IDENTIFYING HAZARDOUS SUBSTANCES IN UK MARINE SEDIMENTS

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Under the OSPAR Hazardous Substances strategy, the UK is committed to the continuous reduction of discharges, emissions and losses of hazardous substances with the ultimate aim of achieving concentrations in the marine environment to near background values for naturally occurring substances and close to zero for man-made synthetic substances. In order to assess the risk posed by hazardous substances present in marine sediments, investigations are required to characterise the most toxicologically significant substances present. This task can represent finding the proverbial needle in a haystack, since, in many industrially impacted areas, estuarine sediments contain a complex mixture of natural and synthetic substances. To help the process, toxicity identification evaluation (TIE) procedures have been developed to isolate and characterise the biologically active compounds present (Figure 1).

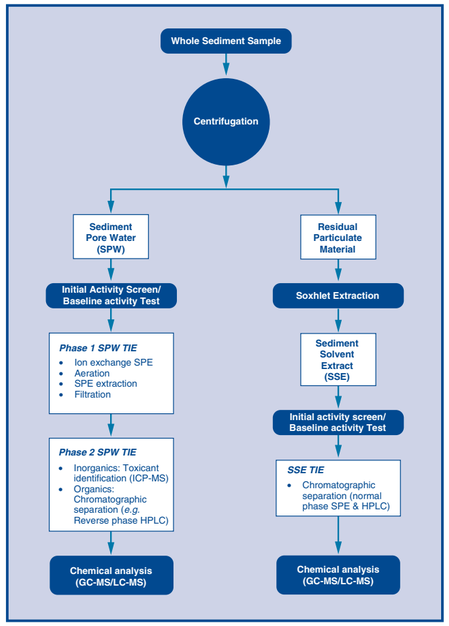

Figure 1 Fractionation scheme for the characterisation of marine sediments

Method validation

The system was evaluated using a series of reference compounds of widely differing polarity and biological activity (Table 1).

Table 1 Reference compounds used to validate sediment TIE protocol

Compound	log K_{OC}	log K_{OW}	Spiked concentration (µg/L)	Expected main effect
phenanthrene	4.6	4.5	40	Mutagenic
TBTO	3.3	3.2	5	Toxic
nonylphenol ethoxylates	4.6	4.5	100	Oestrogenic
nonylphenol	4.2	4.1	100	Oestrogenic
Arochlor 1254	6.2	6.1	50	Mutagenic
oestradiol	3.2	3.1	1	Oestrogenic
cypermethrin	4.5	4.5	20	Toxic
pentachlorophenol	5.3	5.2	10	Toxic
benzo(α)pyrene	5.5	5.4	20	Mutagenic
zinc (sulphate)	-	-	1000	Toxic

Sediment pore water

As a first step in attributing causality, sediment pore waters were screened for toxic, oestrogenic and genotoxic activity (Table 2).

Table 2 Biological response of sediment pore water/sediment pore water extracts

Sample	T. battagliai 48 h TU	YES assay	Mutatox
Spiked pore water	1.2	positive	positive
Tyne pore water	-	negative	negative
Tees pore water	10	positive	negative

Toxic Unit (TU)= 100%/LC_{50}

Where activity was demonstrated, the sample was subjected to a number of simple manipulations to establish the nature of the active compound:

- Filtration to establish whether the active agent was associated with particulate material.
- Aeration to determine how much sample activity can be attributed to volatile compounds.
- C18 SPE to determine the extent of sample activity caused by organic compounds.
- Ion exchange SPE to determine the extent sample activity was caused by certain cationic metals.

Ammonia was determined in samples that were toxic to *T. battagliai*.

To date, the majority of the activity has been associated with organic compounds extracted by C18 SPE. These extracts were then fractionated by reverse phase HPLC (Figure 3) to produce thirty fine fractions. These fine fractions were then tested using the appropriate bioassay (Figure 4). Gas chromatography-mass spectrometry was then used to identify the cause of effect.

