CORRESPONDENCE/MEMORANDUM

State of Wisconsin

DATE: March 26, 1996 FILE REF: 3200

TO: Jim Reyburn - LMD

FROM: Tom Janisch - WR/2 Tom Janisch

SUBJECT: Update on Kewaunee Marsh Sampling Activities

I would like to touch upon two items related to past and upcoming sampling activities at the Kewaunee marsh site. I apologize for the delay in getting back to you with this information.

1. We continue to develop our sampling plans for the upcoming work season and have not finalized them yet. Right now, it looks like our first major sampling trip to the site will be in late May with possibly a trip or two between now and then to try out equipment, locate sampling sites, etc. The sampling plan will be broad in scope including toxicity testing of soil and water, soil and water sampling and analysis for arsenic, plant collection and tissue analysis for arsenic content, macroinvertebrate collections for tissue analysis of arsenic, and possibly more small mammal trapping with associated tissue analysis. I will provide a more detailed sampling plan when we get it more together. Beside Pat and myself, we will have Jim Ruppel and Clay Wible from our Unit assisting in sampling design and collection.

2. Attached are a number of items related to the results of toxicity testing of soils and water we collected at the site in June of last summer. For a discussion of the sample sites and analytical data, see my December 5, 1995, memo to you. Linda Talbot of our unit interpreted the toxicity results and identified statistically significant differences between the controls and study sites, etc.

Tables 1 and 2 that follow provide a general summary of the results of the testing of soils and water, respectively, along with the corresponding arsenic concentration in each media. Included is a map of the sampling locations on the site.

Following the above is Linda's summary write up of the tox testing results. Following that are some bar graphs generated by the Biomonitoring Lab that allows a visual comparison of the results along with the actual test results for sediment. There are no bar graphs for water tox results, only a table of the tox testing results.

Table 1 summarizes the results from the soils where the soil samples are put in beakers, lab water placed over them, and after a time for equilibration, the test organisms are added. There was possible acute toxicity at KM-4 and KM-6. This possibly means arsenic is partitioning from the sediments to the water column and C. Dubia is ingesting it, resulting in toxicity. The significance of potential toxicity at KM-4 is that of the relatively low concentration in the soil (35 mg/kg). In the chronic toxicity testing for

soils involving D. Magna, the field reference site, KM-1, showed that reproduction was significantly impaired. Ideally, this should be the clean site and should not elicit any response. There is possibly some toxicant or other characteristic in these upstream soils other than arsenic that is causing toxicity. The statements about chronic toxicity at the remaining study sites to D. Magna are relative to the laboratory control sediment. The C. Tentans chronic toxicity results may have been influenced by elevated ammonia levels in the test chambers.

While the sediment toxicity results do not give a clear indication of the linkage between arsenic concentrations and the level of effects, there is an indication that the potential is there. The additional toxicity testing we have planned for the upcoming season will hopefully be more definitive in this regard.

Table 2 summarizes the results of the toxicity testing performed on the water samples. KM-1 is a water sample taken in the Kewaunee River upstream of the site. KM-2 was collected in a dug pit south of the railroad tracks. All the rest of the water collection sites was associated with dug ponds on the site. The water collected from the dug pond at KM-5 showed substantial mortality to minnows which is relatable to the high concentration of arsenic in the water (3,400 ug/l).

In the chronic toxicity testing of water at sites KM-2, KM-5, and KM-6, results showed growth and reproduction impairments to both C. Dubia and minnows. These are the three water collection sites with the highest arsenic concentrations. It is significant that toxicity is being elicited at KM-2, which is on the south side of the railroad tracks. The water table at KM-2 was just below the ground surface at the time of collection. Standing water was present in low areas and between hummocks around the area of KM-2. Precipitation, lake seiches, or high river levels could cause the water table levels to rise creating more areas of standing water that could potentially contain chronically toxic levels of arsenic around the area of KM-2 and beyond. The potential for this and how widespread the arsenic contamination is in the water table south of the tracks is something we will try to address in our workplan for the upcoming sampling season. The tox testing performed on the water samples from the site seems to give a more definitive linkage between arsenic and toxicity compared to the first round of sediment toxicity testing. The results also substantiate the requirement that we put into the consent order that called for filling in the dug ponds just to the north and east of the hot area because of the toxicity being demonstrated especially with water in the toxicity test.

The attached also gives the results for Microtox testing performed on the same water samples collected for the water column and benthic organism bioassays discussed above. The Biomonitoring Lab normally uses Microtox testing on wastewater effluents. The Microtox test can be used for an initial screening of acute toxicity in various liquid media (e.g., effluents, groundwater, stormwater, pore water, or water elutriated from sediments or soils). This acute toxicity assay exposes glowing bioluminescent bacterium to the study sample. The amount of light decrease indicates the degree of toxicity of the sample. The light given off by the bacterium is part of their metabolic processes and the toxicant interferes with this metabolism. For acute toxicity to be elicited in the Microtox test, the percent effect must be +25%

or greater. None of the water samples elicited this effect level. The highest effect level was elicited at KM-5, the site that was associated with mortality of the minnow test organisms.

Sometime in the next month we should set up a meeting to discuss the activities at the site, both ours and STSs. If you have any questions on the interpretation of the tox testing results, give Linda Talbot a call at (608) 266-8148. If you want to talk about any other aspects of our sampling activities, please give me a call.

Mike Berger called me on 03/20 and was looking for information on our tox testing results from last summer, and he requested a copy of results when we have them available. Could you please pass along to him the attached dealing with the results of the toxicity testing.

