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DETERMINATION OF SEDIMENT TOXICITY IN WAUKEGAN HARBOR THROUGH PHYTOASSAY METHODS

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ABSTRACT

Phytotoxicity tests that rely on direct contact between plant root systems and contaminated sediment represent an alternative approach to toxicity assessment of these solid substances. The advantage is that this is a direct approach that does not require extraction and separation. The potential application, however, is yet to be tested.

The objective of this study was to assess sediment toxicity by using phytoassay methods. Twenty-one sediment samples were obtained from Waukegan Harbor, Illinois. Phytoassay methods included tests of duckweed, lettuce, and millet.

The most important finding in this study is the disparity of response among test species. Even though the three plant species are all advanced plant life species, they display surprisingly different responses to the same set of sediment samples.

Duckweed is a free-floating macrophyte. It did not exhibit any adverse reactions to the Waukegan Harbor sediments. The lettuce root elongation test is recommended by the U.S. Environmental Protection Agency, the U.S. Food and Drug Administration, and the Organization for Economic Cooperation and Development. This test failed to show harmful effects from the sediment samples, except in the case of a sample from one station (station E). The millet root elongation test was very sensitive in detecting sediment toxicity. Of the 21 sampling stations, 19 stations showed significant phytotoxicity to millet, $P < 0.05$.

The maximum effect was found at station E with 61 percent root length inhibition after three repetitions.

Millet tests that used the growth pouch method were also conducted. The results indicated that the sediment samples were less inhibitory than with the petri dish method, possibly due to the lower sediment concentrations.

INTRODUCTION

Bottom sediment is generally considered a sink for environmental pollutants. Anthropogenic pollutants in the hydrosphere can end up in the sediment and thus be removed from active hydrological cycles. From the environmental point of view, it appears that adsorption of pollutants by sediments is an effective, natural "detoxification" process. Once pollutants are tied down to sediment substances, they fit the description, "Out of sight, out of mind." There are instances, however, indicating that the bottom sediment is not only a depository for pollutants, but also a source of secondary pollution.

Bottom sediment is an active site where benthal organisms thrive. Polluted sediment can be picked up by these organisms, biomagnified, and passed up to successively higher trophic levels, eventually entering humans as the ultimate consumers in the environment. In addition to this route of biomagnification, there is another danger from polluted sediment. Many channels, harbors, and the like are constantly dredged to facilitate navigation. The disturbed sediment can carry and spread pollutants either through the water column or via the relocation of dredged material. It is therefore essential to assess the

toxic potential of sediment material. The results of sediment toxicity assessment can be the basis of intelligent decisions regarding correction strategies, scope of work, priorities, etc.

Extraction Procedure

The currently accepted method for determining sediment toxicity is the Extraction Procedure CEP), published in 43 Federal Register 58956. The procedure uses ambient water as a medium for extraction of water soluble substances. The filtrate is used in toxicity tests with fish, daphnids, algae, etc., as test organisms. The EP has been tested extensively by various investigators, especially Lee and associates and Prater and associates (Lee et al., 1975; Jones and Lee, 1978; Laskowski-Hoke and Prater, 1981; Hoke and Prater, 1980; Prater and Anderson, 1977; Bahnick et al., 1981; Epler et al., 1980).

The extraction step and the subsequent separation step are costly and time consuming, and in addition there is the likelihood that experimental errors will be introduced during these steps. For example, Epler et al. (1980) found that the extraction efficiencies of calcium, chromium, and nickel varied, with the coefficient of variation ranging from 2 to 94 percent. The mean coefficients of variation were 14, 36, and 42 percent for calcium, nickel, and chromium, respectively. These experimental errors are over and above those associated with the bioassay step.

The results of many reports suggest that sediment toxicity tests are still in the stage of early development. Bahnick et al. (1981) conducted a 96-hour bioassay with Hexaoenia limbata.

Daphnia magna. and Pontoporeia affinis and performed chemical analyses of sediments. They also examined Chironomids and Hexaaenia limbata exposed to the sediments for their uptake of chemicals. The results of chemical extraction showed that only small amounts could be readily extracted.

Prater and Anderson (1977) indicated that a correlation could be drawn between the percent mortality of test organisms and chemical data derived from bulk samples. Further study showed that the mortality of the fathead minnow was significantly correlated with the elutriate levels of chloride (Hoke and Prater, 1980). A more recent study was made to correlate 96-hour sediment bioassays with chemical analysis of bulk and elutriate samples. Of the 76 bivariate correlations performed, 14 were considered potentially meaningful (Laskowski-Hoke and Prater, 1981).

Hannan et al. (1976) analyzed 24 elements from sediment and soil samples and correlated algal bioassay results with results of the standard EP. They concluded that the standard EP has limited value; more meaningful data can be obtained from bioassays.

According to Epler et al. (1980), the potential problems associated with the extraction step relate to the introduction of acetic acid, which has a significant phytotoxicity.

