0523
AQUIRE
11789
BARIUM
1/1/86

Environmental Pollution (*Series B*) 11 (1986) 1–14

Toxicity Tests of Aquatic Pollutants by Using Common Duckweed

Wuncheng Wang

Water Quality Section, Illinois State Water Survey,
PO Box 697, Peoria, IL 61652, USA

ABSTRACT

The objective of this study was to develop a phytoassay procedure using common duckweed. The results suggest that the assay is simple, sensitive and very economical.

Those substances evaluated for relative toxicity by the phytoassay included most of the constituents listed in Illinois' general standards for water quality. Based on the duckweed assay, the maximum permissible concentrations of Ba(II), Cd(II), Fe(III), Ni(II) and Se(IV) are lower than the water quality standards. On the other hand, similar limits for B(III), Cr(VI), Cu(II), F(I), Pb(II), Mn(II), SO_4(II) and phenol are higher than those concentrations stipulated in the water quality standards. For Cl(I) and Zn(II) the limits developed by the phytoassay are equal to the standards.

The results suggest that duckweed is a promising indicator of aquatic toxicity. Duckweed assay should be further explored so that its value can be evaluated when more data are available.

INTRODUCTION

Common duckweed *Lemna minor* is a floating, widespread, fast-growing plant. It is small and easy to cultivate. These characteristics make it an ideal candidate for aquatic toxicity tests. Numerous studies have been reported (Haller *et al.*, 1975; Hutchinson & Czyrska, 1975; Walbridge,

Environ. Pollut. Ser. B 0143-148X/86/$03·50 © Elsevier Applied Science Publishers Ltd, England, 1986. Printed in Great Britain

9422

1977; Glandon & McNabb, 1978; Walker & Evans, 1978; Alabaster, 1981; Bishop & Perry, 1981; Davis, 1981; Lockhart *et al.*, 1983).

The profound interest in duckweed bioassay, nevertheless, has no lack of criticism. Kenaga & Moolenaar (1979) claim that 'aquatic animals were more sensitive indicators of toxic effects than were plants and algae'. Consequently, they conclude that 'water quality limitations based on toxicity data for fish and daphnids should be sufficiently restrictive to protect algae and aquatic vascular plants'.

Plants are an essential part of an ecosystem, and a balance of plant–animal–bacteria complex is vital to a healthy environment. Are plants generally less sensitive to toxicity than aquatic animals as indicated by Kenaga and Moolenaar? There is little information on this subject in the literature.

The objectives of this study are (1) to develop a phytoassay procedure using common duckweed and (2) to compare the results of this phytoassay with existing water quality standards.

METHODS

Duckweed sample

Duckweed stock was collected from a pond located inside the Illinois State Water Survey property during the spring of 1982. The stock was maintained in the laboratory in a polypropylene tray with 20 litre capacity. To the tray was added a small amount of sediment, collected from the same pond, 10 litres of pond water and 5 litres of cold tap water. The plants were illuminated with cool-white fluorescent lights. Every 3–4 days cold tap water was added to maintain the water level. The duckweed species was identified as *Lemna minor*.

Twenty-four hours before the toxicity experiment was initiated, duckweed samples were selected for tests. A scoopful of duckweed from the stock was placed in a small tray containing cold tap water. The test specimens were selected from this tray and transferred to a second tray containing only deionized water. The selection of test specimens is very important as the results of bioassay are largely affected by the selected specimens, just like all other bioassays. The test specimens must appear healthy, with two fronds of approximately equal size per colony. Care was exercised so as not to injure the plant.

The selected duckweed colonies were sealed from light and were ready for experiment. The purpose of the light exclusion was to prevent growth during the holding period. The colony was discarded if it grew, or if it appeared unhealthy.

Approximately twice a year, the stock was discarded and restocked with fresh duckweed.

All test chemical compounds were reagent grade.

Experimental procedure

The bioassay experiments were conducted in a series of 200 ml fruit jars. To each jar was added a toxicant and 200 ml of plant nutrient solution, the same as the algal nutrient solution suggested in Standard Methods (1980). The pH of the solution was 7·5. For each toxicant, there were six concentration levels. Unless specified, 20 colonies or 40 fronds of duckweed were transplanted into each jar. The illumination was provided by cool-white fluorescent light with an intensity of 6456 lux. Each jar was covered with a watch glass. Temperature was maintained at 27° ± 2°C and the incubation time was 4 days, unless specified. Each experiment was conducted with 3–6 replicates.

