A 96-Hour Sediment Bioassay of Duluth and Superior Harbor Basins (Minnesota) Using *Hexagenia Limbata, Asellus communis, Daphnia magna,* and *Pimephales promelas* as Test Organisms

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Currently, there is increasing concern about trace levels of toxic substances in our environment. Although analytical procedures allow low levels of toxicants to be measured, the biological significance of these low concentrations is not well understood. Consequently, bioassay procedures will play an increasingly important role in water pollution studies primarily to help answer questions that cannot be answered by water chemistry alone.

The environmental effects of sediments upon water quality and the aquatic ecosystem are currently being investigated by bulk sediment analysis or elutriate procedures, with neither procedure being totally adequate.

Using a sediment bioassay procedure developed at the Central Regional Laboratory, U.S. EPA, Region V by Prater and Anderson (1977), sediment bioassays were conducted on eight sediments collected from Duluth and Superior Harbors in July of 1976. The stations were chosen to reflect areas that were suspected to be heavily polluted, moderately polluted, and nonpolluted. In addition, a correlation was drawn between the percent mortality of biological test organisms (Table 1) and the bulk sediment chemistry data collected by Bowden (1976) and illustrated in Table 2.

TABLE 1

Suggested percent mortality ranges from a 96-hour sediment bioassay for Hexagenia limbata, Asellus communis, Daphnia magna, and Pimephales promelas to be used in sediment classification (Prater, 1976).

Species	Nonpolluted	Moderately Polluted	Heavily Polluted
H. limbata	<10	10-50	>50
A. communis	<10	10-50	>50
D. magna	<10	10-50	>50
P. promelas	<10	10-50	>50

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TABLE II

Ranges used to classify sediments using bulk sediment chemical analysis. All ranges are in mg/kg dry wt. (Bowden, 1976).

Parameter	Nonpolluted	Moderately Polluted	Heavily Polluted
*Volatile solids	<5%	5%-8%	>8%
*COD	<40,000	40,000-80,000	>80,000
*TKN	<1,000	1,000-2,000	>2,000
*Oil & Grease	<1,000	1,000-2,000	>2,000
*Lead	<40	40-60	>60
*Zinc	<90	90-200	>200
*Mercury	<1.0	N.A.	>1.0
**Ammonia	<75	75-200	>200
**Cyanide	<0.10	0.10-0.25	>0.25
**Phosphorus	<420	420-650	>650
**Iron	<17,000	17,000-25,000	>25,000
**Nickel	<20	20-50	>50
**Manganese	<300	300-500	>500
**Arsenic	<3	3-8	>8
**Cadmium	***	***	>6
**Chromium	<25	25-75	>75
**Barium	<20	20-60	>60
**Copper	<25	25-50	>50

*These ranges are based on compilations of data from over 100 different harbors since 1967.

**These ranges are based on 260 samples from 34 harbors sampled during 1975-1976.

***Lower limits not established.

MATERIALS AND METHODS

The bioassay apparatus used was constructed by modifying one described by Fremling (1970). These all-glass toxicity vessels were assembled with silicon glue. Constant recirculation of test water through each test vessel was assured by pumping water via an air lift (made of 4 and 6 mm I.D. glass tubing) from a 4-liter glass jar through the test vessel and into another 4-liter glass jar via an intermittent siphon. A small piece of #60 stainless steel mesh screen covered the siphon to prevent escape of the test organism. Water was returned by a siphon (10 mm I.D. glass tubing) from the second jar to the first. The flow rate was adjusted to insure that the water would recirculate approximately 2 liter/60 minutes. This water velocity did not disturb the substrate and allowed for the maintenance of proper dissolved oxygen (Figure 1).