Phytoassay Methods

A direct method of assessing marine sediment toxicity by using oyster larvae was reported (Chapman and Morgan, 1983). A major difference of this method from EP is that the test species

oyster larvae were placed in a sediment-water mixture, and the extraction and separation steps were omitted. This direct approach is difficult in toxicity tests using fish, daphnids, bacteria, etc., as indicator organisms. It is, however, readily applicable to plant species.

Plant organisms can extend their roots into sediment and respond to its toxicity. This direct method requires no extraction and separation steps. The advantages are enormous. First, the method bypasses the experimental errors associated with the extraction and separation steps. Second, the results can be obtained more economically. Third, phytoassays provide an alternative test method especially suitable for organic-Iaden sediment samples containing herbicidal compounds.

There are indications that aquatic macrophytes may be strong candidates for determination of sediment toxicity. Fekete et al. (1976) reported that the growth of common duckweed was directly related to the phosphorus content of sediment samples. More interestingly, its growth under anaerobic conditions was much greater than under aerobic conditions, indicating the greater release of available phosphorus under anaerobic conditions. A chemical determination of total phosphorus in the sediments was also made. The chemical method, however, was found unreliable for predicting amounts of phosphorus available to duckweed.

Mayes et al. C1977) studied the role of roots in the uptake of the non-essential trace metals cadmium and lead. Plants were grown in two lakes, of which one was a control and the other was treated. Plants grown in the same water but in sediments from

different sources had significantly different concentrations of the two metals. Furthermore, plants grown in the same sediment but in water with different levels of metals also accumulated significantly different amounts of cadmium and lead.

Harding and Whitten (1978) determined the heavy metal budget of the Derwent Reservoir, Northern England. They found that the heavy metals cadmium and lead deposited rapidly in sediments. Macroscopic plants grew only occasionally in this reservoir, due perhaps in part to heavy metal toxicity. Of the two most common submerged species, Nitells flexis probably accumulated almost all its metal content directly from water while Glyceria fluitans derived its heavy metals from sediment.

Common duckweed (Lemna Minor) is a free-floating aquatic macrophyte. It has been used in experiments at the Illinois State Water Survey for several years (Wang, 1986a, 1986b). The results indicate that duckweed is as sensitive to heavy metal toxicity as fish species, and sometimes more so. For example, the water quality criterion for the Zn ion was identical whether obtained by using tests of faunal species or duckweed, while the criteria for the Cd and Ni ions were more stringent when obtained by using tests of duckweed than by using tests of faunal species (Wang, 1986a). For herbicides, duckweed exhibits an EC50 (50 percent effect concentration) value 3 to 4 orders of magnitude more sensitive than that exhibited by bluegill and daphnid (Bishop and Perry, 1981).

The root elongation method can be considered the early-life-stage test of higher plants. It is a part of terrestrial

ecological assays for the Level 1 Bioassay (Brusick and Young, 1982). Wong and Bradshaw (1982) studied rye grass root elongation under the influence of many metal ions. They ranked the metals tested in the following order, from most to least toxic: Cu, Ni, Mn, Pb, Cd, Zn, Al, Hg, and Fe. The U.S. Environmental Protection Agency, the U.S. Food and Drug Administration, and the Organization for Economic Cooperation and Development recommend the use of lettuce, radish, wheat, cucumber, and red clover seeds as test species (Ratsch, 1983; Fletcher et al, 1985; Thomas et al., 1986). In Illinois, millet is commonly found in the Illinois River Valley (Bellrose et al., 1979). Millet tests have been conducted for phenol and chlorophenol toxicity. The millet root elongation test was found to be more sensitive than the biomass test (Wang, 1985a, 1985b).

A comparative toxicology based on the root elongation method has been published (Wang, 1986d). Cucumber and lettuce seeds as recommended by the EPA and OECD and millet seeds as tested at the ISWS were compared concurrently. Phenol and chlorophenols were used. The results showed that millet seeds were consistently more sensitive to phenolic toxicity than cucumber and lettuce seeds. Furthermore, millet seeds exhibited toxic effects in a very regular and predictable fashion, much preferable to the irregular patterns shown by cucumber and lettuce seeds.

In the case of heavy metal toxicity, however, lettuce seeds were more sensitive than either cucumber or millet seeds (Wang, in press). The results suggest that there is no single, most sensitive, representative test organism for environmental

toxicology. A prudent approach, consequently, is to run a battery of tests, using varieties of test organisms. For mixed and complex environmental samples, lettuce and millet seeds are recommended as the phytotoxicity test species (Wang, 1986d, in press).

From the preceding discussion, it can be postulated that phytotoxicity tests can be a potentially useful tool for assessing polluted sediment. It is also postulated that the impact of polluted sediment on the water column can be determined by using duckweed plants, while in the terrestrial environment of sediment slurry, moist soil, or dredged material, the root elongation method with lettuce and millet as the test species may be a relevant approach.

The objective of this study was to assess sediment toxicity through phytoassay methods. Sediment samples from Waukegan Harbor, Illinois, were used as the test materials. The phytoassay tests included tests of duckweed, lettuce, and millet species.

METHODS

Sediment Samples

A total of 21 sediment samples were collected from Waukegan Harbor in two batches. The first batch (9 samples) was taken November 25 and 26, 1985, and the samples were designated A, B, C, D, E, F, G, H, and J. The 12 samples in the second batch, taken June 3, 1986, were designated L-U, W, and X. The locations

of the sample stations are depicted in figure 1. The water depth of these stations varied from 10 to 25 ft (table 1).

The samples were taken by the dredge method. They were placed in glass containers sealed tightly with parafilm-TM and lids, and were kept at 4 C. With this tight-seal method, the sediment samples remained in anaerobic condition throughout the storage period, as evidenced by their typical bluish appearance. The storage period from sample collection to sample testing was 5 months for the first batch samples and 2 weeks for the second batch.

The sediment samples were homogenized, and moisture content was determined. On the basis of the moisture content, a subsample was taken and mixed with deionized water in a ratio of 111 g dry sediment/L water. The mixture was stirred vigorously for 30 minutes with a magnetic stirrer. After settling for 60 minutes, the supernatant was pipetted out without the precipitated portion being disturbed. This supernatant was used to test for phytotoxicity.

For lettuce and millet tests using the growth pouch method, the sediment samples were tested differently. The samples were used in a smaller proportion and without the extraction step. The sediment slurry was diluted into five different concentrations, on a 50 percent dilution scale.

Duckweed Tests

The stock culture used for duckweed tests was obtained from the ground-water recharge pit located inside the property of the Illinois State Water Survey, Peoria, Illinois. The culture has

