Toxicity in Anaerobic Biodegradation of Vegetable Oil in Freshwater Sediment

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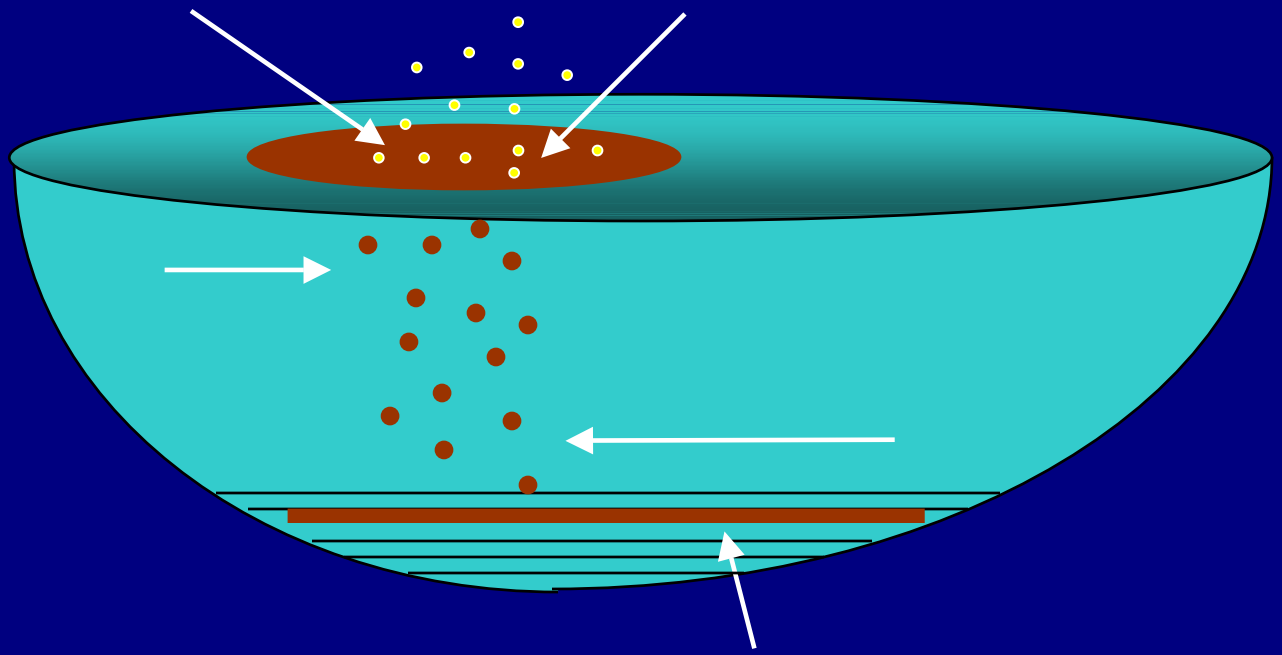
Harmful Effects of Vegetable Oil Spills

- Coating of feathers, fur, and gills with oil
  - absence of smell and sheen might result in reduced avoidance
- High BOD can cause oxygen depletion
- Oxidation of unsaturated oils can foul shorelines with a persistent varnish
  - Polymerization of some vegetable oils (e.g. palm oil)
- Vegetable oil constituents or metabolic products (e.g. free fatty acids) may be toxic