At the end of incubation, the number of fronds in each jar was counted with the aid of a magnifying glass. The increase of frond number was used to indicate duckweed growth and the difference between treated and control samples was used to indicate the toxic effect.

A preliminary experiment was conducted for every substance. A concentration–effect relation was used to determine 50% effect concentration, EC50. The EC50 is a concentration of a toxicant which produces a 50% reduction of duckweed frond increase in comparison with the control sample. In this study each EC50 value represents an overall response of 720 fronds (3 replicates) to 1440 fronds (6 replicates).

RESULTS

Methodology

Two basic questions relating to the procedure of a duckweed bioassay are the number of fronds initially and the length of incubation time.

An experiment was conducted with different initial frond numbers 20,

TABLE I

Growth of Duckweed with Varying Initial Frond Number (IFN) as Determined by Final Frond Number (FFN)

Day	*IFN*	*20*				*30*				*40*				*50*			
4d	FFN	52	46	41	51	67	76	64	71	94	97	86	96	115	125	117	122
	ΔFN	32	26	21	31	37	46	34	41	54	57	46	56	65	75	67	72
	A[a]		28				40				53				70		
	SD		5				5				5				5		
	%		140				130				130				140		
7d	FFN	73	72	75	62	90	108	90	94	125	128	134	118	155	152	176	163
	ΔFN	53	52	55	42	60	78	60	64	85	88	94	78	105	102	126	113
	A		51				66				86				112		
	SD		6				9				7				11		
	%		250				220				220				220		

[a] A—Average increase in frond number. SD—Standard deviation in the increase of frond numbers. %—Percent increase in frond numbers.

30, 40 and 50, and different times of incubation, 4 and 7 days. The results are shown in Table 1. During 4 days' incubation, the growth of duckweed was 130–140 %. By extending to 7 days, the growth became 220–250 %. This growth rate is considered extremely fast in comparison with other plants. For example, two most rapidly growing terrestrial plants, maize and *Amaranthus viridis*, can maintain relative growth rates of 2·3 and 2·6 g g^{-1} $week^{-1}$ for some time (Hillman & Culley, 1978). The growth rate of the common duckweed ranked nearly identical to these fast-growing terrestrial plants.

The variation of results can be shown by using a coefficient of variation (SD/A). During 4 days' incubation, the coefficient of variation was 18 % when initial frond number was 20. By 7 days' exposure, the coefficient of variation decreased to 12 %. For initial frond number 40, they were 9 and 8 %, respectively.

The current trend of bioassay is to conduct acute lethal toxicity up to 4 days and chronic, sublethal toxicity 7 days or longer (Standard Methods, 1980). For this study, the initial frond number was selected at 40 and incubation time 4 days. Further increase of initial frond number and incubation period may create difficulties such as the final frond number being too numerous to count, nutrient depletion, algal competition, etc.

By counting the number of fronds daily, the growth can be plotted linearly as in Fig. 1. In typical population dynamics, the growth of duckweed should follow the conventional growth pattern of S or sigmoid shape. The straight line as depicted in Fig. 1 may not be the line of best fit, but it shows a simplistic relation.

In a toxicological experiment, the time of toxicity exposure has a large effect on biological response. The general rule of thumb is that the longer the exposure time, the less the EC50 value. A good example is Fig. 1 of the study reported by Hutchinson & Czyrska (1975). The EC50 of duckweed *Lemna valdiviana* to cadmium ion was approximately 0·2 mg $litre^{-1}$ during 1 week's exposure. Extending to 2 weeks, the EC50 decreased to 0·03 mg $litre^{-1}$. Variations to this can also be found. Reed & Evans (1981) reported that there was no perceptible difference in LC50 (50 % lethal concentration) of chloride among fish assay with 1-, 4- and 14-day time lengths.

