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REVIEW

Literature Review on Duckweed Toxicity Testing

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Duckweed commonly refers to a group of floating, flowering plants of the family Lemnaceae. Duckweed plants are fast growing and widely distributed. They are easy to culture and to test. Some reports suggest that duckweed plants are tolerant to environmental toxicity. Other studies, however, indicate that duckweed plants are as sensitive to toxicity as other aquatic species. Duckweed plants are especially suitable for use in complex effluent bioassays, and for testing herbicide pollution in the aquatic environment, lake and river pollution, sediment toxicity, and the like. Duckweed and algae represent different levels of complexity in the plant kingdom. They complement each other as phytotoxicity test organisms, instead of mutually excluding each other. Many duckweed species have been studied, primarily of the *Lemna* and *Spirodela* genera. *Lemna minor* and *L. gibba* have been recommended as standard test species. Differences in duckweed test methodology occur with regard to test types, test vessels, control tests, nutrient media, end points, and applications.

INTRODUCTION

Three base-set tests are currently used for freshwater environmental monitoring, effluent toxicity testing, toxicity assessment of a product, etc. They are tests of *Pimephales promelas*, *Daphnia magna* (or *Daphnia pulex*), and *Selenastrum capricornutum* (Peltier and Weber, 1985; Horning and Weber, 1985). These tests are generally applicable to important environmental laws such as the National Pollutant Discharge Elimination System regulations, the Toxic Substance Control Act, and the like. They are used routinely by federal and state regulatory agencies, industries, and consulting laboratories.

Recently the duckweed toxicity test has received much attention. Duckweed is an aquatic plant and is relevant to many aquatic environments, including lakes, streams, effluent, rain, and sediment. Additionally, duckweed is a vascular, flowering plant which provides additional information unlikely to be obtained by the three base-set tests.

The objective of this article is a critical review on the duckweed toxicity test. It is intended to be methodology oriented, rather than an exhaustive literature citation. Other related review articles on duckweed are also available (Hillman, 1961; Hillman and Culley, 1978; Wang, submitted for publication).

FEATURES OF DUCKWEED PLANTS

The term "duckweed" commonly refers to a group of aquatic vascular angiosperms of the family Lemnaceae. Duckweed plants are divided into four genera, *Spirodela*, *Lemna*, *Wolffiella*, and *Wolffia*. There are approximately 40 spe-

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cies worldwide, half of which are found in the United States. Duckweed plants are widely distributed in the world from the tropical to the temperate zones, from freshwater to brackish estuaries, and throughout a wide range of trophic conditions (Hillman and Culley, 1978). Duckweed plants are common in the aquatic environment, especially in quiescent water bodies.

Duckweed plants are composed of two parts, frond and root. The plants are colonial and form aggregates of two or more fronds in a colony. *Lemna minor* has a single rootlet, while *Spirodela* has several rootlets (Correll and Correll, 1972).

Duckweed plants are small. *L. minor* is 2–4 mm across, while *Spirodela* thallus is 3–10 mm long (Correll and Correll, 1972). The plant size is small so that a large laboratory space is not required for culturing or testing. Yet the size is sufficiently large to be visually observed, allowing nondestructive, repeated observations.

Duckweed plants are extremely fast growing (Hillman and Culley, 1978). The plants form a thick mat, frequently dominated by a single species, in a lake or a pond. Fish kills have been reported as a result of duckweed and algal domination (Lewis and Bender, 1961). In an 18-month study, the doubling time for *L. minor* fronds ranged from 1.3 to 2.8 days, as shown in Fig. 1 (Wang, 1987a). The mean value and standard deviation were 1.9 and 0.36 days, respectively. This series of experiments were performed using a nutrient solution containing a double-strength (2×) algal nutrient solution (American Public Health Association *et al.*, 1985). Frick (1985) reported that the frond doubling time for *L. minor* was about 1.4 days. In comparison, Hughes *et al.* (1988) reported the doubling time for *L. gibba* to be 0.7 days. On the basis of results reported by Nasu *et al.* (1984), the doubling time for *L. paucicostata* was calculated to be 0.35 days. Duckweed cultured in the laboratory can grow indefinitely if plant nutrients, light, and water are provided, thus producing unlimited duckweed test specimens for use at any moment.