An Alternative Countermeasure *

floating vegetable oil

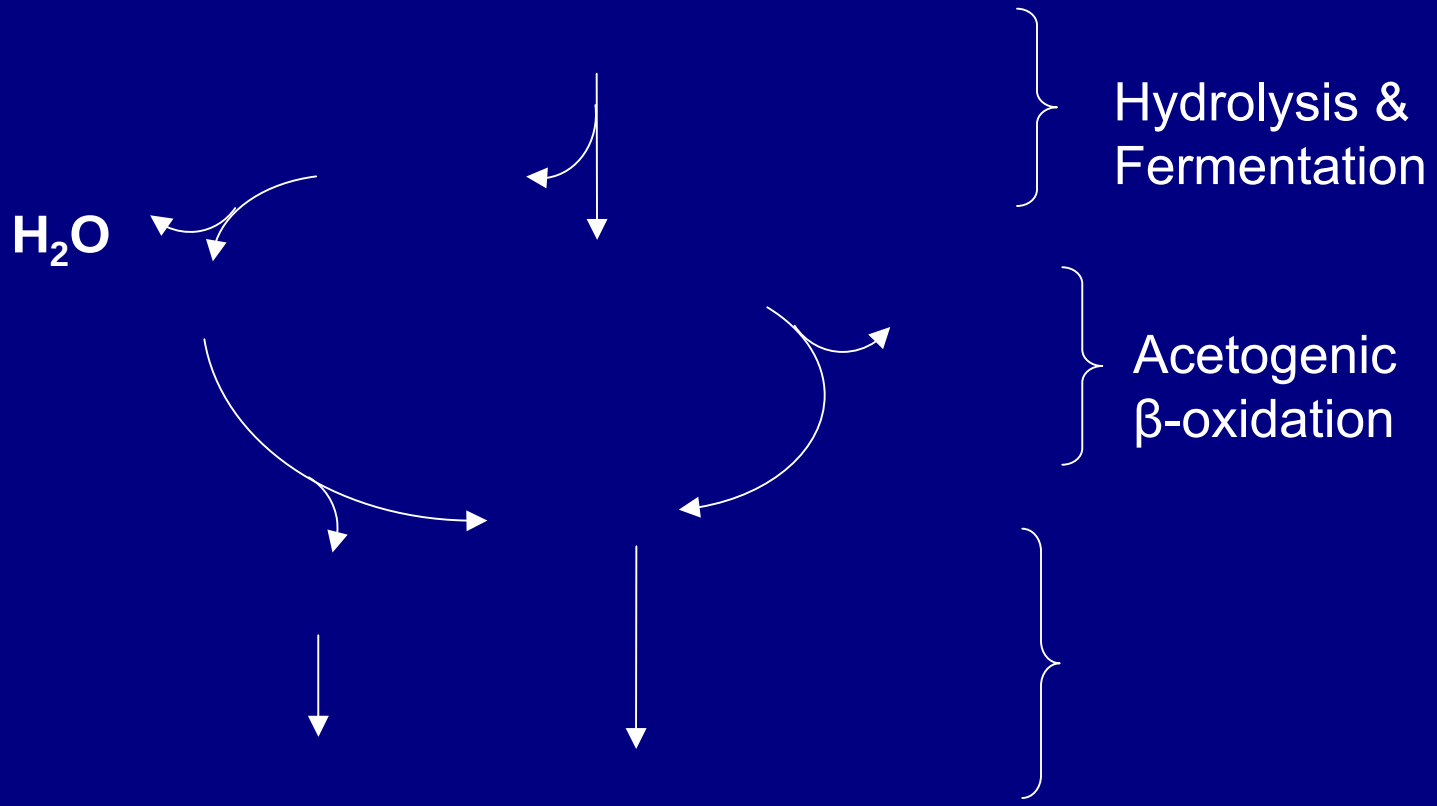
addition of dense minerals



anaerobic biodegradation of oil to CO_2 and CH_4 in sediments

* Wincele, Wrenn, and Venosa. 2004. *J Environ. Eng. ASCE*

Anaerobic Biodegradation of Vegetable Oil in Freshwater Sediments



Environmental Concern

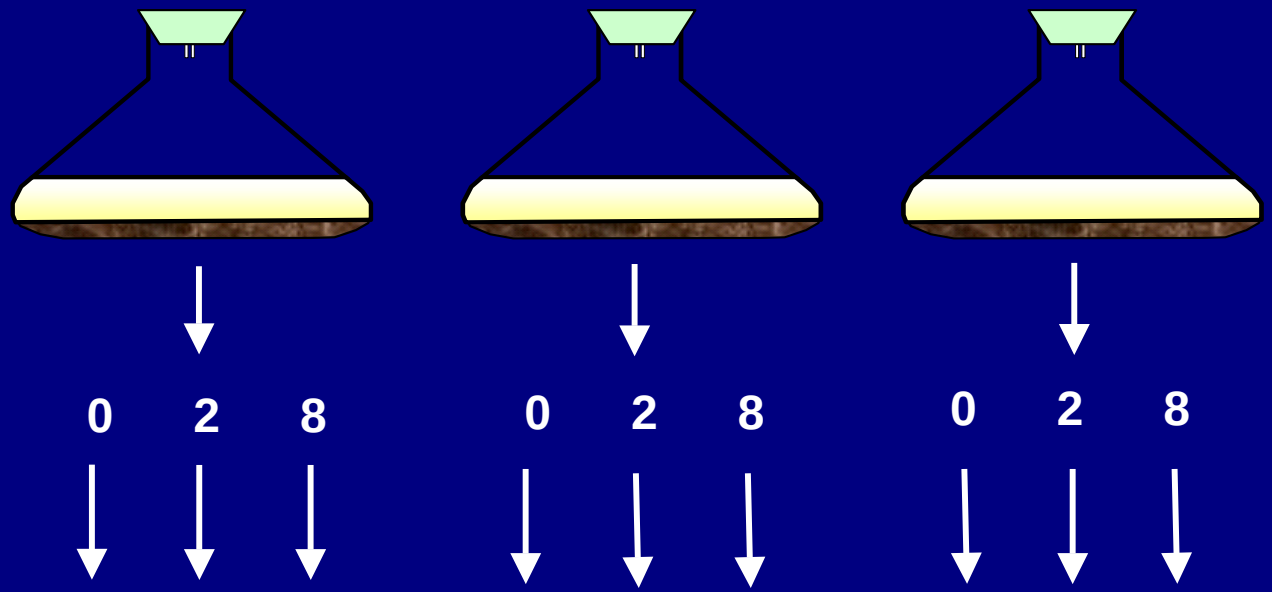
- Potential threat to the benthic ecosystem from free fatty acids that result from the hydrolysis of vegetable triglycerides during biodegradation
- Free fatty acids are known to be toxic to microorganisms due to their ability to disrupt cytoplasmic membranes
- *Therefore, It is essential to evaluate time-related freshwater sediment toxicity from anaerobic vegetable oil degradation*