An experiment was conducted by exposing duckweed to a cadmium solution at 0·2 and 0·4 mg $litre^{-1}$ for specified time periods; duckweed samples were then transferred into standard nutrient solution for one

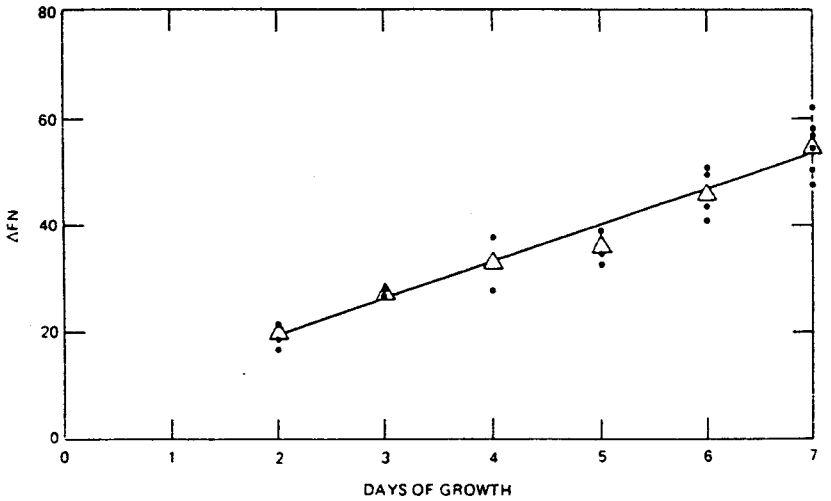

Fig. 1. The growth rate of duckweed.

week. The results are shown in Fig. 2. Cadmium ion is extremely toxic and its EC50 is 0·2 mg litre^{-1} (the following section). Nevertheless, the exposure to 0·2 mg litre^{-1} for 30 min had no adverse effect on the growth of duckweed ($p > 0{\cdot}05$). When the exposure was extended to 1 h or more, the growth of duckweed was very significantly impaired ($p < 0{\cdot}01$). At 0·4 mg litre^{-1} cadmium solution, 30 min exposure also significantly affected the growth of duckweed ($p < 0{\cdot}05$). The merit of this exposure-and-recovery experiment is to be explored.

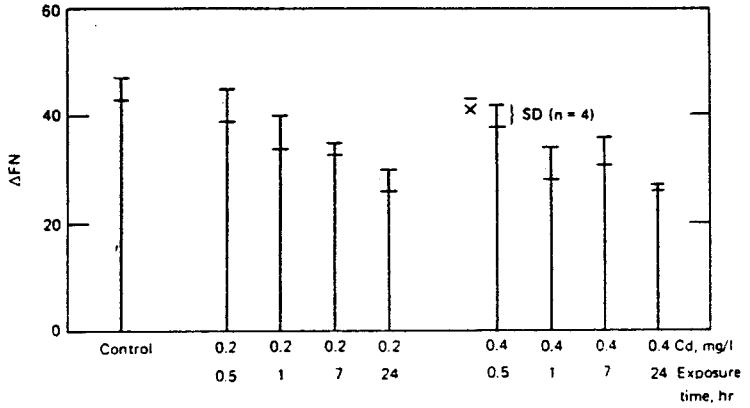

Fig. 2. The effect of varying Cd exposure times on duckweed growth.

During the early stage of this study, it was discovered that the plant nutrient solution prepared according to Standard Methods (1980) by using deionised water produced irregular results. In the first two days, plants appeared unhealthy. Thereafter, they recovered. By comparison, plants grew vigorously in nutrient solution prepared with well water. These results suggested that initially there was/were some unknown toxin(s) in the deionised water inhibiting the growth of duckweed; subsequently it was detoxified during the course of incubation. All the following experiments, therefore, were conducted by using deionised water contained in glass bottles and exposed to sunlight or fluorescent light for more than one week before use. Although well water produced healthy plants, it is not recommended because it might interfere with heavy metal ion activity.

EC50s of various substances

Updated general use water quality standards were issued by the Illinois Environmental Protection Agency (State of Illinois, 1982). These standards are practically the same as the earlier version issued in 1977 except in the case of ammonia and temperature, where these two parameters were defined in greater detail. The objective of the general water quality standards is to protect aquatic life, particularly native fish and/or essential fish food organisms. The standards for 21 chemical constituents are listed in Table 2.

