Centre for Environment Fisheries & Aquaculture Science

Cefas

Tees Sediment Toxicity Testing – Opportune ecotoxicology Microtox testing of Tees Bay Disposal Sites

CSEMP 2021 Survey on Cefas RV Endeavour: Trial of onboard ecotoxicology testing using the Microtox test

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0.1	Redacted		
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1. Introduction

Estimates of the number of chemicals currently in use globally range from around 8,000 to 350,000 and upwards (Erickson, 2017, Wang et al., 2020). It is not possible to chemically analyse samples for all of those chemicals, and so it is commonly accepted to look for known priority chemicals[1]. Babut et al. (2006), working in the freshwater environment, stressed the importance of using a combination of chemical analysis, bioassays, and other toxicity biotests in order to assess the ecological risk of dredged sediments. Toxicity testing has the advantage of measuring the impact of all the chemicals present, and giving an indication of the sum effect, although it does not indicate which chemicals are having the largest impact. During the CSEMP[2] 2021 survey (Figures 1 and 2) in April 2021, Cefas took the opportunity to look at the toxicity of samples already being collected for chemical analysis, using mobile laboratory equipment. The equipment enabled Cefas to carry out an acute screening test, Microtox, aboard the RV Endeavour. It's a well-researched, rapid, test (e.g. Doherty, 2001, Maisto *et al.*, 2011., Pereira *et al.*, 2017), and the portable Microtox FX luminometer was used, as well as associated consumables.

[1] This is common practice across regulatory bodies, such as the OSPAR List of Chemicals for Priority Action (available here: https://www.ospar.org/work-areas/hasec/hazardous-substances/priority-action) and the UK Environment Agency's list of chemicals assessed under the Water Framework Directive (available here: https://www.gov.uk/government/publications/list-of-chemicals-for-water-framework-directive-assessments).

[2] Clean Seas Environment Monitoring Programme, more information available here: https://www.bodc.ac.uk/projects/data_management/uk/merman/project_overview/

2. Methods and Results

The Microtox rapid toxicity detection system is an in vitro test. It uses bioluminescent *Aliivibrio fischeri* bacteria for the detection of toxicity and is used as a screening system to detect the relative toxicity of environmental samples. It responds to chemicals or combinations of chemicals that are toxic to cells or reduce their speed of replication. The test measures reduction in light production by the bacteria as a proxy for reduction in population size. As it is a screening test, it provides an indication on the level of toxicity and not what is driving the toxicity i.e., which chemicals are present.

During the survey, Cefas ran 60 samples of sediment through the Microtox assay. The results are not all from different sites, some are different grabs at the same site whilst some are replicates from the same grab. All the data collected is presented in this report.