TPJ:kk
v:\9605\wr9kewmr.tpj

cc: Dennis Wiesensel - NER
Lee Liebenstein - WR/2
Pat Trochlell - WR/2
Jim Ruppel - WR/2
Clay Wible - WR/2
Linda Talbot - WR/2

Table 1. Results of Exposure of Test Organisms to Kewaunee Marsh Soils in Test Chambers.

		Acute Toxicity Tests			Chronic Toxicity Tests	
Sample Station	**Arsenic mg/kg Soil**	**Daphnia magna**	**Ceriodephnia dubia**	**Hyallela azteca**	**Daphnia magna**	**Chironomus tentans**
KM-1 (Reference Site)	4	NT*	NT	NT	Reproduction significantly impaired	NT
KM-2 (S. of RR tracks)	91	NT	NT	NT	Reproduction significantly impaired significant mortality	Growth impaired but qualified
KM-3 (E. of hot area)	120	NT	NT	NT	Reproduction significantly impaired	Growth impaired but qualified
KM-4 (NE of hot area)	35	NT	Toxic but qualified	NT	Reproduction reduced but not significant	Growth impaired but qualified
KM-5 (N. of hot area)	440	NT	NT	NT	Reproduction reduced but not significant	Growth impaired but qualified
KM-6 (E. of hot area)	230	NT	Toxic but qualified	NT	NT	Growth impaired but qualified

* NT = No Toxicity. No statistically significant differences between control and site results.

corespdc\kewaunee.tj

Table 2. Results of Exposure of Test Organisms to Water Collected from Kewaunee Marsh.

		Acute Toxicity Tests			Chronic Toxicity Tests	
Sample Station	**Arsenic ug/L Water**	**Daphnia magna**	**Ceriodephnia dubia**	**Fathead Minnow**	**Ceriodephnia dubia**	**Fathead Minnow**
KM-1 Kewaunee River	3	100% survival NT	100% survival NT	NT	NT	NT
KM-2 (S. of RR tracks)	500	100% survival NT	100% survival NT	NT	Reproduction significantly impaired	Growth significantly impaired
KM-3 (Dug Pond)	3700	100% survival NT	100% survival NT	NT	NT	NT
KM-4 (Pond #6)	62	100% survival NT	100% survival NT	NT	NT	NT
KM-5 (Pond #12)	3400	100% survival NT	90% survival NT	Toxic but qualified	Reproduction significantly impaired	Significant mortality growth significantly impaired
KM-6 (Dug Pond)	860	100% survival NT	100% survival NT	NT	NT	Growth significantly impaired

* NT = No Toxicity. No statistically significant differences between control and site results.