Fifteen of the 21 constituents were tested with duckweed bioassay. The typical toxic responses are shown in Figs 3–6, using barium, boron, cadmium and chloride as examples. Barium (Fig. 3) is moderately toxic. It substantially suppressed the growth of duckweed expressed by the increase of frond number. The relationship appears to be hyperbolic. At 26 mg litre^{-1} it reduced the growth to 50%, thus the EC50 of barium ion is 26 mg litre^{-1}. The maximum permissible concentration (MPC) is 2·6 mg litre^{-1}, as 10% is a conventional safety factor for this calculation. MPC value for barium 2·6 mg litre^{-1} (column 4) is lower than barium standard 5 mg litre^{-1} (column 2), as shown in Table 2. The result suggests that the barium standard is not stringent enough to protect aquatic plants such as duckweed.

The results of four replications showed that boron (Fig. 4) had a minimal effect on duckweed. Boron up to 60 mg litre^{-1} did not produce any significant toxic effect. Obviously the boron standard 1 mg litre^{-1} is sufficiently stringent to protect duckweed.

TABLE 2
Duckweed Bioassay with Substances Listed in General Water Quality Standards (State of Illinois, 1982)

	Standard mg litre⁻¹	*Duckweed bioassay*			*Standard mg litre⁻¹*	*Duckweed bioassay*	
		EC50	*MPC*[a]			*EC50*	*MPC*[a]
As	1·0			Fe	1·0	3·7	0·37
Ba	5·0	26	2·6	Pb	0·1	8	0·8
B	1·0	>60	—	Mn	1·0	31	3·1
Cd	0·05	0·2	0·02	Hg	0·000 5		
Cl	500	930	500[b]	Ni	1·0	0·45	0·045
Cr(VI)	0·05	35	3·5	Phenol	0·1	>12	—
Cr(III)	1·0			Se	1·0	2·4	0·24
Cu	0·02	1·1	0·11	Ag	0·005		
CN	0·025			SO_4	500	>1000	—
F	1·4	>60	—	TDS	1 000		
				Zn	1·0	10	1·0

[a] MPC—Maximum permissible concentration = 10% × EC50.
[b] See explanation in the text.

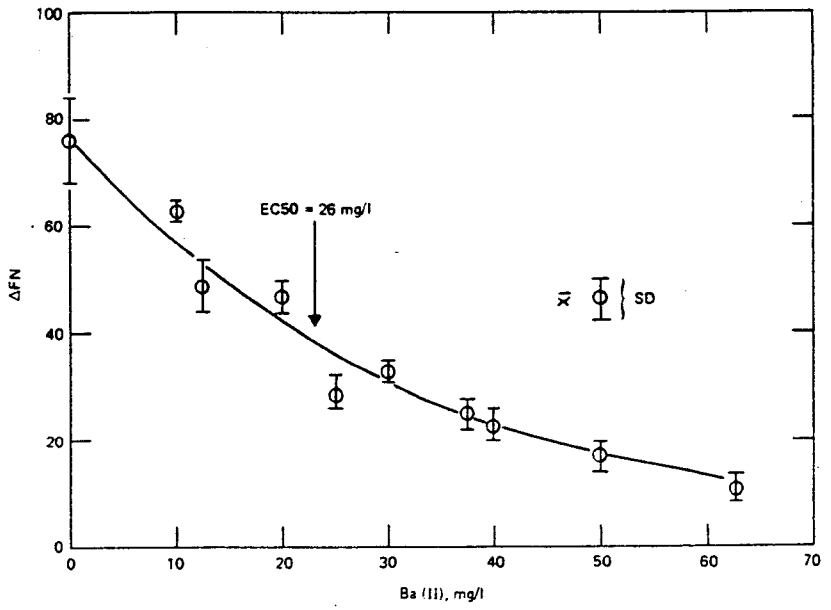

Fig. 3. The effect of Ba on duckweed growth.