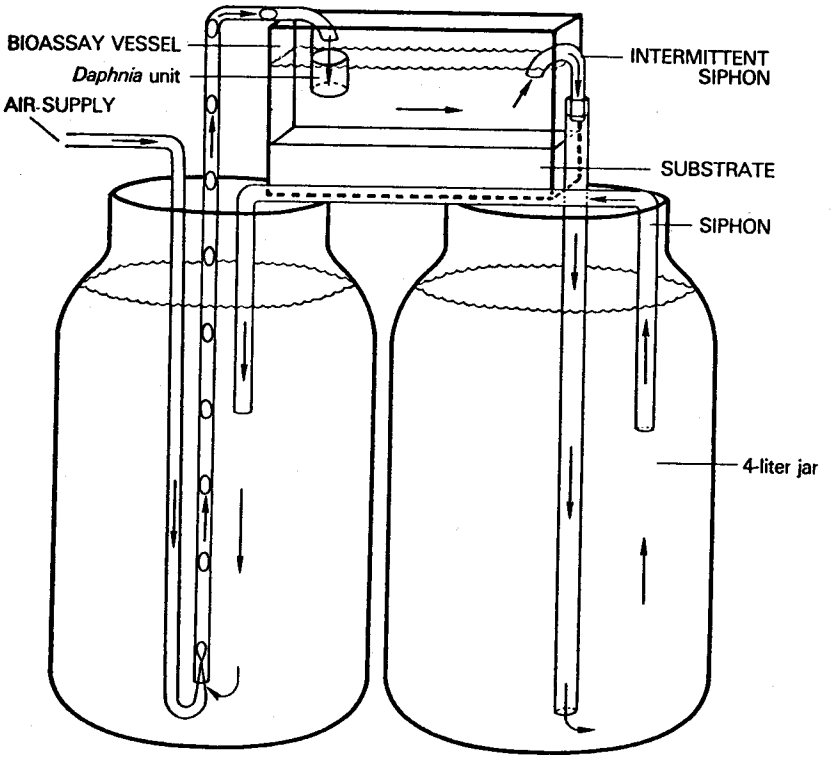

Figure 1. Recycling bioassay apparatus.

Hexagenia limbata and Daphnia magna were selected as test organisms because they are sub-surface and planktonic fauna, respectively. Asellus communis was chosen because it represented a facultative species and spends the majority of its life cycle at the water-substrate interface. Pimephales promelas is an accepted fish for bioassay species and was also used.

The H. limbata nymphs were collected from a river drainage in Northwest Ohio by using the BOP (Prater, et. al., 1977) sampling apparatus and a standard #30 mesh screen. The organisms were placed in a container of water from the source of collection, aerated, and cooled until transport to the laboratory, where they were placed in rearing tanks especially designed for that purpose (Prater and Anderson, 1976). The A. communis were collected from a site near the laboratory, while the D. magna and P. promelas were obtained from laboratory stock cultures.

All test organisms were acclimated at 22°C for 12 hours prior to the test and were not fed during acclimation or testing. All individuals of each test species were approximately the same size, using early to intermediate instars when possible. All fish weighed between 0.5 g and 5.0 g, and the standard length of the longest fish was no more than twice that of the shortest fish.

Sixteen two-jar units were placed in an environmental chamber allowing for a replicate of each sediment type. Eight liters of dechlorinated (pre-test) tap water was added to each bioassay apparatus, and the apparatus was run for 48 hours prior to testing to insure complete mixing, temperature stabilization, and flow calibration.

Prior to starting the bioassay, the air pumps were stopped and the pre-test water siphoned from the bioassay vessel into a glass beaker, and a two-inch layer of sediment was placed on the bottom of the bioassay vessel. The pre-test water that was removed was then returned to the vessel and allowed to settle for 12 hours. The recycling of the water was started, and the flow was checked and recalibrated to insure all bioassay vessels were maintaining approximately the same flow rate.

A special D. magna test apparatus was constructed of 2-inch glass tubing, one end open and the other covered with #60 stainless mesh. One apparatus was suspended below the intake of each vessel allowing a 100 ml volume of water for the D. magna test organisms.

All test organisms were randomly selected and placed into the test vessels, avoiding unnecessary handling. Ten H. limbata, and twenty D. magna were used in each test vessel. Twenty A. communis were used in sediments 76-7 and 76-8 due to a lack of enough individuals of this species for every sediment type. Ten P. promelas were placed in the 4-liter jar that housed the air lift.

The temperature in the environmental chamber was maintained at 22°C (±.5°C) during the test period, and the natural photoperiod of the test region was held constant for the duration of the test. Dissolved oxygen was monitored at regular intervals during the test and is reported as Table III in the results. These measurements were taken directly from the 4-liter jars not holding test organisms to avoid disturbing the test organisms.

After 96 hours, the test was terminated and water samples removed for chemical analysis, which is reported as Tables IV and V in the results. Chemical analysis of the sediments had been completed prior to the bioassay and is reported as Table VI in the results. Each sediment from the bioassay was screened

using a #30 mesh screen to receive and retain the test organisms. Live and dead individuals were recorded for each test vessel and the results reported as percent mortality in the results (Figure 2).

RESULTS

TABLE III

Daily range of dissolved oxygen during 96-hour sediment bioassay of Duluth and Superior Harbor Basins, Minnesota (July 1976). Replicates are indicated as (A) and (B).

	July 22*	July 23	July 24	July 25*
	D.O.	D.O.	D.O.	D.O.
76-1 (A)	6.2	5.9 - 6.7	6.4 - 6.8	7.0
76-1 (B)	5.7	5.8 - 6.4	5.9 - 6.2	6.6
76-2 (A)	6.2	5.9 - 6.4	6.2 - 6.5	6.8
76-2 (B)	6.8	4.1 - 6.6	5.8 - 6.5	7.6
76-3 (A)	5.9	5.7 - 6.2	6.0 - 6.1	6.3
76-3 (B)	5.6	4.9 - 6.4	6.4 - 6.6	6.7
76-4 (A)	6.4	6.2 - 6.9	6.5 - 6.8	6.9
76-4 (B)	6.2	5.4 - 6.4	6.1 - 6.5	6.5
76-5 (A)	5.8	5.7 - 6.2	5.7 - 6.6	6.7
76-5 (B)	5.4	4.8 - 5.6	5.3 - 5.8	6.1
76-6 (A)	6.2	5.8 - 6.2	5.7 - 6.3	6.7
76-6 (B)	6.2	3.7 - 6.9	6.0 - 6.4	6.9
76-7 (A)	7.0	6.4 - 6.7	6.2 - 6.6	6.5
76-7 (B)	6.6	6.4 - 6.7	5.9 - 7.0	6.3
76-8 (A)	6.5	5.8 - 6.5	5.5 - 6.0	5.3
76-8 (B)	6.9	6.4 - 7.1	6.5 - 6.9	6.2

*only one reading taken

DISCUSSION

The biological condition of each station (sediment type) was classified as being either nonpolluted, moderately polluted, or heavily polluted, using Tables I and II and Tables III-VI as well as Figure 2.

Duluth Harbor Stations

Sediment 76-1; death occurred in 10 to 20% of the H. limbata and was the only test species that showed mortality in this sediment. Biologically, this station was classified as nonpolluted. Sediment 76-2; the toxic effect of this sediment on the test organisms wes almost non-existent. Less than 10% of the D. magna died, with no death in the remaining organisms thus the condition of the sediment was considered nonpolluted. Sediment 76-3; a moderately to nonpolluted condition existed. Between 10 to 25% mortality occurred, with D. magna being affected most. Sediment 76-4; the condition of the harbor in the vicinity of where the sample was taken was

TABLE IV Inorganic chemistry of the blank, pre-test and post-test water in mg/l* from 96-hour sediment bioassay of Duluth and Superior harbor basins. Minnesota (July, 1976). Replicates were averaged.

