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TOXICITY ASSESSMENT OF THE AQUATIC ENVIRONMENT USING PHYTOASSAY METHODS

by
Wuncheng Wang

Prepared for the
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EXECUTIVE SUMMARY

Among ecotoxicity tests, higher plant tests are relatively underdeveloped and infrequently used. Phytotoxicity tests using higher plants are simple, sensitive, and cost-effective. In this study, two phytotoxicity tests were employed: common duckweed (Lemna minor) and seed germination/root elongation. Both natural waters and industrial wastewaters were tested.

Lake and river water samples were collected from Buck Creek-Little Wabash River, Court Creek, Lake Loami, and Illinois River-Peoria Lake. These samples were obtained bimonthly and delivered to the laboratory not more than 24 h later. They were either tested immediately for phytotoxicity or stored at 4 C for no more than 48 h and then tested. Ground-water samples also were collected and tested.

The root growth test using millet, oat, and wheat was used to detect phytotoxicity in lake and river waters. Only sporadic test results showed significant phytotoxicity, an indicator of toxic substances and/or other water-quality characteristics inhibiting the development of cabbage and millet. For example, six of 216 tests showed significant phytotoxicity in the Buck Creek-Little Wabash River region, and the phytotoxicity in the other regions was low as well. The low phytotoxicity was possibly due to the particularly dry spell in 1987, which caused lower herbicide runoff than is found in an average year.

The duckweed test was sensitive and could detect phytotoxicity in many ground waters. Among 13 samples collected in June 1987, 10 were found to be phytotoxic. The toxicity ranged from an inhibitory effect of 11 to 23%. Again in July, six samples out of seven were found to be phytotoxic. In August, however, no samples were toxic; cabbage and millet tests did not detect any significant phytotoxicity in any of the samples. The test results, however, did not imply any human health issue.

Eight industrial wastewater samples obtained from three industries were tested. Source A is an industrial wastewater pretreatment plant, and the wastewaters were highly toxic. Duckweed mortality and reproduction tests showed that the effects were nearly total. Source B is an agricultural product utilization plant, and duckweed tests did not indicate significant phytotoxicity from this source. Wastewaters from source C, a specialty chemical industry, were extremely toxic. At 22% effluent concentration or greater, all the duckweed died. By using the moving-average method, the IC50 values (the concentration that caused 50% inhibitory effect) of two source C samples were estimated to be 20 and 2% based on the duckweed test results.

The treatability study of sample A4, from source A, showed that phytotoxicity was effectively removed by either granular or powdered activated carbon. Amberlite XAD-4 and cation exchange resin were partially effective in removing effluent toxicity. Anion exchange resin, silica gel, Sephadex G-75, and insoluble starch xanthate were ineffective. Sample C2, from Source C, was far more toxic. Activated carbon was only partially effective in removing phytotoxicity.

I. INTRODUCTION

Daphnid and fathead minnow mortality test results are generally accepted for regulatory purposes for the freshwater environment regulated by the National Pollutant Discharge Elimination System permit requirements (Peltier and Weber, 1985). The underlying principle is to ensure that the national aquatic environment is suitable for fishing, swimming, and other human use. For example, Section 302.210, Chapter I (Pollution Control Board), Subtitle C (Water Pollution), Title 35 (Environmental Protection) of the Rules and Regulations of the State of Illinois, which deals with substances toxic to aquatic life, requires that "Any substance toxic to aquatic life shall not exceed one-tenth of the 96-hour median tolerance limit (96-hr, TL_m) for native fish or essential fish food organisms ...." (State of Illinois, 1988). Even though fish resources need to be protected and related tests are well established, the question remains, Do other organisms need to be protected and do other tests need to be developed as well?

Phytotoxicity tests using higher plants are seldom used. Environmental scientists have shown negative attitudes toward plant tests (Kenaga and Moolenaar, 1979; Bishop and Perry, 1981). This is unfortunate because higher plants are an essential part of the ecosystem, be it in aquatic or terrestrial environments. They, along with algae, produce oxygen and organic matter that support almost all other life forms. Recently there has been awareness that plant tests are potentially useful for environmental monitoring. This awareness can be seen from the increase in journal articles, the continuation of conference sessions on plant toxicology at the Society of Environmental Toxicology and Chemistry, and especially the inauguration of the First Symposium on Use of Plants for Toxicity Assessment sponsored by the American Society for Testing and Materials in April 1989.

In general, phytotoxicity tests are simple, sensitive, and cost-effective. They can be used for toxicity testing of organic and inorganic pollutants and are particularly useful for monitoring herbicide pollution. Herbicides have been used extensively, and many of the herbicides and their residues have entered rivers, lakes, estuaries, and ground water, causing unacceptable environmental pollution. The use of higher plants for monitoring this class of biocides is essential.

Furthermore, phytotoxicity tests are among relatively few tests suitable for monitoring pollution in soil, sediment, rain, water, wastewater, and solid wastes. They are useful in testing toxicity in water extract as well as in solids. Phytotoxicity tests, therefore, should be considered as a vital part of comparative toxicology.

Considerable evidence has shown that duckweed is an excellent candidate for aquatic phytotoxicity tests; researchers at the Illinois State Water Survey have been conducting duckweed toxicity tests for the last seven years. Duckweed (for example, Lemna minor) is a common floating macrophyte. In the field, the plants grow extremely fast in the spring and summer; in the laboratory, the plants grow continuously under favorable conditions. Duckweed is small enough that large laboratory facilities are not necessary, but large enough that adverse effects can be observed. Because duckweed is a floating macrophyte, it is especially sensitive to surface-active and hydrophobic substances that concentrate at the air-water interface.

The root elongation test, as recommended by the U. S. Environmental Protection Agency (1982) and the Food and Drug Administration (1987), relies on the measurement of each individual root. The test has two major drawbacks. First, each seed is an independent entity and as such, each seed exhibits a high degree of variability. In the same test solution, one seed may fail to germinate, whereas another seed grows well. Because of the response variation, a typical root elongation test employs many seeds in order to obtain a certain degree of statistical confidence. For example, the USEPA (1982) recommends 300 test seeds for each test solution, and the measurement of so many roots is very time-consuming. Second, measuring each root means that the experimenter is constantly exposed to toxic test substances. These two drawbacks can be minimized by using a new technique, the combined dry biomass of the root system, detailed in the methods section.

The objective of this study was to evaluate the use of phytotoxicity tests using higher plants for monitoring the freshwater aquatic environment, including lakes, rivers, and ground waters. In the course of study, a second objective was added, the use of phytotoxicity tests for monitoring industrial wastewaters. Two types of phytotoxicity tests were carried out: duckweed tests and seed germination/root elongation tests.

II. LITERATURE REVIEW

This section contains background information on phytotoxicity and phytotoxicity tests. The presentation is divided into three parts: phytotoxicity in natural waters, duckweed tests for phytotoxicity, and seed germination/root elongation tests for phytotoxicity.

A. PHYTOTOXICITY IN NATURAL WATERS

The U.S. Environmental Protection Agency reports that there was a steady increase in herbicide use between 1964 to 1984, as shown in Figure 1 (Nielsen and Lee, 1987). In 1964, the use of herbicides constituted approximately 45,000 metric tons (100 million pounds) of active ingredients annually; in 1984, use was about 227,000 metric tons (500 million pounds). In contrast, the use of insecticides was constant at 82,000 metric tons (180 million pounds) per year during this period. Figure 1 illustrates that increases in the application of biocides over the past 20 years are largely due to increased herbicide use.

McCall et al. (1984) applied liquid atrazine (2-chloro-4-ethylamino-6-isopropylamino-S-triazine) to a clay loam soil at a rate of 3360 g/ha. The soil was saturated and allowed to drain to field capacity before simulated rainfall events of 1.3 cm/h and 2.5 cm/h. In the worst case, 90% of the atrazine loss was in the runoff water, and the majority of the atrazine loss occurred within 20 minutes of the beginning of the runoff events. Thirty-five percent of applied atrazine was transported from the field for both the 1.3 and 2.5 cm/h runoff events. The desorption and dissolution process appeared to be very slow in comparison with the hydrologic changes.

Glotfelty et al. (1984) studied the Wye River, a tributary of Chesapeake Bay. The river is a shallow, well-mixed estuary surrounded by an agricultural watershed, a large portion of which is planted in corn. The movement of atrazine and simazine (2-chloro-4,6-bis(ethylamino)-S- triazine) showed that the total amount of herbicide reaching the estuary depended upon the quantity applied in the watershed and the timing of runoff with respect to application dates. In a year in which a significant runoff occurred within two weeks of application, 2 to 3% of the atrazine moved to the estuary. In years with less runoff, or runoff delayed longer after application, much smaller quantities reached the estuary.

Muir, Yoo, and Baker (1978) monitored atrazine and N-deethylated atrazine (2-chloro-4-amino-6-isopropyl-amino-S-triazine) in five rivers that drained agricultural areas in the Yamaska River basin of Quebec, Canada. Atrazine and N-deethylated atrazine residues ranged in concentration from 0.01 to 26.9 µig/L to less than 0.01 to 1.34 µg/L, respectively, over the monitoring period. The highest levels of atrazine were observed in July each year and coincided with the region's herbicide spraying season and occasional heavy rainfall events. Losses of atrazine ranged from 0.1 to 2% of the atrazine that was estimated to have been applied in each watershed.

With conservation tillage being encouraged recently, farmers typically keep a portion of crop residues on the soil surface from one growing season to the next. The reduced cultivation requires the use of herbicides to control yield-reducing weeds. Martin et al. (1978) conducted a laboratory

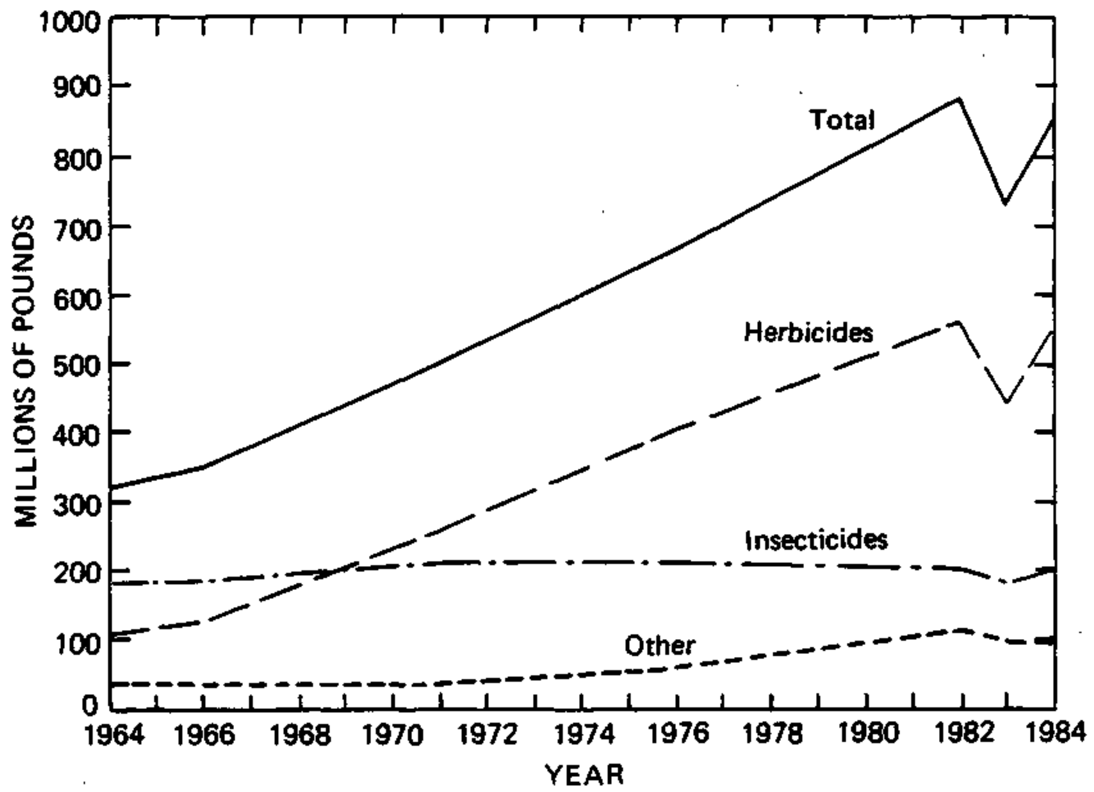

Figure 1. The use of biocides in the United States

simulated rainfall study of washoff of herbicide applied to corn residue. Concentrations in initial washoff water were high for all four test herbicides. At the application rate 1.8 kg/ha, the concentrations were found to exceed 9 mg/L (highest value 35 mg/L). Concentrations decreased rapidly with time; approximately the same amount washed off with the first 0.5 cm of water as did with the next 3.0 cm of water. Unexplained losses of herbicides indicated the possibility of volatilization occurring between application of herbicides and application of washoff water about 12 h later.

Wu et al. (1980) carried out a study on the enrichment of atrazine in the microsurface water (top 100-150 µm) in a subestuary of Chesapeake Bay. They detected atrazine in the water throughout the entire June-December period in both 1977 and 1978. The enrichment of atrazine in the microsurface in comparison with the herbicide in the bulk water at a depth 0.3 m varied from none to 110-fold. Forty-six percent of the 65 samples had an atrazine enrichment factor of five or less. They demonstrated that atrazine in the surface film samples exists partly in the dissolved phase and partly in the solid phase in association with suspended particles.

Wu, Correll, and Remenapp (1983) reported that there were two commonly used herbicides in cornfields of the Rhode River watershed in Maryland: atrazine and alachlor (2-chloro-2'-6'-diethyl-N-methoxymethyl acetanilide). Although alachlor was applied in larger quantities, atrazine was detected more frequently in runoff waters and at higher concentrations than alachlor (0-40 µg/L versus 0-6 µg/L). Atrazine was more persistent and more mobile in watershed soils. A major portion of atrazine was found in dissolved form in runoff water samples collected during storms.

Heavy use of the herbicide atrazine has resulted in concentrations of up to 42 µg/L in various Iowa waters including ground water (Richard et al., 1975), and in the Central Platte Valley of Nebraska (Wehtje et al., 1983; Junk, Spalding, and Richard, 1980). Wilson et al. (1987) reported that two out of four wells in Weld County, Colorado, yielded measurable atrazine concentrations of 1.1 to 2.3 µg/L.

Once atrazine enters a water system, the partitioning of atrazine between the aqueous and solid phases of the water-sediment system largely determines the impact of the herbicide on water quality and aquatic ecosystems. Wolf and Jackson (1982) found that sediments with lower pH values and higher organic matter levels adsorbed higher levels of atrazine than sediments with neutral pH values and lower organic matter levels. It was speculated that organic components of suspended particulate matter might be an active adsorption site.

There have been several studies on the ecological effects of herbicides in natural waters. DeNoyelles, Kettle, and Sinn (1982) compared the biological effects of a single dose of atrazine, 20 and 500 µg/L, with conditions in a control pond for 136 days. At each concentration atrazine depressed phytoplankton growth in the ponds within a few days. This depression was followed by successful changes leading to the establishing of phytoplankton species more resistant to atrazine inhibition. Laboratory studies verified this resistance, which occurred when an atrazine dose was as low as 1-5 µg/L. At 500 µg/L, there was a delayed appearance but eventually a greater biomass of resistant species took over.

DeNoyelles and Kettle (1983) studied the biological impacts of triazine on phytoplankton and macrophyte communities. In treated ponds, communities of phytoplankton quickly developed that were more resistant to atrazine. Macrophytic vegetation was significantly inhibited in treated ponds. DeNoyelles and Kettle detected triazine in many sites in Kansas. In these sites they observed the presence of phytoplankton communities possessing a degree of resistance to triazine, while there were reductions of macrophytic communities in those waters receiving direct runoff from agricultural fields.

Gunkel (1984) studied the bioaccumulation of triazine in a small pond during a vegetation period. High bioconcentration factors (ranging from 27 to 120(D) occurred in phytoplankton during periods of small phytoplankton biomasses. Organisms of high trophic levels reached only small bioconcentration factors, and no food-chain effect was observed. Daphnia pulicaria. a filtering zooplankton organism, accumulated less atrazine than phytoplankton, and had a bioconcentration factor less than 16. Fish, as final consumers, possessed a bioconcentration factor less than 9. At the end of the experiment, atrazine was distributed among water, sediment, atmosphere, phytoplankton, and zooplankton compartments in the ratio 11,400:660:5:2:1.

Peichl, Lay, and Korte (1985) found that the application of atrazine and 2,4-D (2,4-dichlorophenoxyacetic acid) to an outdoor aquatic system resulted in an increase of rotifers. Application of both chemicals maximized the effects and caused a complete mortality of Daphnia pulex. a standard species for toxicity tests.

B. DUCKWEED TESTS

The term "duckweed" commonly refers to the family Lemnaceae. This family has about 40 species worldwide in four genera. About half of the species occur in the United States. Duckweed is a widespread, free-floating aquatic angiosperm, ranging in the world from tropical to temperate zones. Duckweed is a food for waterfowl and small animals and provides food, shelter, and shade for fish. Duckweed also serves as a physical support for a variety of small invertebates.

Wang (1986a) carried out toxicity tests of aquatic pollutants using common duckweed, Lemna minor. He reported that Illinois water quality standards sufficiently stringent to protect duckweed included those for B, Cl, Cr(VI), Cu, Fe, Pb, Mn, phenol, SO_4, and Zn. The standards not sufficiently stringent to protect duckweed were those for Ba, Cd, Fe, Ni, and Se. A comparison of duckweed toxicity test results with the fish test results reported in the literature found without exception that the duckweed sensitivity compared favorably with fish sensitivity.

Wang (1986b) collected a 75 L water sample from the Illinois River and compared metal toxicity in the river water sample and in deionized water. Both waters were spiked with the standard plant nutrients. He discovered that there were three different types of results. Ba was moderately toxic in the deionized water and nontoxic in the river water. Cd was extremely toxic in the deionized water and also substantially toxic, although somewhat less so, in river water. Cr(VI) toxicity was more or less the same in the

river water and in the deionized water. These results point out the importance of site-specific water quality in regulating metal toxicity.

Wang (1986c) further expanded the metal toxicity tests by using 59 water samples encompassing river and lake waters. Water quality of these samples ranged from very soft (hardness 40 mg/L as $CaCO_3$) to very hard (hardness over 300 mg/L as $CaCO_3$). In this series of experiments, Ba, Cr(VI), and Ni were tested. He concluded that Ba was the least toxic among the three metals and was influenced the most by water quality of the test samples. Chromium was moderately toxic among the three metals and was influenced the least by the water quality of the samples. Ni was the most toxic and was influenced moderately by the water quality.

Nasu and Kugimoto (1981) reported that the pH of the test medium, the concentration, the composition of the nutrient in the medium, and the temperature at which cultures were maintained affected the sensitivity of Lemna to heavy metals. They recommended the use of Bonner-Devirian's medium at temperatures above 25 C. The pH of the test solution had strong influence on metal toxicity as metal speciation changed accordingly. They suggested the pH range for toxicity testing of Cd^{+2}, Zn^{+2}, Cr^{+6}, $Mn+^{2}$, $As+^{3}$, Cu^{+2}, and Ag+ to be 6.1 to 7.1.

Hartman and Martin (1984) studied the effect of suspended bentonite clay on the acute toxicity of the herbicide glyphosate (N-(phosphonomethyl)glycine) to Daphnia pulex and Lemna minor. They found that glyphosate toxicity to Daphnia increased when bentonite was added. Apparently the filter-feeding organisms ingested adsorbed glyphosate along with particulate matter. The glyphosate toxicity to Lemna, on the contrary, decreased when bentonite was present, possibly because of the decrease of glyphosate bioavailability.