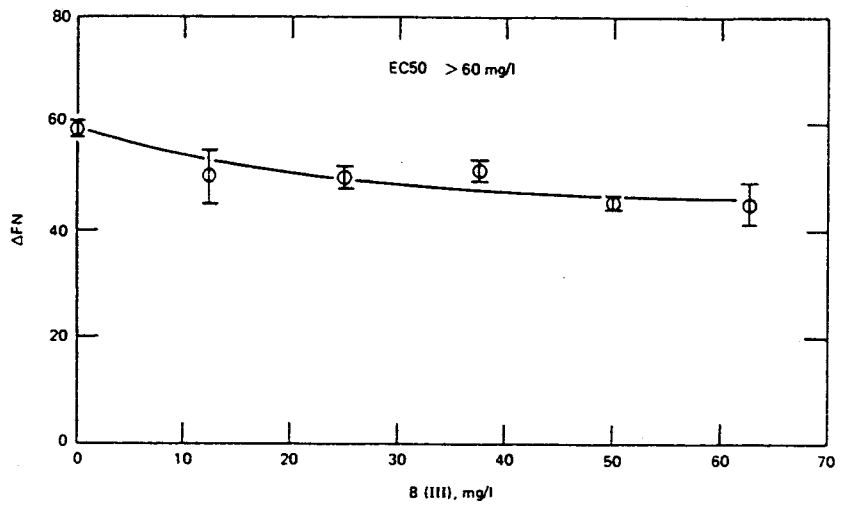

Fig. 4. The effect of B on duckweed growth.

The general water quality standard for total cadmium is 0·05 mg litre^{-1}. A total of six replications were made on cadmium ion. The results in Fig. 5 show that cadmium is extremely toxic. At 0·05 mg litre^{-1} cadmium already displayed a substantial toxic effect, and at 0·2 mg litre^{-1}, 50% reduction of growth. Similar results were reported by Hutchinson & Czyrska (1975). If a 10% safety factor is taken into

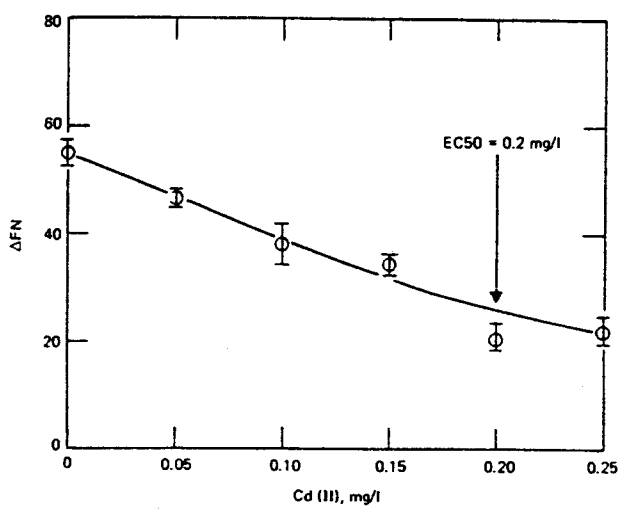

Fig. 5. The effect of Cd on duckweed growth.

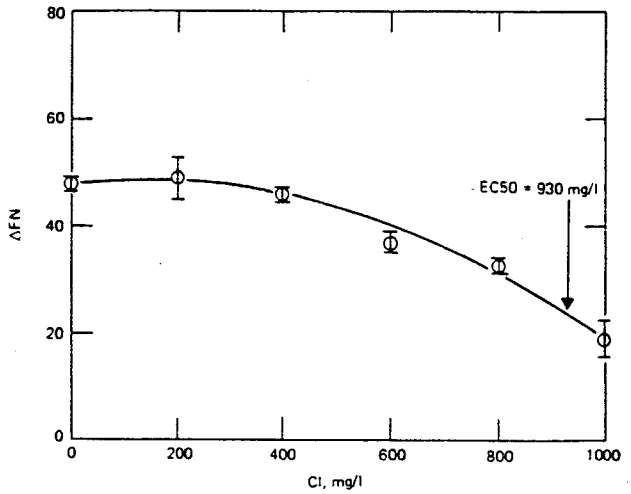

Fig. 6. The effect of Cl on duckweed growth.

account, the MPC is 0·02 mg litre^{-1}, a value lower than the water quality standard, 0·05 mg litre^{-1}. The cadmium standard obviously is not stringent enough for these aquatic plants.

Chloride ion is not generally considered as a toxic substance. At high concentrations, however, it can damage the physiological function of a living organism. Chloride as shown in Fig. 6 had little or no effect on the growth of duckweed at 0–400 mg litre^{-1}. Thereafter, chloride affected the growth at an accelerated rate. The EC50 was 930 mg litre^{-1}. A 10% safety factor gives 93 mg litre^{-1}, obviously a meaningless value because it did not produce a significantly harmful effect on duckweed. The MPC of 500 mg litre^{-1} for chloride (as well as standard) is more realistic as it caused approximately 10% reduction in the growth of duckweed.