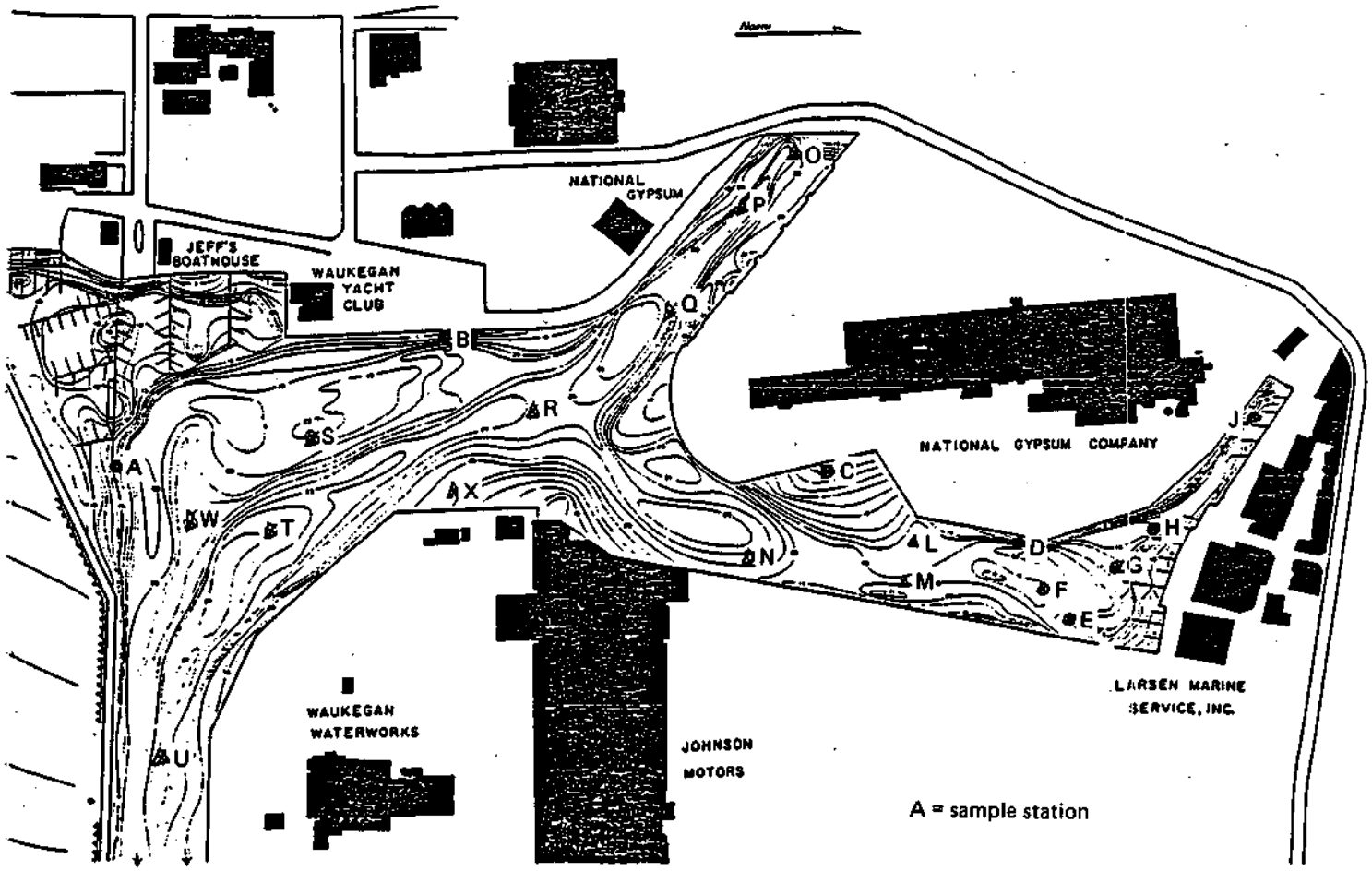

Figure 1. Waukegan Harbor.

Table 1. Sediment Characteristics of Waukegan Harbor

Waukegan sample	Depth (ft)	pH	Solid content (%)	Alkalinity* (mg/L)	Hardness* (mg/L)
A	15	7.52	79.4	89	332
B	15	7.56	74.8	114	288
C	10	7.38	71.3	123	312
D	18	7.32	67.0	102	372
E	18	7.16	54.5	111	350
F	19	7.27	50.6	121	370
G	16	7.48	68.5	76	457
H	14	7.56	74.6	93	263
J	12	7.42	65.9	102	307
L	18	7.02	31.0	123	239
M	20	7.00	30.6	127	233
N	22	7.17	35.2	89	217
0	21	7.07	51.5	119	241
P	23	7.18	46.5	123	255
Q	25	7.35	43.8	140	251
R	24	7.16	37.8	114	249
S	19	7.16	44.0	131	247
T	25	7.01	37.9	127	219
U	25	7.45	60.9	123	235
W	22	6.90	34.4	114	223
X	19	7.15	32.6	123	227

* Expressed as mg/L $CaCO_3$

been maintained in the laboratory since 1980 and is the same stock used in the previous studies (Wang, 1986a, 1986b). Plant nutrient solution was added weekly to the stock culture at double strength (2X) the algal growth medium recommended by Standard Methods (1985), following a previous study (Wang, 1986e). The culture was illuminated with constant, cool-white fluorescent light at 3300 lux. At room temperature, the duckweed plants reproduced continuously and provided test specimens year-round. The duckweed plant, identified as Lemna minor. has been offered as a reference species for duckweed toxicity tests.

Duckweed test specimens were selected from the stock culture 24 hours before the tests began. The selection criteria were that the duckweed specimens be healthy-looking, uniform, and have two fronds of approximately equal size per colony. Plants that were over-sized, under-sized, had irregular shapes, were discolored, or had insect-bite marks or other irregularities were not used. Plastic forks were used for handling instead of forceps to prevent injuries to the duckweed plants. After selection, the test specimens were kept away from light.

The duckweed bioassay experiments were performed with plastic disposable petri dishes, 60 x 15 mm. To an 18-mL sediment extract sample, 36 µL nutrient stock solution (Standard Methods. 1985) was added. This solution was placed into a dish, and 30 fronds, or 15 colonies of duckweed plants, were added to each dish. After lids were placed, the dishes were randomly distributed and a constant light was provided by cool-white fluorescence at an intensity of 6456 lux. A control sample was

also prepared to which only plant nutrients were added, again at double strength the algal growth medium. Each experiment was conducted in triplicate. Temperature was maintained at 26-28 C and the incubation time was 96 hours.

At the end of incubation, the number of fronds in each dish was counted with the aid of a lighted magnifying glass. Each recognizable, protruding bud was counted. The net increase in number of fronds was indicative of the duckweed growth.

Lettuce and Millet Tests

The root elongation tests using lettuce and millet seeds were conducted in a manner similar to that described in previous reports (Wang, 1985a, 1985b, 1986d, in press) with some modification. The lettuce and millet seeds were from the same batches used in the previous studies: Lactuca sativa L. var. Buttercrunch (lettuce) and Panicum miliaceum 1984 (millet). They were kept at -10 C.

The seeds were treated with 10 percent Chlorox-TM solution for 20 minutes and rinsed repeatedly with deionized water. The treated seeds were tested with two methods, the petri dish method and the growth pouch method. For the petri dish method, the test containers were plastic disposable dishes 100 x 15 mm. A 5-mL sediment extract was pipetted into the dishes and a 90-mm, Whatman *1 filter paper was placed on the extract. (Note: no plant nutrients were added in any of the root elongation tests.) Ten treated seeds were placed on the filter paper. In this test, deionized water was used as the control, and each test was performed with six replicates. After 120 hours of incubation in

the dark at 24-25 C, the root elongation of each seed was measured to 1 mm.

