

# DNA AND CHROMOSOME DAMAGING EFFECTS IN MICE EXPOSED TO AN ESTUARY SEDIMENT EXTRACT

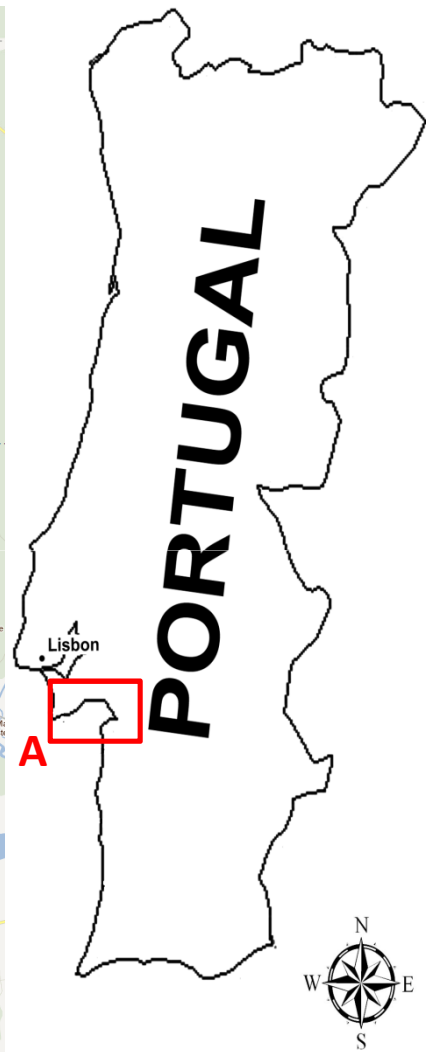
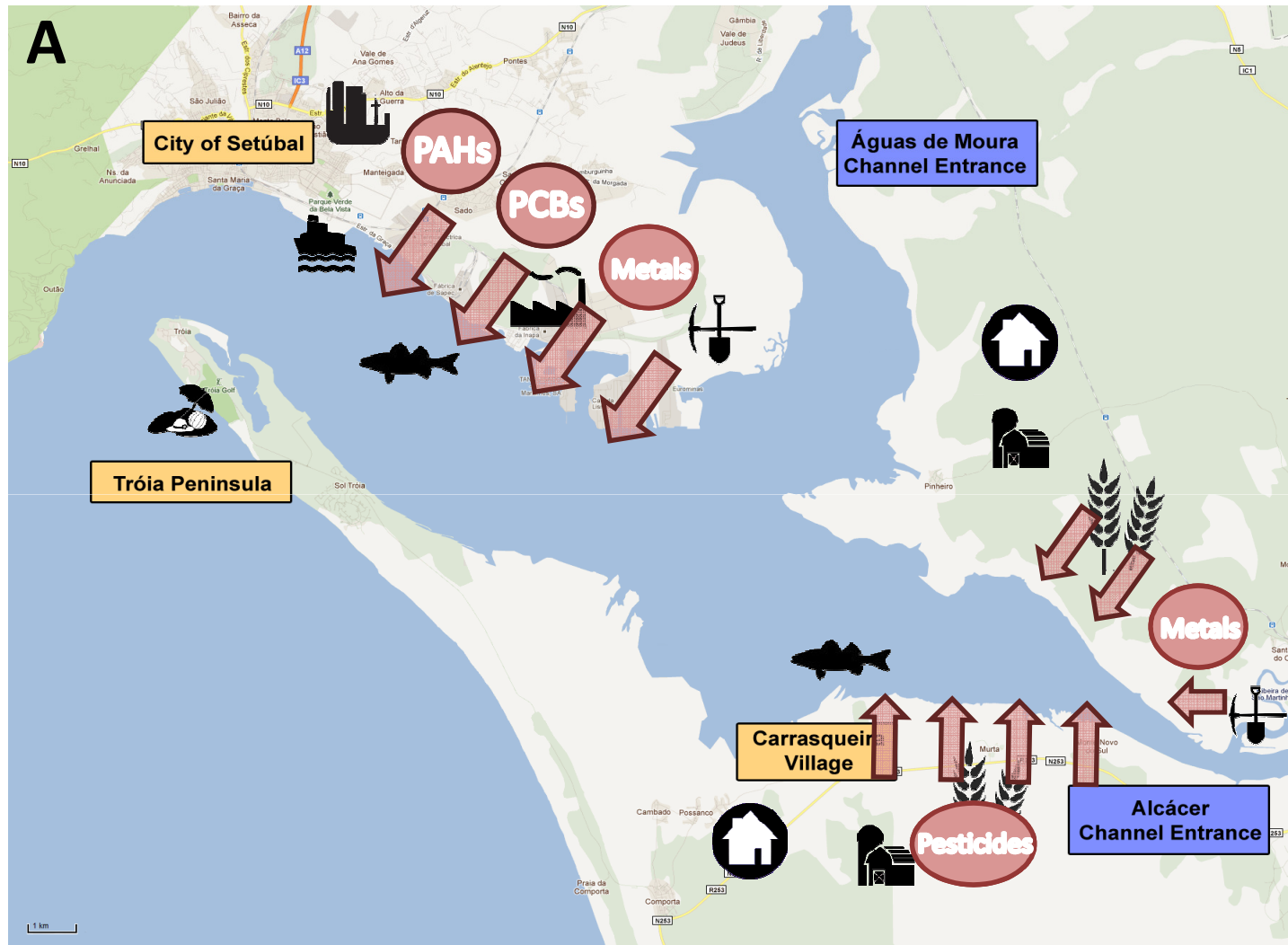
Miguel Pinto<sup>1</sup>; J. Sacadura<sup>1</sup>; H. Louro<sup>1</sup>; P.M. Costa<sup>2</sup>; J. Lavinha<sup>1</sup>; S. Caeiro<sup>3</sup>; M.J. Silva<sup>1</sup>