	Blank	Pre-test water	Post-test SED 76-1	Post-test SED 76-2	Post-test SED 76-3	Post-test SED 76-4	Post-test SED 76-5	Post-test SED 76-6	Post-test SED 76-7	Post-test SED 76-8
Suspended solids (105°C)	<5	<5	38.5	13.0	35.5	21.0	201.5	107.5	36.0	5.5
Dissolved solids (105°C)	<50	190	185.0	250.0	260.0	245.0	215.0	250.0	220.0	195.0
*Phenolics	4	5	7.0	5.5	5.0	7.0	6.5	5.5	10.5	6.5
Cyanide	<.005	<.005	<.005	<.005	<.005	<.005	<.005	<.005	<.005	<.005
Total NO_3 + NO_2	<0.03	0.17	0.08	0.12	0.09	0.15	0.08	0.05	0.05	.12
Dissolved NO_3 + NO_2	<0.03	0.19	0.07	0.12	0.09	0.15	0.08	0.05	0.04	.12
TKN	<0.05	0.31	2.73	1.53	2.68	2.28	3.51	2.46	3.12	1.95
Dissolved TKN	<0.05	0.32	3.06	1.60	2.68	2.35	3.71	2.47	3.15	1.99
Total Ammonia	<0.03	0.03	2.00	.71	1.40	1.35	2.10	1.39	1.78	1.15
Dissolved Ammonia	<0.03	0.03	2.00	.71	1.44	1.41	2.37	1.41	1.90	1.20
Total P	<0.02	0.02	0.11	0.04	0.14	0.08	0.28	0.15	0.17	0.05
Dissolved P	<0.02	0.02	0.13	0.05	0.15	0.10	0.28	0.20	0.18	0.07
Dissolved Ortho P	<0.005	0.007	0.009	0.005	0.020	0.015	0.020	0.016	0.019	0.012
*Turbidity	.60	1.1	41.0	6.5	34.0	17.5	235.0	117.5	35.0	5.0
*Specific Conductance	<10	300	320	335	330	345	320	330	320	325
Sulfate	3	24	27	27	26	30	29	28	27	26
Chloride	<2.0	13.0	12.5	12.0	12.0	12.0	13.5	12.5	13.0	12.0
Total Silica as SiO_2	<0.2	0.2	2.2	0.8	1.3	1.6	4.8	3.0	1.8	1.2
*pH	4.9	8.0	7.6	7.8	7.7	7.3	7.3	7.7	7.6	7.7
COD	<3.0	12.0	20.5	13.0	23.5	16.5	30.6	33.0	28.0	18.5

* Turbidity units in Formazin Turb.units; specific conductance in µmhos/cm at 25°C; phenolics in µg/l; and pH in pH units.

Table V. Metal chemistry of the blank, pre-test and post-test water in µg/l* from 96-hour sediment bioassay of Duluth and Superior harbor basins, Minnesota(July 1976). Replicates were averaged.

		Blank	Pre-test Water	Post-test SED 76-1	Post-test SED 76-2	Post-test SED 76-3	Post-test SED 76-4	Post-test SED 76-5	Post-test SED 76-6	Post-test SED 76-7	Post-test SED 76-8
Aluminum	(T)	<200	242	5465	<200	2765.5	292	4420	1555	973.5	<200
	(D)	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200
Arsenic	(T)	<2	<2	3	<2	2.5	<2	3	20	20	42.5
	(D)	<2	<2	<2	<2	<2	<2	<2	7.5	5.5	27.5
Barium	(T)	<1	11	113.5	19.5	63.5	25.5	100	54	41	26.5
	(D)	<1	10	33.5	18	22.5	21.7	41	33	27.5	22.5
Beryllium	(T)	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
	(D)	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Boron	(T)	<20	28	49.5	32	36.5	26	46	38	35	33
	(D)	<20	32	36	32	27	32.5	36.5	40.5	38.5	32.5
Cadmium	(T)	<24	<24	<24	<24	<24	<24	<24	<24	<24	<24
	(D)	<24	<24	<24	<24	<24	<24	<24	<24	<24	<24
*Calcium	(T)	2.5	41.9	30	<14	17.5	14.4	17.0	<14	<14	<14
	(D)	0.8	55.6	<14	<14	<14	<14	<14	<14	<14	<14
Chromium	(T)	<14	<14	30	<14	17.5	15	17	<14	<14	<14
	(D)	<14	<14	<14	<14	<14	<14	<14	<14	<14	<14
Cobalt	(T)	<8	<8	27	<8	14.5	<8	24.5	10	9.5	<8
	(D)	<8	<8	<8	<8	<8	<8	<8	<8	<8	<8
Copper	(T)	<5	<5	40	<5	15	5.5	16.5	8.5	6.5	6
	(D)	<5	<5	<5	<5	5.5	5	6.6	8.0	6.5	6
Iron	(T)	<10	15	11,110	22.5	5015	573	9115	2900	2450	267.5
	(D)	<10	47	58	<10	34	25.5	178	28.5	56.5	145
Lead	(T)	<58	<58	72.5	<58	<58	<58	<58	<58	<58	66
	(D)	<58	<58	<58	<58	<58	<58	<58	<58	<58	<58
*Magnesium	(T)	0.1	12.5	14.8	12.9	13.8	12.2	14.4	13.4	12.8	13.2
	(D)	<0.1	11.8	11.1	12.6	11.7	12.0	10.7	11.8	11.5	12.4
Manganese	(T)	<4	<4	728.5	<4	158	21.5	957.5	265	291.5	49.5
	(D)	<4	<4	221.5	<4	10.5	6	576	170	227.5	32
Mercury	(T)	<0.1	0.8	7.8	0.4	2.5	0.4	0.5	0.8	2.2	0.3
	(D)	<0.1	<0.2	0.3	0.2	0.2	0.2	0.1	0.2	0.3	0.2
Nickel	(T)	<46	<46	<46	<46	<46	<46	<46	<46	<46	<46
	(D)	<46	<46	<46	<46	<46	<46	<46	<46	<46	<46
Silver	(T)	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	(D)	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Tin	(T)	<50	<50	52	<50	55	<50	<50	<50	<50	<50
	(D)	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50
Titanium	(T)	<4	<4	46.5	<4	38	12.5	37.5	24	24	<4
	(D)	<4	<4	<4	<4	<4	<4	10	5	<4	<4
Zinc	(T)	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50
	(D)	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50

