICITY

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<sub>DCM/met</sub> from 100 mg SEQ/ml (p < 0.05).
- Extracts E<sub>DCM/met</sub>, A<sub>DCM/met</sub> and E<sub>hex</sub> 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<sub>hex</sub>).
- 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<sub>DCM/met</sub>, E<sub>DCM/met</sub> and E<sub>met</sub> 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<sub>DCM/met</sub>, E<sub>DCM/met</sub> and E<sub>met</sub> 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<sub>DCM/met</sub> and P<sub>met</sub> 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<sub>hex</sub> 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<sub>hex</sub> 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<sub>met</sub>, E<sub>met</sub>) which, along with the contamination data, could suggest that these extracts might contain predominantly metals.
- Data indicates that the mixture of DCM:methanol (P<sub>DCM/met</sub>, E<sub>DCM/met</sub> and A<sub>DCM/met</sub>) 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 may be used to estimate the hazard to human health.

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