For the growth pouch method, 15 treated plant seeds were placed in the holding trough at the top of the pouch, and 18 mL sediment slurry was pipetted into the bottom of the pouch. By capillary force, the extract ascended and made contact with the plant seeds. Deionized water was again used as the control, and each test was performed in quadruplicate. Thus each extract was tested with 60 seeds, the same as in the petri dish method. In this method, however, the sediment slurry was at a lower concentration level and the whole sediment sample was used. After 120 hours of incubation in the dark at 24-25 C, the root elongation of each seed was measured to 1 mm.

Chemical Determinations

The pH, alkalinity, and hardness of the sediment extracts were determined according to Standard Methods (1985). The moisture content of the sediment samples was calculated after the samples were heated to 105 C, cooled, and weighed.

RESULTS AND DISCUSSION

Sediment Characteristics

Some sediment characteristics are given in table 1. The pH values were all in the range 7.00 to 7.56, except at station W, which showed pH 6.90. The solid content was greater in the first batch of samples (from 51 to 79 percent) than in the second batch (31 to 61 percent). The alkalinity content ranged from 76 to 140 mg/L as calcium carbonate. The hardness content ranged from 220

to 460 mg/L as calcium carbonate.

Duckweed Tests

The Waukegan sediment samples, as aforementioned, were mixed with deionized water. The supernatant portion was enriched with plant nutrients and tested with duckweed plants. The controls contained nutrient concentrations but no sediment. For comparison, water samples from Lake Eureka, Lake Canton, the Illinois River, and one well located inside the property of the Illinois State Water Survey were also tested. The sediment samples were tested in two batches, A to J and L to X. Each sample was tested in triplicate with the tests designated as I, II, and III. The results are given in tables 2a and 2b.

When compared with the results for the control sample, the results indicated that none of the sediment samples had an adverse effect on duckweed plants. For example, table 2a shows that for the control sample the mean duckweed growth, that is, the net increase of duckweed fronds, was 50 with a standard deviation of 4. The t-tests indicated that none of the samples exhibited either an inhibitory or a stimulatory effect at the 95 percent significance level. In table 2b, the duckweed growth in the control sample was lower: the mean value was 43 duckweed fronds and the standard deviation was 2. Neither the sediment samples nor the water samples showed less growth than this. On the contrary, a significant stimulatory effect occurred, as almost all samples produced greater duckweed growth at the 95 percent confidence level.

Table 2a. Increases in Number of Fronds in Duckweed Tests of Samples A to J

	I	II	III	Mean	S.D.
Control	50	54	47	50	4
Waukegan A	51	51	56	53	3
B	42	56	41	47	8
C	48	50	51	50	2
D	44	53	44	47	5
E	56	54	52	54	2
F	59	55	53	57	3
G	56	60	55	57	3
H	54	47	56	52	5
J	47	48	53	49	3
Lake Eureka	53	55	49	52	3

Table 2b. Increases in Number of Fronds in Duckweed Tests of Samples L to X

	I	II	III	Mean	S.D.
Control	42	45	42	43	2
Waukegan L	51	51	55	52*	2
M	53	55	55	54*	1
N	48	51	45	48*	3
0	51	44	53	49*	5
P	49	45	59	51*	7
0	61	44	49	51*	9
R	52	52	58	54*	3
S	57	51	54	54*	3
T	54	51	51	52*	2
U	47	54	46	49*	4
W	50	42	45	46	4
X	49	56	47	51*	5
Lake Eureka	51	49	46	49*	3
Lake Canton	48	42	45	45	3
Illinois River	39	49	47	45	5
Well water	44	52	53	50*	3

* P < 0.05

A possible explanation of the results is that these sediment samples are not harmful to duckweed plants. Duckweed reared in the laboratory was reported to be as sensitive to heavy metal toxicity as fish species, and occasionally more so (Wang, 1986a). In situ observations, nonetheless, have indicated that duckweed is resistant to environmental stress, as it is the only macrophyte that thrived in wastewater holding ponds (Rodgers et al., 1978). One possible explanation is that duckweed is a fast-growing species (Hillman and Culley, 1978). Its rapid life cycle allows it to acclimate quickly to environmental stress in the field and thus develop tolerance to the adverse conditions. A similar case is algal adaptation and acclimation to zinc toxicity (Wang, 1986c). It is unlikely, however, that duckweed acclimated during this 96-hour acute test. The more plausible interpretation of the data is that duckweed plants are not sensitive to these sediment samples. In other words, these sediment samples caused no harmful effects to duckweed plants.

Lettuce Tests

The lettuce tests using the petri dish method were conducted concurrently with the millet tests (see the following section) and the duckweed tests (see the preceding section). These tests were conducted three times, with the tests designated in tables 3a and 3b as Tests I, II, and III. Each test solution was tested with 60 seeds, and the mean values and standard deviations are given.