Hutchinson and Czyrska (1975) reported that when both duckweed, Lemna valdiviana. and floating fern, Salvinia natans, were present, the addition of 0.01-0.05 mg/L Cd resulted in greater toxicity to Lemna than when the same amount of Cd was added to Lemna alone. In contrast, Salvinia grew more with Lemna in the presence of 0.01-0.05 mg/L Cd than it did when it grew by itself. The possible reason for this discrepancy is species competition. Hutchinson and Czyrska observed that "under the stress of competition Lemna grows less well and cadmium levels are markedly increased in the tissues, while Salvinia grows better and the cadmium concentrations are correspondingly less."

Bishop and Perry (1981) described a flow-through growth inhibition test using common duckweed, Lemna minor. Growth inhibition was measured by using frond count, dry weight, and root length. The test materials included metal ion; anionic, non-ionic, and cationic surfactants; and an aquatic herbicide. They reported that results based on frond count comprised the most useful information.

King and Coley (1985) compared toxicity of natural and synthetic oils by using three species of Lemna: L. pibba. L. minor, and L. perpusilla. Growth was monitored as changes in frond numbers. The results showed that the coal gasification products had a greater acute toxicity to all three species than the natural oils.

C. SEED GERMINATION/ROOT ELONGATION TESTS

Dry plant seeds are in a dormant state. They can withstand a harsh environment without losing viability. When hydrated and under favorable conditions, however, plant seeds undergo rapid changes. Metabolism, nutrient transport, and cell division all take place (Mayer and Poljakoff-Mayer, 1982) and during that period, seeds become highly sensitive to environmental stress. Ah analogous case is that of fish eggs, which are resistant to the environment. Once the eggs are hatched, however, the young fish are more sensitive to stress than at any other stage in their life cycle (Nebeker, Savonen, and Stevens, 1985).

The plant species recommended by the U.S. Environmental Protection Agency (1982), the Food and Drug Administration (1987), and the Organization for Economic Cooperation and Development (1984) for seed germination and root elongation tests include cucumber, lettuce, radish, red clover, and wheat. Other plant species have also been suggested. Oat, corn, cabbage, carrot, soybean, and tomato are mentioned by the USEPA and the FDA. The former also recommends perennial ryegrass and the common onion, and the latter also recommends wheat and beans. Other species such as rice, soybean, mustard, rape, turnip, vetch, fenugreek, and cress are mentioned in the OECD guidelines for terrestrial plant growth tests.

Fletcher et al. (1985) performed a literature review on toxicological data involving higher plants. More than 3,500 publications were surveyed, and the information was entered as the PHYTOTOX computerized database. They reported that although 23 plant species were considered, adequate data existed for only six species: oat, wheat, corn, sorghum, cucumber, and soybean. Oat and wheat, which are monocotyledons, gave the most sensitive responses to the wide range of herbicides. Cucumber was the most sensitive dicotyledon evaluated. Corn and soybeans appeared to be relatively insensitive.

Wong and Bradshaw (1982) conducted toxicity tests using root elongation of ryegrass, *Lolium perenne*. Many metal ions were tested. They reported the descending order of toxicity as Cu^{+2}, Ni^{+2}, Mn^{+2}, Pb^{+2}, Cd^{+2}, Zn^{+2}, Al^{+3}, Hg^{+2}, Cr^{+6}, and Fe^{+2}. With the sole exception of Mn, these results followed the stability constants of metal-organic complexes. The end-point root elongation was more sensitive to toxicity than the end-point shoot growth. Wong and Bradshaw suggested that the results were of value in predicting the metal toxicity in plants growing in contaminated soils.

Millet has been tested at the Illinois State Water Survey for the past seven years. Wang (1985a) used three types of plant seeds (millet, radish, and velvetleaf) for toxicity tests of phenolic compounds. In preliminary experiments, plant seeds were incubated at 23, 28, 30, and 35 C. Wang reported that the seedling growth was identical between 23 and 28 C, while the growth at 30-35 C was significantly less. pH in the range of 5-9 did not have a significant effect. The results showed that the ascending order of toxicity for substituted phenols was phenol, chlorophenol, dichlorophenol, and trichlorophenol.

Wang (1985b) also used millet root elongation for toxicity tests of phenol and seven chlorophenols. Each compound was tested twice, all with a control and six concentration levels. Again the ascending order of toxicity

was identical to that in the previous report (Wang, 1985a). The root elongation method was more sensitive than the biomass method. At low concentration, phenolic compounds stimulated the growth of root, but when the concentration was further increased, the stimulation effect stopped and the inhibition effect took place.

Wang (1986d) compared cucumber, lettuce, and millet for toxicity tests of phenolic compounds. As mentioned earlier, cucumber and lettuce are among the species recommended by the USEPA and the OECD. For testing phenol, 2-chlorophenol, 2,4-dichlorophenol, and 2,4,6-trichlorophenol, millet seeds were shown to be superior over cucumber and lettuce seeds. Millet tests gave regular and predictable toxic responses resulting in an excellent structure-toxicity relationship (R = 0.999). Millet seeds, in addition, were the most sensitive to these organic compounds.

Wang (1987a) further compared cucumber, lettuce, and millet for toxicity tests of metal ions: Cd, Cr(VI), Cu, Mn, Ni, and Zn. Among these plant species, lettuce was found to be most sensitive to metal toxicity, while results for cucumber and millet were mixed. Millet seeds, nevertheless, showed a predictable pattern of response similar to that for phenolic toxicity. After examining the results of toxicity tests of organic and inorganic compounds, Wang (1987b) recommended that lettuce and millet seeds be used together in toxicity tests of unknown, hazardous wastes.

Ratsch (1983) conducted a round-robin study of 10 toxic substances involving four contract laboratories and three USEPA laboratories. Root elongation was used as the test end point after 115 h of incubation. The objective of the research was to determine the precision of the bioassay method used to evaluate environmental effects under the Toxic Substances Control Act. The results showed that the root elongation was a valid and sensitive plant response to toxic exposure. The interlaboratory test procedures were uniform, and the results were reproducible. Most variation was attributed to biological differences between species.

Ratsch and Johndro (1986) used lettuce to compare two different root elongation phytotoxicity test methods with six test substances. Seeds were either germinated in the dark on an inclined filter paper substrate with one end immersed in test solution or germinated in 0.1 strength nutrient solution with a 16:8 h light and dark period and aeration with compressed air. Sodium fluoride, monosodium methanearsonate, and monuron (3-(p-chlorophenyl) -1,1-dimethylurea) affected the root elongation similarly in both methods. Cadmium chloride and 2,4-D inhibited root elongation at concentrations of approximately an order of magnitude smaller in the solution culture method than in the substrate method. Silver nitrate inhibited root elongation with two orders of magnitude difference between the two methods.

A few studies have utilized seed germination/root elongation for effluent toxicity assessment. Perez et al. (1986) compared ryegrass, tomato, green pea, bean, barley, and radish for response to wastewater from olive processing. The results of seed germination and early plant growth showed that raw wastewater, wastewater with organic matter removed, and deionized wastewater were all inhibitory. Among the three wastewaters, the raw wastewater was the most inhibitory, followed by the deionized wastewater

and then the effluent with organic matter removed. The most sensitive plant species was tomato and the least sensitive was barley.

Srivastava and Sahai (1987) tested the performance of Cicer arietinum at various concentrations of distillery effluent. They found that the seed germination was increasingly inhibited with increasing effluent concentration. At 100% concentration there was no germination. It was suggested that the very high BOD load and the presence of excessive concentrations of soluble salts could be responsible for the phytotoxicity in the effluent. Effluent at up to 5% concentration was, however, beneficial to plant growth.

Behera and Misra (1982) studied the effects of molasses distillery effluent on growth and development of rice seedlings. A high concentration of effluent altered the normal pattern of rice seed germination. The seed germination showed an inverse relationship with the effluent concentration. Behera and Misra further reported that the total pigments, proteins, and nucleic acids of rice seedlings declined with an increase in effluent concentration. The loss in contents of macromolecules like deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein was greater in the root than in the shoot. RNA and chlorophyll contents of the seedlings were found to be most susceptible to effluent stress (Behera and Misra, 1983).

Phytotoxicity tests are useful for evaluation of contaminated soil. Thomas and Cline (1985) modified the Neubauer technique by using Ziplock-R plastic enclosures. The use of individual enclosures allowed safe handling and disposal during experiments.

Thomas et al. (1986) used a battery of bioassays to assess chemical waste site contamination. The bioassay organisms were algae, daphnids, microorganisms (dissolved oxygen and Microtox-R), higher plants (cucumber, lettuce, radish, red clover, and wheat), and earthworms. The algal assay generally was most sensitive to pure chemicals of metal ions and insecticides. Bioassays of nine samples of unknown chemical composition from the Rocky Mountain Arsenal site, however, showed that the lettuce root elongation test was most sensitive.

An important application of higher plants for environmental monitoring, although not in the scope of this study, is in studies of acid rain. Seed germination and root elongation have been used extensively to study this environmental problem (Johnston and Shriner, 1985; Tibbitts et al., 1982; Irving, 1985; Wolfenden and Wellburn, 1986; Percy, 1986; Evans, Gmur, and Mancini, 1982).

III. METHODS

A. PHYTOTOXICITY IN NATURAL WATERS

1. Water Samples

Surface water samples were collected from four regions in Central and Southern Illinois from January to December 1987 (Fig. 2). Bimonthly samples were taken from Buck Creek (stations 1 and 2) and the Little Wabash River (station 3). Between station 1 and station 2, there are many oil drilling wells, many of which are still in operation. Water flow in these two stations is influenced by the hydrological cycle and may stop during dry periods in the summer. For each station, a 1.0 L grab-water sample was collected and shipped to Peoria with an ice pack via UPS. The samples usually arrived within 24 h after collection. Upon arrival, they were either tested immediately for phytotoxicity or stored at 4 C for no more than 48 h and then tested.

Sugar Creek is a tributary of Court Creek, which flows into Spoon River (Fig. 2). Station S3 is just below an impoundment, Spoon Valley Lake. Station S2 is midway between S3 and the confluence of Sugar Creek into Court Creek. Court Creek station C2 is just outside the city limits of Dahinda. A station, S1, was designated at a small tributary into Sugar Creek. This station was used especially for monitoring runoff during rain floods and snow-melt floods, when water samples were collected more frequently according to the flood's intensity and duration. A 1.0 L grab-water sample was taken bimonthly as the base-flow. The samples were delivered to the laboratory the day they were collected and kept at 4 C until tested within 48 h.

Three stations were designated in the Illinois River at Peoria Lake. Stations PL-1 and PL-2 are respectively outside and inside an artificial barrier designed to reduce wind and wave action in order to establish aquatic macrophytes. Station PL-C is located in the river channel. A 1.0 L grab-water sample was taken bimonthly and delivered to the laboratory the day it was collected. Samples were kept at 4 C until tested within 48 h.

Lake Loami is a small (approximately 10-acre) impoundment southwest of Springfield. The lake is filled with water pumped in from a nearby stream. There are two basins, East Basin and West Basin. West Basin is relatively shallow, with a maximum depth of 8 feet, whereas East Basin has a maximum depth of 16 feet. A sampling station was designated at the deepest spot in the lake. A 1.0 L grab-water sample was collected at least monthly. During spring and summer 1987, a research project was conducted on the lake's management. Water samples were collected more frequently to coincide with the project. The samples were delivered to the laboratory the day they were collected and kept at 4 C until they were tested within 48 h.

Ground-water samples were collected from wells located in Tazewell County, near Hopedale and Washington. For comparison, the water supply of Illinois-American Water Company, Peoria, was also tested. These wells are in the Sankoty aquifer. The well waters were collected three times: in June, July, and August 1987. A 1.0 L sample was taken and delivered to the laboratory the same day. The samples were kept at 4 C until tested within 48 h.

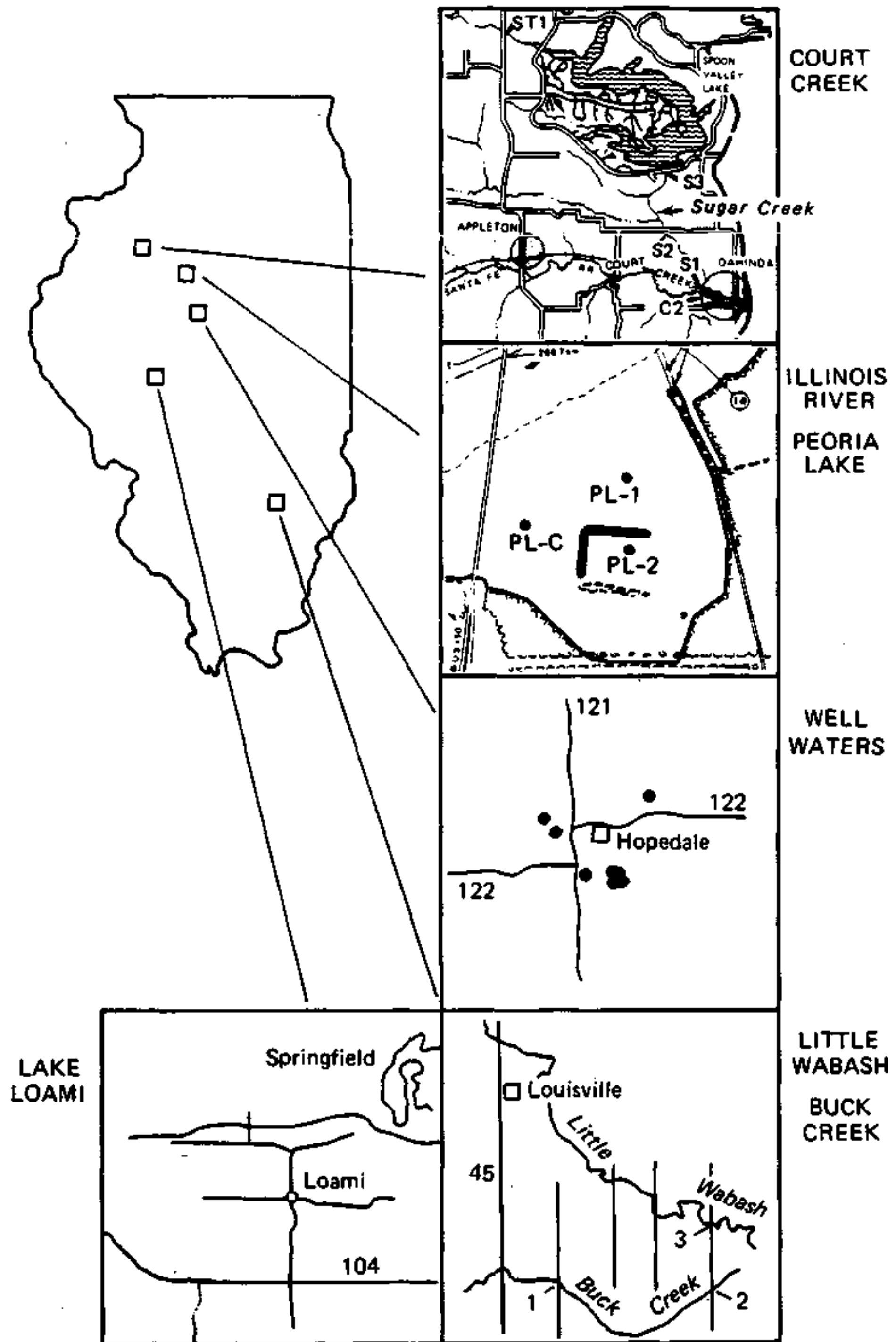

Figure 2. Sampling stations

All these samples were assayed without treatment. For the surface water samples, turbidity, pH, alkalinity, hardness, and chloride were determined by following Standard Methods (1985).

2. Root Growth Tests for Surface Waters

For this study, plant seeds were purchased in bulk and kept at -10 C until use. Millet (*Panicum maliaceum*), oat (*Avena sativa*), and wheat (*Triticum aestivum*) were used. Millet has been tested at the Illinois State Water Survey for a number of years, while oat and wheat are the recommended species by USEPA and FDA.

Plant seeds were treated with diluted hypochlorite solution (1:10 dilution of Chlorox-R:water in V/V) for 20 minutes. The treated seeds were then rinsed repeatedly with deionized water. The experimental conditions are given in Table 1. The test vessel used for this study was the patented Seed-Pack Growth Pouch-R, available from Northrup King in Minneapolis. Fifteen seeds of millet or wheat (12 seeds of oat) were placed in each growth pouch. Twenty mL of test solution was pipetted into each pouch, filling the pouch half-way. The water control was a hard, reconstituted water as recommended in Standard Methods (1985), with hardness 160-180 mg/L as $CaCO_3$. After incubation in the dark for 120 h, roots of all seeds in each growth pouch were cut along the perforated line and seeds were retained. The roots were combined, dried with tissue paper, left to air-dry for 24 h, and weighed to 0.1 mg. There were four replications per test sample.

During this phase of study, experiments were conducted to determine 1) whether 24 h of air-drying was sufficient for root biomass to reach constancy and 2) whether there was significant difference between deionized water and hard, reconstituted water (Standard Methods. 1985) used as test controls.

The precipitation record was obtained from the National Weather Service at the Greater Peoria Airport. The data were used as an indication of general rainfall during the study period.

Reproducibility of test results can be used as an indicator of data quality-control tests. Because each test contained a water control made to be identical from one test to another, it is of interest to pool all control test results. The more reproducible (precise) bioassay, under the same circumstance, would be more desirable.

3. Duckweed and Root Growth Tests for Ground Waters

Two tests were conducted for well water samples: the root growth test and the duckweed test. The root growth test was conducted in the same manner as described in the preceding section. Millet and cabbage (*Brassica oleracea*) were used, and there were five replicates per test sample.

The duckweed tests were modified from those used in previous studies (Wang, 1986a-c). Duckweed (*Lemna minor*) was taken from stock cultures maintained for many years at the Illinois State Water Survey laboratory. The plants are maintained under constant cool-white fluorescent light, and water and plant nutrients are added weekly. Twenty-four hours before a

Table 1. Summary of Test Conditions for Natural Water Samples

	Duckweed Test	Root Growth Test
1. Test type	Static	Static
2. Temperature	25-28 C	25-25.2 C
3. Light quality	Cool-white fluorescent	Dark
4. Light intensity	80 $uE/m^2/s$	No
5. Photoperiod	Constant	No
6. Test vessel	60x15 mm petri dish	Seed-Pack Growth Pouch
7. Test solution volume	18 mL	20 mL
8. Test specimens/vessel	16 fronds (8 colonies)	15 seeds (12 for oat)
9. Replicates	6	4 or 5
10. Plant nutrients	Duckweed growth medium (5x algal growth medium)	No
11. Water control and Dilution water	Duckweed growth medium	Hard standard water
12. Test duration	120 h	120 h
13. End point	Frond increase	Dry root biomass

toxicity test was conducted, duckweed test specimens were selected. The selection criteria were that the plants have two fronds per colony and that the plants be without imperfections such as discoloration and irregular size. Before they were inoculated, these specimens were reexamined and discarded if they showed any sign of ill-health or if they had multiplied.

The experimental conditions are summarized in Table 1. The test vessel used for this study was disposable petri dishes, 60 x 15 mm. Each dish contained 16 fronds (8 colonies). Test samples and water control all contained fivefold algal growth medium as recommended in Standard Methods (1985). Duckweed reproduction as indicated by net frond increase was the test end point.

B. PHYTOTOXICITY IN INDUSTRIAL WASTEWATERS

This study was divided into three parts: duckweed and seed germination tests primarily designed for screening of wastewaters; duckweed reproduction and root elongation tests suitable for a definitive test of wastewaters; and a fractionation/treatability study of wastewater in conjunction with toxicity tests.