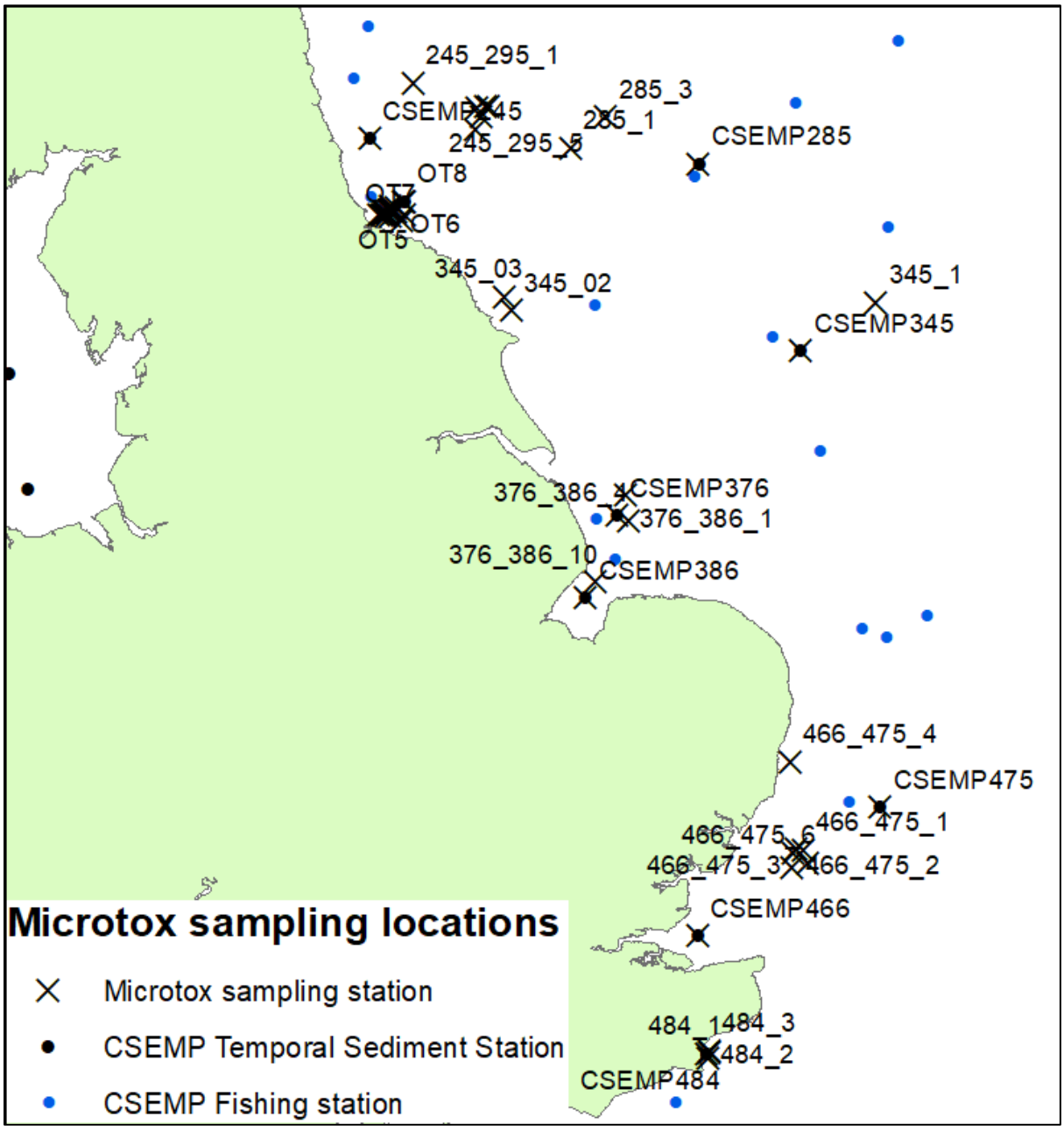


Figure 1. Microtox sampling stations collected onboard CSEMP 2021 – Inner Tees and CSEMP samples, and subsequently from the Outer Tees Disposal monitoring survey HG0121. For Outer Tees and Inner Tees detailed map please see Figure 2