Experimental Procedure

Control (x3)
clay
sediment
culture medium

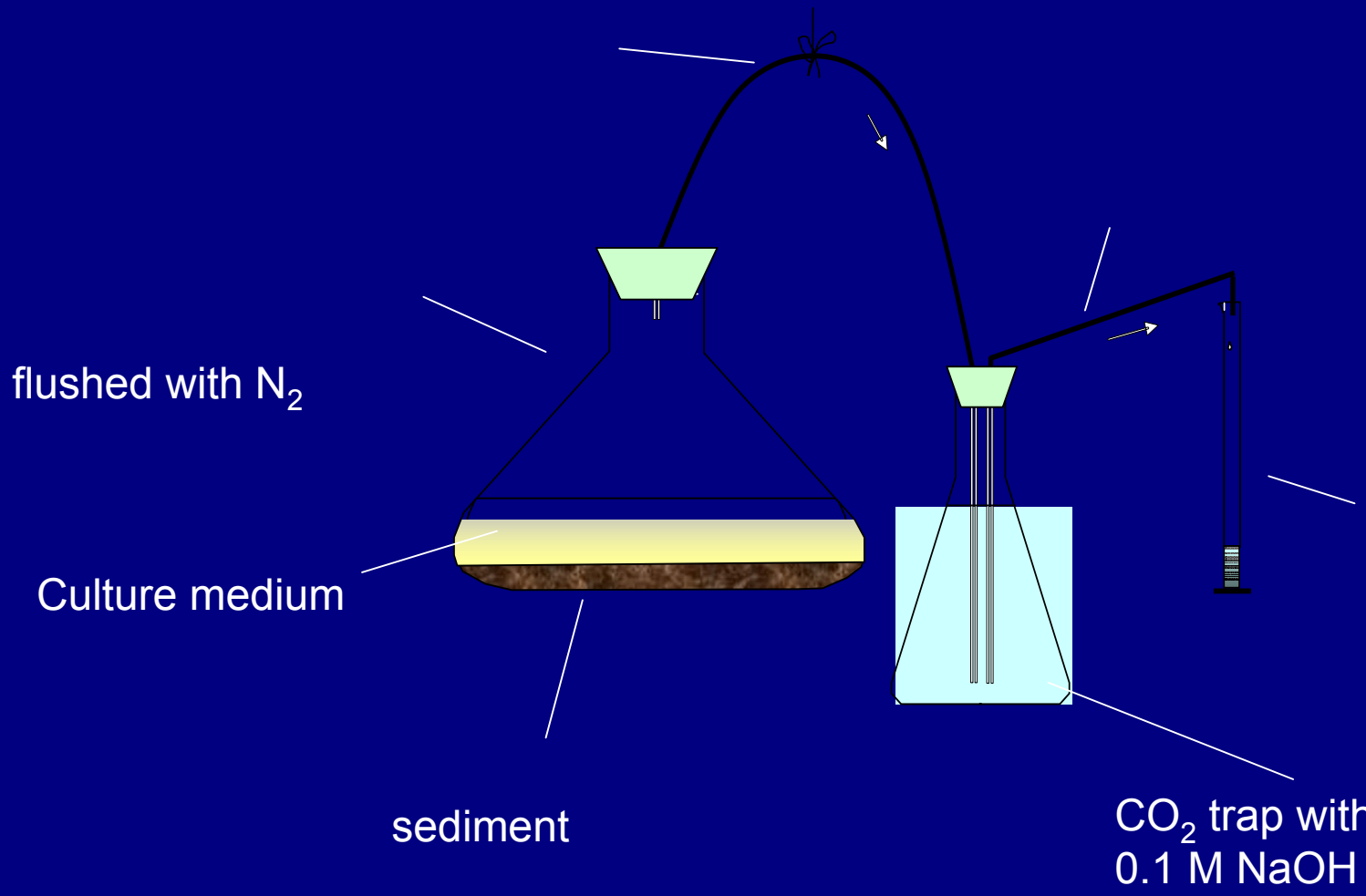
+ 17 g/kg
Canola Oil
(x 3)

+ 35 g/kg
Canola Oil
(x 3)



Chemistry + Toxicity (*Hyalella azteca* + Microtox SPT®)

Closed Reactor for Anaerobic Degradation of Canola Oil and Methane Quantification




T2
20g/kg
Rep 3
T2
40g/kg
Rep 1
T2
40g/kg
Rep 2
T2
40g/kg
Rep 3
T3
40g/kg
Rep

Methods for Toxicity Testing

- Microtox® Solid-Phase assay (SPT) :
  - Based on the suppression of bioluminescence of marine bacterium ***Vibrio fischeri*** on exposure to toxicants (AZUR Environmental, 1999);