Figure 3 HPLC fractionation

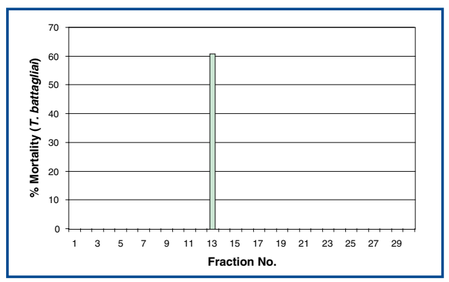

Figure 4 Toxicity of Tees pore water fractions to T.battagliai

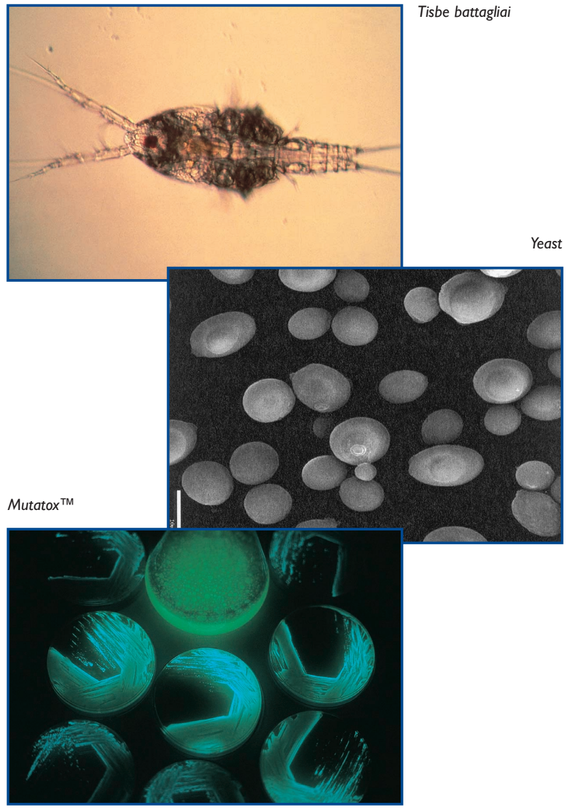

Figure 2 Bioassays used in this study

The TIE procedures have been combined with an acute bioassay using a marine copepod (*Tisbe battagliai*), mutagenic assay (Mutatox™) and an *in vitro* yeast-based screen for oestrogenic activity (Figure 2). The range of endpoints selected allows the procedure to characterise a broad range of compounds with defined biological effects.

Sediment particulate material

The residual particulate material was soxhlet extracted with dichloromethane (DCM) and tested for oestrogenic and genotoxic activity (Figure 2; Table 3).

Table 3 Biological response of sediment extracts

Sample	YES assay	Mutatox
Spiked sediment	positive	positive
Tyne sediment	positive	positive
Tees sediment	positive	positive

Where activity was demonstrated, normal phase SPE (Table 4) and a combination of normal and reverse phase HPLC was used to fractionate the extract (e.g. Figure 5 and 6). GC-MS was used to identify the causal agents.

Table 4 Oestrogenic and mutagenic activity of coarse sediment extract fractions

Sample	Si SPE Fraction	YES assay result	Mutatox result
Spiked sediment	F1 (hexane)	negative	positive
	F2 (DCM)	positive	positive
	F3 (acetone)	positive	positive
	F4 (methanol)	negative	positive
Tyne (Howdon)	F1 (hexane)	negative	positive
	F2 (DCM)	positive	negative
	F3 (acetone)	positive	negative
	F4 (methanol)	negative	positive
Tees (Dabholm Gut)	F1 (hexane)	negative	positive
	F2 (DCM)	positive	negative
	F3 (acetone)	positive	negative
	F4 (methanol)	negative	positive

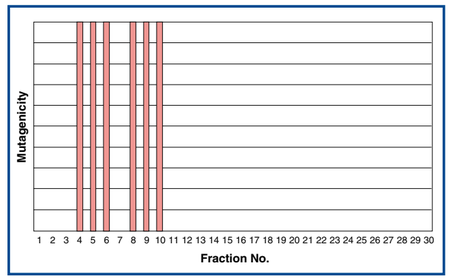

Figure 5 Mutagenic fractions following the normal phase HPLC of Tees sediment extract (F1 coarse fraction)

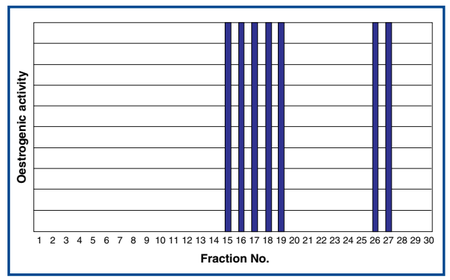

Figure 6 Oestrogenic fractions following the reverse phase HPLC of Tees sediment extract (F3 coarse fraction)

This approach allows compounds with a demonstrated hazard in marine sediments to be identified. The data generated by this study will be fed into the Dynamic Mechanism for Selection and Prioritisation of Hazardous Substances (DYNAMEC) as part of the UKs commitment to OSPAR.

Acknowledgement

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