Interpretations for the remaining constituents can be made in the same manner by comparing column 2 and column 4 of Table 2. Complete results are available upon request. In summary, the relationship between the general water quality standards and maximum permissible concentrations as determined by duckweed bioassay can be delineated as follows:

Standards sufficient to protect duckweed—B, Cr(VI), Cu, F, Pb, Mn, phenol and SO_4.
Standards insufficient to protect duckweed—Ba, Cd, Fe, Ni and Se.
Standards identical—Cl and Zn.

TABLE 3
Metal Toxicity to Duckweed

		EC50, mg litre^{-1}
Extremely toxic	Cd	0·2
	Ni	0·45
Highly toxic	Cu	1·1
	Se	2·4
	Fe	3·7
	Pb	8
	Zn	10
Moderately toxic	Ba	26
	Mn	31
	Cr	35

The toxicity of these substances on duckweed can be classified into extremely toxic, highly toxic and moderately toxic (Table 3).

Comparison of fish and duckweed tests

The results of this study are compared with the results of a published fish bioassay (Table 4). Of available data on seven metal ions, duckweed compared favourably with fish without exception.

TABLE 4
Comparison Between Fish and Duckweed Bioassays to Metal Toxicity (mg litre^{-1})

	Fish[a] *96 h LC50*	*Duckweed 96 h EC50*
Cd	0·92	0·2
Cr(VI)	58·5	35
Cu	0·08–1·2 (3 species)	1·1
Pb	27·8	8
Ni	13·6–48·8 (4 species)	0·45
Se	18·7	2·4
Zn	0·4–55 (6 species)	10

[a] From R. L. Spehar *et al.* (1982).

DISCUSSION

The significance of a phytoassay is that plants are an essential part of an ecosystem, and as such they should be protected in order to achieve a healthy environment. The question has been raised constantly as to whether a phytoassay is comparable to a fish assay. At this early stage of phytoassay experience, the data base is not sufficiently large to draw any conclusion. The educated prediction is that some results of a phytoassay may be parallel to that of a fish assay, while others may be widely different. The available data as listed in Table 4, nevertheless, clearly suggest that duckweed assay is a very promising tool for toxicity testing.

The strength of duckweed assay is that it is simple, inexpensive and sensitive. It requires no special skills and equipments. It can be used for screening or monitoring aquatic toxicity. Any indication of toxicity can then be followed up with a fish assay, if desired. Fish assay is undoubtedly much more expensive. Furthermore, because duckweed is an aquatic plant, it is an excellent choice for detecting herbicide in the aquatic environment. Herbicides are widespread man-made toxins which have received very little attention as environmental pollutants due to their low animal toxicity. This is a rather unfortunate oversight if one considers the quantity of herbicides applied by farmers, homeowners, conservation officials, etc. Herbicides tend to concentrate on the air–water interface (Wu *et al.*, 1980). This makes free-floating duckweed an ideal candidate for toxicity testing.

The Illinois River and Chesapeake Bay both experienced loss of aquatic vegetation (Bellrose *et al.*, 1979; Glotfelty *et al.*, 1984). The cause(s) is/are speculative. Nevertheless, the impact on water quality and animal life must be enormous. Any attempt to study the long-term ecological changes of these waters should address the herbicide issue. Duckweed may provide an ideal key.

Kenaga & Moolenaar (1979) conducted tests of 27 781 chemicals on aquatic plants and 49 082 chemicals on the alga *Chlorella*. Based on these results, they concluded the relative sensitivity between aquatic plants and animals. These huge quantities of data would have been convincing evidence had they obtained them with high quality. There are two major problems with the methodology, however. One, they used chlorosis as the toxicity indicator. Chlorosis is one of many toxic effects. For example, cadmium ion at 0·15 mg litre^{-1} caused chlorosis and reduced duckweed growth to 62 %. Nickel ion at 2·5 mg litre^{-1} reduced duckweed growth to

less than 20%, yet the treated plant was as green as the control. Second, they treated the plant for 24 h and then placed it in untreated water for 3 weeks. This is a recovery test as shown in Fig. 2. Similar procedures were not performed with fish. The variation of experimental procedures between animals and plants, consequently, makes the comparison of relative sensitivity questionable.