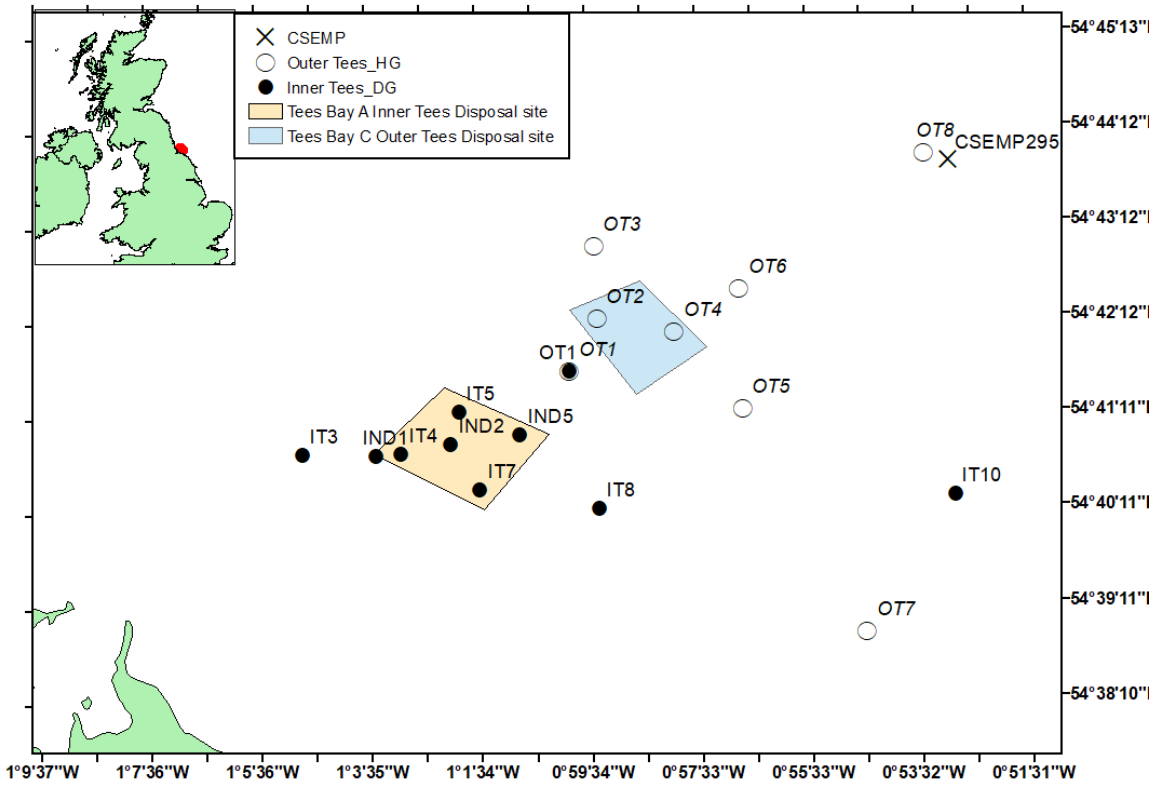


Figure 2. Microtox sampling stations at Inner Tees (collected with day grab(Inner Tees_DG)) and Outer Tees (collected with Hamon grab (Outer Tees_HG)) disposal sites

2.1. Tees Sediment Laboratory Testing using Microtox.

The Microtox assay was also used to assess sediment samples collected from the Outer Tees area (Figure 2) and stored at Cefas laboratory. These were analysed in the laboratory and followed the same process as the used in the testing on the RV Endeavour.

2.2. Method: Toxicity test using *Aliivibrio fischeri*

The methodology used in the *A. fischeri* testing that Cefas carried out follows the principles of ISO 11348-3 (2007) but is adapted for use with the field portable Microtox FX test system and sediment sample elutriates following the instructions of the manufacturer through the 81.9% basic test protocol (Modern Water, 2013). For the analyses carried out on the RV, with the equipment and space available, Cefas opted to work with a simple Water Accommodated Fraction (WAF) approach to assess the bioavailable sediment toxicity. Three grammes of sediment were manually shaken up in 7 mls of water for 30 seconds. These

were allowed to settle until the water was visually clear. The overlying water was then used as the sample in the Microtox test process.

An aliquot of lyophilised *A. fischeri* culture (Microtox Solo reagent, Modern Water plc) is reactivated and added to each empty test vial. The light output from bacteria in these vials is recorded and then 900 µl of sample or control media is added to the vials and they are left to incubate for 15 minutes at ambient temperature, after which time the light output is read again. The luminosity change of each sample is then measured and compared to the luminosity of the control, with the difference expressed as a positive or negative percentage difference from the control.

If the mixture present in the sample has an overall inhibitory effect, leading to less growth of the A. *fischeri* bacteria, then this will lead to reduced luminescence which will be reported as a light loss compared to the control. This can be considered as toxic inhibition of growth.

2.3. Results

60 samples were analysed during the CSEMP 2021 survey. 46 have been shown in figure 3, with replicate samples from the same sites removed to reduce complexity in the figure. All removed sample data would have been green bars in the figure.