corespdc\kewaunee.tj

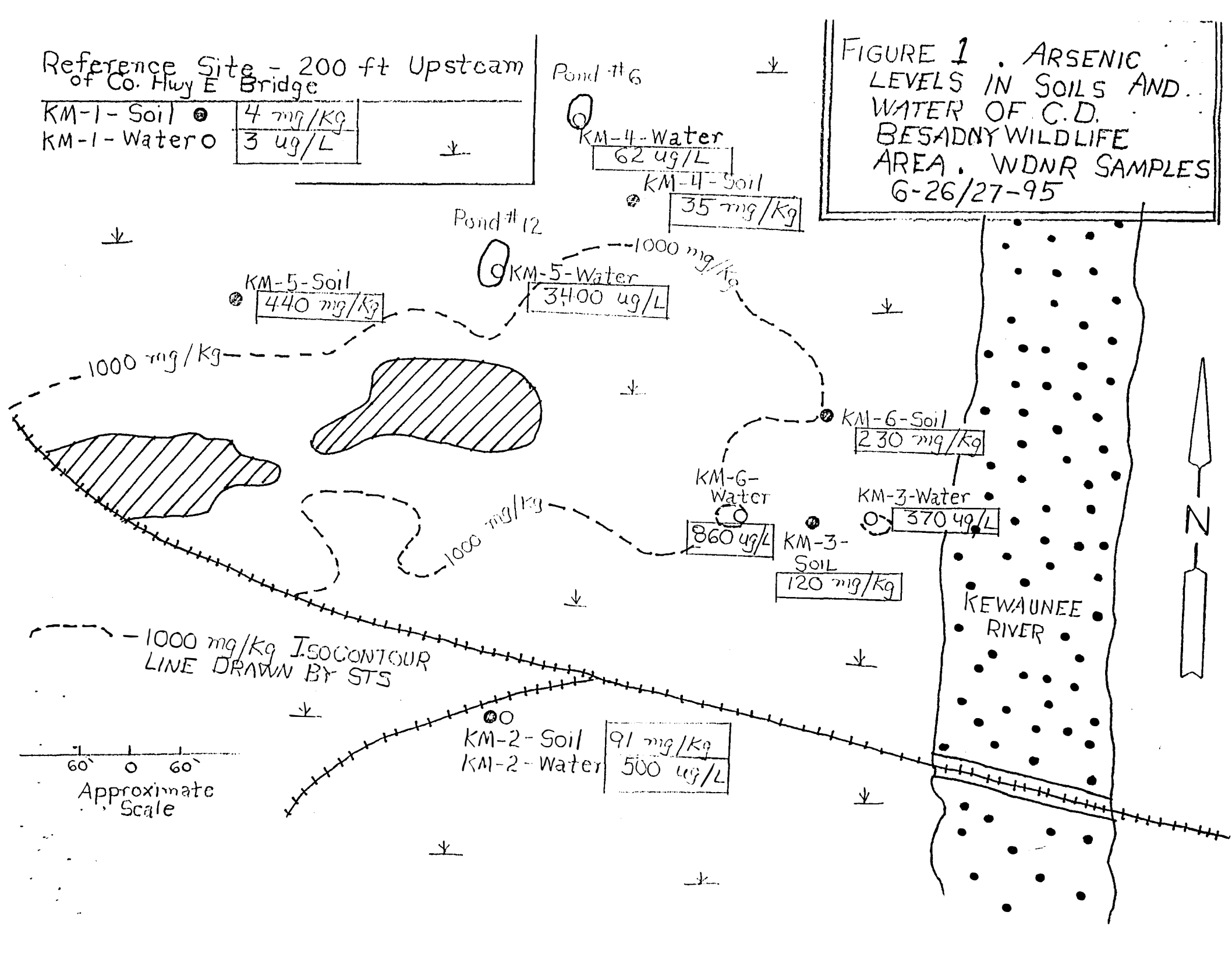


FIGURE 1. ARSENIC LEVELS IN SOILS AND WATER OF C.D. BESADNY WILDLIFE AREA. WDNR SAMPLES 6-26/27-95

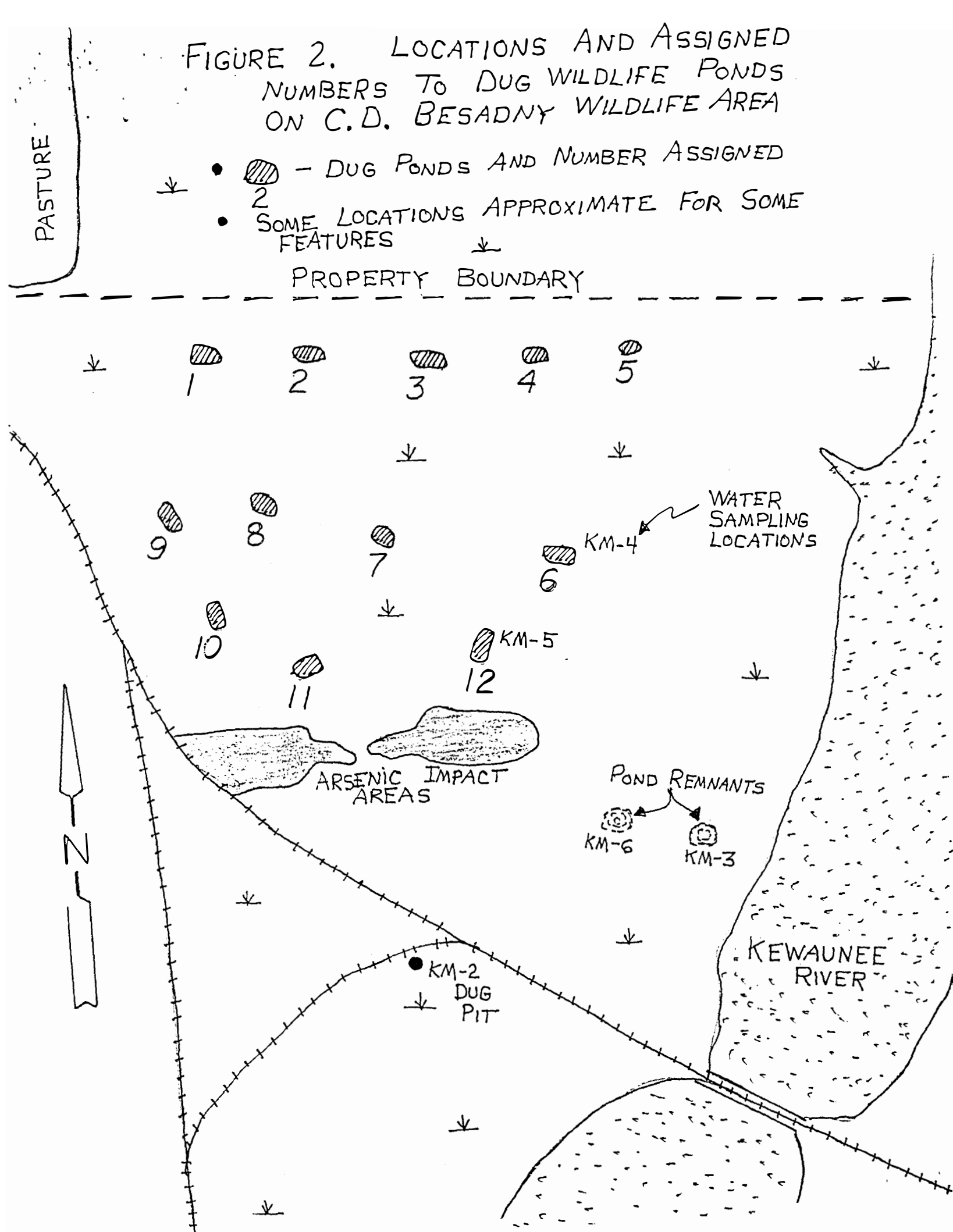


FIGURE 2. LOCATIONS AND ASSIGNED NUMBERS TO DUG WILDLIFE PONDS ON C.D. BESADNY WILDLIFE AREA
- DUG PONDS AND NUMBER ASSIGNED
2
SOME LOCATIONS APPROXIMATE FOR SOME FEATURES
PROPERTY BOUNDARY
PASTURE
1
2
3
4
5
6
7
8
9
10
11
12
KM-4
WATER SAMPLING LOCATIONS
KM-5
ARSENIC IMPACT AREAS
POND REMNANTS
KM-6
KM-3
KM-2
DUG PIT
KEWAUNEE RIVER
N

SEDIMENT TOXICITY RESULTS

SUMMARY OF SEDIMENT TOXICITY TEST RESULTS FOR KEWAUNEE MARSH
(C.D. BESADNY WILDLIFE AREA)

Following is a review of the sediment toxicity data generated July/August 1995 for Kewaunee Marsh. Samples taken from the area are identified as follows: KMC-1, the field reference; KM-2; KM-3; KM-4; KM-5; and KM-6.