Duckweed is a floating organism. It is especially susceptible to surface active

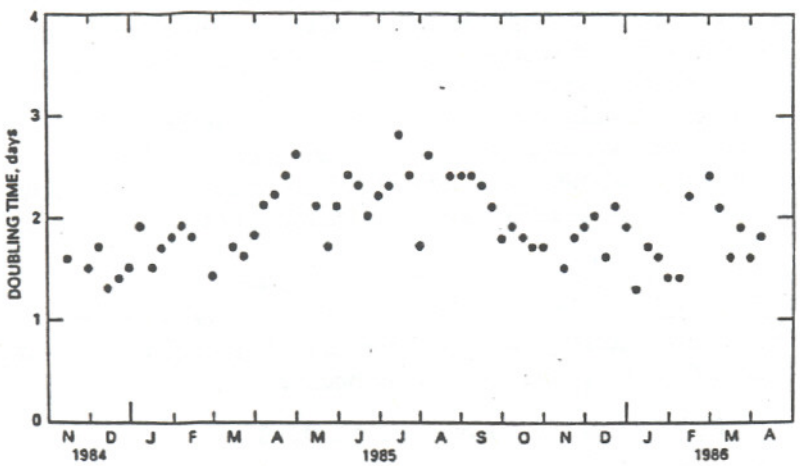

FIG. 1. Quality of control tests as indicated by doubling time of duckweed frond number. Redrawn from Wang (1987a).

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COMPARISON OF FISH AN

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substances, hydrophobic compounds, and the like that concentrate at the air–water interface (Wu *et al.*, 1980).

There are indications that duckweed is tolerant to environmental toxicity, and it is commonly referred to as the "carp" of plant species. Clark *et al.* (1981) reported that duckweed (*L. perpusilla*) was the most abundant macrophyte inhabiting a coal–ash retaining basin of a coal-fired power plant, occupying approximately 95% of the surface area. Gabrielson *et al.* (1980) found that duckweed grew under a wide range of nutrient conditions including high metal concentrations. Seto *et al.* (1979) reported that Cd caused chlorosis and death to *L. gibba*. Cd toxicity, however, was greatly influenced by the nutrient levels in which the specimens were cultured. Duckweed was much more tolerant to Cd when the plant was cultured in a higher-nutrient solution than in a low-nutrient solution.

There are other indications, however, suggesting that duckweed is sensitive to toxicity. Wang (1986a) conducted a series of duckweed toxicity tests on 16 aquatic pollutants. Of special interest is the comparison of the sensitivity to metal toxicity of duckweed and fish species. The results in Table 1 indicate that duckweed was as sensitive or sometimes more sensitive to metal toxicity than fish species.

Duckweed is also sensitive to organic compounds. For example, Rowe *et al.* (1982) reported that duckweed was as sensitive to epoxystearic acid toxicity as juvenile rainbow trout, while the severe toxic effects of catechol compounds on duckweed were comparable with those on daphnids. Furthermore, Hughes *et al.* (1988) reported that the toxic effects of atrazine on *L. gibba*, *Anabaena flos-aqua*, and *Navicula pelliculosa* were comparable. The 50% inhibition concentrations for these species were 0.17, 0.23, and 0.06 mg/liter, respectively. Huber *et al.* (1982) observed the toxic effects of pentachlorophenol on *L. minor* to be comparable to those on grass shrimp, fish, and snails.

The preceding discussion appears to be contradictory. On the one hand duckweed plants are described as tolerant to environmental toxicity, while on the other hand the plants are mentioned as sensitive to toxicity. The contradiction can be explained on the basis that the plants may be highly adaptive. Under optimum culture conditions, free of contamination, the plants are responsive to environmental toxicity when they encounter it. At sublethal range, the duckweed plants

TABLE 1
COMPARISON OF FISH AND DUCKWEED BIOASSAYS ON METAL TOXICITY (mg/liter)

	Fish 96 hr LC_{50}	Duckweed 96 hr EC_{50}
Cd	0.92	0.2
Cr (VI)	58.5	35
Cu	0.08–1.2 (three species)	1.1
Pb	27.8	8
Ni	13.6–48.8 (four species)	0.45
Se	18.7	2.4
Zn	0.4–55 (six species)	10

Note. Reproduced from Wang (1986a) with permission from Elsevier Applied Science Publishers Ltd.

may adapt and/or develop resistance quickly due to their fast growth rate. Several reports (Duncan and Klaverkamp, 1983; Benson and Birge, 1985; Dixon and Sprague, 1981) indicate that fish can develop tolerance. Perhaps the same induced tolerance is the reason that duckweed plants are sometimes insensitive to environmental toxicity.