The root elongation bioassay typically has a large amount of variability. The coefficients of variation for the lettuce test

Table 3a. Mean Root Lengths (in millimeters), Standard Deviations, and Percent Inhibition in Lettuce Tests of Samples A to J (Petri Dish Method)

	Test I Length	S.D.	Test II Length	S.D.	Test III Length	S.D.	Mean inhibition (%)
Water control	44	16	35	16	48	16	
Waukegan A	49	12	41	17	52	16	--
B	42	15	41	16	57	12	--
C	44	16	46	15	54	13	--
D	44	15	49	15	55	17	--
E	31*	12	38	10	45	12	10*
F	44	16	43	13	50	18	--
G	48	13	43	11	48	12	--
H	47	16	47	12	60	16	--
J	45	15	44	13	48	17	--
Lake Canton water			38	15	48	15	--
Lake Eureka water			42	10	45	16	--
Illinois River water			38	11	47	11	--
Well water			34	13	47	12	--

* P < 0.05

Table 3b. Mean Root Lengths (in millimeters), Standard Deviations, and Percent Inhibition in Lettuce Tests of Samples L to X CPetri Dish Method)

	Test I		Test II		Test III		Mean inhibition
	Length	S.D.	Length	S.D.	Length	S.D.	(%)
Water control	45	15	41	17	44	15	
Waukegan L	45	15	48	15	45	15	-
M	47	17	51	15	44	16	-
N	41	17	50	20	49	12	-
O	46	13	46	15	40*	16	-
P	43	16	43	17	42	15	-
Q	46	13	46	19	40*	18	-
R	44	14	46	17	42	15	-
S	44	16	46	16	40	15	-
T	44	13	51	12	41	13	-
U	44	16	47	17	44	12	-
W	45	14	46	17	40*	17	-
X	43	15	44	17	46	14	-
Lake Canton water			39	13	39*	11	-
Lake Eureka water			41	13	42	11	-
Illinois River water			40	11	38*	11	-
Well water			34	13	47	12	-

*P < 0.05

(approximately 30 percent), obtained by dividing the standard deviations by the mean values (tables 3a and 3b), were very close to the results (33-36 percent) of previous studies (Wang, 1986d, in press).

The results of the repeated tests of the 21 sediment samples showed that the sediment samples exhibited significant ($P < 0.05$) inhibitory effect on lettuce root elongation in only four instances (one test each for samples E, 0, 0, and W). After the three tests for each of these samples were averaged, it was determined that only sample E displayed inhibition effects: 10 percent inhibition, significant at the 95 percent confidence level.

These results suggest that the Waukegan Harbor sediments did not have harmful effects on the early-life development of lettuce, except perhaps at station E. Lettuce seed is a species recommended for phytotoxicity tests by the U.S. Environmental Protection Agency, Food and Drug Administration, and the Organization for Economic Cooperation and Development (Fletcher et al., 1985; Miller et al., 1985; Thomas et al., 1986). In comparison with millet seeds, lettuce seeds have been found to be more sensitive to metal toxicity but less sensitive to phenol toxicity (Wang, 1986d, in press).

Millet Tests

The millet tests were conducted concurrently in the same manner as the lettuce tests. Two batches of sediment samples were tested separately and each test was repeated three times. The results are presented in tables 4a and 4b.

Table 4a. Mean Root Lengths (in millimeters). Standard Deviations, and Percent Inhibition in Millet Tests of Samples A to J (Petri Dish Method)

	Test I		Test II		Test III		Mean inhibition
	Length	S.D.	Length	S.D.	Length	S.D.	(%)
Water control	53	27	51	29	46	31	
Waukegan A	37*	22	26*	22	43	25	30*
B	39*	22	31*	20	39	25	27*
C	35*	25	29*	22	31*	26	36*
D	34*	23	31*	25	37*	25	32*
E	18*	9	14*	10	26*	21	61*
F	37*	23	23*	18	37*	24	35*
G	31*	23	26*	20	32*	27	41*
H	43*	32	30*	26	42	34	23*
J	30*	27	26*	18	40	22	36*
Lake Canton water			27*	27	45	28	25*
Lake Eureka water			14*	10	39	22	20*
Illinois River water			30*	19	48	26	45*
Well water			47	35	36*	25	14*

* P < 0.05

Table 4b. Mean Root Lengths (in millimeters), Standard Deviations, and Percent Inhibition in Millet Tests of Samples L to X (Petri Dish Method)

	Test I		Test II		Test III		Mean inhibition
	Length	S.D.	Length	S.D.	Length	S.D.	%
Water control	41	24	40	34	51	29	
L	29*	29	37	34	39*	34	20*
M	29*	22	42	30	25*	29	27*
N	35*	26	48	29	47	31	-
0	31*	24	31*	29	35*	28	27*
P	33*	23	46	32	34*	29	14*
Q	33*	26	37	34	44	31	13*
R	31*	24	40	27	44	25	13*
S	26*	25	35	30	40*	29	23*
T	31*	27	40	27	41*	32	15*
U	34*	28	42	28	37*	27	14*
W	38	29	39	35	48	29	-
X	33*	25	41	28	39*	31	14*
Lake Canton water			39	29	33*	25	20*
Lake Eureka water			34	25	38*	21	21*
Illinois River water			33	24	37*	22	23*
Well water	43	33	38	32	49	37	-