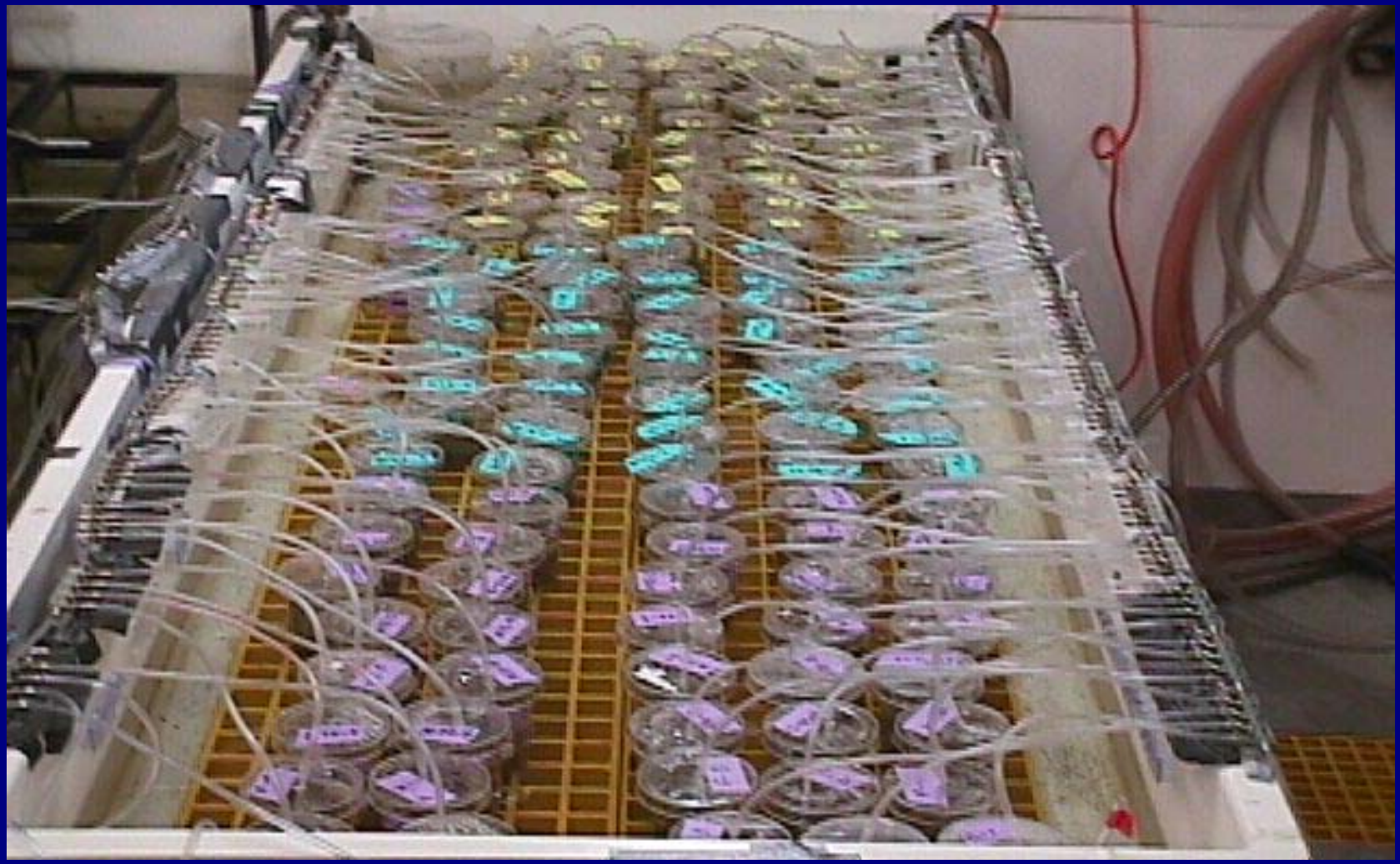
- Endobenthic amphipod *Hyalella azteca* bioassay :
  - Testing was conducted according to Environment Canada standard method *(*EPS 1/RM/33).

Methods for Toxicity Testing: *Hyalella azteca*

- Testing was conducted using 14 day whole sediment toxicity test according to Environment Canada standard method *(*EPS 1/RM/33)
  - Five laboratory replicates for each sample
  - Five replicates of a clean laboratory control
  - Each replicate contained 10 young amphipods (2 to 9 days old).
- Animals were fed daily, and water quality measurements were performed at scheduled times
  - Ammonia was measured in overlying water at the start and end of each test.
- Endpoints were effects on survival and growth (dry weight at end of test).
  - Mean values were calculated for all replicates for each time period.

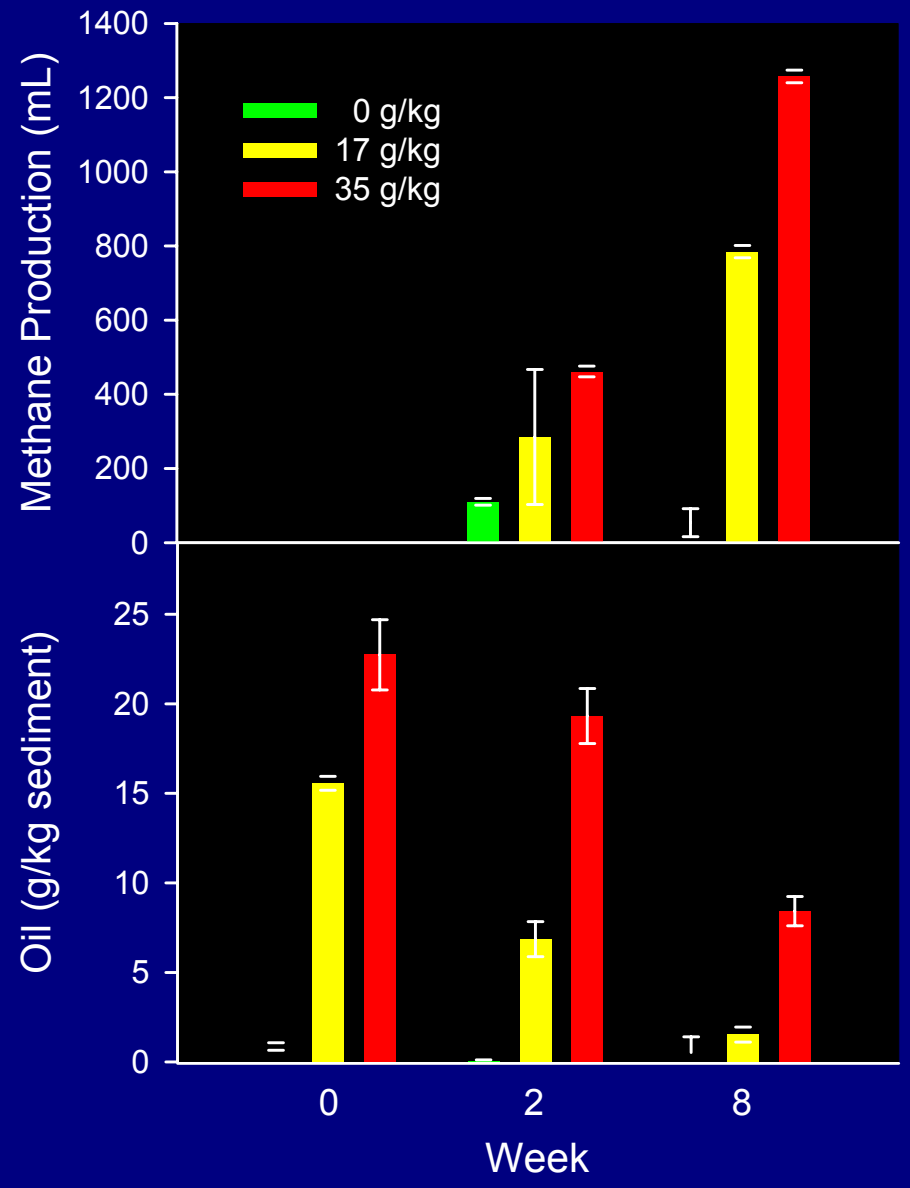
Methods for Laboratory Toxicity Testing

Methods for Laboratory Toxicity Testing

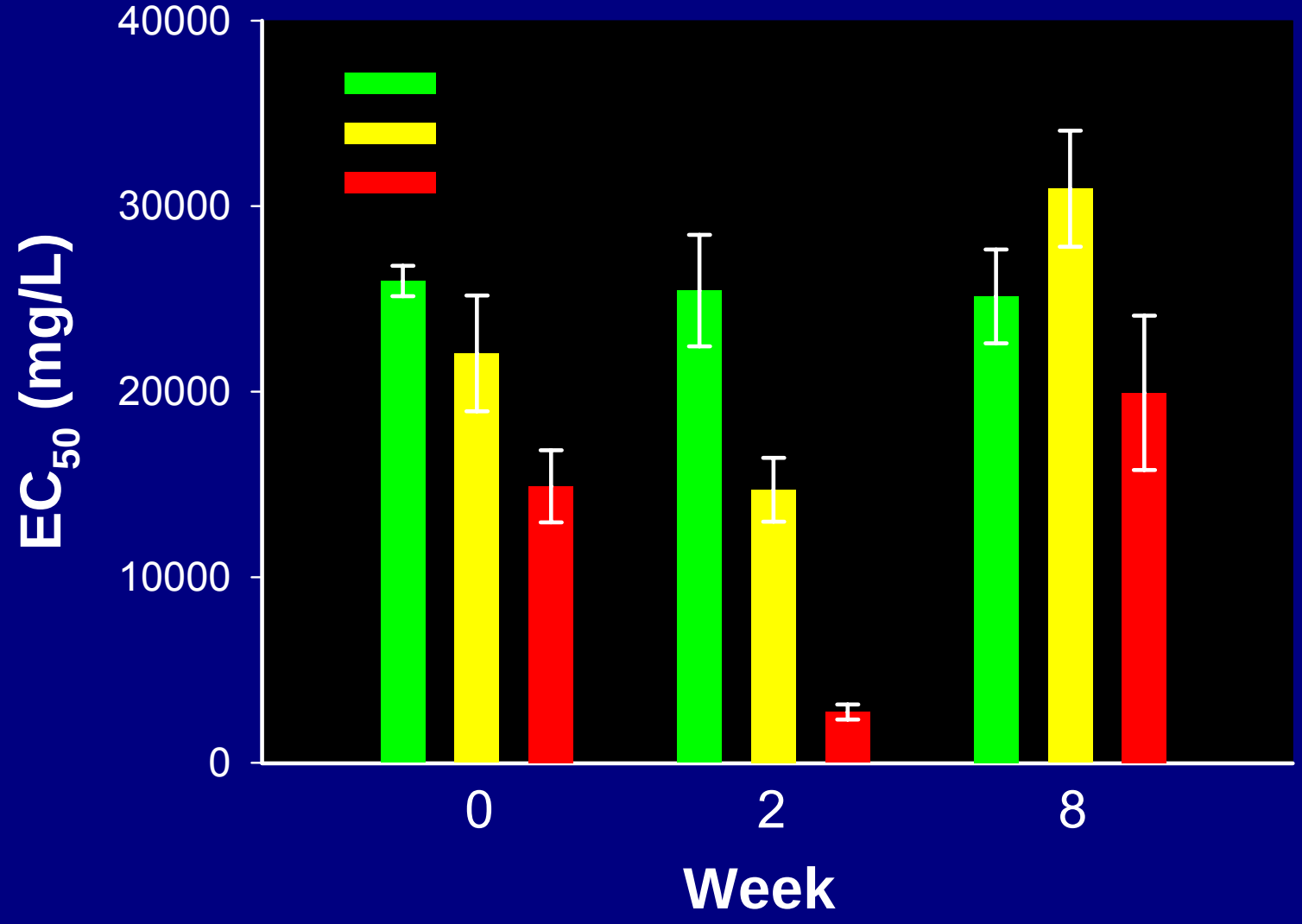


Methods for Laboratory Toxicity Testing

Methane Production & Oil Degradation



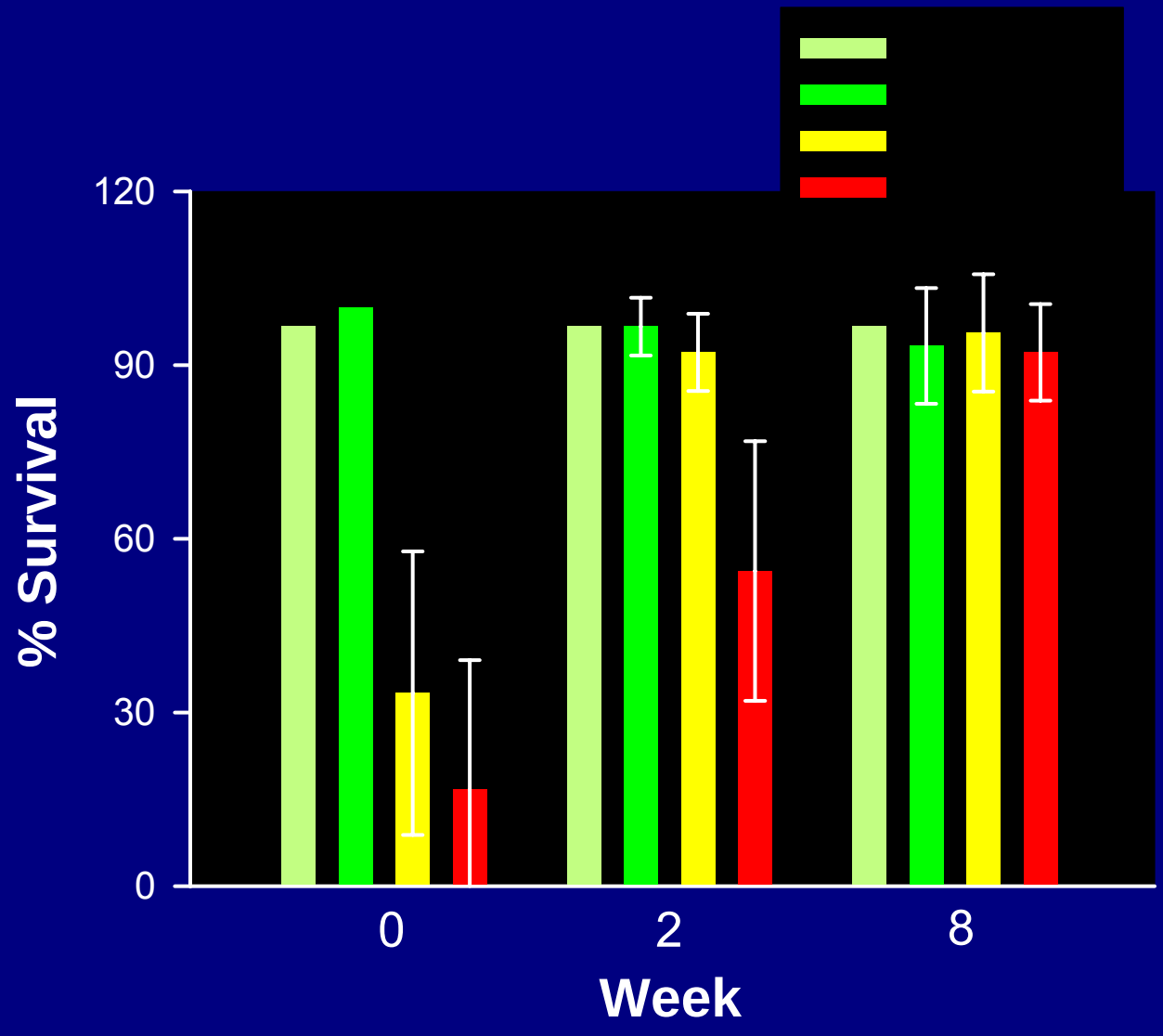
Microtox SPT® EC_{50}



Microtox SPT® EC_{50}

- Sample EC_{50} values at Week 0 decrease with increased oiling;
- Increased toxicity at Week 2 in the oiled treatments may be due to free fatty acids;
- Oiled sample EC_{50} values recovered to the same level as the background at Week 8;
- *Samples deemed non-toxic by EC criteria throughout anaerobic vegetable oil biodegradation* (Environment Canada ocean-dumping guideline threshold for toxicity: 1,000 mg/L)

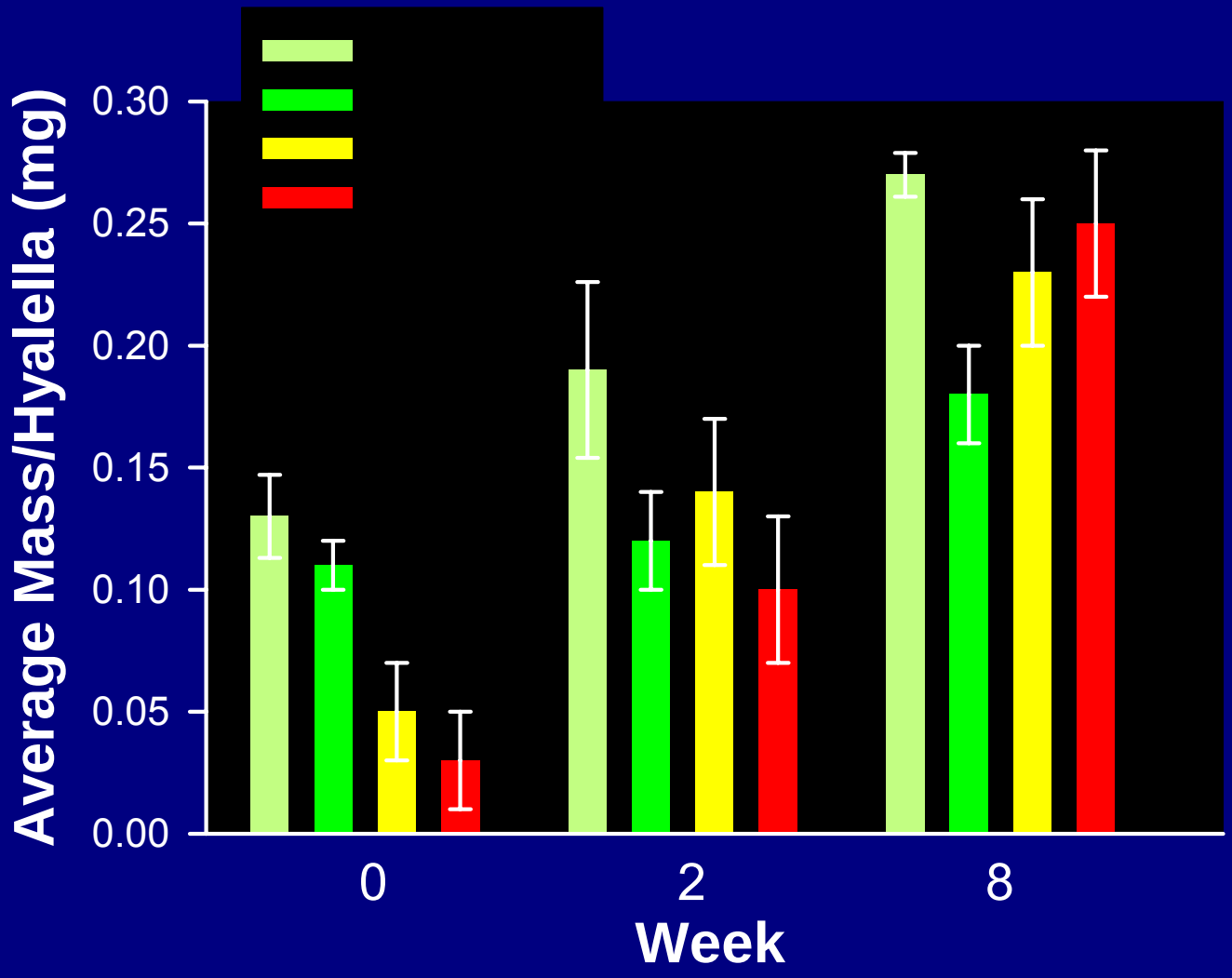
Hyalella azteca % Survival



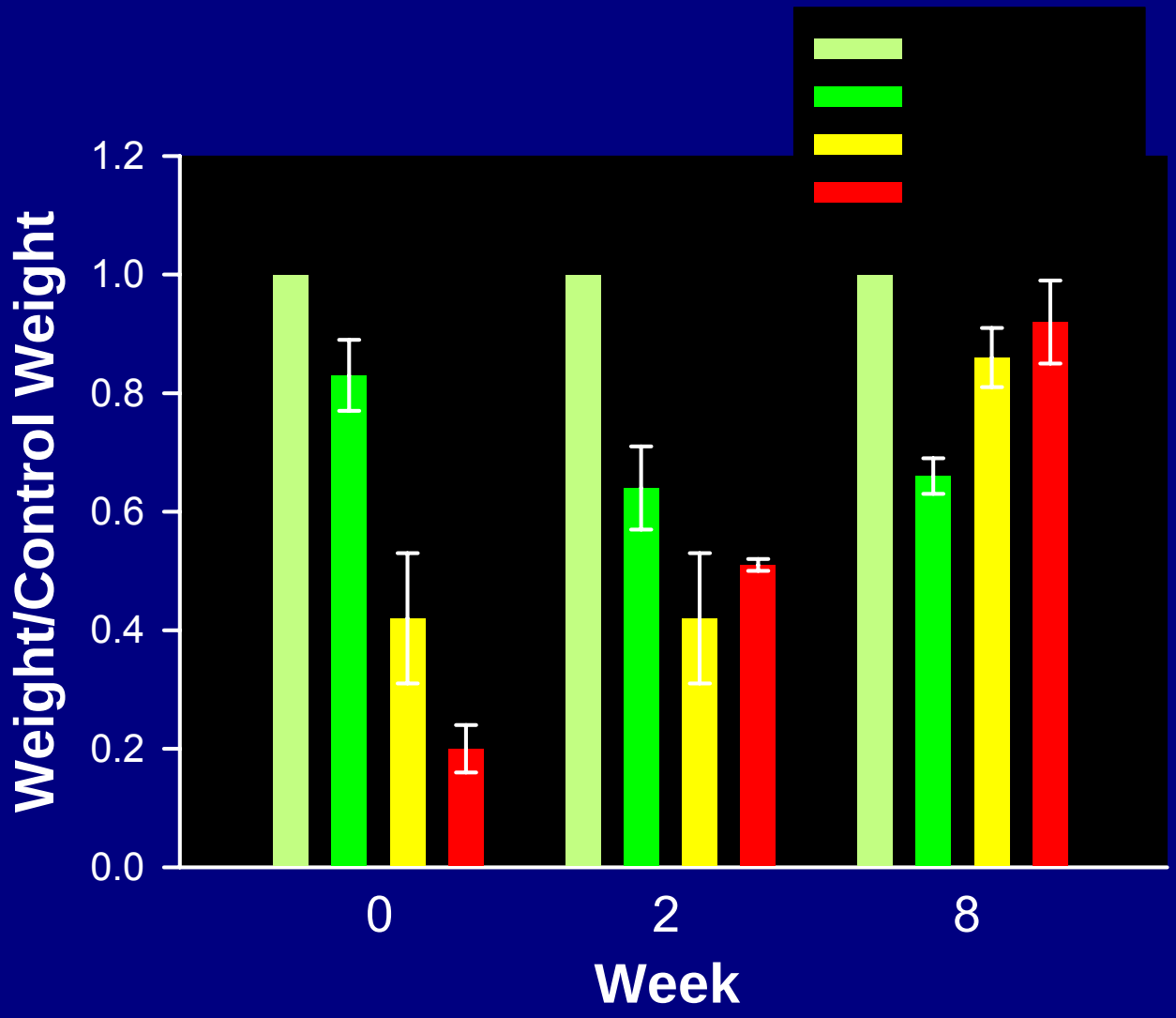
Hyalella azteca % Survival

- Shows toxicity in the oiled treatments at Week 0;
- Reduced toxicity at (17 g/L not toxic) at Week 2;
- No effect on survival at *Week 8*, even in 35 g/L;
- *Anaerobic degradation of vegetable oil in sediments effectively removed sediment toxicity measured by amphipod survival rate.*

Hyalella azteca Growth



Hyalella azteca Growth (Normalized to Lab Control)



Hyalella azteca Growth

- Shows toxicity in oiled samples at *Week 0;*
- Gradual decrease in toxicity over the 8 week study; normalized to lab controls to correct for batch differences in *Hyalella* due to age difference; growth data shows growth of test organisms
- Oiled treatments are similar, or better, than the control (0 g/L) at 8 weeks;
- *Anaerobic degradation of vegetable oil in sediments effectively removed sediment toxicity measured by amphipod growth.*

Conclusions

- Vegetable oil can be mineralized under anaerobic conditions in freshwater sediments even when the initial oil concentration is high;
- Toxic intermediates (presumably free fatty acids) are formed transiently during anaerobic biodegradation of vegetable oil;
- Biotests show that the toxicity of oiled sediments was reduced over time and completely removed due to the anaerobic biodegradation of oil.

Acknowledgements

- U. S. Environmental Protection Agency
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