1. Duckweed and Seed Germination Tests

a. Effluent Samples. Eight effluent samples were obtained from three sources. Source A is an industrial wastewater pretreatment plant that specializes in treating wastewaters from a heavy machinery industry. Samples A1, A2, A3, and A4 were obtained from this source on four different dates (3/23/87, 4/29/87, 5/19/87, and 7/14/87). They were the treated samples. A raw sample entering the pretreatment plant was also collected (3/23/87) and designated as sample A. Source B, where samples B1 and B2 were obtained on two different dates (5/19/87 and 7/14/87), is an agricultural product utilization plant. Samples C1 and C2 were obtained on two different dates (5/19/87 and 7/14/87) from source C, a specialty chemical industry. All eight samples were part of the influent entering the Greater Peoria Sanitary District sewage treatment plant facilities, where 20% of the influent is industrial wastewater. Samples A1 and A2 were grab samples, and the rest were 24 h composite samples. All samples were kept at 4 C until use.

Chemical analyses of these samples were performed according to Standard Methods (1985).

b. Duckweed Tests. The duckweed tests were modifications of those used for surface-water samples. The experimental conditions are given in Table 2. Two types of experiments were performed: screening tests and definitive tests. Screening tests were conducted using 100% effluent samples. After 48 h of exposure, observations were noted on mortality, chlorosis (loss of green pigments), necrosis (local dead tissues), lesion (breakup of colony structure), and loss of buoyancy.

Samples from source C were found to be especially phytotoxic. Consequently, one definitive duckweed experiment was conducted in order to determine the concentration that caused 50% mortality (LC50) as well as 50% inhibition on frond increase (IC50). There were seven diluted samples in

Table 2. Summary of Test Conditions for Industrial Wastewater

		Duckweed Test	Seed Germination Test
1.	Test type	Static	Static
2.	Temperature	25-28 C	25-25.2 C
3.	Light quality	Cool-white fluorescent	Dark
4.	Light intensity	80 uE/m^2/s	No
5.	Photoperiod	Constant	No
6.	Test vessel	60x15 mm petri dish	100x15 mm petri dish Whatman #1 filter paper
7.	Test solution volume	18 mL	5 mL
8.	Test specimens/vessel	16 fronds (8 colonies)	15 seeds
9.	Replicates	5	5
10.	Plant nutrients	Duckweed growth medium (5x algal growth medium)	No
11.	Water control and Dilution water	Duckweed growth medium	Hard standard water
12.	Test duration	48 h, 120 h	48 h, 120 h
13.	End point	Mortality and reproduction	Germination

concentrations of 100, 60, 36, 22, 13, 7.8, and 4.7% effluent concentration, plus a control. The dilution factor 0.6 was used; Standard Methods (1985) recommends 0.5 to 0.6. As in all duckweed tests, each solution was fortified with fivefold strength (5X) algal growth medium as given in Standard Methods (1985) and inoculated with 16 fronds (8 duckweed colonies). For this particular experiment, mortality was determined after 120 h exposure.

c. Seed Germination Tests. The seed germination test was conducted similarly to the test for surface-water samples. The seeds were treated with hypochlorite solution, imbibed, and washed repeatedly. The experimental conditions are given in Table 2. As in the duckweed tests, both screening and definitive tests were performed. In the screening tests, only the 100% wastewater samples were used. After incubation in the dark at 25-25.2 C for 48 and 120 h, each seed was examined to determine whether it had germinated successfully. A seed was determined to have germinated if it showed a 3 mm primary root or greater after a specified time (Food and Drug Administration, 1987).

Because the effluent from source C was highly toxic, a definitive test using cabbage and millet was conducted in order to determine the concentration that caused 50% inhibition on seed germination (IC50). The experiments were conducted twice. In test 1, the dilution factor was 0.8 (concentration range 100-33% effluent concentration), and the results were found not to be in the proper range. In test 2, the dilution factor was 0.6 (concentration range 100-4.7% effluent concentration). There were seven diluted solutions plus a control, all using hard (hardness 160-180 mg/L as $CaCO_3$), reconstituted water as recommended in Standard Methods (1985). After incubation in the dark for 120 h, the seeds were examined to see whether or not they had germinated.

2. Duckweed Reproduction and Root Elongation Tests

a. Effluent Samples. The same eight effluent samples were used in the this study.

b. Duckweed Reproduction Tests. Duckweed tests were conducted similarly to those in the preceding study (Table 2). The major difference is that in this study, serial dilutions of water samples were tested in the manner of the definitive test. There were five to seven diluted concentrations per sample using the dilution factor either 0.5 or 0.6. The objective was to determine IC50 values using duckweed reproduction (frond increase) as the test end point.

c. Root Elongation Tests. The seed germination experiments showed that cabbage and millet were the species most sensitive to effluent toxicity (see Results). These two species were therefore selected for further study. Cabbage and millet seeds are both spherical, are approximately of the same size, and are easy to handle.

Root elongation tests were conducted similarly to those in the preceding study (Table 2). In this study, water samples were examined by employing a definitive test. There were five to seven diluted samples using either 0.8 or 0.9 as the dilution factor. The test vessels were Seed-Pack Growth Pouch-R (Table 1). After incubation in the dark for 120 h, roots of

15 seedlings in each growth pouch were cut, air-dried for 24 h, combined, and weighed to 0.1 mg. There were five replications per concentration.

3. Fractionation/Treatability of Industrial Wastewaters

a. Effluent Samples. Three samples were used for this phase of the study. Grab sample A and 24 h composite sample A4 were from source A, , obtained respectively from influent and effluent at the industrial pretreatment plant. Sample C2 was a 24 h composite sample obtained from source C. Samples A4 and C2 are the same effluents described in the preceding sections.

b. Treatability Study. An exploratory experiment was conducted using a physicochemical treatment method with different substances for phytotoxicity removal. Silica gel (Merck, 35-70 mesh), Sephadex G-75 (for gel filtration, 20-50 micrometer), ionic polymeric adsorbent (Amberlite XAD-4), strong anion exchange resin (Dowex 1x8-100), and strong cation exchange resin (Dowex 50x2-100) were purchased from the Aldrich Chemical Company in Milwaukee. Activated carbon (untreated powder, 250-350 mesh and untreated granules, 14-60 mesh) was purchased from the Sigma Chemical Company in St. Louis. The carbon was heated at 110 C overnight to destroy bacteria, fungi, and spores. Insoluble starch xanthate (ISX/5-CM), a highly cross-linked xanthate starch powder, is commercially available from Stout's Supply (Ainsworth, IA). Its suggested use is for "instantaneous heavy metal cation removal upon contact from industrial and mining process and wastewaters" (technical data sheet, Stout's Supply, undated).

For the exploratory experiment, 50-mL, 50% diluted effluent samples were placed in 250-mL beakers. Into each beaker, 1 g of test substance was added and stirred for 1 h. The mixtures were filtered through Whatman #1 filter papers. The pH of the filtrates ranged from 3.83 with cation exchange resin treatment (sample A4) to 10.44 with starch xanthate treatment (sample C2). The filtrates were adjusted with either 1 N NaOH or HC1 to pH 6.96-7.77 (sample A4) and 7.22-7.72 (sample C2). The modified filtrates were tested for phytotoxicity by using the millet seed germination test. The control was hard, reconstituted water (Standard Methods. 1985). The untreated, 50% diluted effluent samples were also used for the phytotoxicity tests.

c. Adsorption Characteristics. Adsorption characteristics were studied using powdered activated carbon (250-350 mesh). A series of 60-mL portions of samples A4 and C2, at 50% dilution, were placed in 250-mL beakers. Activated carbon was added in the amount of 0.05, 0.1, 0.2, 0.4, 0.7, 1.0, and 1.5 g and was mixed by using a magnetic stirrer for 1 h. The mixtures were filtered through Whatman #1 filter papers, and the filtrates were tested by using the millet seed germination test.

d. Adsorption Kinetics. Three hundred mL of 50% diluted portions of samples 1 and 2 were each placed in a 600-mL beaker. Each was combined with 3 g of activated carbon and stirred. After 2, 5, 10, and 60 minutes of mixing, 35 mL of the mixture was withdrawn and filtered through the Whatman #1 filter paper. The filtrates were tested for phytotoxicity using the millet seed germination test.

e. Adsorption Breakthrough. Granular activated carbon (14-60 mesh) and Amberlite XAD-4 were used for this experiment. The carbon and the resin were pre-wetted with the reconstituted water and packed in chromatographic columns, 2.2 cm (i.d.) x 10 cm. Effluent samples A4 and C2 (250 mL at 50% dilution) were eluted through these columns and eight fractions were collected, 30 mL each. These fractions were tested for phytotoxicity.

f. Phytotoxicitv Tests. Two phytotoxicity tests were performed in this study. A preliminary experiment on the phytotoxicity of samples A and A4 was conducted using common duckweed, *Lemna minor*. The test procedure was similar to that reported in Part III, section A, subsection 3. The effluent samples were diluted with the reconstituted water in a 50% dilution ratio to obtain a series of five dilutions. The test vessels were 100x15 mm plastic petri dishes. Sixteen healthy duckweed fronds were inoculated into 30 mL of test solution or control. Each solution was fortified with duckweed growth medium, which is fivefold (5X) algal growth medium (*Standard Methods*. 1985), and incubated at 25-28 C and 6456 lux for 96 h. The number of new fronds was used as the test end point. Five replicates were used in all the toxicity tests.

For the treatability study of effluent samples A4 and C2, phytotoxicity was measured by using the millet seed germination test. The millet seeds were sterilized, imbibed, and rinsed repeatedly. The test vessels were plastic petri dishes, 100x15 mm, containing 9-cm Whatman #1 filter paper. Each vessel contained 5 mL of test solution or control and 15 millet seeds. The incubation was at 25-25.2 C in the dark for 120 h. The test end point was seed germination, which was determined to be successful when the primary root of a seedling extended 3 mm or longer (Food and Drug Administration, 1987).

C. DATA ANALYSIS

Chi-square analysis was used to determine if there was a significant difference ($p<0.01$) in the treated sample and the control sample. The 1% significance level was selected over the less stringent level 5% because of the high uncertainty of phytotoxicity tests, especially seed germination and root elongation tests. For example, in the same test solution it was observed that some seeds fully developed root systems, while others failed to germinate. By using the more stringent level, an attempt was made to reduce the type I error by rejecting the false null hypothesis.

The moving-average method (Peltier and Weber, 1985) was employed to calculate IC50 values (the concentration causing 50% inhibition expressed by seed germination, root elongation, duckweed mortality, or other biological end points, in comparison with the water control) and 95% confidence levels. A personal computer was used to calculate these values.

IV. RESULTS

A. PHYTOTOXICITY IN NATURAL WATERS

1. Water Samples

Selected quality characteristics of the water samples are given in the Appendix. In the Buck Creek - Little Wabash River basin, water quality fluctuated extensively, typical of a small stream susceptible to hydrological changes. For example, there were several peaks of water turbidity: 374 NTU (nephelometric turbidity units) on June 2 at station #1, 1060 NTU on May 18 at station #2; and 485 NTU on March 2 at station #3, while the background level was in the range 10-50 NTU. Chloride content was generally low, with only one sample exceeding 200 mg/L chloride (202 mg/L). This chloride content was much lower than that found in Contrary Creek, Hamilton County in an earlier study (Butts, Williams, and Evans, undated). In Contrary Creek, 40% of water samples contained greater than 500 mg/L chloride.

Court Creek is a typical midwestern rural stream. The water samples contained relatively low turbidity, except during a rainstorm. Water samples were weakly alkaline; their pH ranged from 7.80 to 8.49. These samples were also hard, generally with hardness greater than 300 mg/L. Chloride content was typically in the range of 10-30 mg/L.

Water quality in Lake Loami was relatively stable. Turbidity ranged from 6 to 18 NTU. pH was above 8. The lake water had relatively high alkalinity.

The Illinois River is a major water resource of the state. Because of the series of locks and dams, water flow is regulated, and pH was greater than 8.00 at all times.

Deionized and reconstituted water were compared for use in control tests; the results are given in Table 3. Among 12 comparisons of root growth in deionized water versus reconstituted water, only one was significantly different, $p<0.01$, using chi-square analysis. The results support the previous study indicating that deionized water was suitable as the water control (Wang, 1985a). The fact remains, nevertheless, that deionized water is not a "natural" water. Thus it was decided to use hard, reconstituted water (Standard Methods. 1985) in the entire project as the water control in seed tests.

The results of the control tests are listed in Table 4. For the 26 sets of data, the average mean values and standard deviations of means for millet, oat, and wheat were 7.1 ± 0.9, 19.4 ± 2.3, and 21.2 ± 2.4 mg, respectively. The coefficients of variation were nearly identical: 12, 12, and 11%, respectively. Given a large number of repeated trials, the coefficients of variation of means do not differ, yet the means of each individual coefficient of variation for a given trial (date) showed that considerable variation existed. The average coefficients of variation for millet, oat, and wheat were 11, 15, and 21%, respectively. Because the coefficient of variation was smallest for millet, it appeared to be the most promising test species among the three species.

Table 3. Comparison of Root Elongation Tests Using Deionized Water and Reconstituted Water as Control Samples

(Results of dry root biomass are given as mean + S.D., in mg/15 seeds [millet and wheat] or mg/12 seeds [oat])

Test	Species	Deionized Water	Reconstituted Water
I	Millet	6.3 ± 0.25	7.6 ± 0.45*
	Oat	18.9 ± 3.86	19.6 ± 1.28
	Wheat	22.7 ± 3.40	21.4 ± 3.17
II	Millet	6.6 ± 0.42	6.9 ± 1.12
	Oat	19.4 ± 2.67	20.3 ± 4.04
	Wheat	18.1 ± 4.10	22.8 ± 3.58
III	Millet	6.8 ± 0.78	6.7 ± 0.84
	Oat	17.8 ± 1.83	20.2 ± 2.09
	Wheat	20.8 ± 6.02	20.2 ± 3.57
IV	Millet	5.6 ± 0.63	6.3 ± 0.43
	Oat	14.9 ± 3.17	19.4 ± 0.90
	Wheat	22.0 ± 7.32	22.0 ± 3.82

* $p < 0.01$

Table 4. Phytotoxicity Control Test Results, Expressed as Dry Root Biomass in mg/15 Seeds (Millet and Wheat) or mg/12 Seeds (Oat)

	Millet			Oat			Wheat		
	Mean mg	S.D. mg	C.V. %	Mean mg	S.D. mg	C.V. %	Mean mg	S.D. mg	C.V. %
1/7/87	5.7	2	35	17.5	1.9	11	20.6	5.7	28
1/16	6.6	1	15	17.8	1.8	10	22.1	3.1	14
1/22	5.3	0.6	11	19.8	2.4	12	23.1	6.1	26
2/4	6.6	0.4	6	19.4	2.7	14	18.1	4.1	23
2/18	6.8	0.8	11	17.8	1.8	10	20.8	6.0	29
3/4	5.6	0.6	11	14.9	3.1	21	22.0	7.3	33
3/18	6.9	0.8	12	16.6	5.4	33	23.2	4.8	21
4/8	7.3	0.4	5	18.8	3.6	19	21.3	3.5	16
4/22	7.2	0.3	4	20.2	2.2	11	20.5	1.3	6
5/7	6.9	1.2	17	18.5	3.5	19	20.5	5.2	25
5/21	5.7	1.1	19	19.5	3.4	17	19.5	4.8	25
6/3	8.0	0.8	10	22.8	4.0	18	26.7	1.8	7
6/10	6.9	0.3	4	-	-	-	-	-	-
6/17	6.1	1.5	25	19.4	1.7	9	18.6	4.5	24
7/8	7.8	0.6	8	18.0	2.7	15	21.8	5.8	27
7/22	7.9	1.3	16	21.9	1.2	5	20.2	4.2	21
8/5	7.8	1.0	13	20.1	5.2	26	25.7	5.6	22
8/19	7.7	0.5	6	16.5	1.1	7	20.8	4.2	20
9/10	8.4	0.7	8	22.4	4.1	18	25.7	4.4	17
9/23	7.8	0.3	4	22.0	4.0	18	19.0	4.8	25
10/9	7.9	0.7	9	17.3	1.4	8	17.7	2.7	15
10/22	8.0	0.7	9	22.0	4.8	22	17.5	4.4	25
11/4	6.3	0.5	8	21.7	3.6	17	19.8	3.8	19
11/18	8.0	0.4	5	19.9	2.6	13	22.0	5.1	23
12/9	6.8	0.5	7	23.8	4.0	17	22.1	2.1	10
12/23	7.4	0.4	5	16.6	1.7	10	20.8	4.7	23
(1) Mean ± S.D.	7.1 ± 0.9			19.4 ± 2.3			21.2 ± 2.4		
C.V. , %	12			12			11		
(2) Mean C.V., %	11			15			21		

(1) Average of daily means and coefficients of variation of average mean

(2) Average of coefficients of variation for each day

The monthly precipitation at Peoria in 1987 is depicted in Figure 3. The year 1987 can be characterized as extremely dry in terms of recent history; for example, rainfall in April was 8.5 cm, which was 37% less than the historic mean rainfall for April (13.5 cm). Rainfall was below average in every month except August, November, and December. On an annual basis, the deficit rainfall amounted to 14 cm. This exceptional dry spell, especially during spring and summer, was expected to affect herbicide runoff and phytotoxicity in the aquatic environment.

2. Root Growth Tests for Surface Waters

The detected phytotoxicity, as measured by root growth inhibition, in Buck Creek and the Little Wabash River is depicted in Figure 4. Among three test species, the millet test was able to detect five samples significantly different from the control, $p<0.01$. The wheat test detected one sample, and the oat test detected none. Among three stations, samples from the Little Wabash River station 3 were phytotoxic on three occasions. The amounts of phytotoxicity were 9, 11, and 25% inhibition using the millet test. Samples from station 2 in Buck Creek were phytotoxic on two occasions, with 11% inhibition on both occasions using the millet test.

The results were interesting in light of the facts that station 1 contained no significant phytotoxicity and that there were occasions on which water samples from stations 2 and 3 contained significant phytotoxicity. Considerable oil drilling activities are concentrated in this region, and these activities may or may not contribute to the stream contamination. It should be cautioned that only three tests (two millet and one wheat) out of 72 possibilities showed positive phytotoxicity.

Phytotoxicity in Court Creek (stat ion C2) and Sugar Creek (stations S2 and S3) is shown in Figure 5. Again, the millet test detected most of the phytotoxicity: six samples with $p<0.01$. The wheat test detected only one sample, and the oat test, none. The highest phytotoxicity was detected on January 12 after a snow-melt-related runoff: 26% inhibition on the wheat test. Among the three stations, station S3 was found to contain phytotoxicity on five occasions (four millet tests and one wheat test), while one sample each was detected at stations C2 and S2.

Phytotoxicity in the Illinois River-Peoria Lake is shown in Figure 6. The millet test was most effective in detecting phytotoxicity: four samples with $p<0.01$. The amounts of phytotoxicity, as determined by the millet test, were 10, 13, 17, and 21% inhibition. The wheat test showed one sample containing phytotoxicity with 29% inhibition. The oat test did not detect any sample to be significantly phytotoxic.

One sample from Lake Loami, taken on August 11, 1987, was phytotoxic, with 18% inhibition of root growth using the millet test.

These results generally indicate that millet is the most sensitive species among the three test species. This is in agreement with the results of a previous study showing that millet is more sensitive to organic pollutants than other species (Wang, 1986d). As an alternative, it is possible that the millet test gave higher false positive results. This possibility, however, is minimal because of the stringent significance level used throughout this study.