Duckweed is uniquely suitable for testing herbicide pollution in the aquatic environment. The use of herbicides has steadily increased in the past 25 years (Nielsen and Lee, 1987), and herbicide runoff into surface waters is widespread (Leonard, 1988). Results obtained by Taraldsen and Norberg-King (in press) demonstrated clearly that tests of duckweed, fathead minnow, and *Ceriodaphnia* complemented each other for testing different types of wastewaters. No single test is likely to be able to detect toxicity in every environmental sample.

Duckweed vs Algal Toxicity Tests

Both algae and higher plants are primary producers and essential parts of a balanced ecosystem. There has been a persistent question as to whether algal species can be a surrogate for plant species and whether the plant kingdom is sufficiently represented by algal species alone as a part of the three base-set tests.

In the aquatic environment, algae and duckweed are competitors. There are reports indicating that algal growth might interfere with the growth of aquatic macrophytes through alleopathic effects (Van Vierssen and Prins, 1985; Kemp *et al.*, 1984). Duckweed plants, being floating organisms, suppress algal growth by blocking sunlight.

There has been some information directly comparing results of duckweed and algal toxicity tests. As mentioned, Hughes *et al.* (1988) reported that *L. gibba*, *Anabaena*, and *Navicula* were almost equally sensitive to atrazine.

There are indications that duckweed is less sensitive to toxicity than algae. Mangi *et al.* (1978) reported that both *L. minor* and *Spirodela polyrhiza* were less sensitive to Cr (VI) than single or filamentous algae. Bioassays of solid waste leachate from coal gasifiers with *Selenastrum capricornutum* and *S. oligorhiza* showed that the alga was more sensitive to toxicity than the duckweed (Klaine, 1985). Klaine reported the 50% inhibition effect concentrations for the test species to be 55 and 76% leachate concentration, respectively.

Rowe *et al.* (1982) compared toxicity test results for spent chlorination and caustic extraction liquors using an alga *Chlorella pyrenoidesa* and a duckweed *L. perpusilla*. They found that the algae tended to be more sensitive than the duckweed to chlorinated phenolic compounds.

These results, however, should be interpreted with caution. As discussed in the following section on end points, the conventional duckweed test grossly underestimates toxic effect. The advancement of a duckweed test protocol will improve the test results in the future. Additionally, Klaine (1985) raised an important point that "Since the alga is distributed throughout the water column and the duckweed only inhabits the water surface, the alga maintains a more constant exposure to the toxicant." Similarly, results were also reported that submerged plant species generally absorbed more Cd, Cu, Pb, and Zn than the floating species, *L. gibba* and *S. polyrhiza* (Van der Werff and Pruyt, 1982). One should therefore bear in

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With regard to terrestrial plant species, it is apparent that higher plants and algae complement each other as toxicity test species when exposed to varieties of toxicants (Miller *et al.*, 1985; Thomas *et al.*, 1986). Garten and Frank (1984) stated that "algal growth inhibition tests cannot be used generally to predict phytotoxicity of herbicides to terrestrial plant species." Perhaps the same conclusion may one day be extrapolated to include aquatic macrophytes in general and duckweed in particular. More research is needed for comparative toxicity tests using algae and duckweed.

Unlike algal toxicity tests, duckweed toxicity tests are especially suitable for effluent biomonitoring. Many industrial and municipal wastewaters are turbid. With these samples, filtration is required to conduct algal tests, resulting in the loss of sample integrity. Duckweed tests, however, can be performed on the sample "as is." More importantly, some industrial and almost all municipal wastewaters are labile because of high organic content and microbial population. These samples require either flow-through or renewal tests. Duckweed can be tested in either manner, while algae cannot.

Duckweed toxicity tests can also reveal effects that cannot be obtained by using algal tests. Nasu *et al.* (1984) observed that Cu suppressed both frond multiplication and frond growth (wet weight increase of each frond) of *L. paucicostata*, while the Cd ion suppressed only frond multiplication and did not affect frond growth. Interesting findings such as these are very important for comparative studies of environmental toxicology.