<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

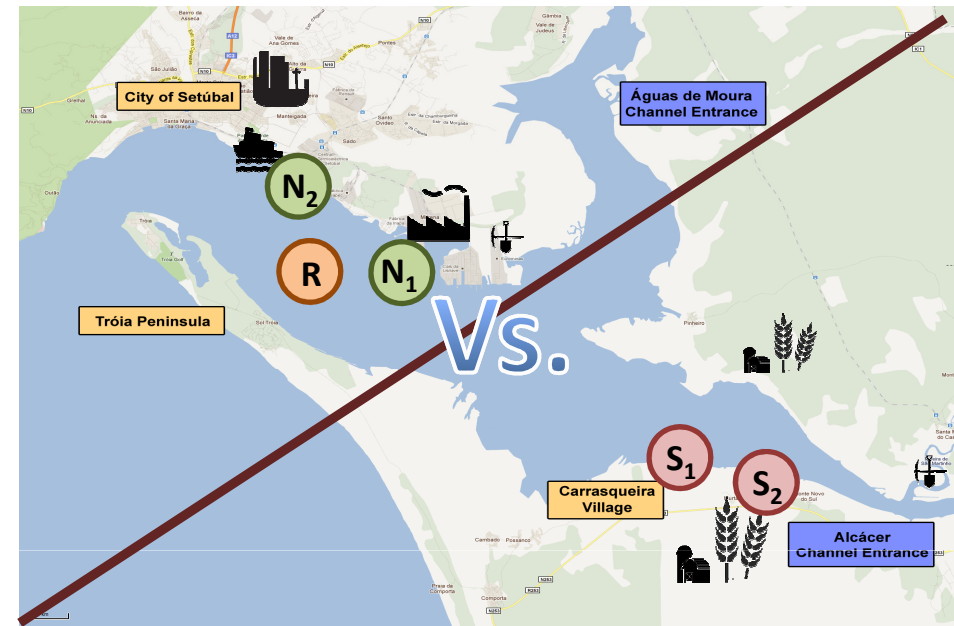
# Study Area – Sado Estuary



An estuary is a semi-enclosed maritime area with limited self renewal capability which makes it particularly capable of retaining contaminants from different sources.

# Background

- Recent studies characterized the cytotoxic and genotoxic effects of 5 sediment sample extracts in a human cell line (HepG2), through the neutral red assays and the comet and micronucleus assays, respectively.
- Reference sample (R), was mainly uncontaminated and failed to induce cyto- and genotoxicity.



## Northern Area

- Industrial and urban area
- Moderate levels of PAHs and metals
- Higher cytotoxicity
- Higher DNA strand breakage
- Higher micronuclei induction

## Southern Area

- Agricultural area
- Mostly contaminated by moderate levels of metals
- Lower cytotoxicity
- Higher oxidative DNA damage
- Lower micronuclei induction

## Main Objective

Assess the potential ecological and human health risk of a contaminated estuarine environment

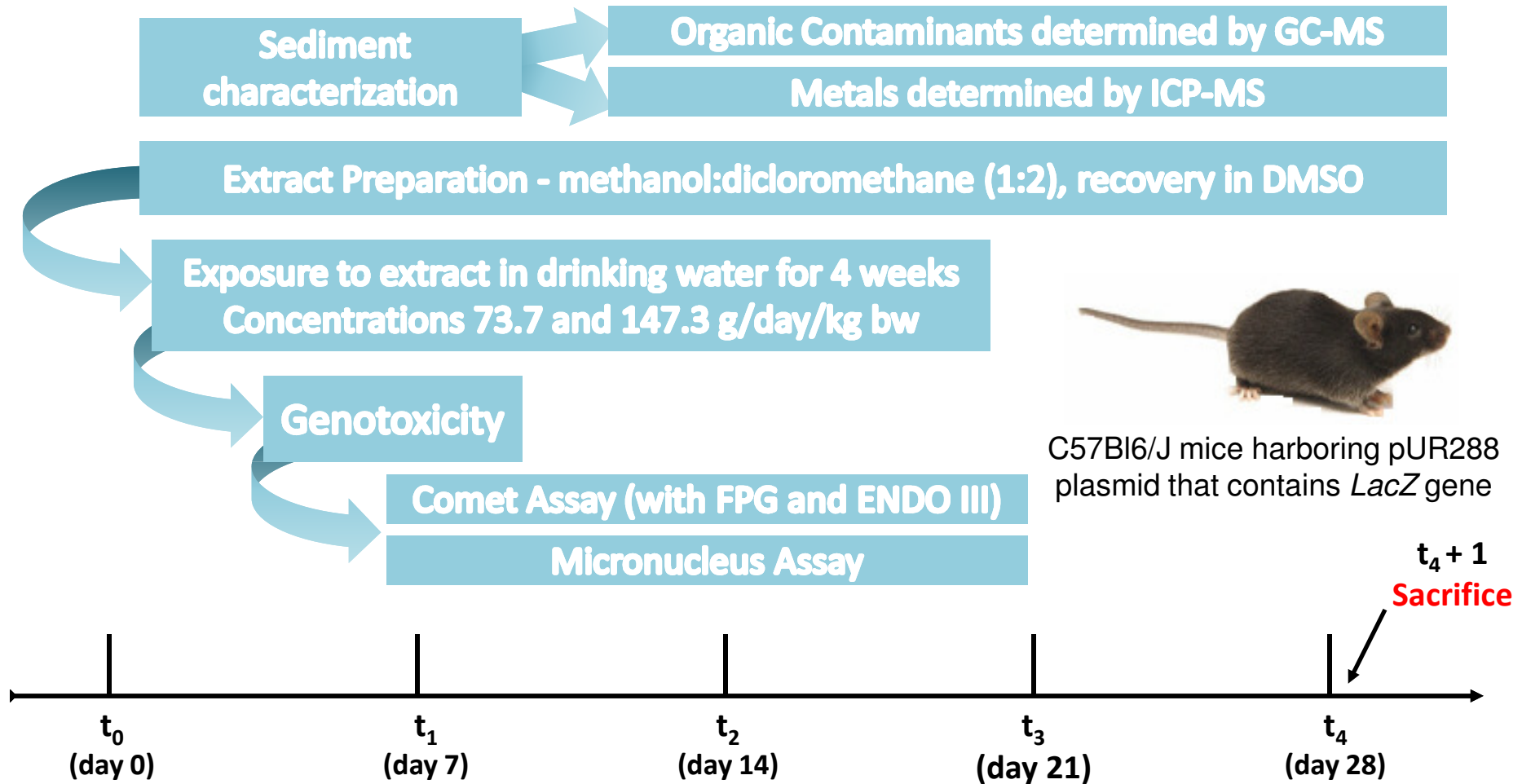
## Particular Objective

Assess the *in vivo* DNA and chromosome damaging potential of a contaminated sediment sample from a local fishing area of the Sado Estuary

# Sediment sampling



# Methods



- 3 groups of 6 mice were used: 1 exposed to DMSO only, and 2 groups exposed to sediment extract at two different concentrations.
- At every time point (t<sub>0</sub> – t<sub>4</sub>), mice were weighed and the comet and micronucleus assays were performed in tail blood.
- At sacrifice, comet assay was performed in liver, kidney and spleen cells.

# Results

---

# Sediment Contaminant Analysis

		Site
		N1
TOM (%)		10.4 ± 0.0
FF (%)		52.9
Contaminant		
Inorganic (µg.g <sup>-1</sup> )		
Non-metal	As	<b>19.7 ± 5.21</b>
	Se	1.92 ± 1.45
Metal	Cr	<b>77.67 ± 4.57</b>
	Ni	<b>16.67 ± 1.1</b>
	Cu	<b>178.64 ± 7.01</b>
	Zn	<b>327.51 ± 1.16</b>
	Cd	0.27 ± 0.03
	Pb	<b>56.45 ± 3.1</b>
Organic (ng.g <sup>-1</sup> )		
PAH		
3-ring	Acenaphthylene	<b>9.77 ± 1.66</b>
	Acenaphthene	<b>9.00 ± 1.53</b>
	Fluorene	8.41 ± 1.43
	Phenanthrene	66.02 ± 11.22
	Anthracene	9.00 ± 1.53
4-ring	Fluoranthene	<b>207.29 ± 35.24</b>
	Pyrene	<b>175.12 ± 29.77</b>
	Benz[a]anthracene	72.16 ± 12.27
5-ring	Chrysene	40.88 ± 6.95
	Benzo[b]fluoranthene	78.17 ± 13.29
	Benzo[k]fluoranthene	49.74 ± 8.46
	Benzo[e]pyrene	70.04 ± 11.91
	Benzo[a]pyrene	86.29 ± 14.67
6-ring	Dibenzo[a,h]anthracene	<b>15.09 ± 2.57</b>
	Indeno[1,2,3-cd]pyrene	101.85 ± 17.31
	Benzo[ghi]perylene	78.14 ± 13.28
tPAH		1.076.98 ± 183.09
PCBs		
tPCBs		5.37 ± 0.91

➤ Moderate levels of metals were also observed (particularly for As, Cr, Ni, Cu, Zn and Pb).

➤ Sediment sample was contaminated with moderate levels of PAHs (particularly acenaphthylene, acenaphthene, fluoranthene, pyrene, dibenzo[a,h]anthracene).

➤ Global evaluation of this sample indicates moderate contamination.



# Conclusions

---

- ✓ Exposure to sediment extract at both tested concentration failed to induce DNA strand breakage and oxidative DNA damage as assessed by the comet assay, in blood and organ cells.
- ✓ Failure to observe DNA damage might be due to DNA repair mechanisms overcoming possible damage, or to the length of exposure period.
- ✓ A significant increase in micronuclei was observed for both exposed group in blood cells, when compared with the control group at different time-points.
- ✓ Data indicates an increase in chromosomal damage along the exposure period, for both exposed groups.
- ✓ This sediment extract was able to induce genotoxicity, nevertheless more studies are need in order to further assess other potential damaging effects.

## Ongoing studies

---

- Analysis of mutation frequencies in *lacZ* gene in liver, spleen, kidney and testis.
- Histological biomarkers in liver, kidney, testis and stomach tissues.

# Acknowledgements



Maria João Silva  
João Lavinha  
Henriqueta Louro  
Joana Sacadura

Sandra Caeiro  
Maria Helena Costa  
Pedro Costa  
Sara Carreira  
Jorge Lobo

# FCT

Fundação para a Ciência e a Tecnologia  
MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E ENSINO SUPERIOR

Work supported by the Foundation  
for Science and Technology  
(ref. PTDC/SAU-ESA/100107/2008)

# Thank you for you attention!