COMPARATIVE SEED GERMINATION TESTS USING TEN PLANT SPECIES FOR TOXICITY ASSESSMENT OF A METAL ENGRAVING EFFLUENT SAMPLE

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Abstract. An effluent sample was collected from the acid bath of a metal engraving plant where the sample had been pretreated with lime to pH 10.6. The sample was adjusted to pH 7.37 (and in a second test to 7.25) and diluted to a series of solutions using hard, standard water. The seed germination method was employed to test the phytotoxicity of the sample to ten plant species. The experimental results of this study compared to previous studies indicated that cabbage, carrot, Japanese millet, oat, and wheat lost some viability during storage. The germination rates of these species fell below 85% in this study, although the rates of cabbage, Japanese millet, and wheat were above 85% in the previous studies. Of the remaining species with adequate germination rates, cucumber and white proso millet were relatively insensitive to the effluent toxicity. The promising candidates for toxicity testing were rice, lettuce, and tomato, based on long shelf life, high germination rate and sensitivity to toxicity.

1. Introduction

Many plant species have been recommended for ecotoxicity tests using seed germination and root elongation methods. Among them cabbage, lettuce, and oats are recommended by the U.S. Environmental Protection Agency (EPA)(1982), the Food and Drug Administration (FDA)(1987), and the Organization for Economic Cooperation and Development (OECD)(1984). Carrot, cucumber, and tomato are also suggested by the EPA and FDA, wheat is accepted by the FDA and OECD, and rice is also mentioned by the OECD. Several other plant species are recommended in each agency document. Although not mentioned in any of these documents, millet has been studied at the Illinois State Water Survey for several years (Wang, 1985a, b, 1986, 1987; Wang and Williams, 1988).

Ratsch (1983) reported results of a round-robin study using cucumber, lettuce, radish, red clover, and wheat. These species were selected to represent important agricultural crops in terms of family size, distribution, and abundance. Ratsch concluded that inhibition of root elongation was a valid and sensitive indicator of environmental toxicity. Miller *et al.* (1985) and Thomas *et al.* (1986) used root elongation tests of the same five species as part of a test battery for hazard assessment of toxic waste sites. They reported that the seed plants were not as sensitive to

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heavy metals and insecticides as algae and daphnids were. The seed plants, however, were far more sensitive to herbicides than algae and daphnids were. More interestingly, the root elongation tests detected toxicity in some well water samples from the hazardous sites, whereas algal and daphnid tests results showed either no effect or stimulation. The results indicated that these plants are valuable as a part of test battery for ecotoxicity testing.

The phytotoxicity tests have several advantages. First, many dry plant seeds remain viable on the shelf for a long time. They require negligible maintenance costs, yet they can be activated at any moment, giving the test permanent stand-by status. This is an immense advantage over many other tests in which specimens are costly to maintain and in some cases are available only seasonally. Second, the test is simple and inexpensive, and does not require major equipment. Third, the test requires only a small volume of test sample, ca. 25 mL, so that exposure to and disposal of the hazardous sample is minimized. The phytotoxicity tests can be employed using either seed germination or root elongation as the test end points.

In a recent study, Wang and Williams (1988) reported that among six species, cabbage and millet were the most sensitive to toxicity of industrial effluents from three sources: the heavy machinery, agricultural product utilization, and specialty chemical industries. In another study, two effluent samples from a metal engraving plant were tested for toxicity, and experiments were conducted to reduce toxicity in the sample using varieties of treatment (Wang, 1990).

In this study, another effluent sample from the same engraving plant was evaluated by means of seed germination tests. Ten plant species were compared: cabbage, carrot, cucumber, lettuce, Japanese millet, white proso millet, oat, rice, tomato, and wheat. The objective was to find plant species suitable for testing effluent toxicity, on the basis of high germination rate, long shelf life, and high sensitivity to toxicity.

2. Methods

Effluent sample

The spent liquor from an acid bath of a metal engraving plant is discharged into the sewer system of the Greater Peoria Sanitary District. The Sanitary District required the plant to pretreat the waste water to meet federal pretreatment standards (Federal Register, 1983). The plant initiated lime treatment in July 1987 to raise the pH to 9 or above.

A 5 L grab sample of the spent liquor was obtained on August 29, 1988, and was kept at 4 °C until use. Detailed chemical analyses were made as required by the national Pollutant Discharge Elimination System permit requirement. The analyses were performed by personnel of an accredited consulting laboratory.

Seed germination tests

The seeds of ten plant species were purchased and used in this study. Their cultivars

TABLE I

Seed germination of test species in water control, all expressed in percent

	Cultivar	Guaranteed	Previous studies	This study
Cabbage	Golden Acre	90	92[a]	81
Carrot	Chantenay	80	NT	62
Cucumber	Chicago Pickler	90	91[a]	86
Lettuce	Butter Crunch	88	99[a,b]	99,95,96[d]
Millet	Japanese	85	89[a]	75
Millet	White Proso	NA	92[a],93[b],94[c]	95,90,95[d]
Oat	Larry	90	NT	23
Rice	NA	NA	89[a]	94,89[d]
Tomato	Rutger	85	NT	88
Wheat	Caldwell	90	88[a]	75

NA – not available
NT – not tested
[a] Wang and Williams (1988)
[b] Wang (1987)
[c] Wang (1986)
[d] Results of repeated tests

TABLE II

Seed germination test conditions

1. Test type	Static
2. Pretreatment	20 min, 10% Chlorox-TM (3.33 g/L OCl^-
3. Temperature	24.5–25 °C
4. Light	No
5. Test vessel	100×10 mm culture dish plus Watman #1 filter paper
6. Test volume	5 mL/dish
7. # seeds	16/dish
8. Replicates	5
9. Water control, dilution water	Hard, standard water
10. Test duration	120 hr
11. End point	Germination, primary root equal to or greater than 5 mm

are given in Table I, except for that of the rice, which was unknown. None of the seeds was treated with fungicides. They were guaranteed to germinate at the rate of 80% or better, except for white proso millet and rice, for which the rates were unknown. The seeds were all kept in loosely capped plastic bottles and at –10 °C until use.

The seed germination tests that were conducted were similar to the test used in a previous study (Wang and Williams, 1988). The test procedure is summarized in Table II. Each test species was treated with hypochlorite solution for 20 min and rinsed repeatedly. Hard, standard water, hardness 160 to 180 mg L^{-1} as $CaCO_3$ (*Standard Methods*, 1985), was used as the water control and dilution water for all tests. After 120 hr incubation in the dark, the seeds were examined to determine whether they had germinated. A 5-mm primary root was used as the operational definition of germination (U.S. Environmental Protection Agency, 1982).

In the first batch of experiments, 1 L effluent sample was adjusted to pH 7.37 using 1 N HCl. It was the diluted with dilution water into a series of solutions in ratios of 100:70:49:34:24:17:12:8. The solutions were found to be too toxic to some plant species, resulting in toxic effects skewed to one side. In the second batch of experiments, therefore, the effluent sample was adjusted to pH 7.25 and diluted into a series of solutions in ratios of 50:30:18:11:6.5:3.9.

Data analysis

The binomial method (Peltier and Weber, 1985) was used to calculate the IC50 values (the concentration causing 50% inhibition of seed germination relative to the water control) and the 95% confidence limits.

3. Results

Effluent sample

This sample was clear, brownish, and odorous, similar to the previous study (Wang, 1990). The pH was 10.6, higher than the pH values of 6.2 and 9.4 of the two previous samples. The Zn concentration was 3.8 mg L^{-1} (Table III), comparable to the 4.0 mg L^{-1} Zn concentration of sample B in the previous study. The Zn level was in excess of the federal Zn pretreatment standard, 2.61 mg L^{-1}. The other chemical quality compared favorably with the federal pretreatment standards (Federal Register, 1983). Cyanide concentration was slightly elevated, 0.41 mg L^{-1}; the concentration was below the pretreatment standard 1.2 mg L^{-1}.

TABLE III

Effluent water quality, all expressed in mg L^{-1} except pH

	Sample	Pretreatment standards for existing sources[a]
pH	10.6	
CN	0.41	1.20
Cd	<0.005	0.60
Cr(VI)	0.045	2.77
Cu	0.053	3.38
Fe	0.055	
Pb	0.023	0.69
Mn	<0.010	
Ni	0.020	3.98
Ag	<0.010	0.43
Zn	3.80	2.61

[a] Federal Register (1983)

Seed germination rate

An important criterion of a good phytotoxicity test is high germination rate; the higher the germination rate of a test species in control water, the lower the uncertainty of test results. As mentioned, all seeds were quaranteed to germinate at rates of 80% or better, except for white proso millet and rice. Cabbage, cucumber, oat, and wheat were guaranteed to germinate at 90% or better. The same batch of seeds was used in three previous studies (Wang and Wiliams, 1988; Wang, 1987, 1986), and the germination results in these studies were close to the guaranteed values (Table I). For example, cabbage was guaranteed to have a 90% germination rate, and the results were 92% germination (Wang and Williams, 1988). Wheat was guaranteed to have 90% germination rate, and the test results were 88%. All test species exceeded the 85% germination rate, which was adopted as the cutoff point for acceptance of test data (Wang, 1987, 1986).

Another important criterion of a good phytotoxicity test is long shelf life. The seeds used in this study had been stored since the time of the previous studies, a period of about 4 yr. Table I shows the current germination rates of test species. The rates for cucumber, lettuce, white proso millet, rice, and tomato were practically unchanged during that period, while the others (cabbage, carrot, Japanese millet, oat, and wheat) fell below the acceptable level of 85%. The decrease of germination rate is likely to be gradual, rather than precipitous. Nevertheless, the species that lose viability during storage are not desirable for general phytotoxicity tests.