* P < 0.05

One measure of quality assurance of this study is the comparison of data in this study with previously reported values. In previous control millet tests the root length was reported to be 44 ± 28 mm (Wang, 1986d) and 45 ± 28 mm (Wang, in press). These values compared favorably with the control value in this study, 47 ± 27 mm. There was a slight variation between the control test results for the first and second batches: 50 ± 29 mm and 44 ± 29 mm, respectively. The variation, however, was not statistically significant.

Of the sediment samples from the 21 stations in Waukegan Harbor, all the samples except those from two stations (N and W) were found to be harmful to millet plants at the 95 percent significance level. The highest inhibition effect was 61 percent from station E sediments, and the lowest was 13 percent from stations Q and R. The distribution pattern of the inhibitory effect in the harbor is depicted in figure 2. It can be seen that the phytotoxicity of the sediment samples is rather widespread, with sediments from the northern end of the harbor appearing to be more phytotoxic than those from the southern end. If the harbor is divided into two approximately equal parts by a line drawn south of stations C and N as shown in figure 2, the average inhibition effect in the southern end (10 stations, excluding station W) was 19 percent with a standard deviation of 7, and the average in the northern end (9 stations, excluding station N) was 35 percent with a standard deviation of 12 percent. The "hot spot" of the harbor was apparently in the region surrounding stations E, F, and G. The near-shore stations