Total (T); Dissolved (D) * in mg/l

TABLE VI

Inorganic and metals chemistry of the sediments in mg/kg from a 96-hour sediment bioassay of Duluth and Superior harbor basins, Minnesota (July 1976). Based on dry weight.

	SED 76-1	SED 76-2	SED 76-3	SED 76-4	SED 76-5	SED 76-6	SED 76-7	SED 76-8*
Ammonia	46	19	10	10	56	24	27	--
COD	70,000	7,000	20,000	5,000	54,000	11,000	67,000	--
Mercury	0.1	<0.1	<0.1	<0.1	0.1	<0.1	<0.1	--
Phosphorus	510	210	260	270	780	280	450	--
TKN	1,000	43	350	160	1,300	180	1,000	--
Total solids (%)	63.3	84.6	78.6	82.1	53.8	86.4	71.4	--
Total volatile solids (%)	6.0	<1	1.0	<1	5.6	<1	3.2	--
Arsenic	<2	<2	<2	<2	3	<2	<2	<2
Cadmium	<1	<1	<1	<1	<1	<1	<1	<1
Chromium	14	3.0	<2	5.0	46	18	14	3.0
Copper	17.0	3.0	5.0	5.0	30.0	8.0	16.0	<2
Iron	14,000	6,300	5,600	3,800	28,400	7,900	9,100	4,800
Lead	15	<5	<5	<5	<5	<5	10	<5
Magnesium	4,100	1,600	1,500	1,200	17,000	3,300	3,500	1,500
Manganese	220	79	72	74	600	120	200	74
Nickel	<10	<10	<10	<10	30	<10	<10	<10
Zinc	83	8.0	17	20	90	26	61	<0.5

