Genotoxicity and oxidative stress induced by sediments from the Sado Estuary and potential antimutagenic effects of quercetin

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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

- **To study genotoxic effects of sediments from the Sado Estuary in a human liver-derived cell line**
- **To attempt to prevent induction of oxidative DNA damage with a co-exposure to quercetin, as an antioxidant.**

METHODS

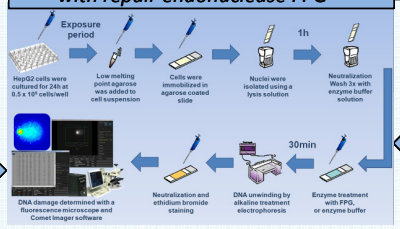
Sediment sampling
P (Industrial area, North)
C (Reference, North)
A (Rural/riverine area, South)
E (Rural/riverine area, South)

Extraction with(3)
dichloromethane:methanol (2:1)
Recovery in DMSO

HepG2 cells, 48h exposure
to non-cytotoxic concentrations
of sediment extracts

Sediment Analysis(2)
Organic contaminants determined by GC MS
Metals and Metalloids determined by ICP-MS

Alkaline Comet Assay
with repair endonuclease FPG(4)



If samples are positive for oxidative DNA damage (revealed by FPG)

48h Co-Exposure to Quercetin(5)

Positive and solvent controls were used in every assay.

SEDIMENT CONTAMINANT ANALYSIS

- Sediment sample P was especially contaminated with PAH (namely acenaphthene, pyrene, fluoranthene and acenaphthylene) and metals (namely As, Cr, Ni Cu and Pb).
- Sediment samples A and E were mainly contaminated with metals (particularly As, Cr, Ni, Pb and Zn).
- Sample C, consisting of a sandy sediment, in an area with high oceanic influence, showed low levels of contaminants.

Data obtained from (2).

GENOTOXICTY RESULTS

	Sample C (Reference)	*Sample P (Industrial Area)*	*Sample A (Rural Area)*	*Sample E (Rural Area)*
48h exposure to extracts				
48h co-exposure to extracts and quercetin				

0 mg SEQ/ml corresponds to 2% DMSO for all samples. ♦ - 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).

- Reference sample C failed to induce genotoxicity in both experimental conditions.
- Extract P significantly increased DNA damage particularly at concentrations of 50 and 100 mg SEQ/ml with FPG when compared with solvent control ($p < 0.01$)
- Extract A and E revealed a significant increase in DNA strand breakage at concentrations of 100 and 200 mg SEQ/ml with FPG treatment ($p < 0.01$)
- Co-exposure with quercetin did not reduce (and for P and E slightly increased) the overall strand breakage with and without FPG, when compared with the extract alone.

CONCLUSIONS

- **The differential results observed in samples from the northern (P) and southern areas (E and A) of the Sado Estuary probably reflect different pressures from an industrial-urban area *versus* an rural-agricultural area, respectively.**
- **Genotoxicity results are in accordance with sediment contamination, particularly in relation to the difference of organic contaminants (more present in sample P).**
- **Predominant oxidative DNA damage in samples E and A might be mediated by metal-induced oxidative stress.**
- **Sample C appears to be a good reference sample of the Sado Estuary.**
- **Co-exposure to quercetin failed to revert the observed oxidative DNA damage (revealed by FPG), and the slight increase in DNA strand breakage (P and E) might suggest potential interactions between the contaminants present in the sediments and the anti-oxidant.**

References

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