CYTOTOXIC AND GENOTOXIC POTENTIAL OF SEDIMENTS FROM THE PORTUGUESE MIRA RIVER ESTUARY

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INTRODUCTION

➢ The accumulation of environmental contaminants in estuary sediments can pose a public health problem, considering that 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.

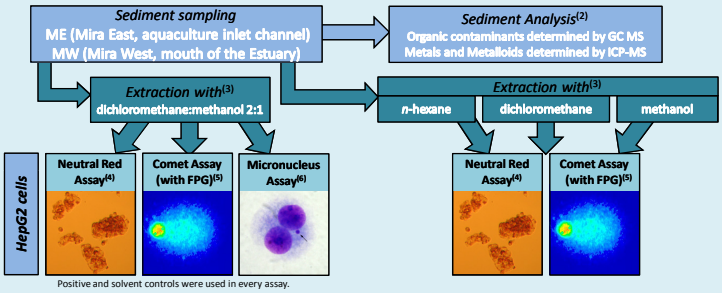
➢ As a case study we selected the Mira River Estuary, located in a protected area of the western coast of Portugal. Its main anthropogenic pressures arise from the small village of Vila Nova de Milfontes, located at the mouth of the estuary, with seasonal tourism, as well as activities related with agriculture, animal production, such as livestock and aquaculture, along the Estuary.

OBJECTIVES

➢ **To characterize the potential cytotoxic and genotoxic effects of Mira River Estuary sediments in a human cell line.**

➢ **To attempt to associate the type/level of contaminants in each sediment extract and the observed effects, in order to uncover its potential impact on human health.**

METHODS



Positive and solvent controls were used in every assay.

CONTAMINANTS IN SEDIMENTS

➢ Sediment sample ME was especially contaminated with metals (particularly As, Cu, Cr and Ni) as well as with moderate levels of organic contaminants.

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

Data obtained from (2).

RESULTS - CYTOTOXICITY

Sediment sample	Extraction Solvent	Cytotoxicity
ME	DCM:methanol (2:1) ($ME_{DCM/met}$)	+++
	n-hexane (ME_{hex})	++
	DCM (ME_{DCM})	-
	methanol (ME_{met})	+++
MW	DCM:methanol (2:1) ($MW_{DCM/met}$)	-
	n-hexane (MW_{hex})	-
	DCM (MW_{DCM})	-
	methanol (MWE_{met})	-

+++, POSITIVE - Significant dose-dependent increase, ≥2 significant doses.
++, POSITIVE - Significant dose-dependent increase, high dose significant.
-, NEGATIVE.

➢ The highest cytotoxicity was observed for extracts $ME_{DCM/met}$ and ME_{met}, from 100 and 50 mg SEQ/ml, respectively ($p < 0.05$).

➢ Extract ME_{hex} reduced cell viability up to approximately 65% with statistical significance from 175 mg SEQ/ml ($p < 0.05$).

➢ Sediment sample MW was not cytotoxic.

➢ Dose response curves yielded significant correlations (Spearman's *R*) for sediment ME extracts ($p < 0.05$), ranked as: and $ME_{met} > ME_{DCM/met} > ME_{hex} > ME_{DCM}$.

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

RESULTS - GENOTOXICITY

COMET ASSAY – SAMPLE ME

♦- Statistical significant difference between treatment with and without FPG, at the same concentration. ✱- Statistical significant difference over the solvent control (without FPG treatment). ▲ - Statistical significant difference over the solvent control (with FPG treatment). Concentration 0 mg SEQ/ml refers to DMSO 2% v/v.

COMET ASSAY – MW $_{DCM/met}$

MICRONUCLEUS ASSAY

*Statistical significance over control.
Concentration 0 mg SEQ/ml refers to DMSO 2% v/v.

➢ Extract $ME_{DCM/met}$ raised significantly the level of DNA damage at concentrations 25 and 50 mg SEQ/ml ($p = 0.020$ and $p = 0.015$, respectively), only after FPG treatment.

➢ Extracts ME_{hex} and ME_{DCM} exhibited significant DNA damage over solvent control, particularly at the highest concentration tested of 200 mg SEQ/ml, with and without FPG ($p < 0.05$).

➢ Overall, all extracts from sample MW, as well as extract ME_{met} failed to induce significant DNA damage in HepG2 cells.

➢ Extract $ME_{DCM/met}$ induced a 2-fold significant increase in micronucleus frequency.

➢ In contrast, extract $MW_{DCM/met}$ was unable to induce micronuclei above the solvent control.

CONCLUSIONS

➢ Both sediment samples differ significantly, presenting high to moderate (sample ME) and low to non-existing (sample MW) 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 and higher levels of DNA strand breakage (ME_{hex} and ME_{DCM}).

➢ High cytotoxicity but no genotoxicity was observed with the methanol extraction, which, along with the nature of the collection area, could suggest that this extract (ME_{met}) might contain unsurveyed products used, e.g. in aquaculture practices.

➢ Data indicates that the mixture of DCM:methanol ($ME_{DCM/met}$) might be the more appropriate extraction method to determine the overall effects of a complex environmental sample.

➢ The fractioning with solvents of different polarities might allow to establish an association between a set of contaminants and its particular biological effects.

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

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