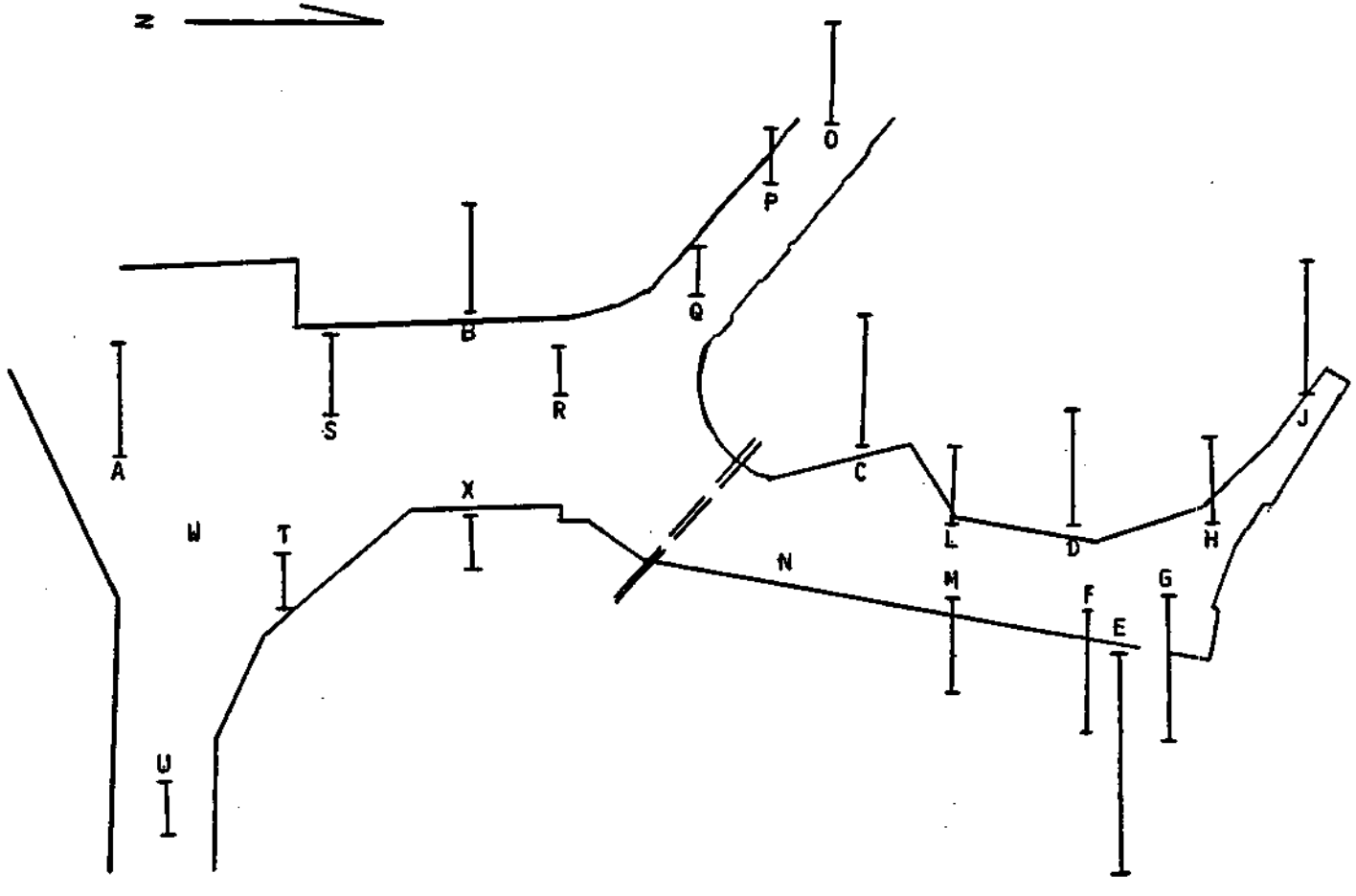

Figure 2. Phytotoxicity distribution pattern in Waukegan Harbor, in relative units.

C, D, and J also contained relatively high phytotoxicity.

Growth Pouch Method

Lettuce and millet tests were conducted concurrently with the growth pouch method. Only the first set of samples, A to J, were tested. Because the whole sediment sample was used, it was tested at a much lower sediment concentration and also in a series of concentrations. The results are given in table 5.

The results of the lettuce tests indicated that these sediment samples did not cause significant deleterious effects. This is not surprising in light of the fact that even at greater sediment concentrations, these sediment samples were not inhibitory to the same plant species (table 3). Even with the sample from station E, there was no significant effect.

The results of the millet tests were uneven. Only stations A, F, and G displayed significant phytotoxic effects ($P < 0.05$), as seen in table 5. In contrast, all nine stations showed adverse effects in the other millet tests employing the petri dish method Ctable 4).

SUMMARY

The most important finding in this study is the disparity of responses among test species. Even though the three test species are all advanced plant life species, they displayed surprisingly different responses to the same set of sediment samples.

Duckweed is a free-floating macrophyte. It has been reported to be sensitive to toxicity of heavy metals and

Table 5. Mean Root Length (in millimeters) and Standard Deviations in Lettuce and Millet Tests of Samples A to J (Growth Pouch Method)

	Lettuce		Millet	
	Length	S.O.	Length	S.D.
Waukegan A				
0	48	11	85	31
1.91	45	11	85	27
3.82	48	11	90	27
7.64	48	12	82	37
15.27	49	10	80	38
30.55	45	11	71* (17%)+	40
WaukeganB				
0	45	10	82	28
2.80	44	10	83	29
4.67	42	12	85	30
7.78	42	12	90	31
12.97	43	11	81	30
21.62	41	9	86	33
Waukegan C				
0	41	10	76	28
2.74	38	11	85	28
4.57	40	13	82	35
7.62	45	12	84	27
12.71	46	10	91	31
21.18	45	13	82	24
Waukegan D				
0	46	14	79	29
1.45	46	9.86	80	26
2.91	44	11.61	84	37
5.82	45	10	87	25
11.64	44	12	79	32
23.27	46	12	86	28
Waukegan E				
0	42	14	81	29
1.95	42	12	80	30
3.91	43	29	73	35
7.81	46	14	79	33
15.63	50	10	78	34
31.25	47	13	79	28

Table 5. Coneluded

	Lettuce		Millet	
	Length	S.D.	Length	S.D.
Waukegan F				
0	47	13	76	30
2.21	48	10	84	30
4.43	46	14	88	29
8.86	46	8	83	30
17.72	47	15	78	30
35.43	46	12	66* (14%)+	21
Waukegan G				
0	46	17	86	26
1.57	48	9	82	27
3.14	46	12	84	33
6.29	44	14	92	21
12.58	46	11	83	28
25.15	41	14	78* (9%)+	25
Waukegan H				
0	44	16	72	34
0.74	48	15	74	27
1.49	47	15	82	34
2.97	46	16	90	28
5.95	48	13	80	37
11.89	47	15	86	27
WaukeganJ				
0	49	14	79	23
0.69	50	14	84	25
1.39	48	15	92	27
2.77	51	12	91	32
5.54	50	15	82	39
11.08	52	10	82	38

Note: The values in the first column indicate sediment concentrations in g/L

* $P < 0.05$
+ Percent inhibition

herbicides. This plant, however, did not exhibit an adverse reaction to the Waukegan Harbor sediments.

The lettuce root elongation test is recommended by USEPA, FDA, and OECD. It has been reported that this species is very sensitive to heavy metal toxicity. The tests in this study, however, failed to show harmful effects of the sediment samples, except for a sample from station E.

The millet root elongation tests were very effective in detecting sediment toxicity. Of 21 stations, 19 stations exhibited significant phytotoxicity to millet, $P < 0.05$. The maximum effect was found at station E with 61 percent inhibition after 3 repeated tests.

In the millet tests using the growth pouch method, the sediment concentration was much lower than in the petri dish tests. The results indicated that the sediment samples were less inhibitory to millet root elongation with the growth pouch method than with the petri dish method.

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