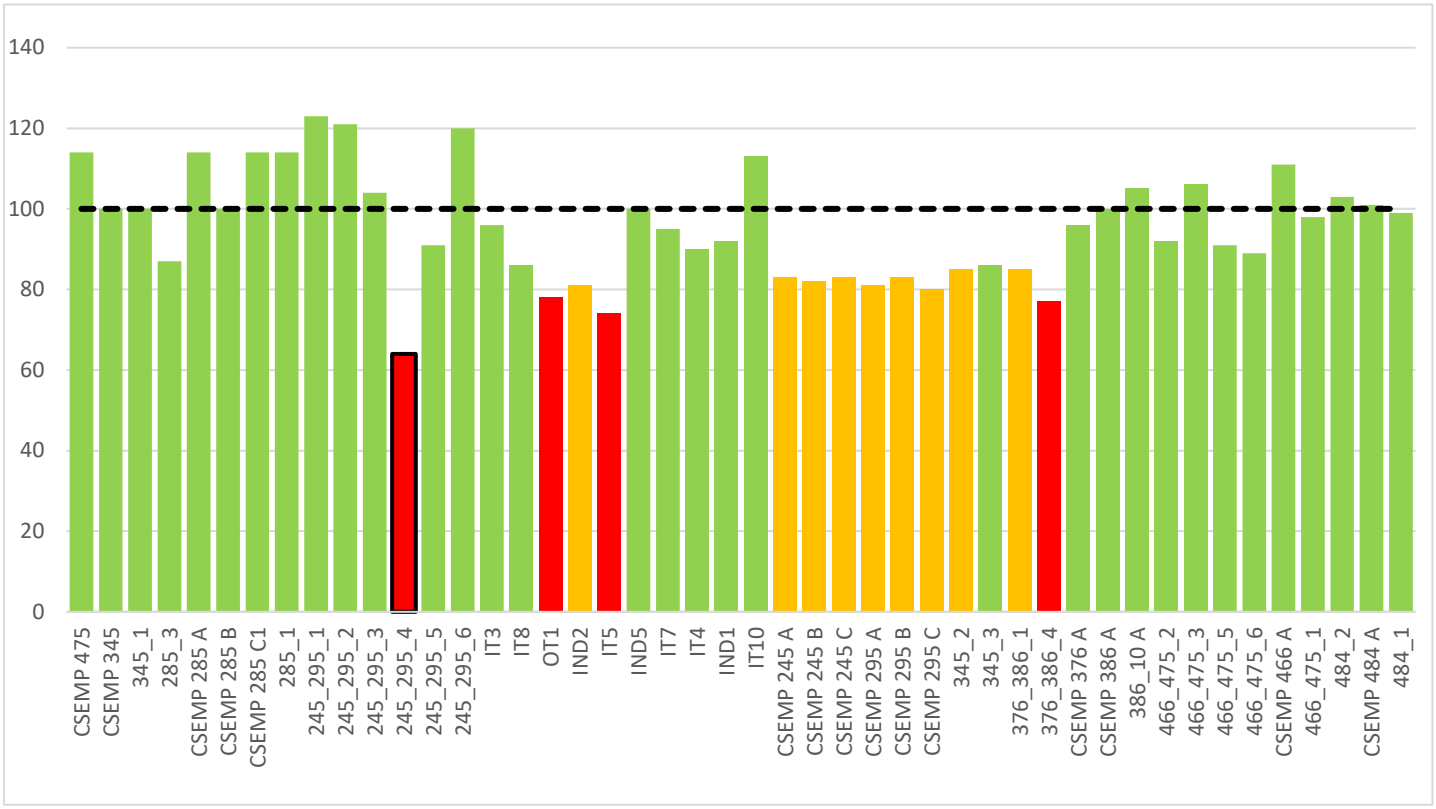


Figure 3. Samples analysed during the CSEMP 2021 Survey. Y axis shows % expected luminescence. Normal growth is represented here as >85%% expected luminescence. Values over 100% indicate where bacteria

have grown more than expected in the samples possibly due to extra nutrients from the sediment. There is an amount of variation in the response for the test, so we have indicated samples showing a 15-20% reduction in expected light production as amber bars, and those with more than 20% reduction with red bars. This is a purely nominal pair of thresholds to allow the most affected samples to be identified easily.