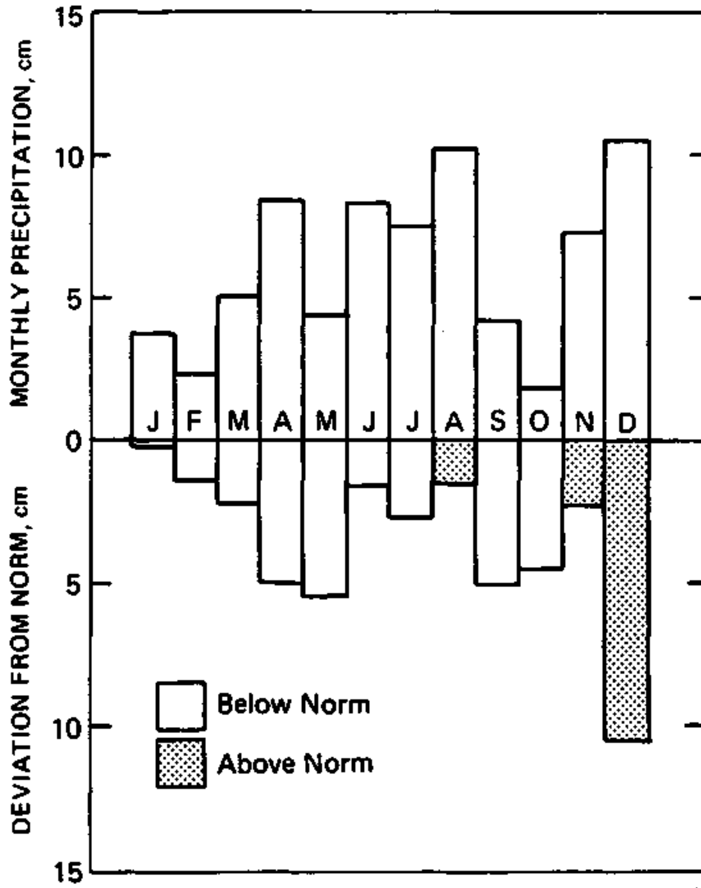

Figure 3. Monthly precipitation and precipitation deviations from the norm in Peoria, IL, in 1987

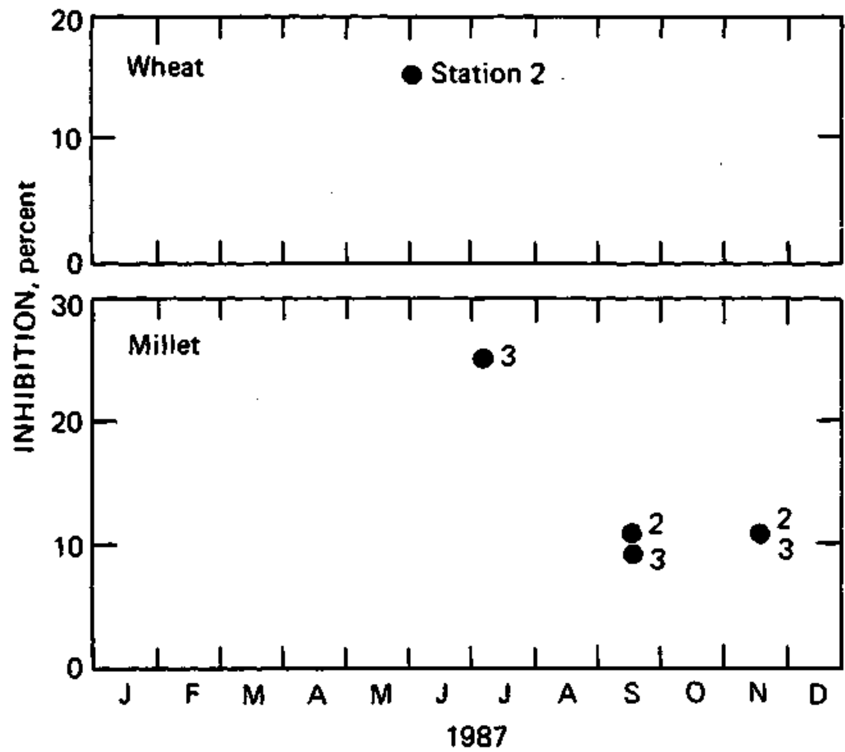

Figure 4. Root growth inhibition in samples from Buck Creek (station 2) and the Little Wabash River (station 3)

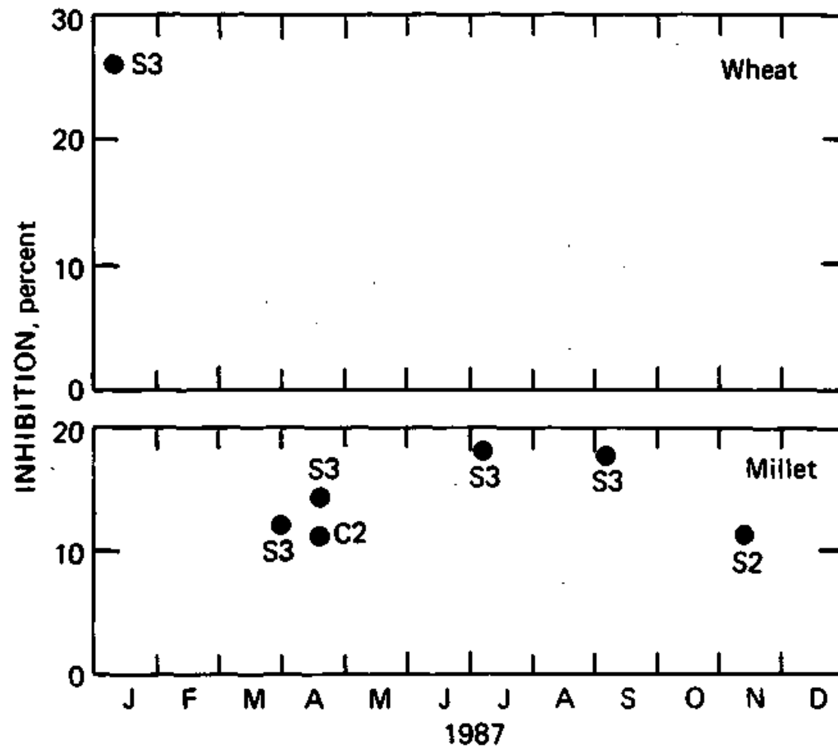

Figure 5. Root growth inhibition in samples from Sugar Creek (stations S2 and S3) and Court Creek (station C2)

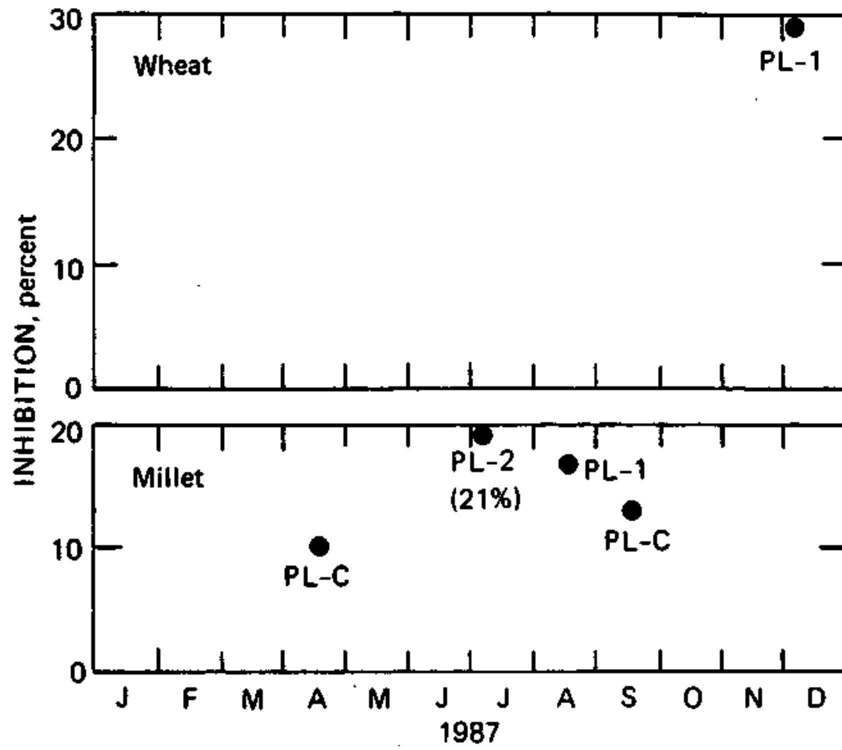

Figure 6. Root growth inhibition in samples from Illinois River - Peoria Lake

The results generally indicated that significant phytotoxicity was sporadic in these rural waters. Because of the unexpected dry spell, apparently no major herbicidal pollution occurred during the study period. Nevertheless, chronic, low-level herbicide contamination is suspected to be present in the aquatic environment.

3. Duckweed and Root Growth Tests for Ground Waters

The results of phytotoxicity tests of ground waters are given in Table 5. Among three tests conducted, the duckweed test appeared to be the most effective in detecting phytotoxicity, especially with the June and July samples. Among 13 samples collected in June, 10 were found to be phytotoxic, $p<0.01$. The inhibitory effect ranged from 11 to 23%. Again in July, six samples out of seven were found to be phytotoxic. In August, however, all samples were found not to be significantly toxic. Cabbage and millet tests did not detect any significant phytotoxicity in any of the samples.

The results are significant because they show that the ground waters in the Sankoty aquifer may be phytotoxic and that phytotoxicity may be seasonal. The results, however, should not be construed to imply a human health issue.

B. PHYTOTOXICITY IN INDUSTRIAL WASTEWATERS

1. Duckweed and Seed Germination Tests

<u>a. Effluent Samples.</u> The quality of the effluent samples can be characterized as highly complex (Table 6). Both sources A and C contained high NH_3, COD (chemical oxygen demand), and total solids. Source A samples were weakly acidic, whereas source B and C samples were neutral to weakly alkaline. Source B samples were characterized by low NH_3 and NO_3-N and relatively low COD and chloride. The source A samples contained elevated concentrations of Zn, grease/oil, and phenol. The Fe concentrations were high in samples A1, A2, A3, A4, C1, and C2.

<u>b. Duckweed Tests.</u> In the duckweed tests, the eight effluent samples were tested in two batches: samples A1, A2, A3, B1, C1 and A4, B2, and C2.

Duckweed exhibited an all-or-nothing effect as indicated by Peltier and Weber (1985). With samples B1 and B2, duckweed mortality was 4 and 0, respectively, out of 80 fronds originally inoculated. These results were not significantly different from those for the control samples (2 and 0, respectively). It can be concluded from the duckweed test that the source B effluent samples contained little or no phytotoxicity.

The duckweed plants were extremely sensitive to 100% effluent from source C. The plants showed 100% mortality in these samples, with symptoms of lesion and complete loss of green pigments. Duckweed death was complete, and no new protruding buds developed during the test period.

Effluent A was nearly as toxic as effluent C. In samples A1, A2, and A3, the mortality was complete, while in sample A4, five of 80 fronds remained green and healthy-looking. The survivors were the small fronds of

Table 5. Percentage Decreases in Plant Growth in Ground-Water Samples from Growth in Control Samples

Sources of Samples	6/9-10/87			7/7-9/87			8/4-5/87		
	Dkwd.	Cabbage	Millet	Dkwd.	Cabbage	Millet	Dkwd.	Cabbage	Millet
Birkey	14*	S	NS	NT	NT	NT	S	S	NS
Bleck	11*	S	S	11*	S	NS	NS	S	NS
Eichellberger	15*	S	S	15*	S	NS	NS	S	NS
Evans	11*	S	NS	NT	NT	NT	NT	NT	NT
Henderson	18*	S	S	NT	NT	NT	NS	S	NS
D. Litwiller	21*	S	S	NS	S	NS	NS	S	NS
L. Litwiller	NS	S	NS	NT	NT	NT	NS	S	NS
McMullin	23*	S	S	15*	S	NS	NS	S	NS
Robison	23*	S	NS	15*	S	S	S	S	NS
Schmitgell	17*	S	S	21*	S	S	NS	S	NS
Schnepper	NS	S	S	NT	NT	NT	NT	NT	NT
Steiger	14*	S	S	13*	S	S	S	S	NS
Illinois-American W.C.	NS	NS	NS	NT	NT	NT	NT	NT	NT

S - stimulatory
NS - not significantly inhibitory, $p > 0.01$
NT - not tested
* - $p < 0.01$

Table 6. Characteristics of Effluent Samples*

(Al and A2 were grab samples, and the rest were 24-hour composite samples)

Sample	Al	A2	A3	A4	Bl	B2	C1	C2
NH_3	200	220	93	219	0.1	0.2	33	14.4
pH	5.5	5.9	5.5	6.7	7.3	6.4	7.6	9.2
Grease/oil	50	68	82	73 .	-	-	-	-
COD,$x10^{-3}$	15	14	12	19	2	5.8	8	5.3
Alkalinity,$x10^3$**	1	0.8	0.5	1.8	0.4	0.56	2	2.9
Chloride,$x10^3$	0.5	0.4	0.2	0.3	0.08	0.05	3.5	4.2
Sus. solids,$x10^3$	0.15	0.8	0.7	0.15	0.05	0.17	0.04	0.04
Total solids,$x10^3$	10	11	9.7	13	1.2	2	15	17
Vol. solids,$x10^3$	5	4.6	-	5.8	0.04	0.15	0.027	0.02
$N0_3$-N,$x10^3$	1	0.7	1.6	4	4.6x10-5	$1.5x10^{-3}$	$2x10^{-3}$	$5.9x10^{-3}$
Total P	-	-	-	-	0.16	5.26	31.5	92
Phenol	1.5	1.1	0.7	1.3	-	-	-	-
Cyanide	0.1	0.03	0.021	0.26	-	-	-	-
Sb	0.2	0.1	1.4	0.6	-	-	-	-
Cd	-	0.006	0.009	0.006	0.005	<0.0002	0.019	0.025
Cr	-	<0.1	0.04	0.2	<0.003	0.012	0.024	0.046
Cu	-	<0.04	<0.04	0.4	0.006	0.046	0.036	0.039
Pb	-	<0.1	<0.1	<0.1	0.067	<0.01	0.133	0.27
Hg	-	<0.0001	<0.0005	<0.0005	-	-	-	-
Fe	20	7.9	16.2	31	0.47	0.813	0.259	5.88
Mn	0.8	0.5	1.2	1.9	0.063	0.061	-	0.162
Ni	0.3	0.2	0.13	0.46	0.018	0.004	4.6	1.2
Zn	3.0	0.6	2.2	4.2	0.085	0.132	0.191	0.18

* all in mg/L except pH (pH scale)

** as $CaCO_3$

duckweed colonies. The signs of stress of the duckweed plants in these samples were different from those in effluent C. The plants showed necrosis, but the colony structure remained intact and green pigments were not bleached.

The duckweed test was also modified for definitive, in-depth characterization of effluent toxicity for source C samples. The test end points included duckweed mortality and reproduction. The results of samples C1 and C2 are depicted in Figures 7a and b. Mortality as a test end point had a drawback: the uncertainty of mortality at the low effluent concentrations. This is probably the cause of the scatter of data seen in Figure 7a. Reproduction as the test end point gave more consistent results.

By using the moving-average method (Peltier and Weber, 1985), the IC50 value of sample C1 using frond increase as the test end point was calculated to be 20% effluent concentration (95% confidence limit 16-30%); the LC50 value using frond mortality as the test end point was 42% (95% confidence limit 39-45%).

Sample C2 was extremely toxic. At the 22% effluent concentration or greater, the duckweed died (100%) and did not reproduce. At 13% and below, some plants reproduced but died thereafter, so that the numbers of dead fronds were greater than the 80 initial fronds. At 4.7% effluent concentration, 16 new fronds developed from the 80 initial fronds. These fronds all died later, resulting in 96 dead fronds. Both the IC50 and LC50 values were estimated to be less than 2% concentration.

c. Seed Germination Tests. In this study, it was observed that after exposure to some effluent samples, Japanese millet had a well-developed shoot system, while the root system was nonexistent. More than 40% of the Japanese millet seeds displayed this particular phenomenon in sample C1. Some seeds also developed a short primary root of less than 3 mm and did not develop further. These seeds were considered ungerminated according to the operational definition.

The results of the seed germination tests are given in Table 7. For all the control samples, seed germination was 85% or greater after 120 h of incubation (i.e., 11 or fewer ungerminated seeds), except for wheat in the second batch of tests. The results show that there were considerable variations of seed germination from 48 h to 120 h. The results suggest that a 120 h incubation time was required to allow plant seeds sufficient time to germinate.

Cabbage, cucumber, and millet seeds were capable of detecting phytotoxicity in all samples. It is especially notable that the germination of these seeds was completely (or nearly so) inhibited in samples A2, A3, A4, C1, and C2. The results of the cabbage and millet germination tests showed that samples B1 and B2 were also phytotoxic, although to a much lesser extent than the other effluent samples.

Cabbage, cucumber, and millet were more sensitive to effluent toxicity than Japanese millet, rice, and wheat. This can be seen especially from the results of their responses to samples A1, A2, and A3 (Table 7). The inhibition of germination was much less in the second plant group than in the first plant group. The reason(s) for this deviation is unknown. The

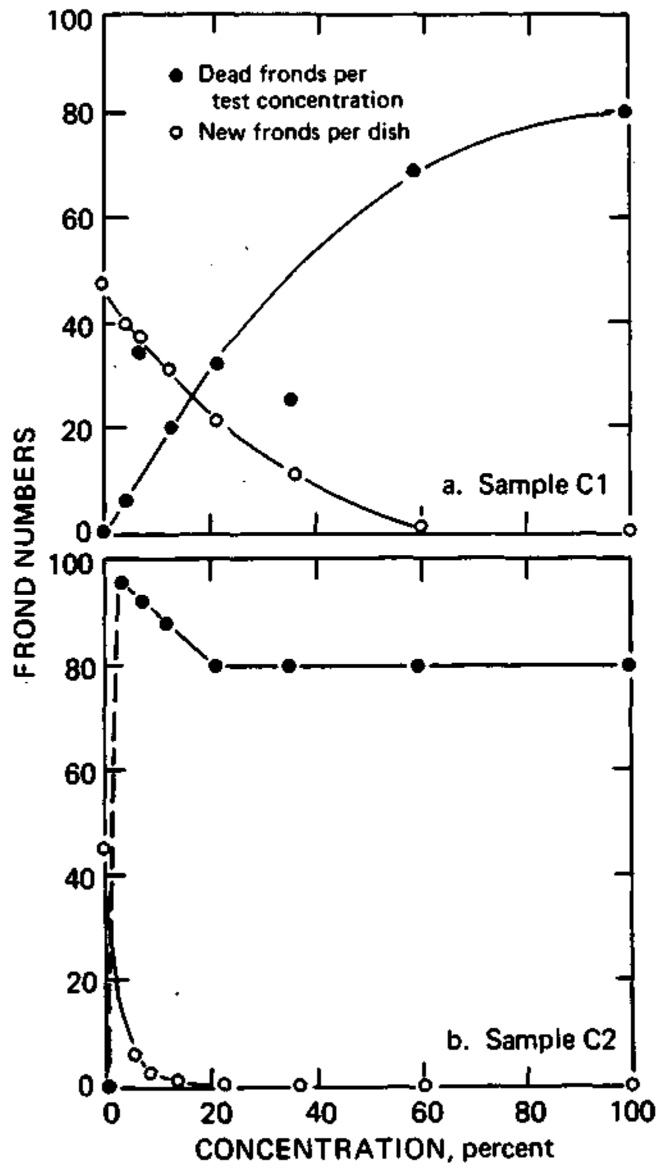

Figure 7. Duckweed test results for effluent samples C1 and C2

Table 7. Results of the Seed Germination Tests of Effluent Toxicity

(The numbers indicate seeds ungerminated out of 75 seeds/test solution)+

	Cabbage		Cucumber		Millet		Japanese millet		Rice		Wheat	
	48 h	120 h	48 h	120 h	48 h	120 h	48 h	120 h	48 h	120 h	48 h	120
Control I	9	4	5	2	4	3	11	10	13	8	33	6
Sample Al	74*	74*	61*	14*	20*	,67*	19	15	31*	29*	73	34*
A2	75*	75*	54*	49*	55*	71*	31*	19*	31*	21*	75	24*
A3	74*	75*	64*	59*	70*	73*	46*	24*	52*	46*	75	31*
Bl	33*	17*	11	7*	28*	14*	15	12	19	13	57	8
C1	75*	75*	75*	75*	75*	75*	75*	73*	48*	75*	75	75*
Control II	10	8	19	11	24	9	24	7	57	11	21	12
Sample A4	75*	75*	75*	75*	75	75*	75	74*	75	75*	75	38*
B2	73*	29*	32*	19	51	30*	39	12	64	12*	27	15
C2	75*	75*	75*	75*	75	75*	75	75*	75	.75*	75	75*

+ 5 replicates of 15 seeds

* p<0.01 using chl-square analysis

results, nevertheless, showed that cabbage, cucumber, and millet are promising as phytotoxicity test species.