Acute

Acute toxicity tests were conducted with *Daphnia magna, Ceriodaphnia dubia,* and *Hyallela azteca*. Test results were analyzed using methods described in "Methods for Measuring the Toxicity and Bioaccumulation of Sediment Associated Contaminants with Freshwater Invertebrates".

No significant mortality was observed for the exposure of *D. magna*. Survival exceeded 87% for all treatments and the greatest difference from lab control was only 10%.

The test acceptability requirement for 90% or greater survival in the control exposure of *C. dubia* was not met. There was 80% survival in the lab control. The variability of recovery within treatments was also too great to demonstrate any statistically significant difference between treatments and the control or reference using the statistical tests of hypothesis.

In an alternate method of analysis using the 25% Inhibition Concentration (criterion), currently applied to wastewater discharge data, the individual site exposure summary results are compared to 75% of the lab control response and any response >25% inhibition relative to the control is considered a failure or toxic. The occurrence of *Ceriodaphnia dubia* mortality being 25% or more greater than the control in treatments KM-4 and KM-6 would typically be interpreted as toxic. In this test the same conclusion is not as definite due to the survival difficulties experienced in the lab control.

The test acceptability requirement of 80% or greater survival of *Hyallela azteca* also was not met. No toxicity was observed as survival in the treatments was similar to (greatest inhibition at only 11% different) or better than the control and reference.

Chronic

Ten-day chronic toxicity exposures were conducted with *Daphnia magna* (survival and reproduction endpoints) and *Chironomus tentans* (survival and weight endpoints). The data were analyzed using "Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates" (EPA, 1994) and "Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms" (EPA, 1994a).

SEDIMENT

D. magna experienced significant mortality (P=0.05) over treatment KM-2. Survival of *D. magna* exceeded 80% in all other exposures. (80% survival is also the test acceptability criterion in the lab control.)

D. magna reproduction was significantly impaired in the field reference (KMC-1) and treatments KM-2 and KM-3. Also, the summary data indicates average reproduction in treatments KM-4 and KM-5 (as well as KM-2 and KM-3) was reduced >25% relative to the control; however, the variability in the number of young produced over replicate treatments for samples KM-4 and KM-5 precludes a significant difference determination. Ammonia levels remained within required limits for all treatments. All other test acceptability requirements were met except for a pH change in treatment KM-2 from an initial 7.16 to final 8.33 and substantial changes in alkalinity and hardness, which may have a role in the significant mortality and the subsequent lack of young produced.

The Kewaunee Marsh treatments were not toxic to survivability of *C. tentans* over 10 days; however, *C. tentans* growth was significantly inhibited (P=0.05) over all treatments, excluding the field reference KMC-1. The ammonia results confound these results as ammonia concentrations increased substantially over the duration of the *C. tentans* test for all of the toxic treatments and may have affected the growth results.

WATER TOXICITY RESULTS

SUMMARY OF WATER COLUMN TOXICITY TEST RESULTS FOR KEWAUNEE MARSH (C.D. BESADNY WILDLIFE AREA)

Following is a review of the water column toxicity test data generated July/August 1995 for Kewaunee March. Samples taken from the area are identified with field descriptor and lab number as KMC-1-TOX, the field reference water sample with lab number 95069; KM-2-TOX = 95070; KM-3-TOX = 95071; KM-4-TOX = 95072; KM-5-TOX = 95073; KM-6-TOX = 95074. The lab control water is Black Earth Creek water (BEC)=LC.

ACUTE

Acute toxicity tests were conducted for 48 hours with *Ceriodaphnia dubia* and *Daphnia magna*. A four-day acute toxicity test was conducted with *Pimephales promelas*. Test results were analyzed using methods described in "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms" (EPA, 1993).

Over 48 hours *Ceriodaphnia dubia* survival was 100% over all treatments except KM-5, which experienced only a 10% loss. *Daphnia magna* survival was 100% over all treatments.

In the 4-day acute exposure of *Pimephales promelas*, the only substantial mortality occurred over KM-5 with a 25% loss relative to the control. This difference is not statistically significant. However, using the criterion currently applied to wastewater discharge data (the Inhibition Concentration at which there is 25% inhibition relative to the control), this response is considered a failure or toxic.

CHRONIC

Survival of *Ceriodaphnia dubia* over the 7-day chronic test was greater than or equal to 90%. *Ceriodaphnia dubia* reproduction was significantly impaired (P=0.05) over treatments KM-2 and KM-5.

Significant mortality of *Pimephales promelas* over 7 days occurred over treatment KM-5. All other treatments saw 93% or greater survival. Growth (weight) was significantly impaired over treatments KM-2, KM-5, and KM-6. The inhibition summary also indicates these sites were 27% or more different than the control.

WATER

SUMMARY

No significant acute toxicity was observed in any of the Kewaunee Marsh water column samples. However KM-5 caused substantial mortality (25%) to *Pimephales promelas* in the last day of the 4-day acute test.