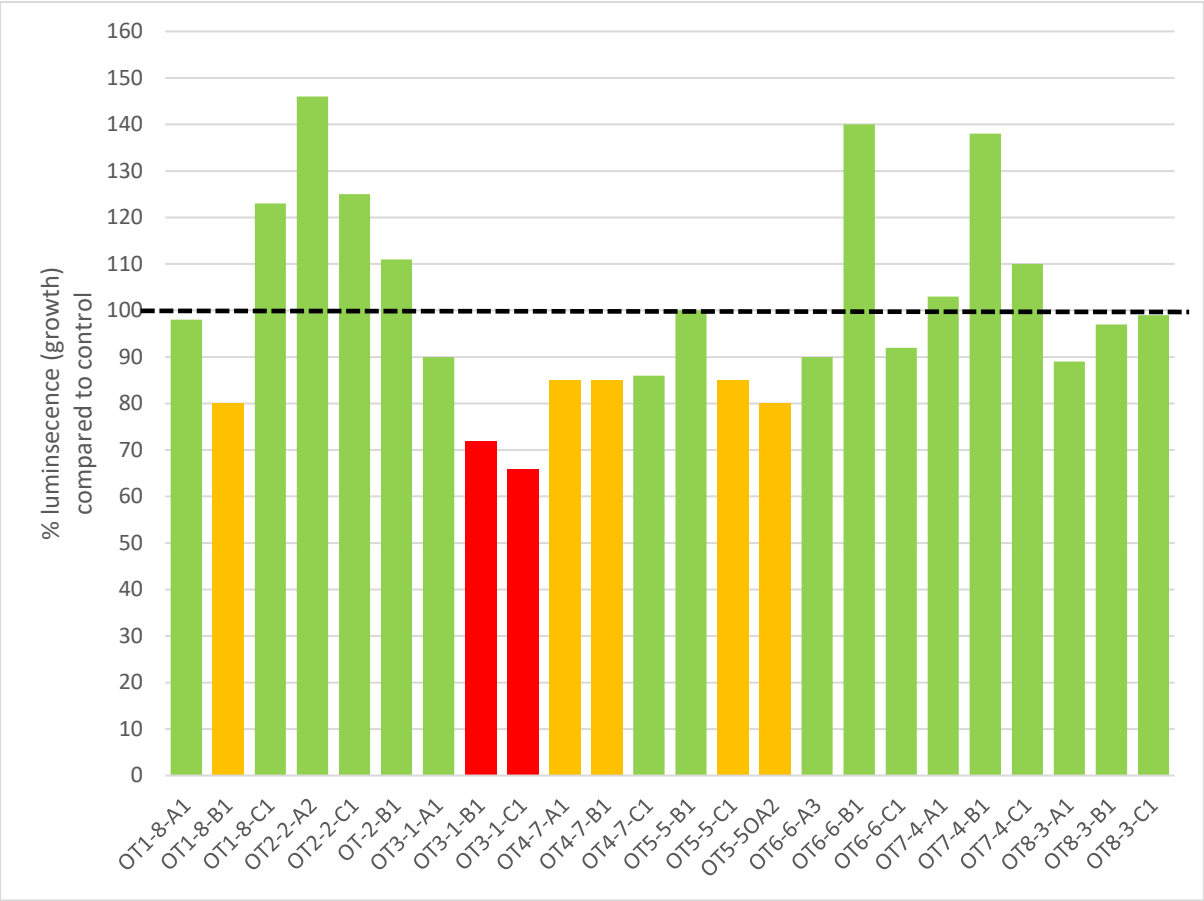