The results of the definitive test using cabbage and millet seeds are presented in Figures 8a and b. Both samples C1 and C2 were tested twice. Even though the dilution factor was different (0.8 and 0.6), the results are very close. One line of best fit can be used for each test species in an effluent sample. The numbers of ungerminated cabbage seeds in the control samples in this series of experiments were 15, 15, 10, and 9, whereas the numbers for millet were 4, 8, 6, and 6.

The results of the definitive tests are summarized in Table 8. The use of millet seed germination as the test end point for samples C1 and C2 gave the IC50 values of 17 and 12% effluent concentrations, respectively, whereas the use of cabbage seed germination gave IC50 values of 8 and 6%, respectively. Among the three species, cabbage was the most sensitive species to sample C1, and duckweed was the most sensitive species to sample C2.

2. Duckweed Reproduction and Root Elongation Tests

a. Effluent Samples. The same eight effluent samples were used in this series of experiments as in the previous experiments (Part IV, Section B, Subsection 1).

b. Duckweed Reproduction Tests. Results of duckweed tests varied in a wide range: 25-51 new fronds (Table 9). The old duckweed culture used in the first four tests exhibited a much lower growth of 25-32 new fronds. After these tests, the culture was restocked and allowed two weeks to reestablish before the next experiments were conducted. The control samples in subsequent tests (test 7 and thereafter) showed a much greater growth: 37-51 new fronds.

The results of the effluent toxicity tests are depicted in Figure 9. Cabbage, duckweed, and millet tests were all performed for samples Al, A2, A3, B2, and C1. The cabbage test was omitted for samples A4, B2, and C2. The concentration-toxicity relationships were fitted graphically and can be expressed as nearly linear to sigmoid curves.

The precision of the duckweed test is shown by the results for sample A2. This sample was tested twice, and the results are shown in Figure 9. The test results are very close, even though the control samples for the two tests are significantly different: 3 0 + 4 and 48 ± 8 , respectively, for test 1 and test 2.

Among these three test species, duckweed was invariably more sensitive to effluent toxicity than cabbage or millet. This was especially obvious when the effluent samples were less toxic (samples Bl and B2, Figure 9) and when the effluent samples were diluted (Figure 9). In samples Al, A2, A3, A4, C1, and C2, duckweed specimens showed visible signs of injuries in 24 h, including chlorosis and lesion. At that point, the plants probably passed the stage of stress and might have been damaged irreparably. The duckweed mortality was total in 96 h of exposure to these samples. The reason that duckweed plants were more sensitive to effluent toxicity than cabbage and millet may possibly be that during the test period, duckweed went through

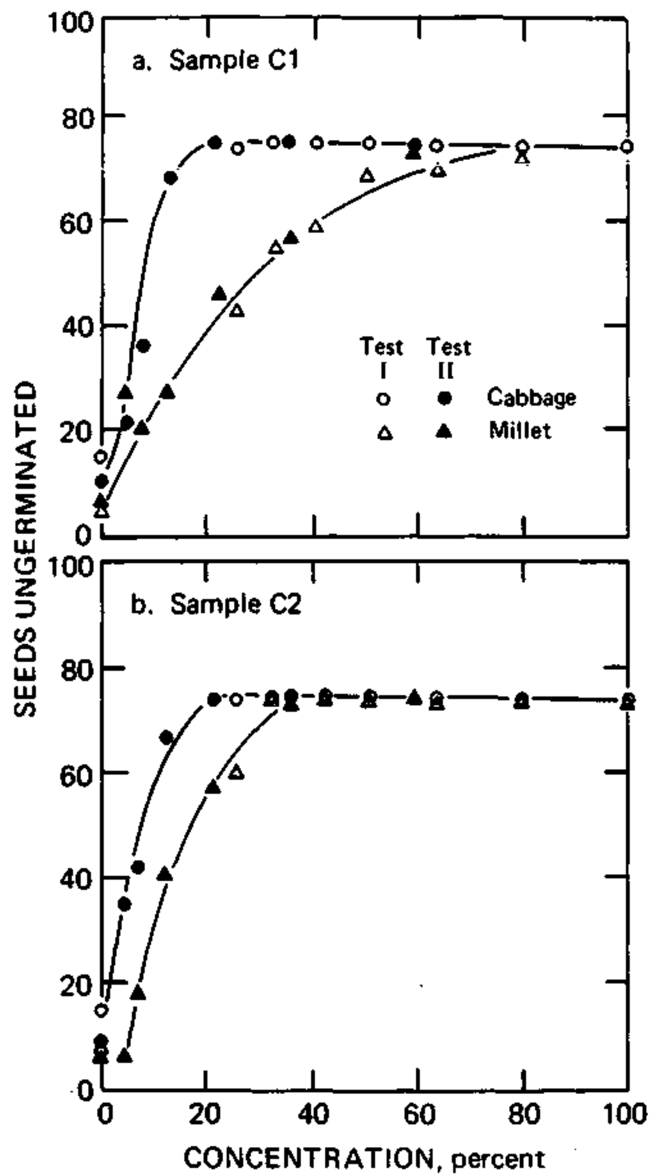

Figure 8. *Seed germination test results for effluent samples C1 and C2*

Table 8. Fifty Percent Effect Concentrations and 95% Confidence Limits of Effluent Samples C1 and C2

(Calculated by using the moving-average method, all expressed in percent effluent concentration)

	C1		C2	
	50% Effect Conc.	95% C. L.	50% Effect Conc.	95% C. L.
Duckweed				
Reproduction	20	16-30	<2	-
Mortality	42	39-45	<2	-
Millet				
Germination	17	14-22	12	11-16
Cabbage				
Germination	8	7-9	6	0-100

Table 9. Test Results of Control Samples in Duckweed Growth Medium or in Hard Standard Water (N = 5)

	In Duckweed Growth Medium, Fronds	In Hard Standard Water, 0.1 mg	
Test	Duckweed Test	Cabbage Test	Millet Test
1.	25 ± 3	46 ± 5	73 ± 9
2.	NT	47 ± 3	71 ± 4
3.	32 ± 3	42 ± 6	79 ± 7
4.	30 ± 5	45 ± 3	75 ± 8
5.	NT	45 ± 4	68 ± 5
6.	30 ± 4	39 ± 7	75 ± 5
7.	48 ± 8 (new culture)	44 ± 10	72 ± 9
8.	43 ± 12	45 ± 2	74 ± 4
9.	51 ± 8	46 ± 4	70 ± 12
10.	46 ± 6	44 ± 7	74 ± 5
11.	50 ± 5	44 ± 5[a]	72 ± 7[a]
12.	NT	NT	65 ± 12
13.	41 ± 3	47 ± 3	72 ± 9
14.	47 ± 4	NT	76 ± 10
15.	NT	41 ± 3	75 ± 4
16.	41 ± 3	NT	67 ± 10
17.	42 ± 1	NT	66 ± 10
18.	37 ± 3	NT	69 ± 5

[a]N = 4

NT = not tested

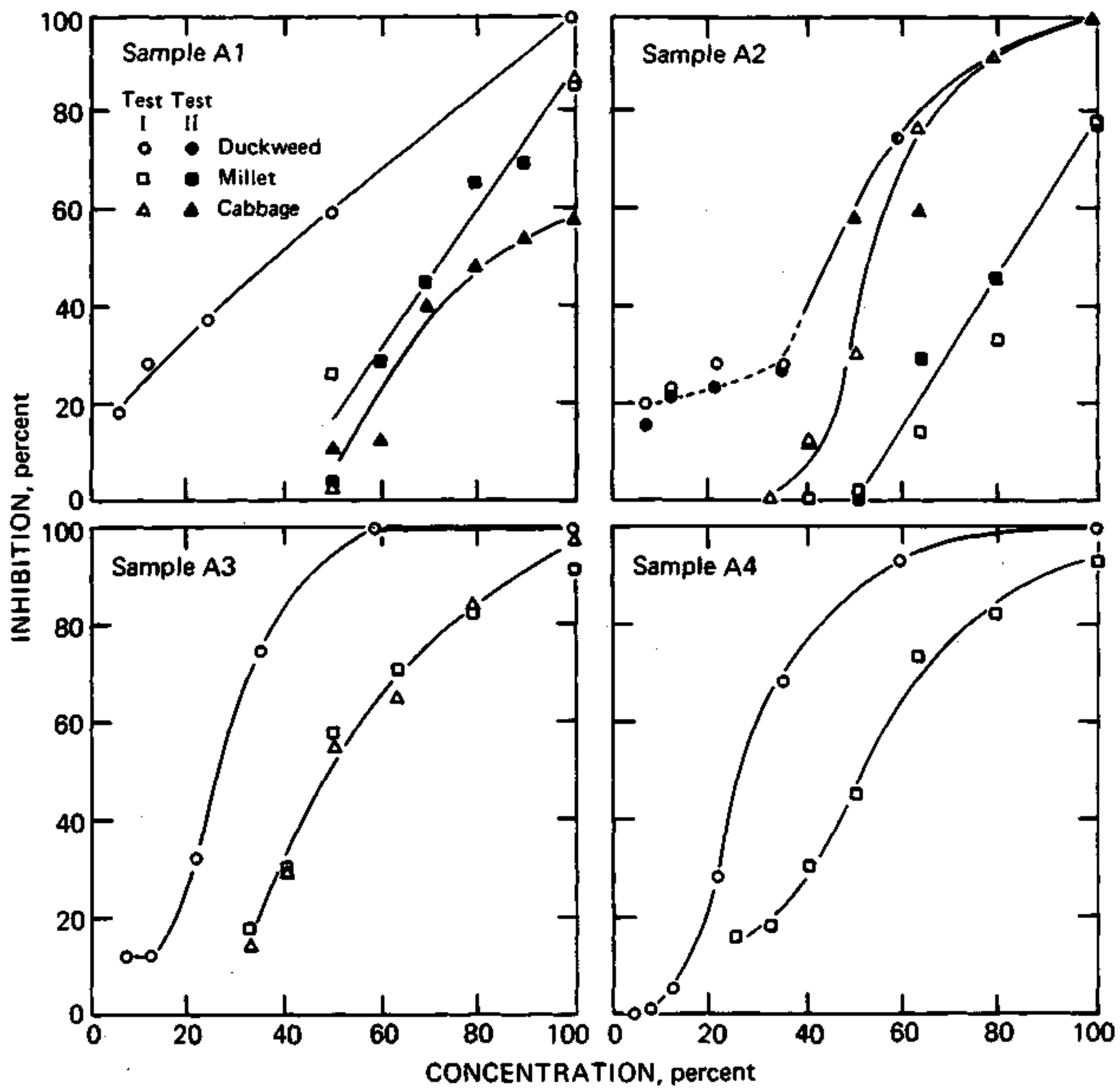

Figure 9. Effluent toxicity test results for duckweed, cabbage, and millet

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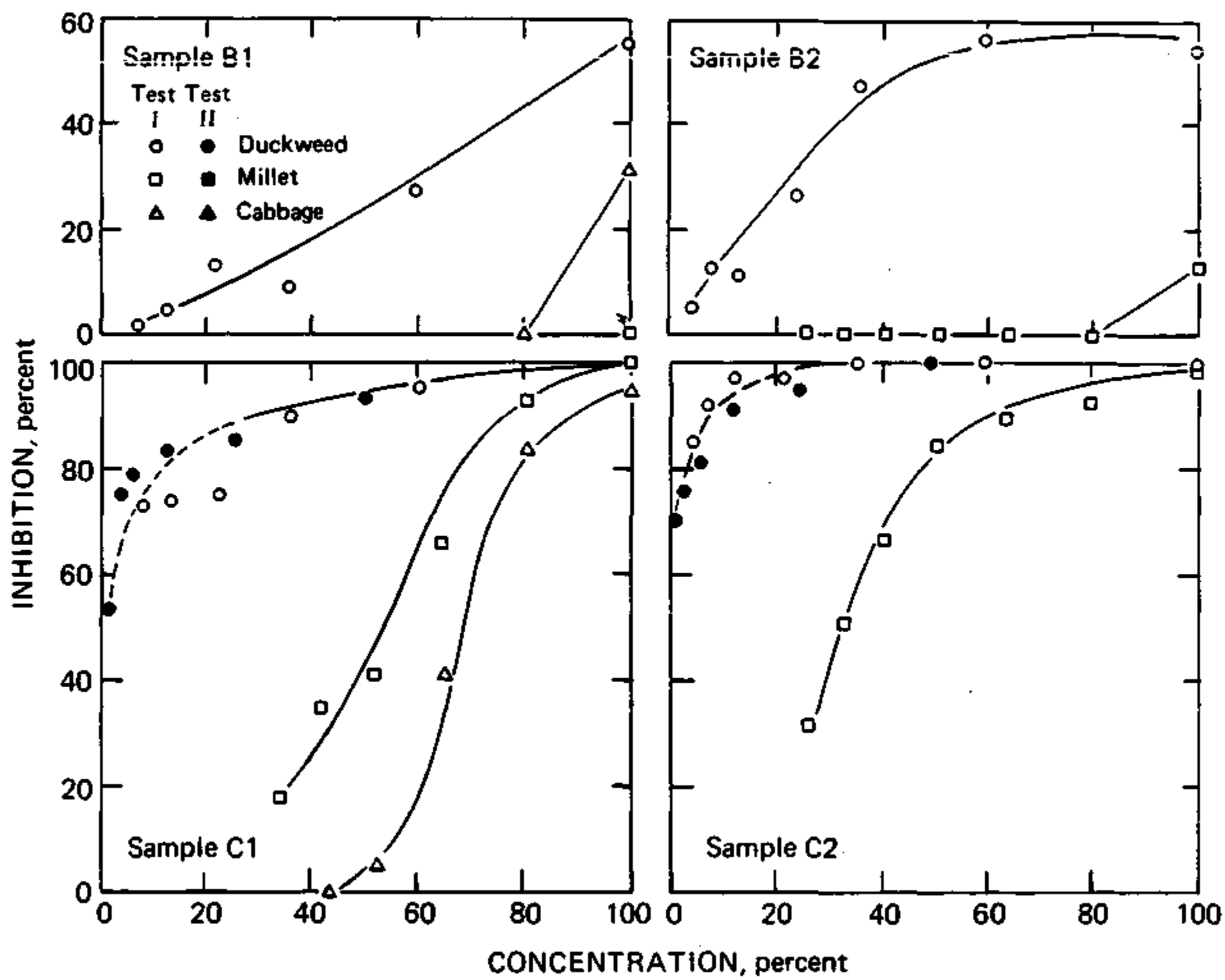

Figure 9. Concluded

the stages of its life cycle involving growth, development, metabolism, photosynthesis, and reproduction, whereas cabbage and millet went through only the processes of growth, development, and metabolism. It is possible that the more living processes the test organism is involved in during the test period, the more susceptible the organism tends to be to toxic effects because each process may be inhibited by different toxicants, or by the same toxicant in varying degrees. The combined toxic effect of all toxicants is what causes the test organism to respond as indicated at the test end point.

The definitive test as reported here provides more information than a screening test using only a single effluent concentration. A case in point can be explained by using the duckweed test results shown for samples A1 and C2, Figure 9. At 100% effluent concentration for samples Al and C2, the duckweed growth was inhibited 100%. Yet the dilution of effluent samples showed that sample C2 was inherently more toxic than sample Al. The IC50 values were less than 1.6% for sample C2, and 44% for sample Al.

c. Root Elongation Tests. The control test results were pooled and are presented in Table 9. The control samples for cabbage and millet varied in a relatively small range: 3.9-4.7 and 6.5-7.9 mg/15 seedlings, respectively. The coefficients of variation of the millet tests were in the range of 5-20%, a considerable improvement over the previous results of 59-67% and 54-68% (Wang, 1986d; 1987a-b). The millet test results compared favorably with the last series of tests, 7.1 + 0.9 mg/15 seedlings (Part IV, Section A, Subsection 1).

The precision of the root elongation tests is shown in Table 10. Of particular interest are the millet test results for sample A2. Four tests (for this and companion studies) were conducted on four days, and the results for the control samples varied: 7.5±0.5; 7.2 ±0.9; 6.5± 0.9; and 8.4 + 0.8 mg. Root growth inhibition results were nearly identical: 79, 78, 79, and 79%. Other millet test results are also highly reproducible, regardless of the absolute value for the control samples. Cabbage tests were not conducted as extensively. In Table 10, a greater variability can be seen for sample Al than for sample A2.

The gravimetric method for root measurement was found especially useful with cabbage and millet seeds, which develop an extensive primary root that is easily cut and combined. Other seeds such as oat and wheat seeds develop extensive lateral secondary roots retained inside the trough. It would be a laborious task to cut the individual roots of these seeds.

The toxic effects were mixed for the cabbage and millet species. These two species exhibited nearly identical responses to sample A3, whereas their responses to the other samples varied.

Both the cabbage and millet tests showed narrower toxic response than the duckweed tests. This was the reason that the dilution factor used in these tests was 0.8-0.9, instead of the more conventional factor 0.5 or 0.6 (Standard Methods. 1985). Samples Al and A2 were each tested twice using the cabbage and millet tests. The IC50 values (Table 11) of these tests were very close.

Table 10. Reproducibility of Root Growth Test, Expressed as Inhibitory Effect in the Effluent Samples

Sample	Cabbage		Millet	
	Control 0.1 mg	Inhibition %	Control 0.1 mg	Inhibition %
A1	46 ± 5	68	73 ± 9	87
	42 ± 6	88	79 ± 7	86
	45 ± 4	59	68 ± 5	88
A2	39 ± 7	100	75 ± 5	79
	44 ± 10	100	72 ± 9	78
	NT		65 ± 9[a]	79
	NT		84 ± 8	79
A3	45 ± 2	98	74 ± 4	92
	NT		65 ± 12[a]	92
	44 ± 7	95	74 ± 5	100
A4	NT		67 ± 10	94
	NT		80 ± 5[b]	93
C1	NT		69 ± 5	98
	NT		80 ± 5[b]	99

[a,b] One control was used on one test date

NT = Not tested

Table 11. IC50 Values and 95% Confidence Limits of Effluent Samples

(All expressed in % effluent concentrations)

	Cabbage		Millet		Duckweed	
Sample	IC50	95% C.L.	IC50	95% C.L.	IC50	95% C.L.
Al	76	72 - 79	65	60 - 70	38	29 - 52
	82	0 - 100	72	66 - 75	NT	
A2	56	54 - 58	87	83 - 90	49	44 - 55
	49	48 - 51	82	76 - 86	49	45 - 55
A3	49	46 - 55	48	45 - 52	22	25 - 29
A4	NT		53	48 - 56	29	26 - 31
Bl	NA		NA		91	80 -100
B2	NT		NA		43	0 -100
C1	67	64 - 69	55	50-59	<1.6	
C2	NT		33	30-46	<1.6	

NA = not available (100% effluent caused less than 50% inhibition)

NT = not tested

3. Fractionation/Treatability of Industrial Wastewaters

a. Preliminary Phytotoxicitv Test. The objective of the preliminary test was to measure the intrinsic toxicity of the samples. Influent and effluent from source A were tested, and the results are depicted in Figure 10. At 100% concentration, both influent and effluent samples completely inhibited duckweed reproduction. In fact, all duckweed plants died within 48 h. At 50% concentration, the influent and effluent samples caused 100 and 60% inhibition, respectively. The shaded area in Figure 10 shows the detoxification effect achieved by the industrial pretreatment process. The IC50s were 20% and 38% for influent and effluent, respectively. These results confirmed that the effluent sample was highly toxic and that the phytotoxicity test was a simple and sensitive test for detecting effluent toxicity.

There were two reasons for switching from the duckweed test to the millet test to determine phytotoxicity in the remaining studies. First, the millet test requires 5 mL per test vessel in contrast to the 18 mL required for the duckweed test. The smaller test volume required for the seed test permits greater flexibility for experimentation and for completion of this and companion studies. Second, the millet test can be initiated at any moment and takes considerably less time to start than the duckweed test. Although the millet test was found to be less sensitive than the duckweed test (unpublished data), a previous report (Wang, 1987a) showed that the millet test was especially useful for organic pollutants.

An experiment was performed to measure the phytotoxicity of samples A4 and C2 by using the millet test (Figure 11). The samples were each diluted with the reconstituted water with a dilution factor of 0.6. At 100% concentration, samples A4 and C2 completely inhibited seed germination. The control samples in both tests contained six ungerminated seeds; the germination rate was thus 92%. On the basis of chi-square analysis, the critical value that a test sample had to surpass to be considered significantly different ($p<0.01$) from the control was 13 ungerminated seeds. The IC50 values (and 95% confidence limits) for samples A4 and C2 were calculated to be 17% (14-22%) and 12% (11-16%), respectively. Because both samples were highly toxic, the treatability study was conducted using effluent samples diluted 50% with the reconstituted water.

Quality assurance of test results is a major concern in toxicity testing. One measure of quality assurance is precision of results for control samples, which by design are tested under identical conditions from one test to another (Table 12). The millet seed germination rate in this series of experiments ranged from 87 to 95%. The net inhibition of sample A4 (50% dilution) to millet germination ranged from 69 to 87%, whereas that of sample C2 ranged from 97 to 100%.

b. Effluent Samples. The pH values of the three effluent samples (Al, A4, and C2) were 5.5, 6.7, and 9.2. Although a previous study (Wang, 1985a) indicated that pH in the range of 5-9 did not affect early seedling growth, two experiments were conducted to further ascertain pH effect on millet seed germination. In experiment 1, reconstituted water was modified to pH 4.1, 5.25, 6.31, 7.22, and 8.28. No significant differences in test results were found among these solutions. In experiment 2, the pH of effluent sample A4

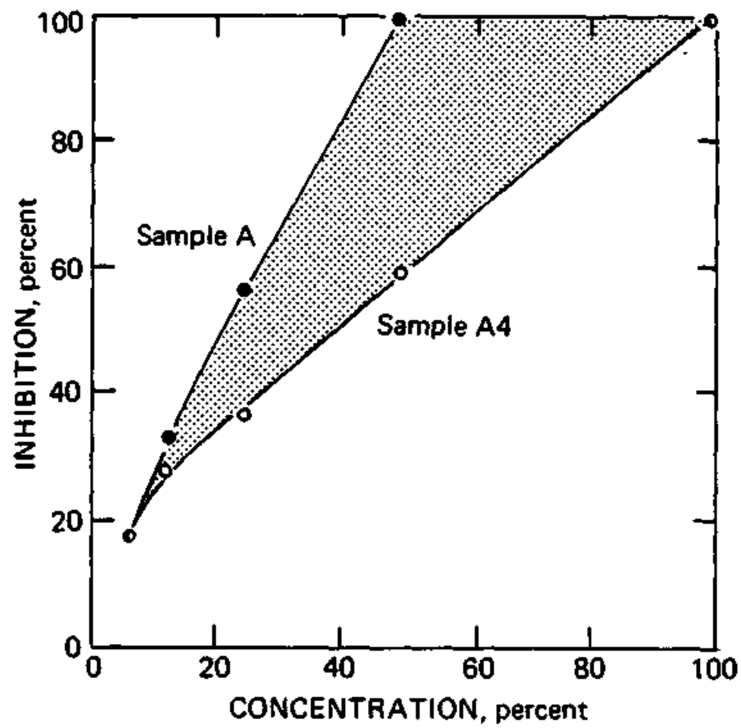

Figure 10. Toxicity test results of wastewater samples from an industrial pretreatment plant, source A, determined by using the duckweed test (Samples A and A4 are influent and effluent, respectively, and the shaded area denotes toxicity reduction)

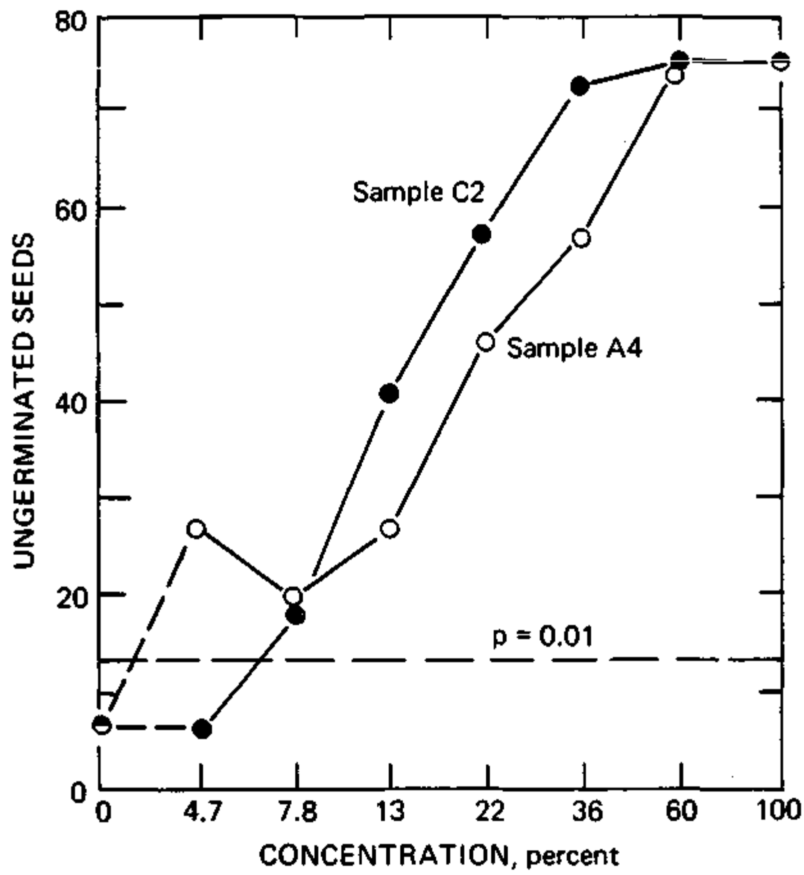

Figure 11. Toxicity test results of effluent samples A4 and C2, determined by using the millet seed germination test

Table 12. Quality Assurance Results for Millet Seed Germination Tests, Expressed as Inhibitory Effect in 50% Diluted Effluent Samples

Effluent Sample	Germination in Control Sample, %	Net Inhibition, %
A4	87	69
	88	73
	95	82
	93	87
	92	72
C2	88	98
	93	100
	91	99
	93	100
	89	100
	91	97

was adjusted from 6.7 to 7.95. Again, the millet test showed no significant difference in seed germination in the effluent sample before and after pH adjustment.

Neutralization, or pH adjustment, is a simple and economic means of preteating wastewater (U.S. Environmental Protection Agency, 1980). The results of these experiments, however, showed that pH adjustment would not alleviate phytotoxicity in these effluent samples. Another approach is required for toxicity removal.

c. Treatability Study. Figure 12 shows different treatments of effluent samples and the resultant phytotoxicity. The number of ungerminated seeds in the control samples for samples A4 and C2 were 9 and 10, respectively; the critical values of ungerminated seeds for the treated samples to be considered significantly different ($p<0.01$) from the controls were 17 and 19. From the results in Figure 12, it can be concluded that both granular and powdered activated carbon almost completely removed phytotoxicity from the effluent samples, while the other treatments were either only partially effective or ineffective in toxicity removal.

Toxic effect is typically expressed by taking into account the results of the control sample (American Society for Testing and Materials, 1987). The net inhibition of a test sample is defined as [1 - (number of germinated seeds in a test sample/number of germinated seeds in a control)] x 100. The calculated net inhibitions are given in Table 13. Although samples A4 and C2 were not physically mixed, the overall toxic effect of the test samples can be conceptualized by taking their average, called "mean inhibition." The results in Table 13 clearly show that treatments with anion exchange resin, silica gel, Sephadex G-75, and insoluble starch xanthate had no effect on phytotoxicity removal (76-85% mean inhibition for these four treatments versus 84% inhibition for the positive control). Treatments with XAD-4 and cation exchange resin were partially effective (44 and 57% mean inhibition, respectively). Powdered and granular activated carbon both nearly completely removed phytotoxicity. Consequently, further experiments were conducted using these substrates.

d. Adsorption Characteristics. One common approach for expressing adsorption characteristics is to use the empirical Freundlich adsorption isotherm, a log-log plot of the amount of residual substance after equilibrium versus the amount of adsorbed substance per unit of adsorbent, for a given temperature and other conditions.

The experimental results were first plotted in the Freundlich adsorption isotherm. The plot scattered widely. Although Scheindorf, Rebhun, and Scheintuch (1982) proposed a modified Freundlich isotherm for multicomponent systems, the modified isotherm is unlikely to be applicable to the uncontrolled, complex effluent samples. Several other plots were tried. Among them, the linear plot of carbon dose-residual toxicity appeared to be most satisfactory (Figure 13). There was an outlier point for sample A4: carbon dose 0.7 g and inhibition 20%. If this point is discounted, then the five doses in the range of 0.1 and 1.5 g carbon have a linear relationship with the residual phytotoxicity. Through linear extrapolation, approximately 1.7 g carbon were required to remove the phytotoxicity of sample A4 completely. There appeared to be a bi-phasic change at 0.04 g activated carbon. The removal of phytotoxicity per unit of

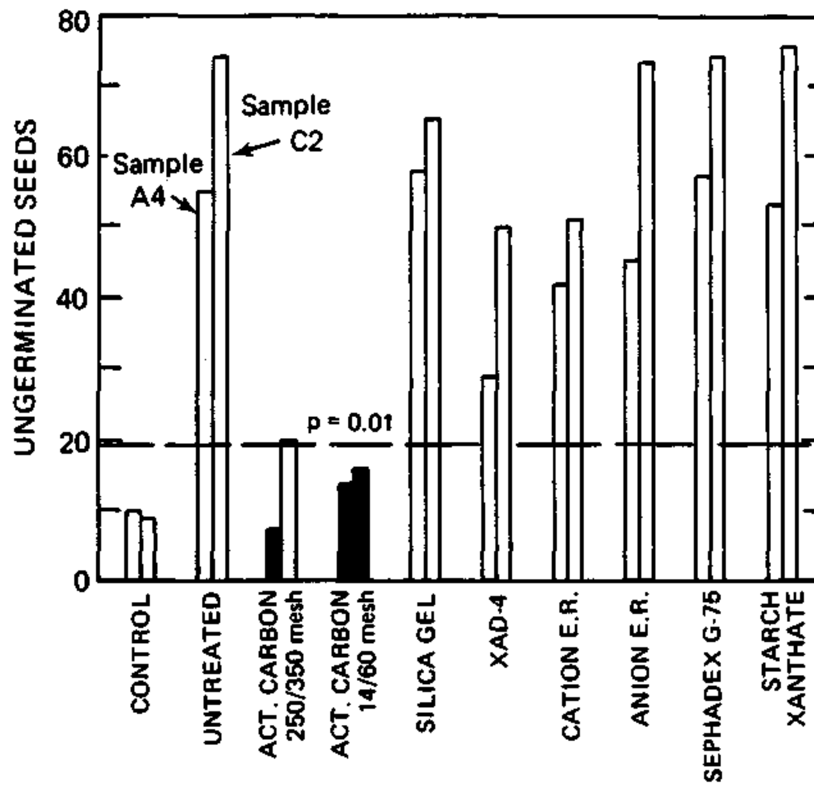

Figure 12. Results of treating 50% diluted effluent samples A4 and C2 with various substrates

Table 13. Inhibitory Effect of 50% Diluted Effluent Samples on Seed Germination after Treatment with 20 g/L Substrates

(All expressed in percent inhibition)

	Net inhibition†		Mean inhibition*
	Sample A4	Sample C2	
Untreated	69	98	84
Activated carbon (powdered)	0	17	9
(granular)	6	11	9
XAD-4	29	59	44
Cation exchange resin	49	64	57
Anion exchange resin	54	97	76
Silica gel	74	85	80
Sephadex G-75	72	98	85
Insoluble starch xanthate	66	100	83

† Net inhibition = $\left(1 - \frac{\text{number of germinated seeds in test sample}}{\text{number of germinated seeds in control}}\right) \times 100$

* Mean inhibition = mean of net inhibitions of samples A4 and C2

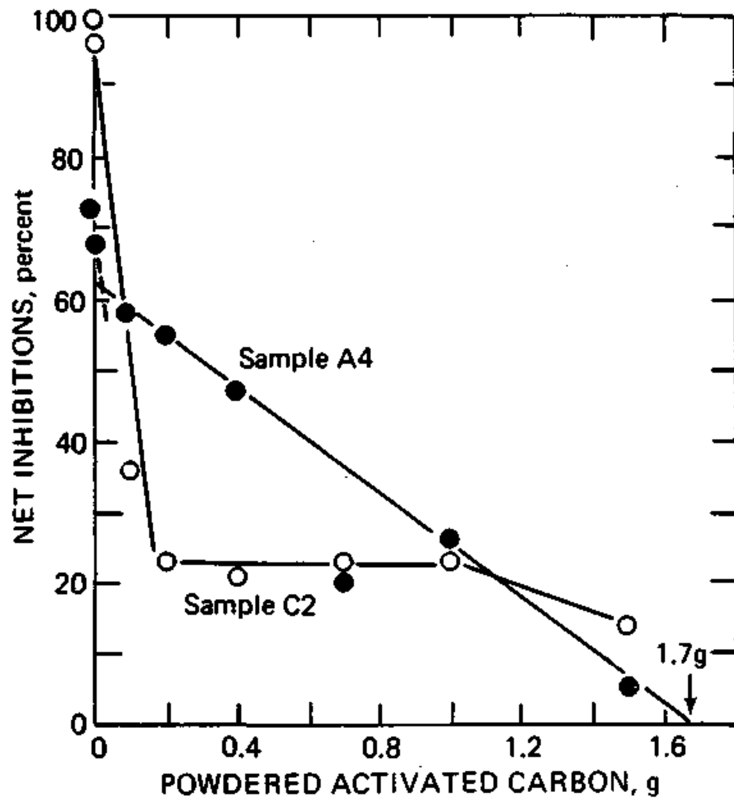

Figure *13. Toxicity test results of 50% diluted effluent samples after treatment with different amounts of powdered activated carbon*

carbon was greater in the dosages of 0.04 g carbon or less than in dosages of 0.04 g or more. There did not appear to be a threshold dosage of the activated carbon for toxicity removal; any amount of activated carbon resulted in a corresponding toxicity removal.

The results of carbon treatment of sample C2 were different (Fig. 13). When carbon dosages ranged from 0.2 to 1.0 g, there was practically no difference in net inhibition (21-23%). At dosages of less than 0.2 g, the removal of phytotoxicity per unit of carbon had a linear relationship with the carbon dosage.

At the lower carbon dosages (0-0.04 and 0-0.2 g for samples A4 and C2, respectively), there was a parallel between samples A4 and C2. The results suggest that the activated carbon was equally effective for toxicity removal of both effluent samples.

The bi-phasic adsorption characteristics shown in Figure 13 are difficult to explain without an in-depth, mechanistic study. In complex effluent samples it is unlikely that a toxicant exists alone. The qualities and quantities of toxicants in these samples might be drastically different. Furthermore, the matrix of constituents may potentiate or attenuate the phytotoxicity considerably. All these and other unknown reasons might have caused the divergent results shown in Figure 13.

e. Adsorption Kinetics. The removal of phytotoxicity by activated carbon (10 g/L) is depicted in Figure 14. The controls for these two tests had five and eight ungerminated seeds, respectively. The results of chi-square analysis showed that every data point in Figure 14 was significantly different from the data points of the controls ($p<0.01$), suggesting the presence of residual phytotoxicity even after 60 minutes of contact time. The phytotoxicity removal of sample A4 reached 77% [(1-20%/87%)X100] in 20 minutes of contact time, whereas that of sample C2 reached 81% [(1-19%/100%)X100] in 2 minutes. The causes for the different rates of phytotoxicity removal can possibly be explained on the basis of effluent characteristics shown in Table 6. Sample A4 contained 73 mg/L grease/oil, whereas sample C2 contained none. The large molecular size of grease/oil in sample A4 is likely to be adsorbed relatively slowly and to hinder the adsorption capacity of activated carbon (Martin and Ng, 1985).

The results given in Figures 13 and 14 resemble each other. The removal of phytotoxicity in sample C2 had a noticeable plateau at which increasing the carbon dosage as well as the contact time had no effect on phytotoxicity removal. In sample A4, the removal was gradual in terms of both the carbon dosage and contact time.

f. Adsorption Breakthrough. Samples A4 and C2 were each eluted through granular activated carbon and XAD-4 columns. Eight fractions (30 mL each) were collected. The blackened areas in Figure 15 show the fractions that were not significantly different ($p<0.01$) from the controls, suggesting that they were not phytotoxic.

The results in Figure 15 show that the first fractions of the four tests contained insignificant phytotoxicity. With only one exception, every fraction afterward contained significant amounts of phytotoxicity. After eight fractions were collected by elution from sample A4, the mean

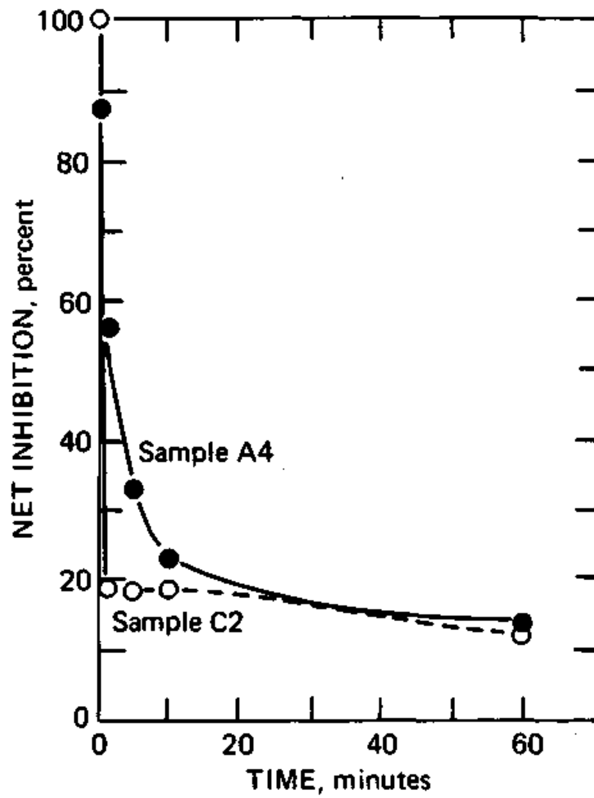

Figure 14. Toxicity test results of 50% diluted effluent samples after treatment with powdered activated carbon (10 g/L) for different amounts of time

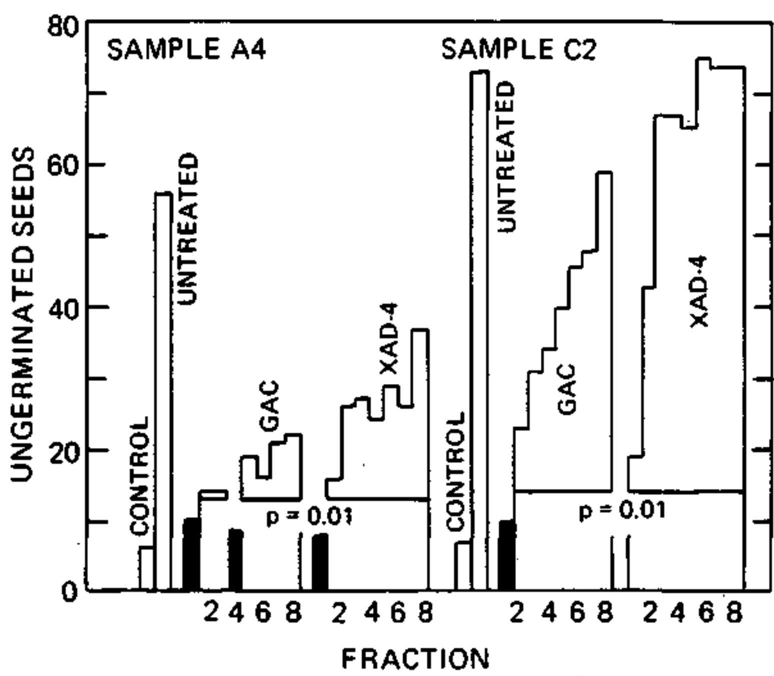

Figure 15. Adsorption breakthrough of 50% diluted effluent samples A4 and C2, using granular activated carbon (GAC) and Amberlite XAD-4 columns, 2.2x10 cm

phytotoxicity removals were calculated to be 57 and 46% for activated carbon and XAD-4 columns, respectively. The mean phytotoxicity removals for sample C4 were 54 and 18% for activated carbon and XAD-4 columns, respectively.

V. DISCUSSION

Phytotoxicity is a measured phenomenon, but not a property of a sample. During toxicity testing, various biological indicators can be used to denote the presence of toxicity, such as mortality, growth inhibition, or germination inhibition. In a single compound toxicity test, the cause-effect relationship is clear-cut, while in complex effluents, toxicity can be affected by such factors as inhibitor(s), interaction of inhibitors, matrix of constituents, speciation, temperature, and pH (Wang, 1987c). In this study, phytotoxicity was used as a general term to denote the presence of adversity in a test sample in comparison with the water control.

The important criteria for selecting biomonitoring tests for complex effluents are speed, sensitivity, and cost-effectiveness. In general, daphnid and fathead minnow mortality tests fit these criteria, and the results are generally accepted for regulatory purposes for the freshwater environment (Peltier and Weber, 1985). These and other faunal tests are not appropriate, however, for testing effluent samples containing herbicides, herbicidal active compounds, or algicides. Gersich and Mayes (1986), for example, reported that 2-(2,4,5-trichlorophenoxy) propionic acid (silvex) was practically nontoxic to daphnids (LC50>140 mg/L).

On the basis of this information, the requirements established for the National Pollutant Discharge Elimination System (NPDES) can either be rather lenient or else not needed at all. The problem with this conclusion is that silvex is a selective, phenoxy herbicide (Ashton and Crafts, 1981). The toxicity of herbicides to plants is typically a thousandfold greater than to animals (Bishop and Perry, 1981). Silvex, although nontoxic to daphnids, certainly will injure or kill plants. When it reaches non-target areas, the herbicide will create unacceptable environmental risks to floral species.

The literature abounds with reports assessing environmental effects of herbicides on faunal species (Presing and Ponyi, 1986; Gersich, Hopkins, and Milazzo, 1985; Naqvi, Davis, and Hawkins, 1985; Call et al. 1984; Spencer, 1984; Berry, 1984). Currently, state and federal regulatory agencies appear to accept results of this sort as the NPDES requirements (Gersich and Mayes, 1986). However, it is not ideal to test herbicides on faunal species, just as it is not ideal to test insecticides on plants.

Higher plants as toxicity test species are relatively underdeveloped and infrequently used in ecotoxicity tests. Previous reports indicated that common duckweed and other aquatic macrophytes are less sensitive to chemicals than are faunal species (Bishop and Perry, 1981; Kenaga and Moolenaar, 1979). It was then concluded that fish and daphnids could be used as surrogates for plant species. This conclusion was later disputed (Wang, 1984).

Algal species have been well developed and widely tested. There is a common perception that algal species can be used as surrogates for aquatic higher plants. Until recently, little information has existed to support or refute this perception. Thomas et al. (1986) reported that some water samples were inhibitory to lettuce seed germination/root elongation, whereas the same samples were stimulatory to *Selenastrum capricornutum*. The authors did not offer any explanation for the discrepancy. Regardless of the cause(s), it is obvious that higher and lower plant organisms may not always

respond alike. In this study, the stated objective was to develop higher plants as toxicity test organisms for aquatic environmental monitoring. No attempt was made to compare these tests with algal tests at this time. Comparative studies are required to relate algal test results to higher plant test results.

There are perhaps two reasons why phototoxicity tests are not widely used: 1) they are difficult to conduct, and 2) they are not generally accepted because researchers are not familiar with them.

Phytotoxicity tests as presented here and elsewhere (Ratsch and Johndro, 1986; Wong and Bradshaw, 1982) are simple, rapid, and cost-effective. Both duckweed tests and seed germination tests, furthermore, are sensitive and capable of detecting effluent toxicity. For example, the eight effluent samples discussed previously nearly all met state water quality criteria (State of Illinois, 1988). However, the duckweed mortality in samples A1, A2, A3, A4, C1, and C2 was nearly total in 48 h. The seed germination test also showed that the above-mentioned effluent samples were significantly different from the water controls.

The toxicants present in source A are speculated to be Ni ion and other unknown constituent(s). The toxicants present in source C are speculated to be Ni ion, biocides in the form of quarternary amines, and others. Wang (1987b) reported that Ni was highly toxic to common duckweed. Nickel ion at 1 mg/L inhibited duckweed growth 70 and 30% in very soft (hardness 40-80 mg/L as $CaCO_3$) and hard (hardness 100-400 mg/L as $CaCO_3$) waters, respectively. Nickel ion was also toxic to other higher plants. The IC50 values of Ni ion obtained by using cucumber, lettuce, and millet root elongation tests were 11, 0.83, and 3 mg/L, respectively (Wang, 1987a). In complex effluents as reported here, the phytotoxicity may be the result of the presence of two or more toxic substances and the resulting toxicant interactions (Marking, 1985). Recently, Wang (1987c) presented a literature review on the factors influencing metal toxicity to aquatic organisms. The results of solution matrix and toxicant interaction in these effluent samples all are likely to affect the phytotoxicity.

The results of this study indicated that phytotoxicity was sporadic in the natural waters. During this year-long survey, only six tests out of 216 were found to contain significant phytotoxicity, $p<0.01$, in the Buck Creek - Little Wabash River region. The same trend of low phytotoxicity was found in the Court Creek Basin, the Illinois River - Peoria Lake region, and Lake Loami. Because the year 1987 was exceptionally dry, the minimal runoff during the planting season could explain the low phytotoxicity in the lake and river waters.

The phytotoxicity tests of the well waters were not conclusive: some well waters exhibited phytotoxicity, although the toxicity was not consistent. Ground water contamination is currently an important environmental concern because of human health issues. The presence of phytotoxicity in ground water suggested possible contamination, as either a natural or a man-made phenomenon. There are two important points to be emphasized. First, the results as shown here are from a preliminary study. A more thorough study is recommended for an in-depth assessment of ground-water contamination. Second, phytotoxicity can be used as an indicator of environmental contamination, especially herbicidal

contamination. The results, however, should not be applied to human health issues.

An important application of phytotoxicity tests is to monitor industrial wastewaters as a part of NPDES permit requirements. Phytotoxicity tests are especially useful for wastewaters containing herbicides, herbicidal active compounds, and even general toxicity. Phytotoxicity tests are generally simple, sensitive, and cost-effective. The maintenance cost is minimum. For example, common duckweed can grow indefinitely with water, plant nutrients, and light provided. Dry seeds usually have a long shelf-life. These test species thus are on a stand-by basis; they can be activated at any time. This is an advantage over other test species, which are available only seasonally, or which require high costs of upkeep.

The results in Table 11 show that the IC50 values for the duckweed tests were smaller than those for the root growth tests. Duckweed plants were so sensitive to samples C1 and C2 that the IC50 values were less than 1.6% of effluent concentration, while IC50 values using the millet test for these two samples were 55 and 33%, respectively. Samples Bl and B2 were the least toxic among the samples. It can be seen from Table 11 that, for these samples, both the cabbage and millet tests failed to obtain IC50 values and the duckweed test showed relatively high IC50 values, 91 and 43%, respectively.

A major difference between bioassay and chemical analysis for a hazard assessment is that the former relies on the measurement of biological response(s) to the combined effects of all toxicants, while chemical analysis relies on the speciation and quantification of toxicant. These two approaches are both essential for environmental protection. Because a complete chemical analysis is very expensive compared to bioassay, a prudent approach is to perform a series of screening bioassays including daphnids, algae, fathead minnows, and higher plants. If a sample exhibits strong toxicity, then chemical analysis can be employed to identify and quantify toxic substance(s).

Several studies have combined fractionation schemes with toxicity tests (Parkhurst, Gehrs, and Rubin, 1979; Samoiloff et al., 1983; Walsh and Garnas, 1983; Doi and Grothe, 1987). The first step is usually to separate liquid wastes into organic and inorganic fractions. Walsh and Garnas (1983) employed various approaches including filtration, XAD-4 resin elution, cation and anion exchange resin, and solvent extraction with pH adjustments for acid, base, and neutral fractionation. Doi and Grothe (1987) used activated carbon, silica gel, and cation and anion exchange resin in a general fractionation scheme. They used the sequential approach of activated carbon followed by silica gel to remove less and more polar organic compounds, respectively. The trend of environmental toxicology is to combine bioassay and chemical analysis to help protect the environment.

VI. CONCLUSION

Higher plant tests are relatively underdeveloped and seldom used. Phytotoxicity tests using higher plants are simple, sensitive, and cost-effective. In this study, two phytotoxicity tests were employed, common duckweed growth/mortality and seed germination/root elongation. Both natural waters and industrial wastewaters were tested.

The root growth test using millet, oat, and wheat was used to detect phytotoxicity in lake and river waters. Only sporadic test results showed significant phytotoxicity. For example, six of 216 tests showed significant phytotoxicity in the Buck Creek-Little Wabash River region. The phytotoxicity in the other regions was also low, perhaps because of lower-than-average rainfall in 1987.

The phytotoxicity in ground water was tested with the duckweed test and cabbage and millet tests. Among these three tests, the duckweed test appeared to be the most sensitive in detecting phytotoxicity. The phytotoxicity test results in well water suggested possible contamination of the ground-water resource.

Eight industrial wastewater samples obtained from three industries were tested. Source A is an industrial wastewater pretreatment plant, and the waste waters contained substantial phytotoxicity. Duckweed mortality and reproduction tests showed that the effects were nearly total. Among six plant species, cabbage, cucumber, and millet were more sensitive to the effluent toxicity than Japanese millet, rice, and wheat. The IC50 values using millet root elongation ranged from 48-87% effluent concentration (four samples), while that using the duckweed reproduction test ranged from 22-49%. Source B is an agricultural product utilization plant, and duckweed tests indicated that there was no significant phytotoxicity from this source. Source C is a specialty chemical industry where wastewaters were extremely toxic. At the 22% effluent concentration or greater, all duckweed plants died. At 13% and less, some plants reproduced but died thereafter. The inhibition on cabbage and millet seed germination was also total. The IC50 values using millet root elongation ranged from 33-55%, while that using the duckweed reproduction test was <1.6%.

The treatability study of sample A4 showed that phytotoxicity in this sample was effectively removed by using either granular or powdered activated carbon. Other resins were not effective. It is suggested that activated carbon be incorporated in the pretreatment processes to further remove effluent toxicity. Sample C2 was far more toxic and required other advanced treatment technologies (or combination of technologies) for toxicity removal.

REFERENCES

American Society for Testing and Materials. 1987. Standard practice for conducting static acute toxicity tests with larvae of four species of bivalve molluscs. Annual Book of ASTM Standard, Vol. 11.04, E724-80, Philadelphia, PA.

Ashton, F.M., and A.S. Crafts. 1981. Mode of action of herbicides. John Wiley, N.Y.

Behera, B.K., and B.N. Misra. 1982. Analysis of the effect of industrial effluent on growth and development of rice seedlings. Environ. Res. 28: 10-20.

Behera, B.K., and B.N. Misra. 1983. Analysis of the effect of industrial effluent on pigments, proteins, nucleic acids, and the 2,6-dichlorophenol indophenol Hill reaction of rice seedlings. Environ. Res. 31: 381-389.

Berry, C.R. 1984. Toxicity of the herbicides diquat and endothall to gold fish. Environ. Poll. (Ser. A), 34: 251-258.

Bishop, W.E., and R.L. Perry. 1981. Development and evaluation of a flow-through growth inhibition test with duckweed (Lemna minor). Aquatic Toxicology and Hazard Assessment ASTM STP 737, Amer. Soc. Testing & Materials, Philadelphia, PA.

Butts, T.A., J.W. Williams, and R.L. Evans. Undated. Water quality evaluation of the Rector Creek - North Fork Saline River drainage basin. Illinois State Water Survey Contract Report Prepared for Soil Conservation Service, U.S. Dept. Agriculture, Champaign, IL.

Call, D.J., L.T. Brooke, R.J. Kent, S.H. Poirier, and M.L. Knuth. 1984. Toxicity, uptake, and elimination of the herbicides Alachlor and Dinoseb in freshwater fish. J. Environ. Qual. 13: 493-498.

deNoyelles, F., and W.D. Kettle. 1983. Site studies to determine the extent and potential impact of herbicide contamination in Kansas waters. Natl. Tech. Inform. Service. PB84-140631, Springfield, VA. 37 p.

deNoyelles, F., W.D. Kettle, and D.E. Sinn. 1982. The responses of plankton communities in experimental ponds to atrazine, the most heavily used pesticide in the United States. Ecol. 63: 1285-1293.

Doi, J., and D.R. Grothe. 1987. Use of fractionation/chemical analysis schemes for plant effluent toxicity evaluation. 11th ASTM Symposium Aquatic Toxicology and Hazard Assessment, May 11, 1987, Cincinnati, OH.

Evans, L.S., N.F. Gmur, and D. Mancini. 1982. Effects of simulated acidic rain on yields of Raphanus sativus, Lactuca sativa, Triticum aestivum and Medicado sativa. Environ. Expt. Bot. 22: 445-453.

Fletcher, J.S., M.J. Muhitch, D.R. Vann, J.C. McFarlane, and F.S. Benanati 1985. PHYTOTOX database evaluation of surrogate plant species recommended by the U.S. Environmental Protection Agency and the

Organization for Economic Cooperation and Development. Environ. Toxicol. Chem. 4: 523-532.

Food and Drug Administration. 1987. Seed germination and root elongation. Environmental Assessment Technical Assistance Document 406. Center for Food Safety and Applied Nutrition, Center for Veterinary Medicine, Washington D.C.

Gersich, F.M., D.L. Hopkins, and D.P. Milazzo. 1985. Acute and chronic toxicity of technical picloram (4-aminp-3,5,6-trichloropicolinic acid) to Daphnia magna Straus. Bull. Environ. Contam. Toxicol. 35: 121-126.

Gersich, F.M., and M.A. Mayes. 1986. Acute toxicity test with Daphnia magna Straus and Pimephales promelas Rafinesque in support of National Pollutant Discharge Elimination permit requirements. Water Res. 20: 939-941.

Glotfelty, D.E., A.W. Taylor, A.R. Isensee, J. Jersey, and S. Glenn. 1984. Atrazine and simazine movement to Wye River estuary. J. Environ. Qual. 13: 115-121.

Gunkel, V.G. 1984. Investigations of the ecotoxicological effects of a herbicide in an aquatic model ecosystem; II. Food chain significance and pesticide balance. Archiv fur hydrobiologie Suppl. 69: 130-168.

Hartman, W.A., and D.B. Martin. 1984. Effect of suspended bentonite clay on the acute toxicity of glyphosate to Daphnia pulex and Lemna minor. Bull. Environ. Contam. Toxicol. 33: 355-361.

Hutchinson, T.C., and H. Czyrska. 1975. Heavy metal toxicity and synergism to floating aquatic weeds. Verh. Internat. Verein. Limnol. 19: 2102-2111.

Irving, P.M. 1985. Modeling the response of greenhouse-grown radish plants to acid rain. Environ. Expt. Bot. 25: 327-338.

Johnston, J.W., and D.S. Shriner. 1985. Responses of three wheat cultivars to simulated acid rain. Environ. Expt. Bot. 25: 348-353.

Junk, G.A., R.F. Spalding, and J.J. Richard. 1980. Areal, vertical, and temporal differences in ground water chemistry. II. Organic constitutents. J. Environ. Qual. 9: 479-483.

Kenaga, E. and J.J. Moolenaar. 1979. Fish and daphnia toxicity as surrogates for aquatic vascular plants and algae. Environ. Sci. Tech. 13: 1479-1480.

King, J.M. and K.S. Coley. 1985. Toxicity of aqueous extracts of natural and synthetic oils to three species of Lemna. Aquatic Toxicology and Hazard Assessment ASTM STP 891, Amer. Soc. Testing & Materials, Philadelphia, PA.

Marking, L.L. 1985. Toxicity of chemical mixtures. IN: Fundamentals of Aquatic Toxicology, ed. by G.M. Rand and S.R. Petrocell; Hemisphere Publ. Corp., Washington, D.C.

Martin, C.D., J.L. Baker, D.C. Erbach, and H.P. Johnson. 1978. Washoff of herbicides applied to corn residues. Trans. Amer. Soc. Agr. Eng. 21: 1164-1168.

Martin, R.J., and W.J. Ng. 1985. Chemical regeneration of exhausted activated carbon-II. Water Res. 19: 1527-1535.

Mayer, A.M., and A. Poljakoff-Mayer. 1982. The germination of seeds, 3d ed. Pergamon Press, Oxford, England.

McCall, E.C., D.D. Lans, and A.D. Parr. 1984. Transport modeling of an agricultural herbicide, a laboratory simulation. Natl. Tech. Inform. Service PB84-140623. 25 p.

Muir, D.C.G., J.Y. Yoo, and B.E. Baker. 1978. Residues of atrazine and N-deethylated atrazine in water from five agricultural watersheds in Quebec. Arch. Environ. Contain. Toxicol. 7: 221-235.

Naqvi, S.M., V.O. Davis, and R.M. Hawkins. 1985. Percent mortalities and LC50 values for selected microcrustaceans exposed to Treflan, Cutrine-plus, and MSMA herbicides. Bull. Environ. Contam. Toxicol. 35: 127-132.

Nasu, Y., and M. Kugimoto. 1981. Lemna (duckweed) as an indicator of water pollution. I. The sensitivity of Lemna pancicostata to heavy metals. Arch. Environ. Contam. Toxicol. 10: 159-169.

Nebeker, A.V., C. Savonen, and D.G. Stevens. 1985. Sensitivity of rainbow trout early life stages to nickel chloride. Environ. Toxicol. Chem. 4: 233-239.

Nielsen, E.G., and L.K. Lee. 1987. The magnitude and costs of ground water contamination from agricultural chemicals. U.S. Dept. of Agriculture, Economic Res. Service, Natural Resource Economics Division. Washington, D.C. 54 p.

Organization for Economic Cooperation and Development. 1984. Terrestrial plants: Growth test. OECD guideline for testing of chemicals, No. 208, Paris.

Parkhurst, B.R., C.W. Gehrs, and I.B. Rubin. 1979. Value of chemical fractionation for identifying the toxic components of complex aqueous effluents. ASTM STP 667, Amer. Soc. Testing and Materials, Philadelphia, PA, 122-130.

Peichl, L., J.P. Lay, and F. Korte. 1985. Effects of atrazine and 2,4-dichlorophenoxyacetic acid to the population density of phyto- and zooplankton in an aquatic outdoor system. Zeitschrift fur Wasser- and Abwasser Forschung 18: 217-222.

Peltier, W.H., and C.I. Weber. eds. 1985. Methods for measuring the acute toxicity of effluents to freshwater and marine organisms, 3d ed. EPA/600/4-85/013, U.S. Environ. Protection Agency, Cincinnati, OH.

Percy, K. 1986. Effects of simulated acid rain on germination capacity,

growth and morphology of forest tree seedlings. New Phytol. 104: 473-484.

Perez, J.D., E. Esteban, M. Gomez, and F. Gallardo-Lara. 1986. Effects of wastewater from olive processing on seed germination and early plant growth of different vegetable species. Jour. Environ. Sci. Health B21: 349-357.

Presing, M., and J.E. Ponyi. 1986. Studies on the acute and chronic effect of a 2,4-D- containing herbicide (Dikonirt) on Eudiaptomus gracilis (G.O. Sars) (Crustacea, copepoda). Arch. fur Hydrobiologie 106: 275-286.

Ratsch, H.C. 1983. Interlaboratory root elongation testing of toxic substances on selected plant species. U.S. Environ. Protection Agency EPA-600/S3-83-051.

Ratsch, H.C, and D. Johndro. 1986. Comparative toxicity of six test chemicals to lettuce using two root elongation test methods. Environ. Monit. Assessment 6: 267-276.

Richard, J.J., G.A. Junk, M.J. Avery, N.L. Nehring, J.S. Fritz, and H.J. Svec. 1975. Analysis of various Iowa waters for selected pesticides: atrazine, DDe, and dieldrin -- 1974. Pest. Monit. J. 9: 117-123.

Samoiloff, M.R., J. Bell, D.A. Birkholz, G.R. Webster, E.G. Arnott, R. Pulak and Madrid, A. 1983. Combined bioassay-chemical fractionation scheme for the determination and ranking of toxic chemicals in sediments. Environ. Sci. Tech. 17: 329-334.

Scheindorf, C, M. Rebhun, and M. Scheintuch. 1982. Organic pollutants adsorption from multicomponent systems modeled by Freundlich type isotherm. Water Res. 16: 357-362.

Spencer, D.F. 1984. Oxygen consumption by the crayfish Orconectes propinguus (Girard) exposed to aquashade. Bull. Environ. Contain. Toxicol. 33: 373-378.

Srivastava, N., and R. Sahai. 1987. Effects of distillery waste on performance of Cicer arietinum L. Environ. Poll. 43: 91-102.

Standard methods for the examination of water and wastewater. 1985. American Public Health Association, American Water Works Association, and Water Pollution Control Federation, Washington, D.C. 1268 p.

State of Illinois. 1988. Rules and regulations. Title 35: Environmental Protection, Subtitle C: Water pollution, Chapter 1: Pollution Control Board. Springfield, IL.

Thomas, J.M., and J.R. Cline. 1985. Modification of the Neubauer technique to assess toxicity of hazardous chemicals in soils. Environ. Toxicol. Chem. 4: 201-207.

Thomas, J.M., J.R. Skalski, J.F. Cline, M.C. McShane, J.C. Simpson, W.E.

Miller, S.A. Peterson, C.A. Callahan, and J.C. Greens. 1986. Characterization of chemical waste site contamination and determination of its extent using bioassays. Environ. Toxicol. Chem. 5: 487-501.

Tibbitts, T.W., S. Will-Wolf, D.F. Karnowsky, and D.M. Olszyk. 1982. Impact of air pollution on vegetation near the Columbia Generating Station: Wisconsin Power Plant Impact Study. Natl. Tech. Infor. Service PB82-258591, Springfield, VA.

U.S. Environmental Protection Agency. 1980 Treatability manual, III, Technologies for control/removal of pollutants. EPA-600/8-80-042, Washington, D.C.

U.S. Environmental Protection Agency. 1982. Seed germination/root elongation toxicity tests. EG-12, Office of Toxic Substances, Washington, D.C.

Walsh, G.E. and R.L. Garnas. 1983. Determination of bioactivity of chemical fractions of liquid wastes using freshwater and saltwater algae and crustaceans. Environ. Sci. Tech. 17: 180-182.

Wang, W. 1984. Editorial: Uses of aquatic plants for ecotoxicology. Environ. Intl. 10: i-iii.

Wang, W. 1985a. The use of plant seeds in toxicity tests of phenolic compounds. Environ. Internat. 11: 49-55.

Wang, W. 1985b. Use of millet root elongation for toxicity tests of phenolic compounds. Environ. Internat. 11: 95-98.

Wang, W. 1986a. Toxicity tests of aquatic pollutants by using common duckweed. Environ. Poll. (Ser. B) 11: 1-14.

Wang, W. 1986b. The effect of river water on phytotoxicity of Ba, Cd, and Cr. Environ. Poll. (Ser. B) 11: 193-204.

Wang, W. 1986c. Site-specific phytotoxicity of heavy metals. Illinois State Water Survey Contract Report 403 to the U.S. Environ. Protection Agency. 85 p.

Wang, W. 1986d. Comparative toxicology of phenolic compounds using root elongation method. Environ. Toxicol. Chem. 5: 891-896.

Wang, W. 1987a. Root elongation method for toxicity testing of organic and inorganic pollutants. Environ. Toxicol. Chem. 6: 409-414.

Wang, W. 1987b. Toxicity of nickel to common duckweed (Lemna minor). Environ. Toxicol. Chem. 6: 961-967.

Wang, W. 1987c. Factors affecting metal toxicity to (and accumulation by) aquatic organisms - Overview. Environ. Intl. In press.

Wehtje, G.R., R.F. Spalding, O.C. Burnside, S.R. Lowry, and R.C. Leavitt. 1983. Biological significance and fate of atrazine under aquifer conditions. Weed Sci. 31: 610-618.

Wilson, M.P., E.P. Savage, D.D. Adrian, M.J. Aaronson, T.J. Keefe, D.H. Hamar, and J.T. Tessari. 1987. Ground water transport of the herbicide, atrazine, Weld County, Colorado. Bull. Environ. Contain. Toxicol. 39: 807-814.

Wolf, D.C., and R.L. Jackson. 1982. Atrazine degradation, sorption, and bioconcentration in water systems. Natl. Tech. Inform. Service PB83-150151. 45 p.

Wolfenden, J., and H.R. Wellburn. 1986. Cellular readjustment of barley seedlings to simulated acid rain. New Phytol. 104: 97-109.

Wong, M.H., and A.D. Bradshaw. 1982. A comparison of the toxicity of heavy metals, using root elongation of rye grass, Lolium perenne. New Phytol. 91: 255-261.

Wu, T.L., D.L. Correll, and A.E.H. Remenapp. 1983. Herbicide runoff from experimental watersheds. J. Environ. Qual. 12: 330-336.

Wu, T.L., L. Lambert, D. Hastings, and D. Banning. 1980. Enrichment of the agricultural herbicide atrazine in the microsurface water of an estuary. Bull. Environ. Contam. Toxicol. 24: 411-412.

Appendix: Quality of Surface Water Samples

Buck Creek #1

Date	Turb. NTU	pH	Alk. mg/1*	Hard. mg/1*	Chloride mg/1
1-5-87	78	7.72	81	214	103
2-19	18	7.74	109	236	132
3-2	224	7.35	56	104	21
3-16	106	7.98	198	357	35
4-6	12	8.10	130	-	158
4-13	75	7.65	131	-	162
4-20	62	7.61	95	-	86
5-4	34	7.40	151	276	202
5-18	27	7.69	165	234	177
6-2	374	7.46	34	56	21
6-15	50	8.02	76	180	39
7-6	208	7.28	34	58	15
7-20	70	7.35	73	120	45
8-3	52	7.40	81	110	52
8-17	15	7.42	102	220	157
9-8	13	7.81	120	238	149
9-21	35	7.88	131	198	135
10-5	97	7.59	125	179	118
10-19	154	7.11	144	189	107
11-2	42	7.22	182	209	101
11-16		Insufficient amount of sample			
12-7	53	7.29	66	153	107
12-21	237	6.81	28	63	18

* as $CaCO_3$

Appendix. (Cont'd.)

Buck Creek #2

Date	Turb. NTU	pH	Alk. mg/1*	Hard. mg/1*	Chloride mg/1
1-5-87	60	7.74	85	149	103
2-19	15	7.85	101	159	64
3-2	191	7.50	56	101	19
3-16	127	7.42	95	158	79
4-6	16	7.99	115	-	125
4-13	344	7.28	65	-	71
4-20	35	7.71	92	-	64
5-4	17	8.05	139	188	141
5-18	1060	7.70	53	83	80
6-2	217	7.30	38	53	13
6-15	48	8.05	106	94	18
7-6	188	7.13	33	39	8
7-20	31	7.52	87	108	19
8-3	30	7.42	75	74	11
8-17	17	7.47	131	124	10
9-8	19	7.77	142	136	14
9-21	30	7.90	134	112	13
10-5	56	7.61	115	108	12
10-19	22	7.52	94	106	8
11-2	45	7.65	100	115	9
11-16	100	7.74	84	112	8
12-7	40	7.41	77	128	21
12-21	210	6.90	29	62	16

* as $CaCO_3$

Appendix. (Cont'd.)

Little Wabash River

Date	Turb. NTU	pH	Alk. mg/1*	Hard. mg/1*	Chloride mg/1
1-5-87	19	8.10	214	316	36
2-19	14	8.05	223	304	35
3-2	485	7.50	87	149	25
3-16	45	7.62	117	289	178
4-6	23	8.25	257	-	46
4-13	45	8.07	246	-	47
4-20	38	8.01	182	-	32
5-4	131	7.71	181	303	28
5-18	130	7.92	208	254	32
6-2	308	7.48	55	83	15
6-15	60	8.20	208	220	28
7-6	206	7.45	78	114	14
7-20	86	8.03	161	185	21
8-3	70	7.63	100	105	12
8-17	53	8.05	199	208	28
9-8	39	8.00	224	226	35
9-21	35	8.11	223	228	54
10-5	37	7.91	210	215	81
10-19	25	7.69	168	195	76
11-2	19	7.74	187	199	27
11-16	24	7.89	201	218	63
12-7	33	7.70	131	192	35
12-21	203	7.09	44	73	9

* as $CaCO_3$

Appendix. (Cont'd.)

Court Creek C2

Date		Turb. NTU	pH	Alk. mg/1*	Hard. mg/1*	Chloride mg/1
1-5-87		14	8.11	218	344	15
1-16		11	8.05	227	332	18
2-2		42	8.14	224	297	22
2-16		22	8.15	160	333	15
3-2		22	8.19	254	331	32
3-16		28	8.08	249	327	22
3-30		89	8.08	229	-	21
4-13		32	7.92	247	295	26
4-20		22	8.10	251	221	24
5-4		20	7.73	249	340	24
5-18		12	8.10	265	335	25
5-20		173	8.01	246	306	22
6-1		89	8.13	276	348	26
6-2	(set 1)	6264	7.80	123	152	9
6-2	(set 2)	1720	7.90	182	216	19
6-15		28	8.10	256	306	26
7-6		26	8.00	254	324	22
7-20		33	8.36	242	345	15
8-3		33	8.11	222	318	13
8-17		160	8.00	166	370	24
9-8		38	8.31	254	320	25
9-21		45	8.22	243	300	23
10-5		21	8.12	276	353	21
10-19		25	7.89	295	337	59
10-26		25	8.04	211	340	20
11-2		70	8.00	215	298	27
11-16		17	8.35	171	314	12
12-7		34	8.35	256	360	25
12-21		44	7.82	171	270	38

* as $CaC0_3$

Appendix. (Cont'd.)

Court Creek S2

Date		Turb. NTU	pH	Alk. mg/1*	Hard. mg/1*	Chloride mg/1
1-5-87		20	8.11	180	320	7
1-16		9	8.12	178	273	8
2-2		23	8.12	186	315	8
2-16		16	8.12	192	327	9
3-2		18	8.00	229	306	17
3-16		63	8.08	216	300	11
3-30		47	7.98	208	-	14
4-13		102	8.00	221	295	14
4-20		27	8.21	265	294	35
5-4		25	8.31	235	312	13
5-18		14	8.07	225	348	11
5-20		238	7.74	172	276	8
6-1		13	8.00	214	345	11
6-2	(set 1)	15	8.04	180	299	9
6-2	(set 2)	13	8.02	219	312	10
6-15		20	8.10	216	353	11
7-6		55	7.90	210	361	12
7-20		25	8.40	190	354	9
8-3		21	8.22	198	335	8
8-17		220	8.49	177	387	22
9-8		15	8.21	206	334	12
9-21		34	8.19	213	327	11
10-5		20	8.17	214	358	11
10-19		30	8.21	318	271	110
10-26		24	8.11	167	323	11
11-2		18	8.29	173	321	10
11-16		13	8.39	161	316	9
12-7		48	8.18	200	323	13
12-21		15	8.00	168	297	12

* as $CaC0_3$

Appendix. (Cont'd.)

Court Creek S3

Date		Turb. NTU	pH	Alk. mg/1*	Hard. mg/1*	Chloride mg/1
1-5-87		3	8.14	159	315	7
1-16		6	8.15	170	324	6
2-2		2	8.10	175	323	8
2-16		7	8.12	171	320	6
3-2		25	7.70	362	445	4
3-16		38	7.96	152	282	5
4-13		13	8.17	172	309	10
4-20		8	8.22	174	327	8
5-4		8	8.36	183	331	10
5-18		6	8.28	184	338	8
5-20		11	8.10	172	329	10
6-1		12	8.37	279	332	10
6-2	(set 1)	9	8.48	178	324	9
6-2	(set 2)	7	8.10	173	326	9
6-15		16	8.29	172	326	13
7-6		17	8.29	170	333	11
7-20		14	7.88	230	377	9
8-3		15	8.34	157	301	9
8-17		11	8.38	152	522	9
9-8		12	8.28	154	306	9
9-21		10	8.34	158	302	9
10-5		15	8.30	164	312	10
10-19		13	8.20	154	313	14
10-26		6	8.19	327	317	12
11-2		8	3.13	159	311	9
11-16		7	8.41	165	322	9
12-7		27	8.02	122	239	7
12-21		7	8.20	164	321	11

* as $CaC0_3$

Appendix. (Cont'd.)

Sugar Creek Tributaries

	Date	Turb. NTU	pH	Alk. mg/1*	Hard. mg/1*	Chloride mg/1
ST-i	1-16-87	26	8.05	299	555	10
	4-13	19	8.25	267	364	13
	5-20	8	7.90	237	492	15
ST-2	1-16-87	11	8.05	283	349	21
	5-20	29	7.88	242	284	16

* as $CaC0_3$

Appendix. (Cont'd.)

Peoria Lake PL-1

Date	Turb. NTU	pH	Alk. mg/1*	Hard. mg/1*	Chloride mg/1
1-5-87	114	8.22	250	347	53
3-2	112	8.45	223	321	64
3-15	96	8.42	214	312	68
3-9	137	8.42	219	-	73
3-30	113	8.20	220	-	79
4-20	105	8.32	211	-	71
5-4	88	8.62	220	311	57
5-18	135	8.74	214	296	68
6-1	113	8.49	178	253	35
6-15	59	8.47	199	278	55
7-6	102	8.88	174	239	54
7-20	133	8.72	178	243	58
8-3	66	8.71	170	218	57
8-16	125	8.90	166	226	59
8-31	79	8.31	155	202	43
9-8	110	8.15	200	258	48
9-21	73	8.35	184	245	52
10-5	340	8.60	200	259	53
10-19	62	8.52	190	271	61
11-2	130	8.40	184	262	66
11-16	128	8.31	202	282	62
12-7	126	8.22	205	299	57
12-23	59	8.09	198	314	52

* as $CaC0_3$

Appendix. (Cont'd.)

Peoria Lake - PL-2

Date	Turb. NTU	pH	Alk. mg/1*	Hard. mg/1*	Chloride mg/1
1-5-87	53	8.29	264	354	51
3-2	101	8.49	224	325	63
3-15	80	8.45	220	308	60
3-9	107	8.59	219	-	73
3-30	109	8.21	224	-	73
4-20	100	8.32	217	-	70
5-4	89	8.68	220	311	57
5-18	107	8.80	216	297	69
6-1	88	8.43	176	255	36
6-15	47	8.55	197	273	43
7-6	89	8.92	174	340	52
7-20	129	8.74	180	243	58
8-3	103	8.72	166	234	56
8-16	123	8.82	168	222	59
8-31	90	8.19	149	199	40
9-8	12	8.28	154	306	9
9-21	78	8.51	190	252	54
10-5	113	8.80	208	265	55
10-19	75	8.57	191	271	60
11-2	133	8.61	185	265	67
11-16	119	8.31	197	275	60
12-7	83	8.22	205	303	56
12-23	42	8.01	195	309	48

* as $CaC0_3$

Appendix. (Cont'd.)

Peoria Lake - PL-C

Date	Turb. NTU	pH	Alk. mg/1*	Hard. mg/1*	Chloride mg/1
1-5-87	104	8.15	243	347	55
3-2	97	8.45	226	328	64
3-9	121	8.35	220	-	73
3-30	106	8.20	223	-	79
4-20	97	8.31	214	-	70
5-4	82	8.62	220	311	57
5-18	88	8.60	216	302	71
6-1	88	8.48	182	262	39
6-15	80	8.54	195	278	46
7-6	131	8.71	172	237	54
7-20	117	8.71	178	241	58
8-3	70	8.83	180	220	58
8-16	114	8.58	165	221	59
8-31	93	8.21	148	194	40
9-8	84	8.22	201	261	49
9-21	77	8.30	181	238	52
10-5	202	8.68	201	261	53
10-19	67	8.51	190	271	61
11-2	131	8.31	183	267	68
11-16	107	8.35	184	276	60
12-7	77	8.22	216	303	56
12-23	62	8.05	199	316	53

* as $CaC0_3$

Appendix. (Concluded)

Lake Loami

Date	Turb. NTU	pH	Alk. mgl*	Hard. mg/1*	Chloride mg/1	
2-19-87	-	8.18	176	227	14	
3-25	18	8.18	175	222	13	
4-22	17	8.30	171	202	16	Not tested
5-6		8.55	167	203		
5-20	6	9.30	98	143	15	
6-3	7	9.51	102	146	16	
7-1	17	8.69	108	126	13	Not tested
7-14	10	8.80	119	143	14	
7-28	8	9.42	110	137	16	
8-11	10	9.41	111	132	16	
8-25	13	8.00	124	143	18	Not tested
9-8	8	8.54	130	152	18	
9-23	16	8.87	138	159	21	
10-13	10	8.92	141	175	21	
11-10	15	9.18	136	165	19	
12-7	13	8.42	132	178		

* as $CaC0_3$