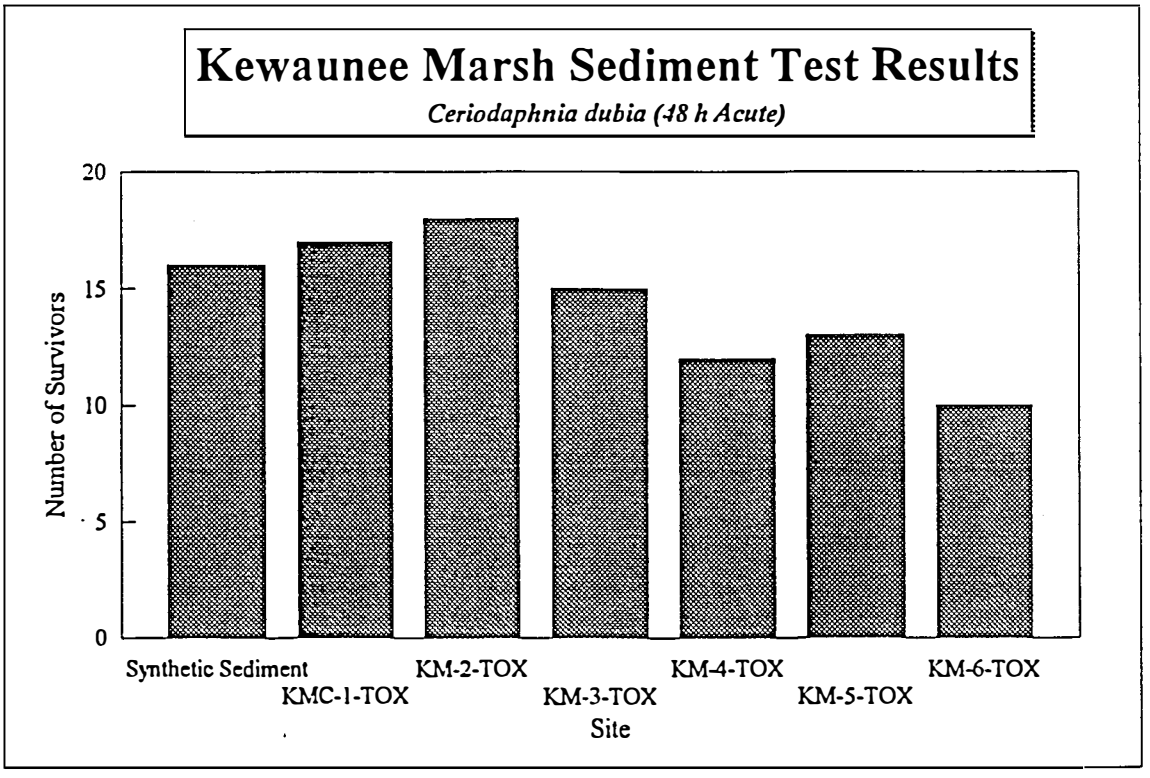
Over the 7-day chronic test, KM-5 did cause significant mortality to the fathead larvae.

Water samples from KM-2 and KM-5 were chronically toxic to *Ceriodaphnia dubia* reproduction and *Pimephales promelas* growth. *Pimephales promelas* growth was also significantly reduced over KM-6.

Kewaunee Marsh Sediment Test Results

Ceriodaphnia dubia (48 h Acute)

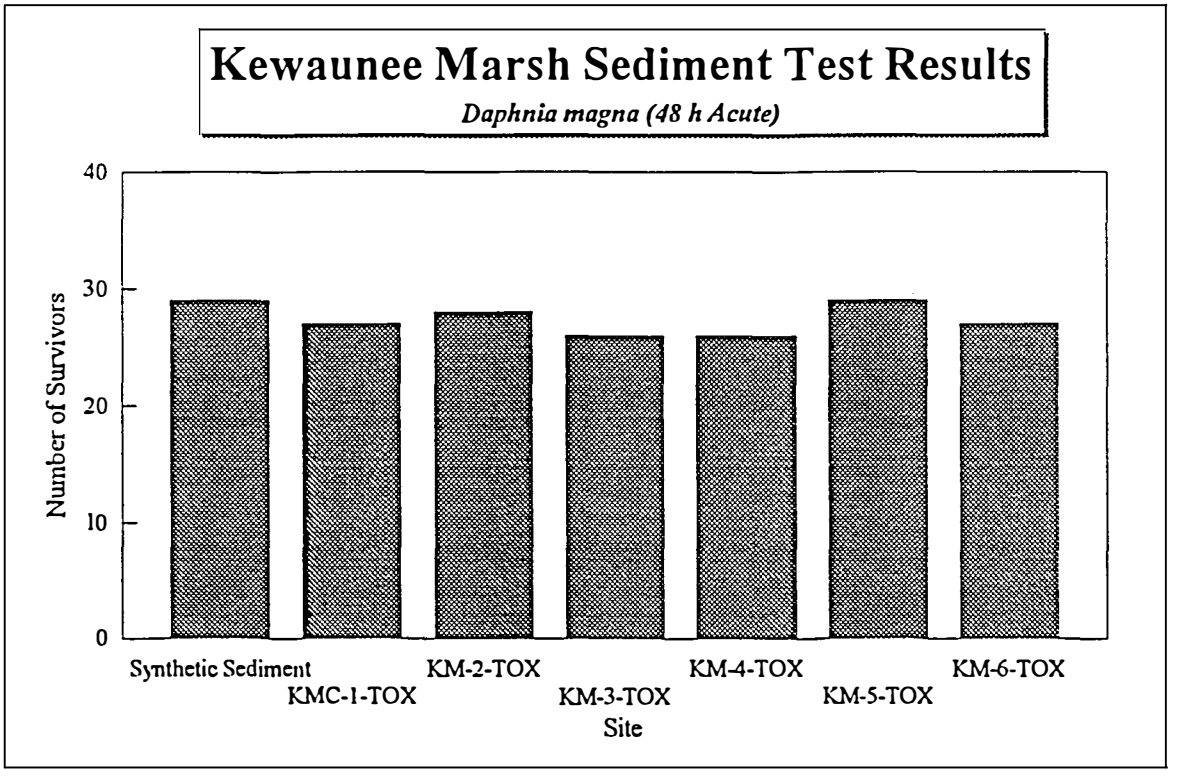
Site	Number of Survivors	Percent Survival (%)	Difference from Lab Control (%)
Synthetic Sediment	16	80%	—
KMC-1-TOX	17	85%	6%
KM-2-TOX	18	90%	13%
KM-3-TOX	15	75%	-6%
KM-4-TOX	12	60%	-25%
KM-5-TOX	13	65%	-19%
KM-6-TOX	10	50%	-38%



Kewaunee Marsh Sediment Test Results

Daphnia magna (48 h Acute)

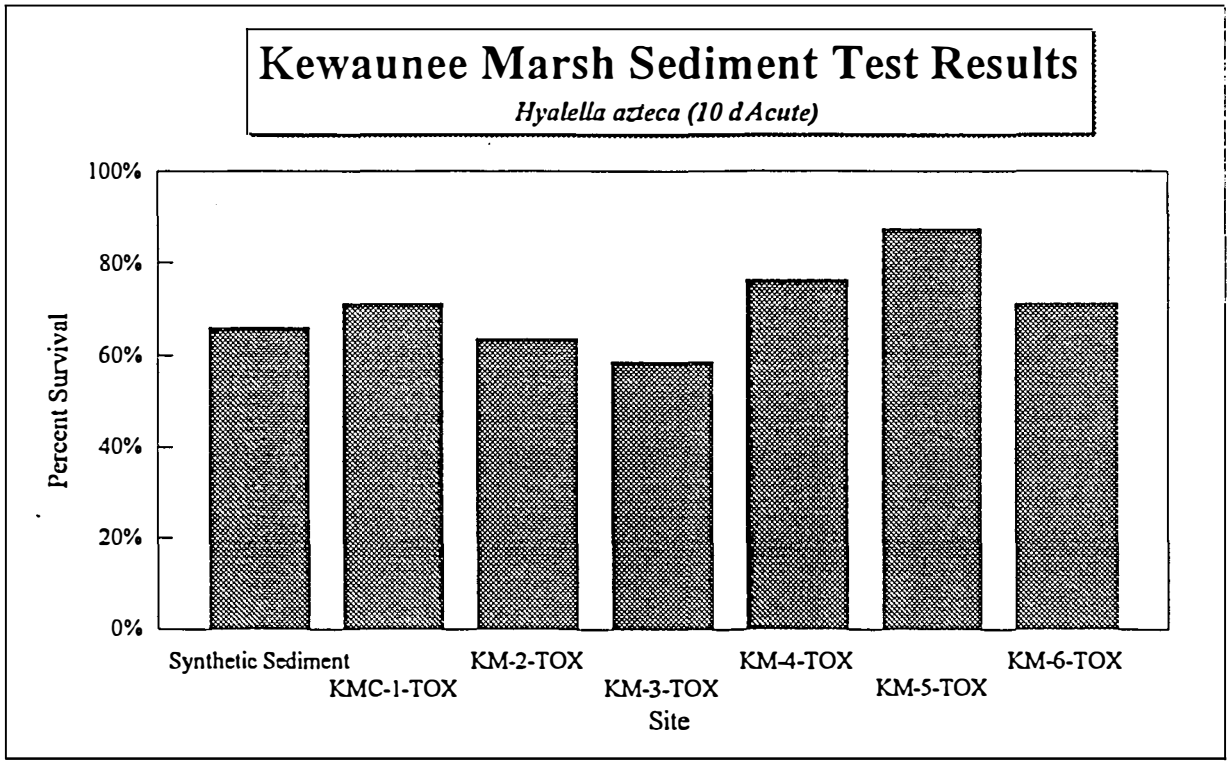
Site	Number of Survivors	Percent Survival (%)	Difference from Lab Control (%)
Synthetic Sediment	29	97%	---
KMC-1-TOX	27	90%	-7%
KM-2-TOX	28	93%	-3%
KM-3-TOX	26	87%	-10%
KM-4-TOX	26	87%	-10%
KM-5-TOX	29	97%	0%
KM-6-TOX	27	90%	-7%



Kewaunee Marsh Sediment Test Results

Hyalella azteca (10 d Acute)

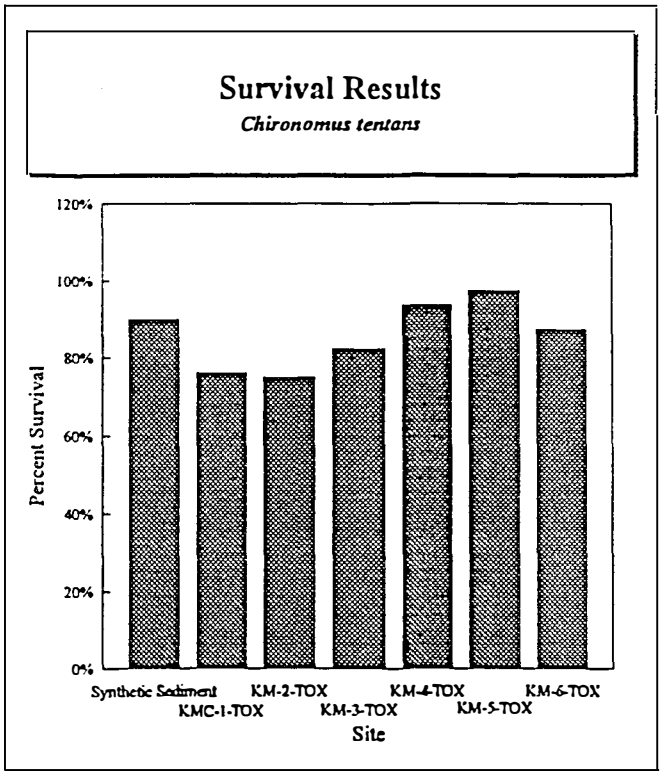
Site	Mean Number of Survivors	STDV	Percent Survival (%)	Difference from Lab Control (%)
Synthetic Sediment	7	2	66%	---
KMC-1-TOX	7	2	71%	8%
KM-2-TOX	6	4	64%	-4%
KM-3-TOX	6	4	59%	-11%
KM-4-TOX	8	3	76%	15%
KM-5-TOX	9	2	88%	32%
KM-6-TOX	7	3	71%	8%

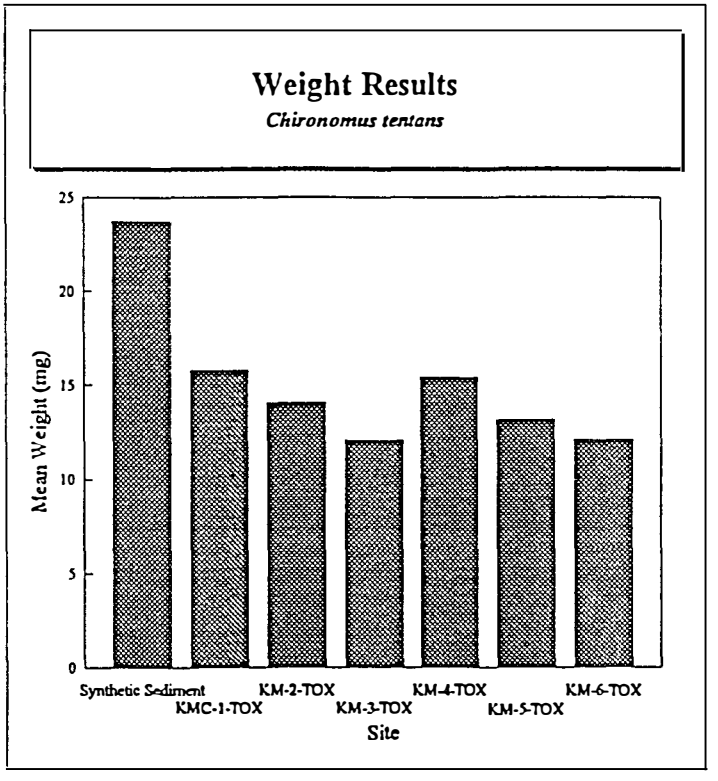


Kewaunee Marsh Sediment Test Results

Chironomus tentans (10 d Chronic)

Site	Mean Number of Survivors	STDV	Percent Survival (%)	Difference from Lab Control (%)	Mean Weight (mg)	STDV	Difference from Lab Control (%)
Synthetic Sediment	9	1	90%	—	23.70	4.92	—
KMC-1-TOX	8	2	76%	-15%	15.81	4.35	-33%
KM-2-TOX	8	2	75%	-17%	14.09	2.52	-41%
KM-3-TOX	8	1	83%	-8%	12.10	1.72	-49%
KM-4-TOX	9	1	94%	4%	15.43	1.82	-35%
KM-5-TOX	10	1	98%	8%	13.22	1.64	-44%
KM-6-TOX	9	2	88%	-3%	12.12	3.02	-49%

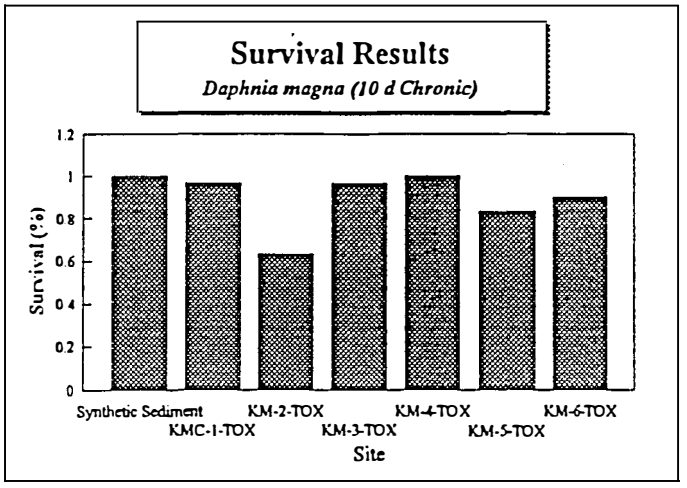


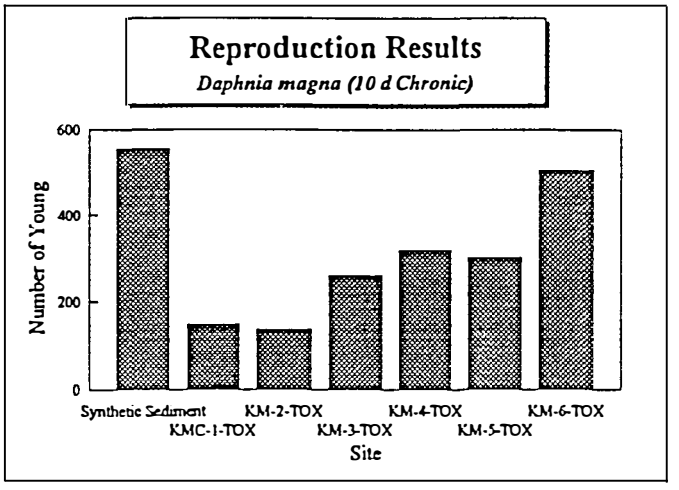


Kewaunee Marsh Sediment Test Results

Daphnia magna (10 d Chronic)

Site	Number of Survivors	Percent Survival (%)	Difference from Lab Control (%)	Number of Young	Difference from Lab Control (%)
Synthetic Sediment	30	100%	---	555	---
KMC-1-TOX	29	97%	-3%	149	-73%
KM-2-TOX	19	63%	-37%	137	-75%
KM-3-TOX	29	97%	-3%	262	-53%
KM-4-TOX	30	100%	0%	320	-42%
KM-5-TOX	25	83%	-17%	302	-46%
KM-6-TOX	27	90%	-10%	505	-9%





Acute And Chronic Water Toxicity Test Results

Summary of Acute Toxicity Test Results on Besadny Wildlife Area Overlying Waters

Site	Lab Number	DO (mg/L)	pH (su)	Cond (µmhos/cm)	Alk (mg/L)	Hard (mg/L)	Total Ammonia (mg/L)	Percent Survival 48 h *C. dubia*	48 h *D. magna*	96h *P. promelas*
Black Earth Creek	LC	8.0	8.54	521	257	297	0.21	100%	100%	100%
KMC-1-TOX	95069	7.5	8.33	599	245	286	0.14	100%	100%	100%
KM-2-TOX	95070	6.5	7.41	373	192	214	0.23	100%	100%	95%
KM-3-TOX	95071	6.6	7.81	519	236	356	0.64	100%	100%	95%
KM-4-TOX	95072	7.1	7.98	335	159	182	0.49	100%	100%	95%
KM-5-TOX	95073	7.5	7.94	381	197	226	0.33	100%	100%	75%
KM-6-TOX	95074	6.8	7.65	440	226	257	0.25	90%	100%	100%

Summary of Chronic Toxicity Test Results on Besadny Wildlife Area Overlying Waters

Site	Lab Number	DO (mg/L)	pH (su)	Cond (µmhos/cm)	Alk (mg/L)	Hard (mg/L)	Total Ammonia (mg/L)	Percent Survival *C. dubia*	Mean Number of Neonates	Difference from Lab Control (%)	Percent Survival *P. promelas*	Mean Weight (µg)	Difference from Lab Control (%)
Black Earth Creek	LC	7.8	8.57	409	257	297	0.21	100%	15	—	100%	605	—
KMC-1-TOX	95069	7.6	8.36	597	245	286	0.14	100%	22	45%	98%	521	-14%
KM-2-TOX	95070	6.8	7.63	382	192	214	0.23	100%	4	-77%	95%	365	-40%
KM-3-TOX	95071	6.6	7.82	512	236	356	0.64	90%	20	28%	95%	531	-12%
KM-4-TOX	95072	7.2	8.09	335	159	182	0.49	90%	17	12%	100%	510	-16%
KM-5-TOX	95073	7.5	8.10	370	197	226	0.33	90%	3	-84%	78%	342	-43%
KM-6-TOX	95074	7.0	7.80	437	226	257	0.25	90%	19	20%	93%	444	-27%

Microtox Test Results On Water Samples Collected June 1995

Test Date: 30 June 95
Lab Numbers: 95069-95074

		Results after 5 minutes								Results after 15 minutes							
Site	Lab Number	1	2	3	4	5	Mean	STDV	% Effect	1	2	3	4	5	Mean	STDV	% Effect
Blank	LC	90	82	82	81	81	82	0.50	—	73	67	65	65	65	66	0.87	—
KMC-1-TOX	95069	83	80	81	83	80	81	1.22	0.61%	70	69	68	70	66	68	1.48	-4.20%
KM-2-TOX	95070	79	72	69	68	66	69	2.17	15.64%	80	75	72	73	71	73	1.48	-11.07%

		Results after 5 minutes								Results after 15 minutes							
Site	Lab Number	1	2	3	4	5	Mean	STDV	% Effect	1	2	3	4	5	Mean	STDV	% Effect
Blank	LC	90	79	78	79	78	79	0.50	—	74	68	64	64	65	65	1.64	—
KM-3-TOX	95071	83	84	84	83	83	84	0.50	-6.37%	86	68	86	86	86	87	0.87	-32.57%
KM-4-TOX	95072	85	82	81	80	81	81	0.71	-3.18%	75	73	71	69	71	71	1.41	-8.81%

		Results after 5 minutes								Results after 15 minutes							
Site	Lab Number	1	2	3	4	5	Mean	STDV	% Effect	1	2	3	4	5	Mean	STDV	% Effect
Blank	LC	90	79	78	77	76	78	1.12	—	78	64	64	62	62	63	1.00	—
KM-5-TOX	95073	67	66	65	67	65	66	0.83	15.16%	61	61	58	59	60	60	1.12	5.56%
KM-6-TOX	95074	80	74	74	74	73	74	0.43	4.84%	83	78	78	78	77	78	0.43	-23.41%

		Results after 5 minutes								Results after 15 minutes							
Site	Lab Number	1	2	3	4	5	Mean	STDV	% Effect	1	2	3	4	5	Mean	STDV	% Effect
Blank	LC	90	77	76	74	75	76	1.12	—	75	66	64	61	63	64	1.80	—
KM-3-TOX-DUP	95071D	80	81	77	76	74	77	2.55	-1.99%	82	83	79	78	75	79	2.86	-24.02%
							ERR	ERR	ERR						ERR	ERR	ERR

Comments:

To Demonstrate Acute Toxicity, % Effect Level must exceed +25%