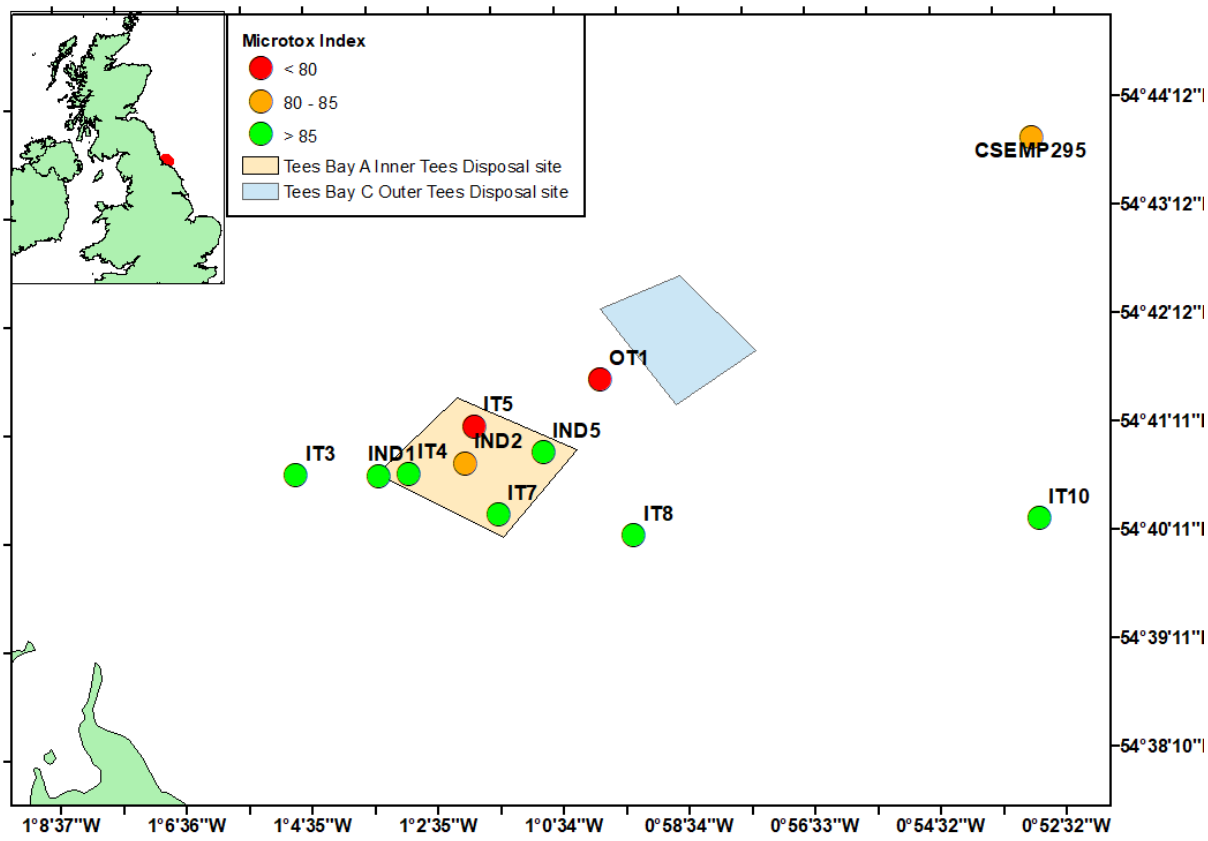