DUCKWEED TOXICITY TESTS

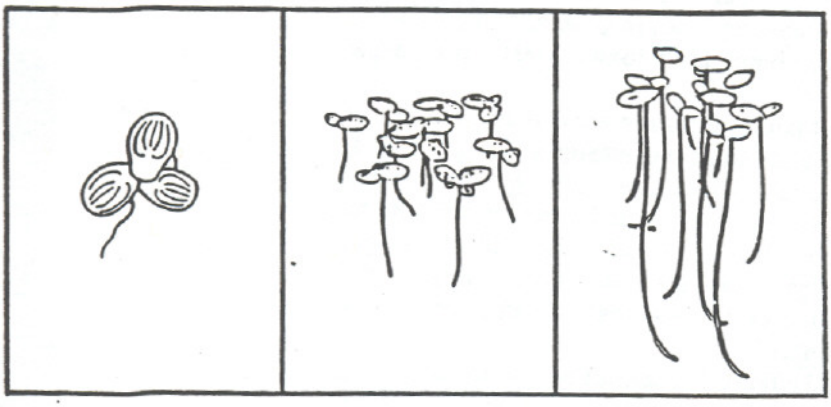
Species

Many duckweed species have been studied: *L. minor, L. gibba, L. valdiviana, L. polyrrhiza, L. perpusilla, L. paucicostata, S. polyrhiza, S. punctata,* and *S. oligorhiza*. Features of three common duckweed species are depicted in Fig. 2.

There are relatively few data for comparative studies on different species. King and Coley (1985) compared the sensitivity of *L. gibba, L. minor,* and *L. perpusilla* to aqueous extracts of natural and synthetic oils, as well as to coal distillate. They reported that *L. gibba* was the most resistant among the three species. The 10% extract of coal distillate completely inhibited the growth of *L. minor* and *L. perpusilla,* whereas complete inhibition of *L. gibba* was noted only in the 15% extract.

Under low irradiance, Takemoto and Noble (1986) found that sulfite toxicity was most pronounced for *L. gibba,* less marked for *S. oligorhiza,* and not observed with *L. valdiviana*. Staves and Knaus (1985) reported that *S. polyrhiza* was least tolerant to Cr, while *S. punctata* and *L. gibba* were more tolerant to concentrations above 10 mg/liter.

The selection of test species is usually based on specimen availability, sensitivity to the toxicant, historical data, and the like. To encourage test standardization and comparability, two species have been recommended. The American



Spirodela polyrhiza, x 2.4 Lemna minor, x 2.4 Lemna perpusilla, x 2.4

FIG. 2. Features of *Spirodela polyrhiza*, *Lemna minor*, and *Lemna perpusilla*. Reproduced from Correll and Correll (1972).

Society for Testing and Materials (in press) recommends *L. gibba*, while *Standard Methods for Examination of Water and Wastewater* in its 17th Edition Supplement (American Public Health Association *et al.*, in press) endorses *L. minor*. Although the use of standard species is encouraged, other species should be tested so that new information may help to evolve test methods in the future.

Test Types

Duckweed toxicity tests can be used in static, renewal, or flow-through experiments. Static experiments are simple and economical. They are especially useful for screening tests of unknown samples or samples which contain toxic metals (Wang, 1986a, b, 1987a, b, 1988; Wang and Williams, 1988). Typical test conditions for conducting duckweed static experiment are given in Table 2. Flow-through and renewal experiments have also been reported (Bishop and Perry,

TABLE 2
TYPICAL DUCKWEED STATIC TEST

Temperature	27–28°C
Light quality	Cool white fluorescent
Light intensity	86 $\mu E/m^2/sec$
Photoperiod	Continuous
Test vessel	60 × 15-mm culture dish
Test solution/vessel	15 ml
Test specimens/vessel	20 fronds (10 colonies)
Replicates	4
Water control and dilution water	Duckweed nutrient medium, i.e., 10-fold algal nutrient medium
Test duration	120 hr
End point	Frond increase/vessel

Note. Modified from *Standard Methods* (American Public Health Association *et al.*, in press).

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Test Vessel

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Control Tests

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Threader and Houston,
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toxicity to *L. minor* wa

1981; Davis, 1981; Walbridge, 1977). In general, flow-through and renewal experiments are useful for samples containing volatile or biodegradable compounds.

Test Vessel

Several different test vessels have been used: glass beakers, flat-bottomed test tubes, jars, Erlenmeyer flasks, and culture dishes (Fekete *et al.*, 1976; Nasu and Kugimoto, 1981; Hutchinson and Czyrska, 1975; Stanley and Madewell, 1976; Wang, 1986a, b). In general, only one type of test vessel should be used in an experiment. Plastic vessels are not recommended because of strong adherence of duckweed plants to the plastic walls. The test vessel should be covered to avoid excessive evaporation of water and test sample.

The test solution in a vessel in general should have a liquid height of 5 cm or more to accommodate duckweed root length. Either 100 or 200 ml of test solution can be used. Recently the author reduced the test solution to 15 ml by using 60 × 15-mm culture dishes. The liquid depth was 5 mm. