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Toxicity of Uranium Adsorbent Materials using the Microtox Toxicity Test

DOE-NE Milestone Report M3FT-15PN0310056

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Li-Jung Kuo George Bonheyo

October 2014

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Richland, Washington 99352

Executive Summary

The Marine Sciences Laboratory at the Pacific Northwest National Laboratory evaluated the toxicity of a diverse range of natural and synthetic materials used to extract uranium from seawater. The uranium adsorbent materials are being developed as part of the U. S. Department of Energy, Office of Nuclear Energy, Fuel Resources Program. The goal of this effort was to identify whether deployment of a farm of these materials into the marine environment would have any toxic effects on marine organisms. Toxicity was evaluated using the well-recognized and accepted Microtox® toxicity test. The Microtox® assay uses a microorganism found in seawater that is susceptible to a wide range of toxins. This established toxicity assay is quick, reliable and cost-efficient.

Direct contact with 5 out of 10 adsorbents were toxic (reached EC50), but only when the adsorbent material was at very high concentrations. No toxicity was observed with seawater that was in contact with the absorbent material from 63 different adsorbent materials. Based on testing to date, there does not appear to be any significant concerns with toxicity of adsorbent materials; Extraction from seawater could be performed with minimal impact to marine environment.

Materials and Methods

The toxicity of adsorbent materials were evaluated using the Microtox® assay to determine if the supplied adsorbent materials and seawater effluent from uranium extraction columns have any toxic effects on marine organisms, The assay measures the luminescence of the bioluminescent marine bacterium *Aliivibrio fischeri* (ATCC 49387), an indicator of metabolism in the organism, after a 30-minute (1) exposure to the sample material. The Microtox® assay is a useful tool to evaluate the toxicity of a wide range of metals (2, 3) and organic (4, 5) compounds that are either in liquid or solid phase. In addition, the Microtox® test is low-cost and easy to operate and has a long history of use for determining toxicity in environmental samples (6-8). The Advanced Monitoring Systems (AMS) Center that is part of the Environmental Technology Verification (ETV) Program has evaluated, tested and validated the Microtox® assay for assessing various contaminants in water samples. The ETV is supported by the U.S. Environmental Protection Agency (EPA) (9). The Microtox® assay has also been used by U.S. Geological Survey and National Oceanic and Atmospheric Administration (NOAA) to assay sediment from the Delaware Bay and surrounding areas for toxicity (10).

Aliivibrio fischeri culture was grown overnight in ALNa Broth (1) at 22°C with vigorous shaking. The overnight culture was used to inoculate 10 ml of ALNa broth, and the culture was incubated with vigorous shaking at 22°C. Cells were grown until the optical density at 590 nm (OD_{590}) was 0.25. Cells were harvested by centrifugation at 4150 rpm for 5 min followed by washing in 10ml 3% NaCl; pH 7. Cells were then resuspended in 10 mL 3% NaCl at pH 7. The cell suspension was mixed with test materials for 30 minutes, and the luminescence was measured using a Synergy HT microplate reader (Biotek, Winooski, VT, USA).

The decrease in bacterial luminescence (INH%) (11) was calculated as shown below. If the luminescence decreased more than 50% after exposure to sample materials for 30 minutes, the concentration of toxicant is designated as the effective concentration (EC_{50}) (11). Values obtained with cells only control sample (suspended in 3% NaCl or seawater) was set to 100%, and the luminescence of each sample was represented as a percentage of cells only value. $ZnSO_4$ (12) was used as control toxicant (0.625, 1.25, 2.5, and 5µg/mL to validate the Microtox® assay results.

The decrease in bacterial luminescence (INH%)

$$\mathrm{INH\%} = 100 - \left(\frac{\mathrm{IT}_T}{\mathrm{IT}_0 \times \mathrm{KF}}\right) \times 100$$

$$\mathrm{KF} = \frac{\mathrm{IC}_T}{\mathrm{IC}_0}$$

IC_0: luminescence of control at t = 0
IC_T: luminescence of control after '*T*' minutes exposure time
IT_0: luminescence of test sample at t = 0
IT_T: luminescence of test sample after '*T*' minutes exposure time
KF: correction factor based on the control/blank

A positive response means a decrease in luminescence, while a negative response means the luminescence increased.

EC_{50} (50% Effective Concentration)
The concentration of the toxicant (mg/L) that causes a 50% reduction in light after exposure for '*T*' minutes

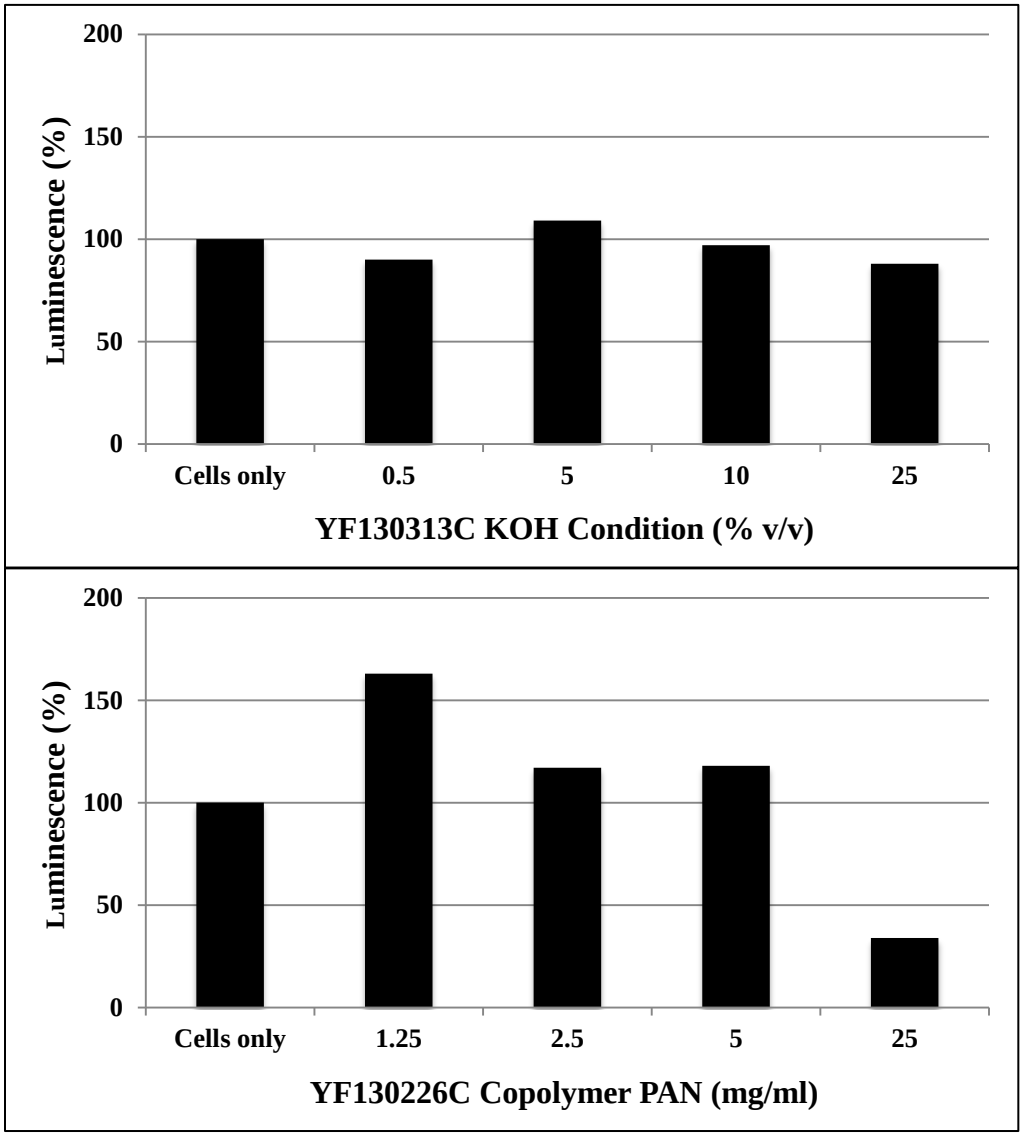
Results and Discussion

1. Toxicity of adsorbent materials via direct contact

Supplied adsorbent materials were tested for direct contact toxicity using increased concentrations. Due to limited number of adsorbent materials currently available, only 10 samples were tested, and hence the results for direct testing should be considered preliminary.

1) Oak Ridge National Laboratory (Richard Mayes)

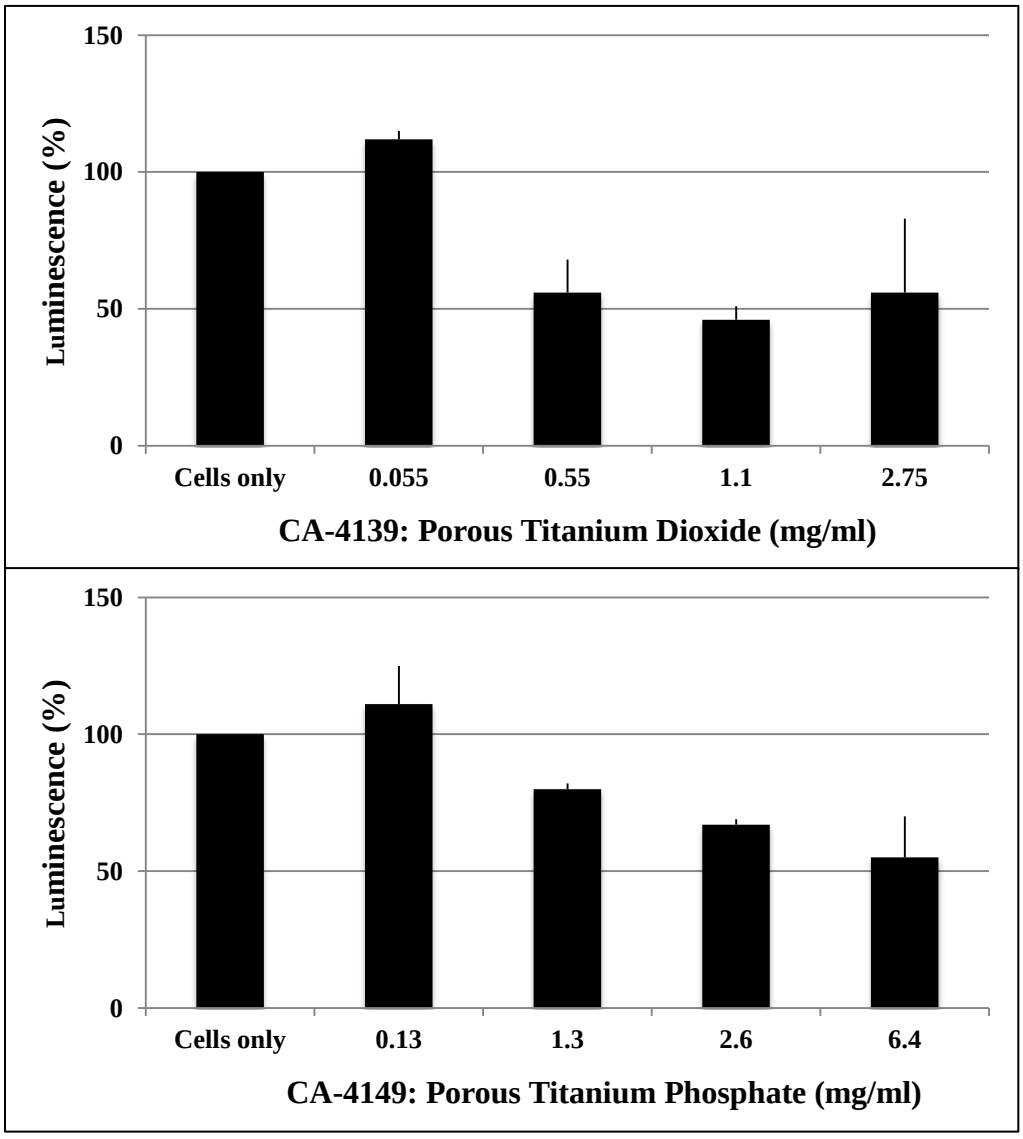
- YF130313C KOH Condition did not have an EC_{50} up to 25% v/v.
- YF130226C Copolymer-PAN had an EC_{50} at 25mg/ml.



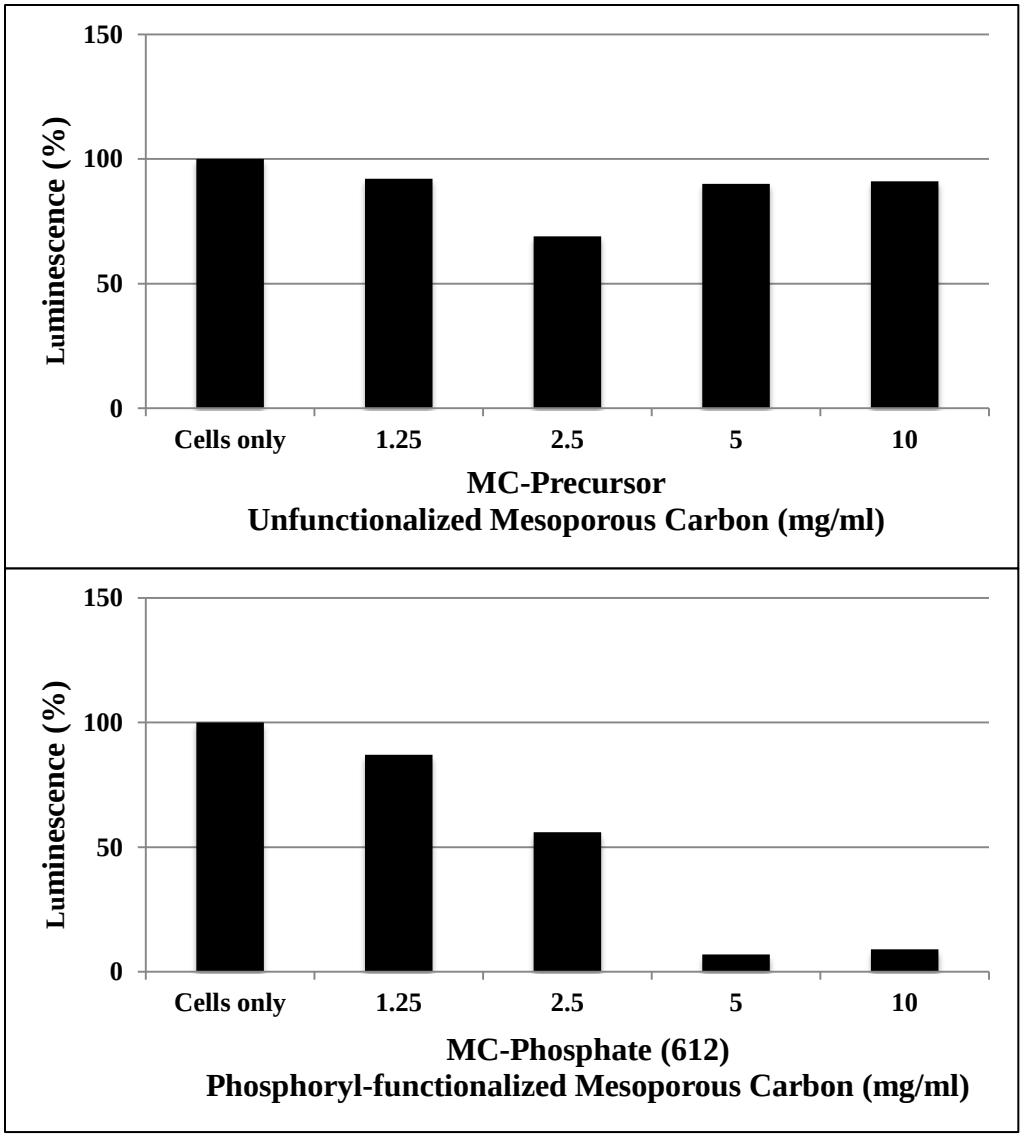
- The Microtox® assays on YF130313C KOH Condition and YF130226C Copolymer-PAN were done one time due to the limited amount of supplied sample materials.

2) University of North Carolina (Carter Abney/Lin group)

- CA-4139: Porous Titanium Dioxide had an EC_{50} at 1.1mg/ml.
- CA-4149: Porous Titanium Phosphate did not have an EC_{50} up to 6.4mg/ml.
- MC-Precursor Unfunctionalized Mesoporous Carbon did not have an EC_{50} up to 10mg/ml.
- MC-Phosphate (612) Phosphoryl-functionalized Mesoporous Carbon had an EC_{50} at 5mg/ml.



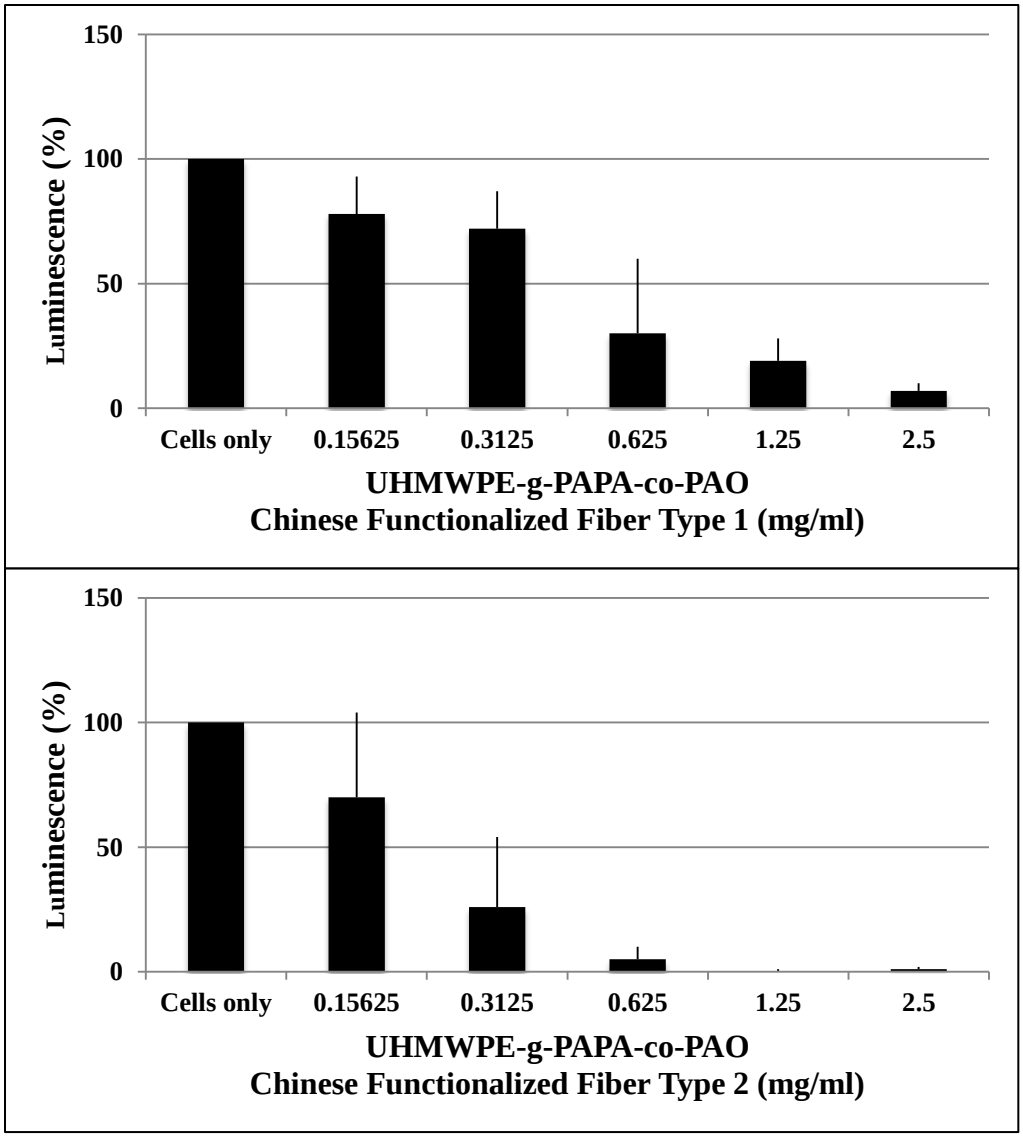
- CA-4139: Porous Titanium Dioxide and CA-4149: Porous Titanium Phosphate were tested in duplicated experiments. Graphs show the mean value and standard deviation is shown as error bars.

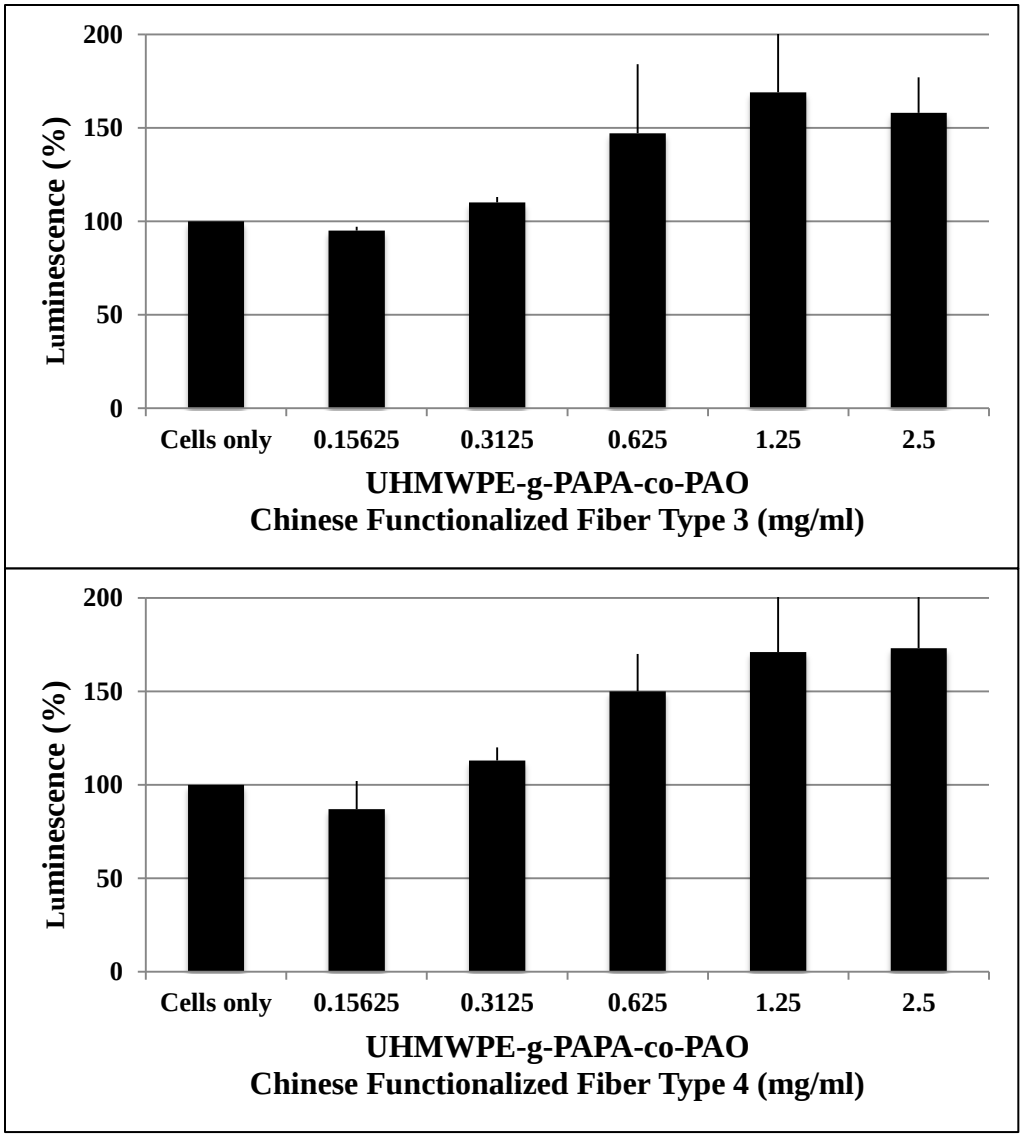


- The Microtox® assays on MC-Precursor Unfunctionalized Mesoporous Carbon and MC-Phosphate Phosphoryl-functionalized Mesoporous Carbon were done one time due to the limited amount of supplied sample materials.

3) Chinese Academy of Science

- UHMWPE-g-PAPA-co-PAO #1 had an EC_{50} at 0.625mg/ml.
- UHMWPE-g-PAPA-co-PAO #2 had an EC_{50} up to 0.3125mg/ml.
- UHMWPE-g-PAPA-co-PAO #3 did not have an EC_{50} up to 2.5mg/ml.
- UHMWPE-g-PAPA-co-PAO #4 did not have an EC_{50} up to 2.5mg/ml.
  All fiber types 1~4 were tested in triplicate. Graphs show the mean value and standard deviation is shown as error bars.


150
100
50
0
Luminescence (%)
Cells only
0.15625
0.3125
0.625
1.25
2.5
UHMWPE-g-PAPA-co-PAO
Chinese Functionalized Fiber Type 1 (mg/ml)
150
100
50
0
Luminescence (%)
Cells only
0.15625
0.3125
0.625
1.25
2.5
UHMWPE-g-PAPA-co-PAO
Chinese Functionalized Fiber Type 2 (mg/ml)



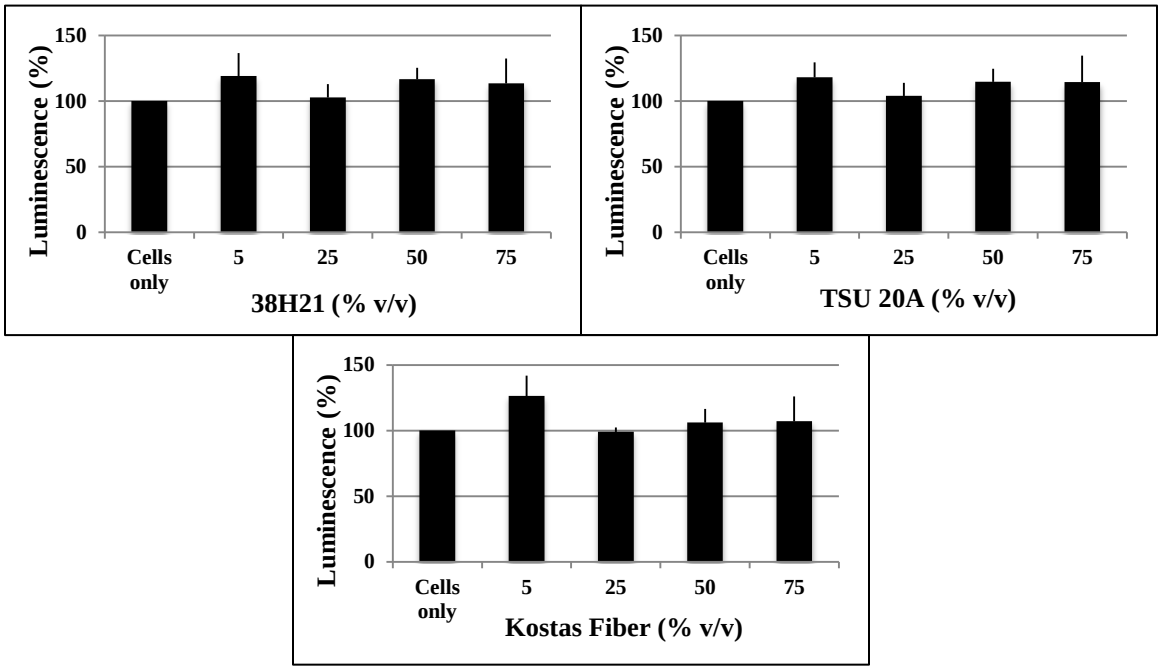
- UHMWPE-g-PAPA-co-PAO Chinese Functionalized Fiber Type 3 and 4 showed no signs of toxicity. Increase in luminescence is likely due to compound present on functionalized fiber. Some select compounds have been shown to increase luminescence at high concentrations.

2. Toxicity of seawater effluent collected from uranium extraction columns

Toxicity was evaluated in seawater that passed through columns containing adsorbent materials (effluent test). The Microtox® assay was performed with increasing amounts of seawater effluent up to 75% v/v because a minimum amount of *Aliivibrio fischeri* cells were required to measure luminescence. None of seawater effluent samples had an EC_{50}. The experiments were done in triplicate. Mean values are shown and standard deviations are indicated as error bars.

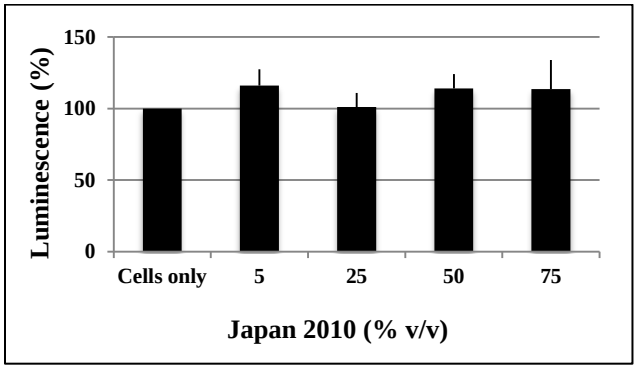
1) Oak Ridge National Laboratory

- 38H21: 3 days
- TSU 20A: 1 week
- Costas Fiber: 8 weeks



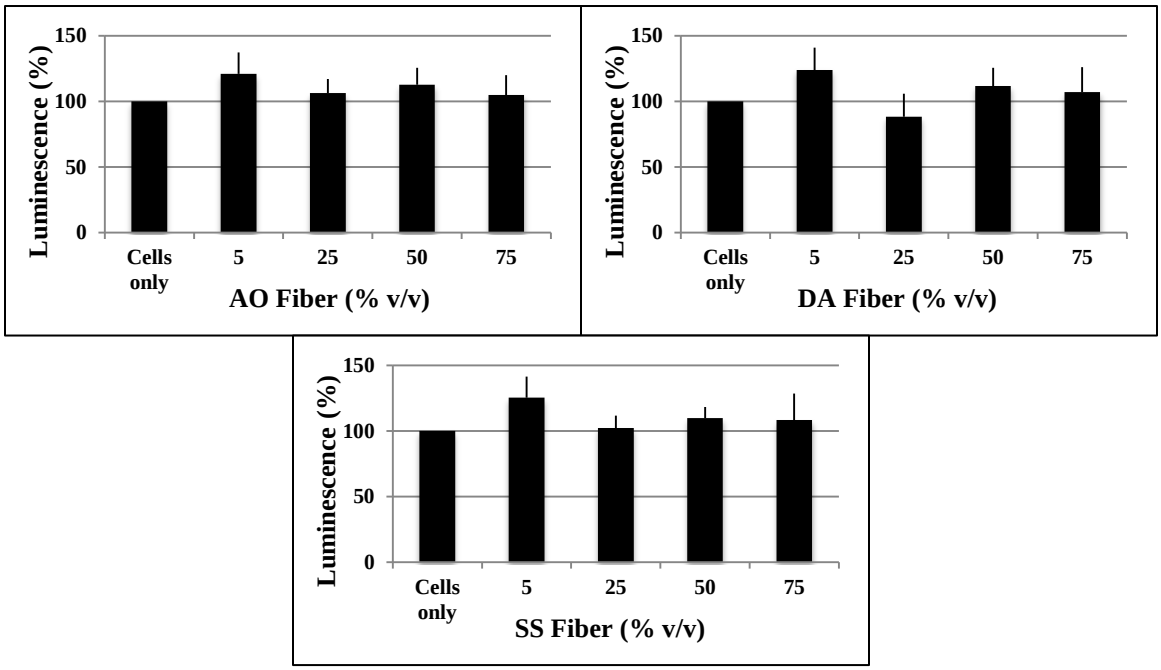
2) Japan

- Japan 2010: 3 days



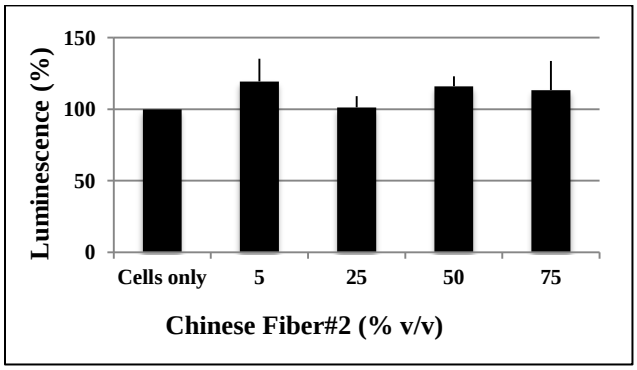
3) University of Alabama

- AO Fiber: 6 weeks
- DA Fiber: 6 weeks
- SS Fiber: 6 weeks



4) Chinese Academy of Science

- Chinese Fiber #2: 3 days

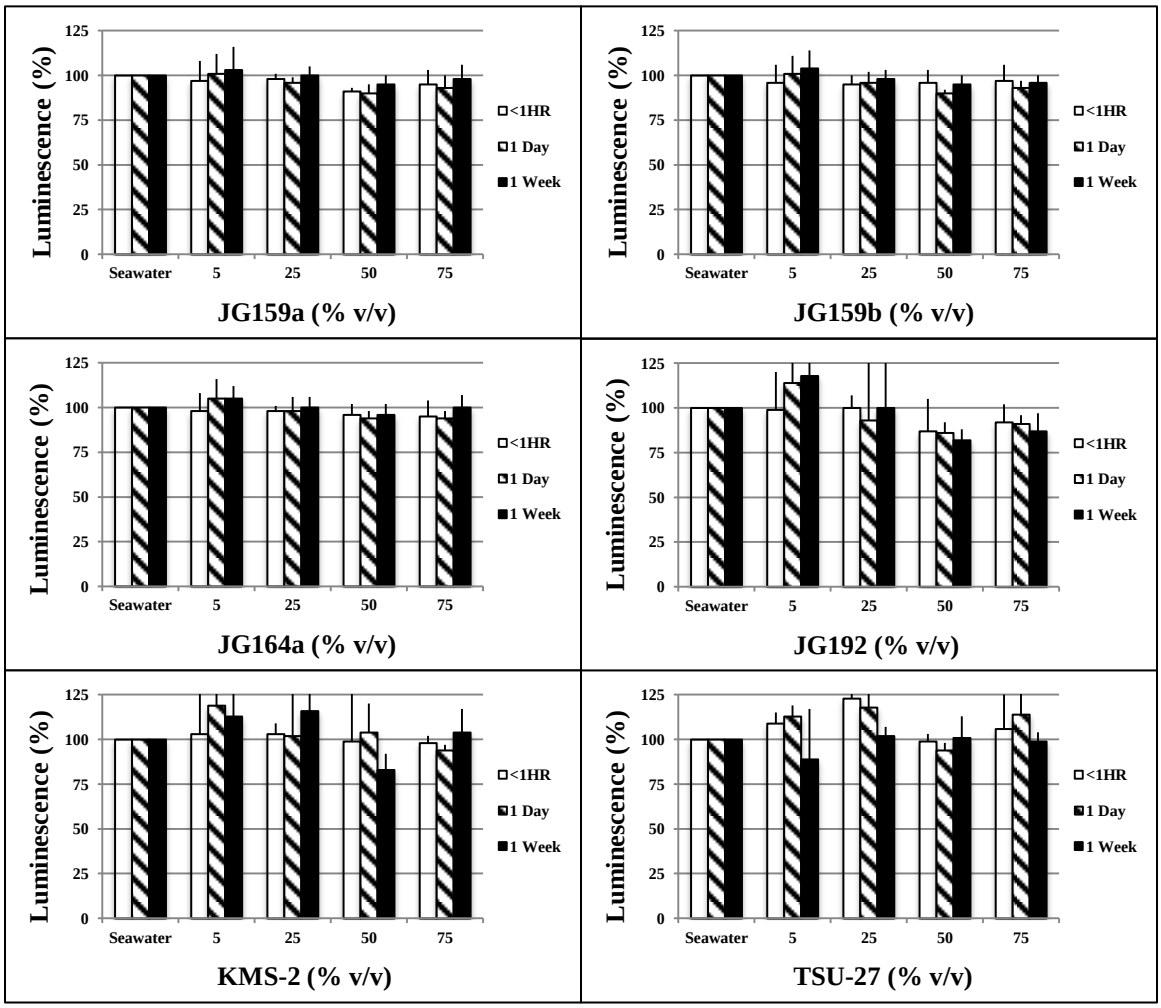


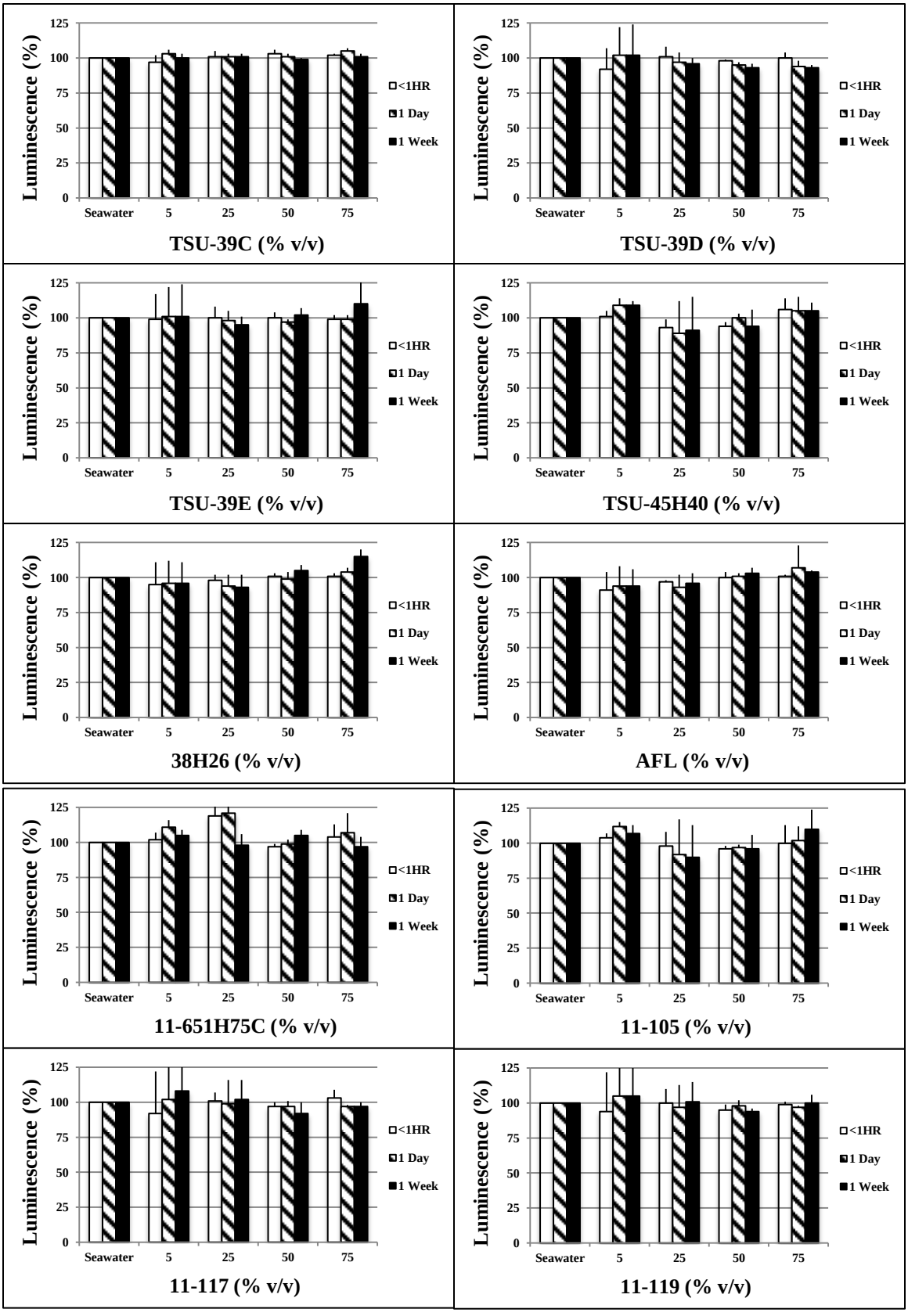
3. Time-course experiments to determine toxicity of seawater effluent collected from uranium extraction columns

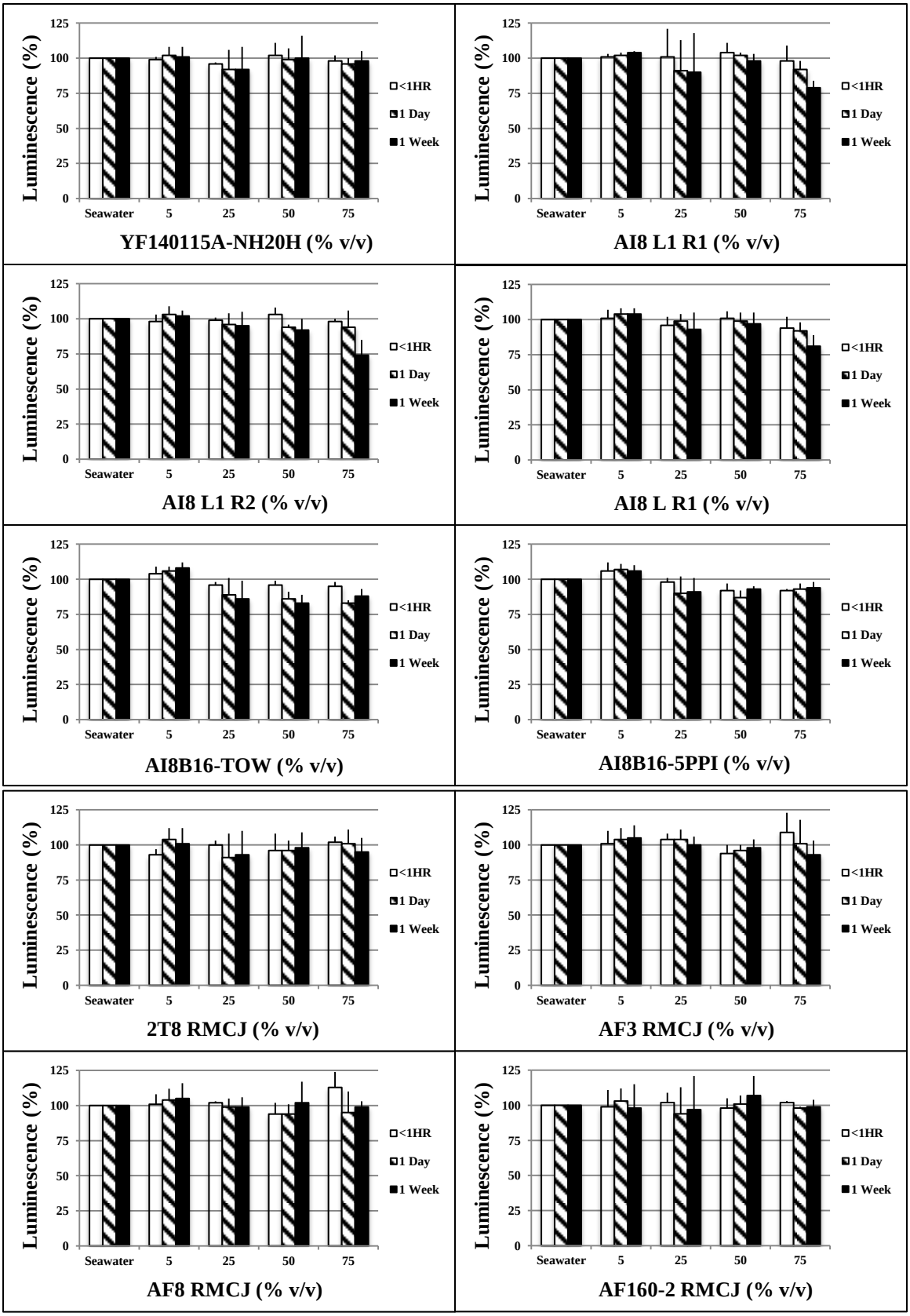
In order to determine whether a longer exposure of the adsorbent to seawater could have toxic effects on marine organisms, time-course experiments were done on seawater effluents samples that were in contact with 55 different adsorbent materials. Seawater effluent samples were collected at three different time points; 1 hour, 1 day, and 1 week after exposure to adsorbent materials in uranium uptake columns. Samples up to 75% v/v were tested in order to measure luminescence from *Allivibrio fischeri* to

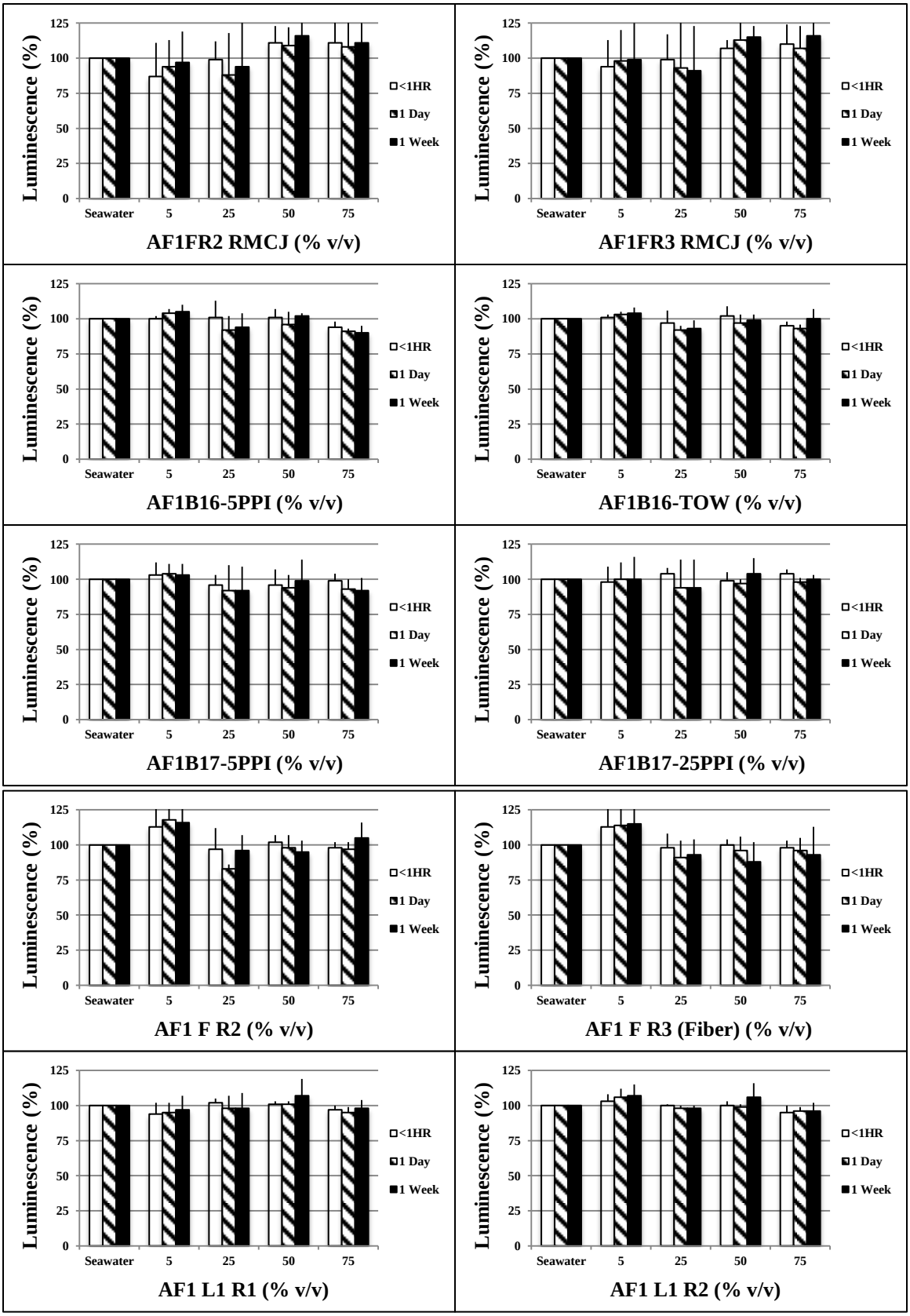
evaluate toxicity of the sample. Three independent experiments were done on these samples, and all samples up to 75% v/v did not have an EC_{50}. The experiments were done in triplicate Mean values are shown and standard deviations are indicated as error bars.

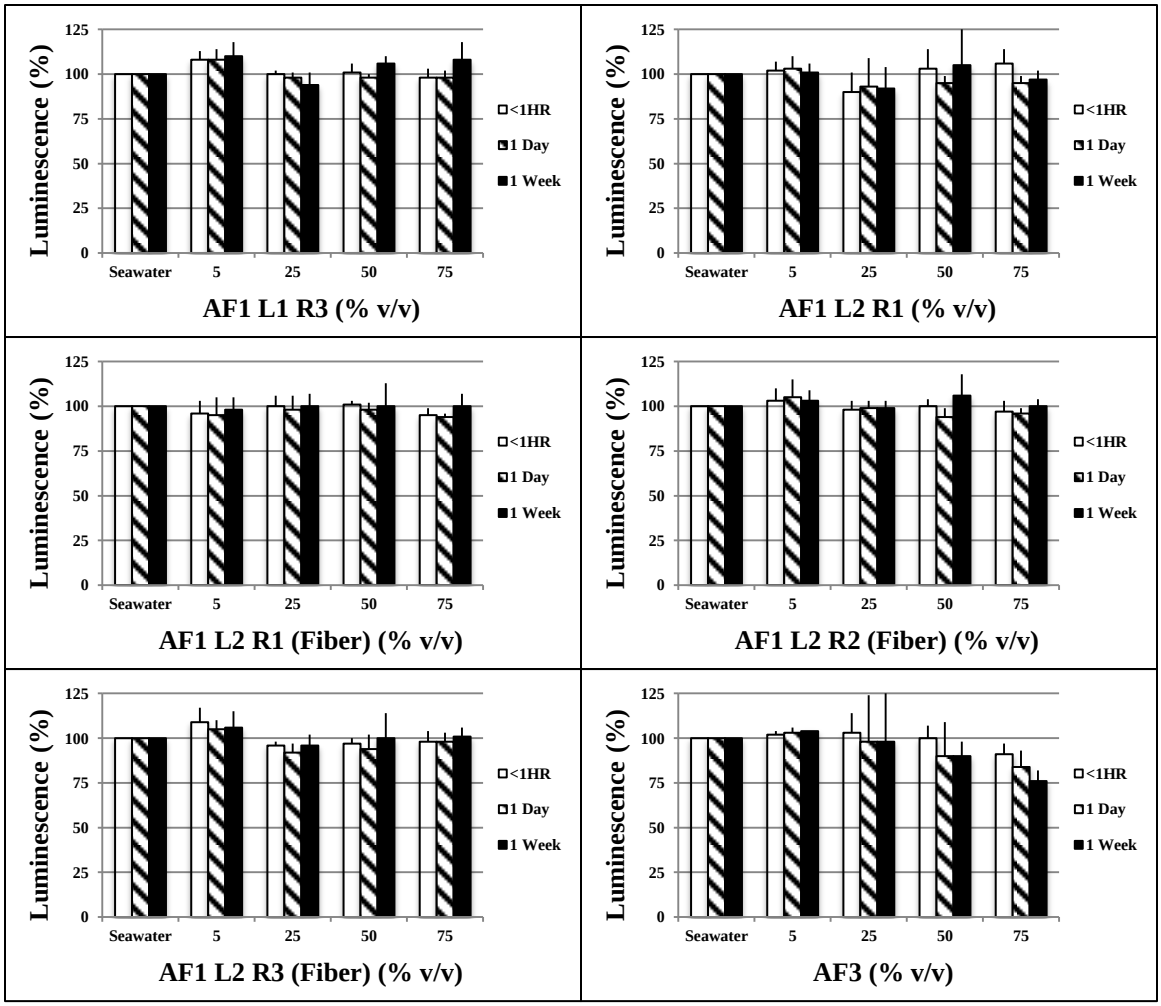
1) Oak Ridge National Laboratory (42 adsorbents)



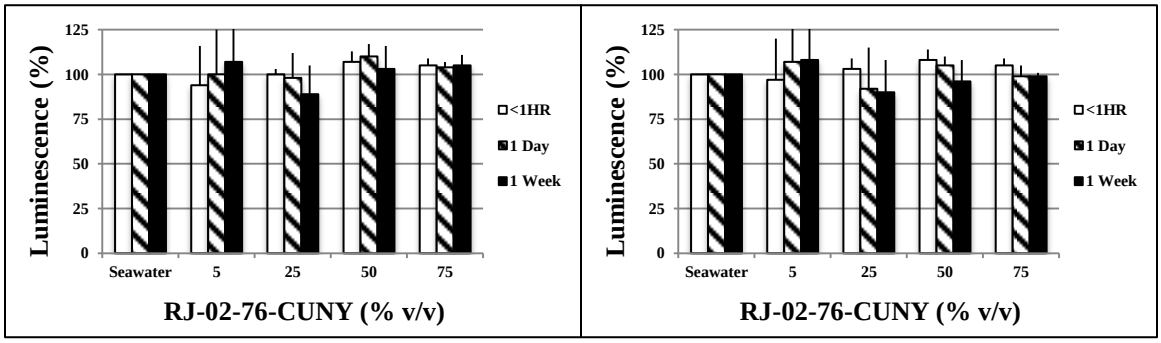

Luminescence (%)
125
100
75
50
25
0
Seawater
5
25
50
75
<1HR
1 Day
1 Week
TSU-39C (% v/v)
TSU-39D (% v/v)
TSU-39E (% v/v)
TSU-45H40 (% v/v)
38H26 (% v/v)
AFL (% v/v)
11-651H75C (% v/v)
11-105 (% v/v)
11-117 (% v/v)
11-119 (% v/v)


Luminescence (%)
125
100
75
50
25
0
Seawater
5
25
50
75
<1HR
1 Day
1 Week
YF140115A-NH20H (% v/v)
AI8 L1 R1 (% v/v)
AI8 L1 R2 (% v/v)
AI8 L R1 (% v/v)
AI8B16-TOW (% v/v)
AI8B16-5PPI (% v/v)
2T8 RMCJ (% v/v)
AF3 RMCJ (% v/v)
AF8 RMCJ (% v/v)
AF160-2 RMCJ (% v/v)

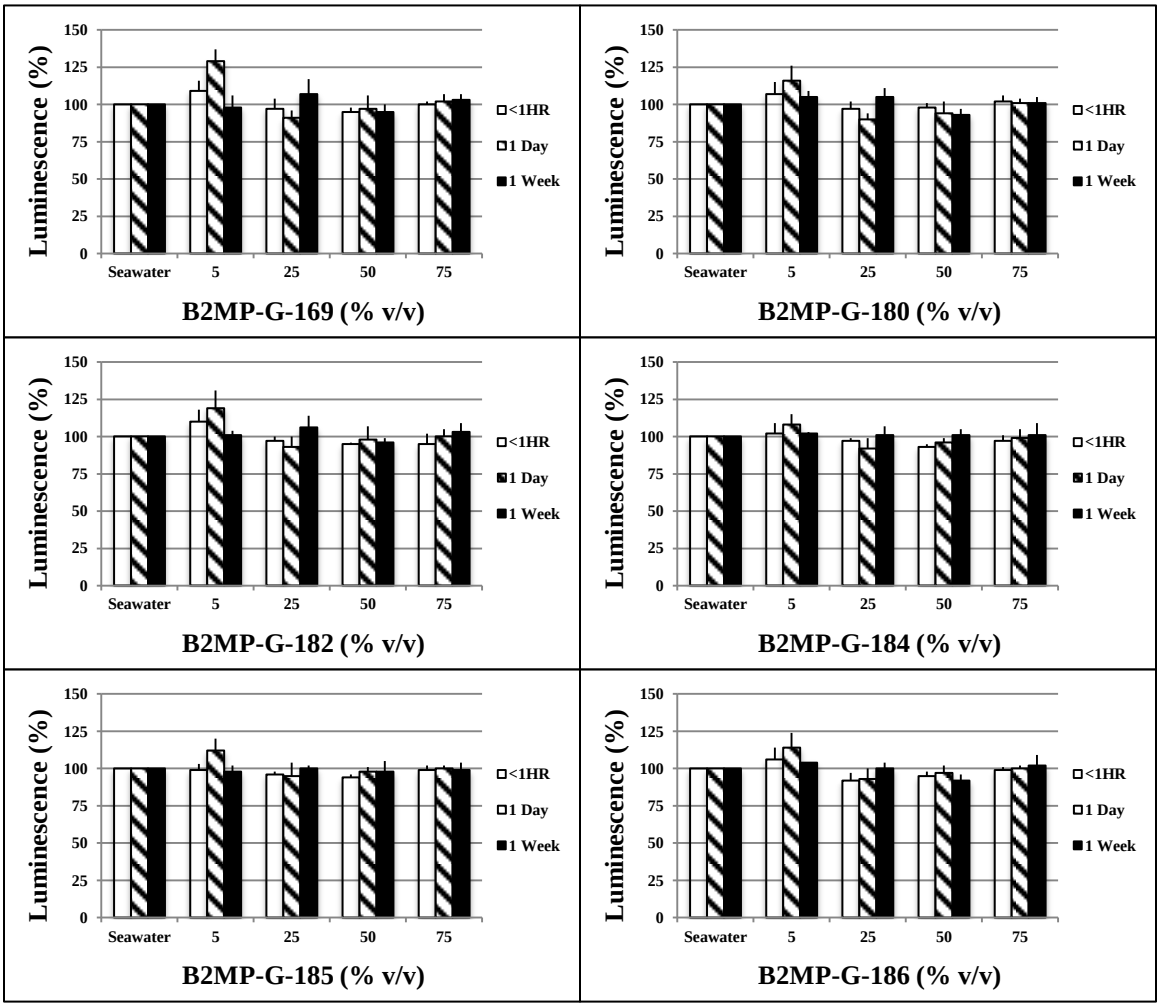

Luminescence (%)
125
100
75
50
25
0
Seawater
5
25
50
75
<1HR
1 Day
1 Week
AF1FR2 RMCJ (% v/v)
AF1FR3 RMCJ (% v/v)
AF1B16-5PPI (% v/v)
AF1B16-TOW (% v/v)
AF1B17-5PPI (% v/v)
AF1B17-25PPI (% v/v)
AF1 F R2 (% v/v)
AF1 F R3 (Fiber) (% v/v)
AF1 L1 R1 (% v/v)
AF1 L1 R2 (% v/v)

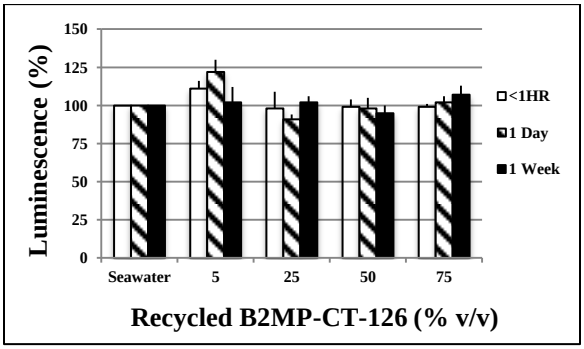


2) The City University of New York (2 adsorbents)

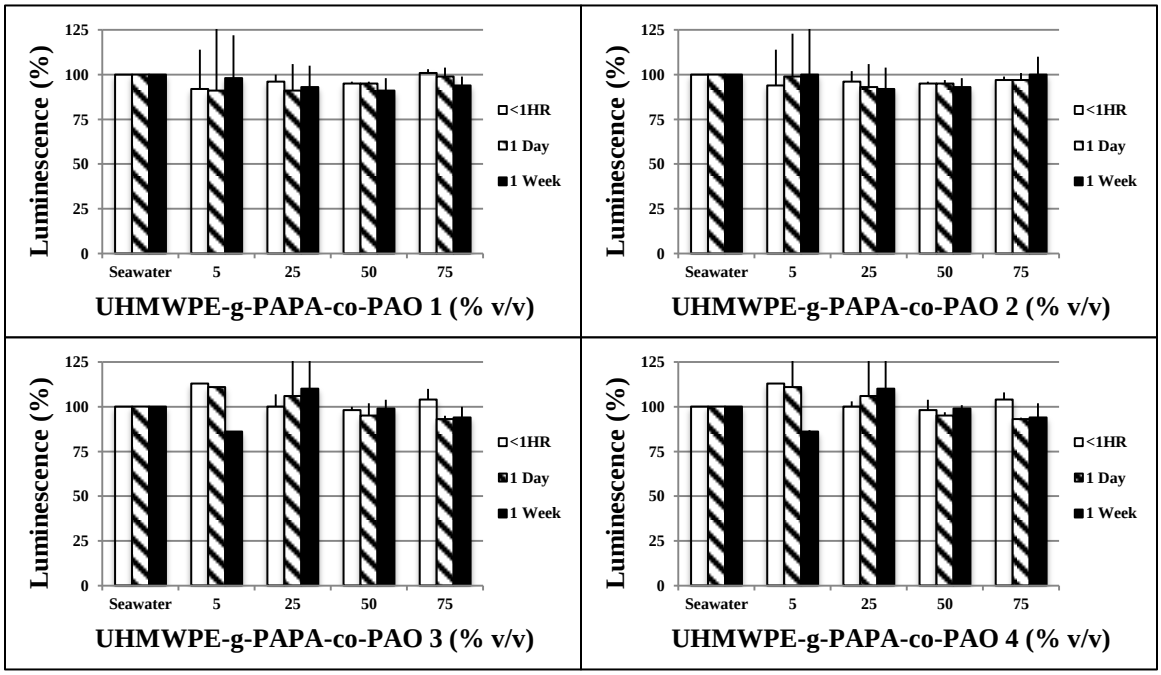


3) University of Maryland (7 adsorbents)





4) Chinese Academy of Science (4 adsorbents)



Summary

We tested the toxicity of direct contact with 10 adsorbent materials at different concentrations. Due to the limited amount of supplied adsorbent samples, our results are preliminary.

Adsorbent	EC_{50} at	Source
YF130313C KOH Condition	No up to 25% v/v	Oak Ridge National Laboratory Richard Mayes
YF130226C Copolymer-PAN	Yes ≥ 25mg/ml	
CA-4139 Porous Titanium Dioxide	Yes ≥ 1.1mg/ml	University of North Carolina Carter Abney/Lin group
CA-4149 Porous Titanium Phosphate	No up to 6.4mg/ml	
MC-Precursor Unfunctionalized Mesoporous Carbon	No up to 10mg/ml	
MC-Phosphate (612) Phosphoryl-functionalized Mesoporous Carbon	Yes ≥ 2.5mg/ml	
UHMWPE-g-PAPA-co-PAO Chinese functionalized fiber type 1	Yes ≥ 0.625mg/ml	Chinese Academy of Science
UHMWPE-g-PAPA-co-PAO Chinese functionalized fiber type 2	Yes ≥ 0.3125mg/ml	
UHMWPE-g-PAPA-co-PAO Chinese functionalized fiber type 3	No up to 2.5mg/ml	
UHMWPE-g-PAPA-co-PAO Chinese functionalized fiber type 4	No up to 2.5mg/ml	

We also tested seawater effluent that came in contact with adsorbent materials in uranium extraction columns. The Microtox® assay was done at multiple concentrations and time points. All 63 samples listed below showed no toxic effects.

Adsorbent	Time Points	Source
38H21	3 Days	Oak Ridge National Laboratory
TSU 20A	1 Week	
Kostas Fiber	8 Weeks	
JG159a	1 Hr, 1 Day, & 1 Week	
JG159b		
JG164a		
JG192		
KMS-2		
TSU-27		
TSU-39C		
TSU-39D		
TSU-39E		
TSU-45H40		
38H26		
AFL		
11-651H75C		
11-105		
11-117		
11-119		
YF140115A-NH20H		
AI8 L1 R1		
AI8 L1 R2		
AI8 L2 R1		
AI8B16-TOW		
AI8B16-5PPI		
2T8 RMCJ		
AF3 RMCJ		
AF8 RMCJ		
AF160-2 RMCJ		
AF1FR2 RMCJ		
AF1FR3 RMCJ		
AF1B16-5PPI		
AF1B16-TOW		
AF1B17-5PPI		
AF1B17-25PPI		

Adsorbent	Time Points	Source
AF1 F R2	1 Hr, 1 Day, & 1 Week	Oak Ridge National Laboratory
AF1 F R3		
AF1 L1 R1		
AF1 L1 R2		
AF1 L1 R3		
AF1 L2 R1		
AF1 L2 R1 (Fiber)		
AF1 L2 R2 (Fiber)		
AF1 L2 R3 (Fiber)		
AF3		
AO Fiber	6 Weeks	University of Alabama
DA Fiber		
SS Fiber		
RJ-02-76-CUNY	1 Hr, 1 Day, & 1 Week	The City University of New York
RJ-03-66-CUNY		
B2MP-G-169	1 Hr, 1 Day, & 1 Week	University of Maryland
B2MP-G-180		
B2MP-G-182		
B2MP-G-184		
B2MP-G-185		
B2MP-G-186		
Recycled B2MP-CT-126		
Japan 2010	3 Days	Japan Atomic Energy Agency
Chinese Fiber #2	3 Days	Chinese Academy of Science
UHMWPE-g-PAPA-co-PAO 1	1 Hr, 1 Day, & 1 Week	
UHMWPE-g-PAPA-co-PAO 2		
UHMWPE-g-PAPA-co-PAO 3		
UHMWPE-g-PAPA-co-PAO 4		

Conclusion

Uranium adsorbent materials had little or no toxicity even when assayed with direct contact at pp-thousand levels. It is important to note that these adsorbent materials will be exposed in the marine environment at pp-billion or lower levels. Hence these results provide preliminary evidence that uranium extraction from seawater could be performed with minimal impact to marine fauna. We conclude that the adsorbent materials used to extract uranium from seawater are relatively non-toxic and likely to have minimal contact with marine organisms on a large scale

References

1. Girotti SL, Bolelli L, Roda A, Gentilomi G, & Musiani M (2002) Improved detection of toxic checmicals using bioluminescent bacteria. *Analytical Chimica Acta* 473:113-120.
2. Fulladosa E, Murat JC, & Villaescusa I (2005) Study on the toxicity of binary equitoxic mixtures of metals using the luminescent bacteria Vibrio fischeri as a biological target. *Chemosphere* 58(5):551-557.
3. Tsiridis V, *et al.* (2006) Interactive toxic effects of heavy metals and humic acids on Vibrio fischeri. *Ecotoxicology and environmental safety* 63(1):158-167.
4. Backhaus T, Scholze M, & Grimme LH (2000) The single substance and mixture toxicity of quinolones to the bioluminescent bacterium Vibrio fischeri. *Aquat Toxicol* 49(1-2):49-61.
5. Lei L & Aoyama I (2010) Effect-directed investigation and interactive effect of organic toxicants in landfill leachates combining Microtox test with RP-HPLC fractionation and GC/MS analysis. *Ecotoxicology* 19(7):1268-1276.
6. De Zwart D & Sloof W (1983) The Microtox as an alternative assay in the acute toxicity assessment of water pollutants. *Aquatic Toxicology* 4:129-138.
7. Chang JC, Taylor PB, & Leach FR (1981) Use of the Microtox assay system for environmental samples. *Bulletin of Environmental Contamination and Toxicology* 26:150-156.
8. Ankley GT, Hoke RA, Giesy JP, & Winger PV (1989) Evaluation of the toxicity of marine sediments and dredge spoils with the Microtox bioassay. *Chemosphere* 18:2069-2075.
9. Anonymous (2003) ETV Joint Verification Statement. (U.S. Environmental Protection Agenecy and Battelle).
10. Station USGSBRDMER (1998) Toxicity Testing of Sediment from delaware Bay and surrounding Areas. *National Oceanic and Atmospheric Administration Office of Ocean Resources Conservation Assessment*.
11. Parvez S, Venkataraman C, & Mukherji S (2006) A review on advantages of implementing luminescence inhibition test (Vibrio fischeri) for acute toxicity prediction of chemicals. *Environment international* 32(2):265-268.
12. Dutka BJ & Kwan KK (1982) Application of four bacterial screening procedures to assess changes in the toxicity of chemicals in mixtures. *Environmental Pollution* 29:125-134.

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