Figure 4. Outer Tees sediment samples analysed at the Cefas laboratory Normal growth is represented here as >85%% expected luminescence. Values over 100% indicate where bacteria have grown more than expected in the samples possibly due to extra nutrients from the sediment. There is an amount of variation in the response for the test, so we have indicated samples showing a 15-20% reduction in expected light production as amber bars, and those with more than 20% reduction with red bars. This is a purely nominal pair of thresholds to allow the most affected samples to be identified easily


Microtox Index
< 80
80 - 85
> 85
Tees Bay A Inner Tees Disposal site
Tees Bay C Outer Tees Disposal site
CSEMP295
OT1
IT5
IND5
IND2
IT3
IND1
IT4
IT7
IT8
IT10
54°44'12"
54°43'12"
54°42'12"
54°41'11"
54°40'11"
54°39'11"
54°38'10"
1°8'37"W
1°6'36"W
1°4'35"W
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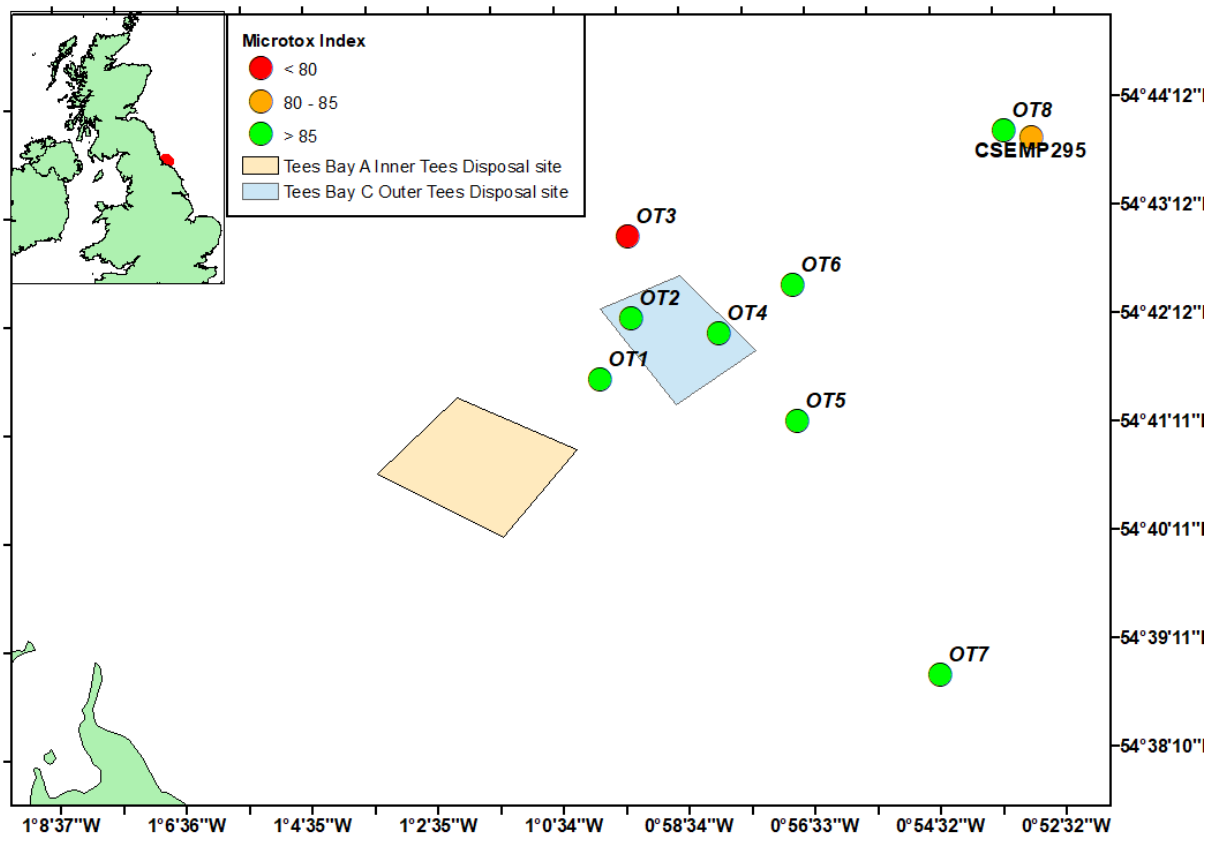
Microtox Index
< 80
80 - 85
> 85
Tees Bay A Inner Tees Disposal site
Tees Bay C Outer Tees Disposal site
OT8
CSEMP295
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OT6
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OT4
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OT7
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3. Discussion

The CSEMP samples were taken purely as a research experiment to investigate whether real time toxicity analysis at sea is feasible. The Outer Tees samples were taken for other purposes and the samples at the Cefas laboratory were subsequently also tested using the Microtox method. Samples were only analysed once each, so no within-sample variation is presented. These data were generated as screening tests to find some rapid indication of differences between sites, as well as whether sediment toxicity could be detected using such a quick and simple 'extraction' approach.

Sediment samples potentially contain a mixture of chemicals, and these will have complex interactions with each other, with the sediment, and with any organisms that come into contact. Some chemicals will be strongly associated with organic material in the sediment, some will be in the water and others will achieve some sort of equilibrium between the two, which will be disturbed during the sampling process and subsequent disturbance of the sediment sample.

There are definite indicators of toxic impacts occurring for some of the samples from some of the sites. This is a measure of the cumulative effect of all the chemicals in the sample that have positive, negative, or no discernible effect on the growth of *A. fischeri* population. Some of these will be chemicals that Cefas or other institutions test for. Some will be chemicals that Cefas are not aware of, nor are not routinely tested for. Using a toxicity test gives an indicator of whether the overall situation is harmful or not, with limits of interpretation imposed by only using one species in a short-term test.

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Centre for Environment Fisheries & Aquaculture Science

Cefas

World Class Science for the Marine and Freshwater Environment

We are the government's marine and freshwater science experts. We help keep our seas, oceans and rivers healthy and productive and our seafood safe and sustainable by providing data and advice to the UK Government and our overseas partners. We are passionate about what we do because our work helps tackle the serious global problems of climate change, marine litter, over-fishing and pollution in support of the UK's commitments to a better future (for example the UN Sustainable Development Goals and Defra's 25 year Environment Plan).

We work in partnership with our colleagues in Defra and across UK government, and with international governments, business, maritime and fishing industry, non-governmental organisations, research institutes, universities, civil society and schools to collate and share knowledge. Together we can understand and value our seas to secure a sustainable blue future for us all, and help create a greater place for living.

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