*sample lost

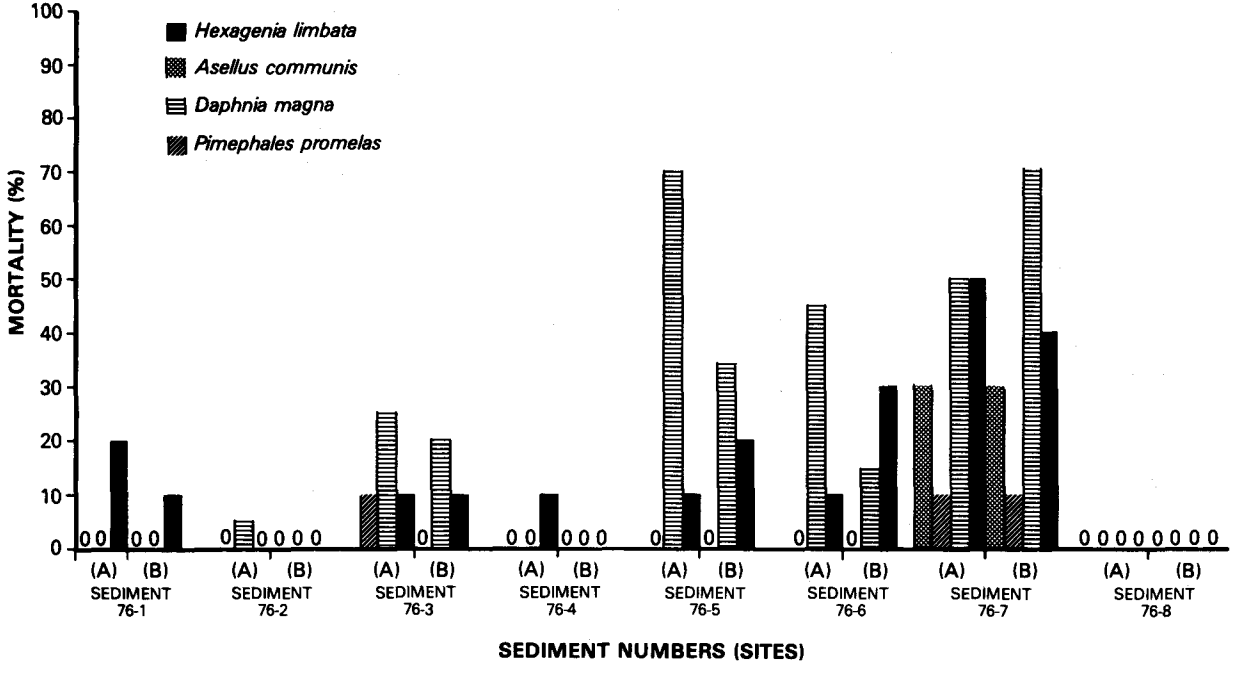

Figure 2. Percent mortality of Hexagenia limbata, Daphnia magna, Pimephales promelas during a 96-hour sediment bioassay of Duluth and Superior Harbor Basins, Minnesota (July 1976). Mortality Rates for Asellus communis are given for sediments 76-7 and 76-8 only. Replicates are indicated as (A) and (B).

considered to be nonpolluted by previous bulk chemistry sediment analysis. Zero mortality was observed in all organisms with the exception of H. limbata where 10% were killed. Sediment 76-7; the sediment at this station showed the greatest amount of toxicity to the test species of any collected from the Duluth Harbor. D. magna, the most sensitive of the organisms used, showed between 50 and 75% mortality. The burrowing mayfly H. limbata had a mortality of between 40 to 50%, while 30% of the A. communis died. In addition, 10% of the fathead minnows died, the only mortality of this species in the entire test. From a biological point of view, this station was considered to be moderate to heavily polluted. An evaluation of the chemistry data did not show any one parameter to be excessively high compared to the other stations so as to indicate what might have caused the death of the organisms. Sediment 76-8; 100% of all test organisms lived in the sediment during the course of the 96-hour sediment bioassay test, resulting in a nonpolluted classification.

Superior Harbor Stations

Sediment 76-5; the D. magna showed a mortality of 45 to 70%. The H. limbata showed a mortality of 10 to 20%. No death occurred among the fathead minnows. Based on these data, it was concluded that this station was moderately polluted. Sediment 76-6; conditions here were somewhat similar to Sediment 76-5. D. magna's mortality was 15 to 45% with H. limbata 10 to 30%. No death occurred among the fathead minnows. This station was also considered to be moderately polluted.

A comparison was made between the bulk sediment chemistry classification (Table II) and the sediment bioassay (Table I) result to determine if there was a correlation between the two. It appeared that six of the eight stations showed a similar classification, using the two methods. Stations 76-6 and 76-7 were the two stations that were dissimilar.

A review of the chemistry data did not indicate that any one parameter analyzed was responsible for the mortality of the test organisms. The concentrations varied from one station to another and did not always appear highest at those stations where mortality was greatest. An assumption was made that either a synergistic or antagonistic effect was occuring which was lethal to the organisms, or chemical parameters that were not measured such as organics were present in high enough concentrations to cause mortality.

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