Effluent toxicity

Another important criterion of a good phytotoxicity test is its sensitivity to toxicity. The test results are expressed as effluent concentration *vs* percent inhibition (Figure 1). In Figure 1a, the results for cabbage, carrot, cucumber, and lettuce are given, and Figure 1b, the results for the remaining species. The oat results were not plotted because of the low germination rate of 23% in the control sample, as shown in Table I.

The experimental results in the linear plot depicted in Figure 1 are typically in a sigmoid relation. At effluent concentrations of 70% and above, germination failed in all species, attesting to the extreme toxicity of this sample, even though only Zn concentration of the sample violated the pretreatment standard (Table III). The toxicity was not related to pH, as the effluent sample was neutralized before dilution.

Among nine species, rice was the most sensitive, with an IC50 value of 12% effluent concentration (Table IV). The next tier of species, with IC50 values of 14 to 23% effluent concentrations included carrot, lettuce, and tomato. Cucumber, cabbage, millet, and wheat were relatively insensitive to the effluent toxicity, with IC50 values of 30% effluent concentration or greater.

Phytotoxicity tests for effluent toxicity testing are a relatively new approach in comparison with conventional tests using the fathead minnow, *Daphnia magna*,

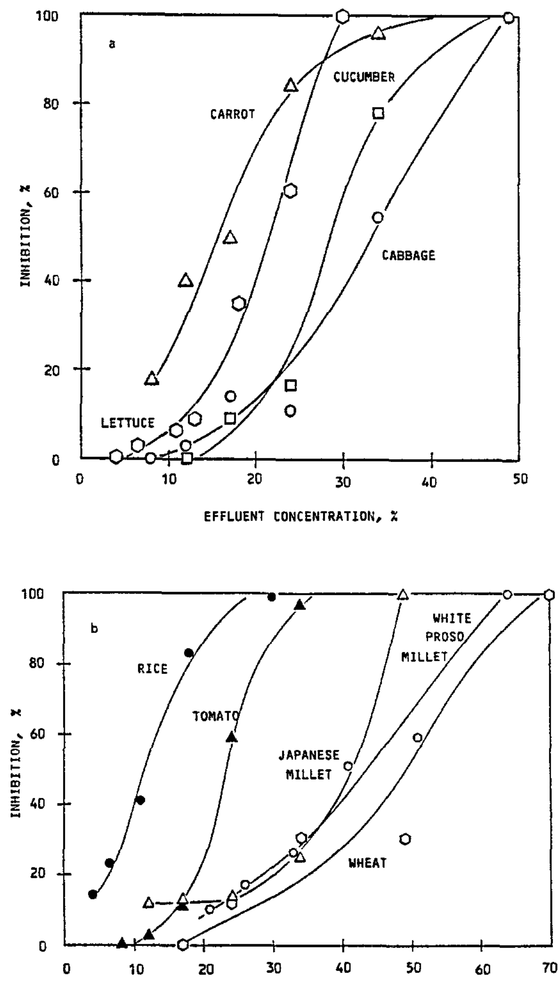

Fig. 1. Seed germination test results of a metal engraving effluent sample, a cabbage, carrot, cucumber, and lettuce and b Japanese millet, white proso millet, rice, tomato, and wheat

or green alga (Peltier and Weber, 1985). Many recent studies have explored the potential of using higher plants for ecotoxicological studies (Perez *et al.*, 1986; Srivastava and Sahai, 1987; Behera and Misra, 1982; Thomas *et al.*, 1986; Wang and Williams, 1988; Wang, 1990). More efforts are required so that phytotoxicity tests can be evaluated as an integral part of ecotoxicology.

4. Summary and Conclusion

There are three important criteria for good phytotoxicity tests; high germination rate, long shelf life, and high sensitivity to toxicity. On the basis of the criteria

TABLE IV

IC50 values (the concentration causing 50% inhibitory effect) and 95% confidence limits for a metal engraving sample, all expressed as percent effluent concentration

	IC50	95% C.L.
Rice	12	11–18
Carrot	17	17–24
Lettuce	21	18–24
Tomato	23	17–24
Cucumber	30	24–34
Cabbage	33	24–34
Millet (Japanese)	37	34–49
Millet (white proso)	41	34–41
Wheat	53	49–70

of 85% germination rate as the cutoff point for acceptance of test results and 2 yr or longer as the acceptable shelf life, it was found cucumber, lettuce, white proso millet, rice, and tomato are suitable test species. If sensitivity to effluent toxicity is also taken into consideration, then rice, lettuce and tomato are the most promising candidates for effluent toxicity testing. More effluent samples should be tested to evaluate the effluent-specificity of plant species.

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