The results of this study suggest that duckweed is a promising indicator of aquatic toxicity. In a recent talk the author advocated that duckweed assay should be further explored so that its value can be evaluated when more data are available (Wang, 1984*a*). In a recent article, the author has explored the use of duckweed for ecotoxicology in greater detail (Wang, 1984*b*).

REFERENCES

Alabaster, J. S. (1981). Joint action of mixture of toxicants on aquatic organisms. *Chemy Ind.*, **15**, 529–34.

Bellrose, F. C., Paveglio, Jr, F. C. & Steffect, D. W. (1979). Waterfowl populations and the changing environment of the Illinois River valley. *Bull. Ill. nat. Hist. Surv.*, **32**, Article 1.

Bishop, W. E. & Perry, R. L. (1981). Development and evaluation of a flow-through growth inhibition test with duckweed (*Lemna minor*). ASTM STP 737, *Am. Soc. Test. & Mater.*, 421–35.

Davis, J. A. (1981). *Comparison of static-replacement and flow-through bioassays using duckweed*, Lemma gibba *G-3*. US EPA 560/6-81-003. Washington, DC.

Glandon, R. T. & McNabb, C. D. (1978). The uptake of boron by *Lemna minor*. *Aquat. Bot.*, **4**, 53–64.

Glotfelty, D. E., Taylor, A. W., Isensee, A. R., Jersey, J. & Glenn, S. (1984). Atrazine and simazine movement to Wye River estuary. *J. environ. Qual.*, **13**, 115–21.

Haller, W. E., Sutton, D. L. & Barlowe, W. C. (1975). Effects of salinity on growth of several aquatic macrophytes. *Ecology*, **55**, 891–4.

Hillman, W. S. & Culley, D. D., Jr (1978). The use of duckweed. *Am. Scient.*, **66**, 442–51.

Hutchinson, T. C. & Czyrska, H. (1975). Heavy metal toxicity and synergism to floating aquatic weeds. *Verh. int. Verein. Limnol.*, **19**, 2101–11.

Kenaga, E. & Moolenaar, J. J. (1979). Fish and daphnia toxicity as surrogates for aquatic vascular plants and algae. *Environ. Sci. Technol.*, **13**, 1479–80

Lockhart, W. L., Billeck, B. N., deMarch, G. G. E. & Muir, D. C. G. (1983). Uptake and toxicity of organic compounds. ASTM STP 802, *Am. Soc. Test. & Mater.*, 460–8.

Reed, P. & Evans, R. L. (1981). Acute toxicity of chlorides, sulfates, and total dissolved solids to some fishes in Illinois. *Illinois State Water Survey, Contract Report*, **283**.

Spehar, R. L., Christensen, G. M., Curtis, C., Lemke, A. G., Norberg, T. J. & Pickering, G. H. (1982). Effects of pollution on freshwater fish (review). *J. Wat. Pollut. Control Fed.*, **54**, 877–922.

Standard Methods (1980). *Standard methods for the examination of water and wastewater 15th edn.* Washington, DC, American Public Health Association.

State of Illinois (1982). *Rules and regulations*. Title 35, Subtitle C, Chapter 1, Pollution Control Board.

Walbridge, C. T. (1977). *A flow-through testing procedure with duckweed* (Lemna minor *L.*), US EPA-600/3-77-108. Washington, DC.

Walker, J. R. L. & Evans, S. (1978). Effects of quaternary ammonium compounds on some aquatic plants. *Mar. Pollut. Bull.*, **9**, 136–7.

Wang, W. (1984*a*). The role of phytoassay in the derivation of numerical national water quality criteria. ASTM STP 854, *Am. Soc. Test. & Mater.*, 548–50.

Wang, W. (1984*b*). Guest editorial: Uses of aquatic plants in ecotoxicology. *Environ. Int.*, **10**, i–iii.

Wu, T. L., Lambert, L., Hastings, D. & Banning, D. (1980). Enrichment of agricultural herbicide atrazine in the microsurface water of an estuary. *Bull. environ. Contam. & Toxicol.*, **24**, 411–14.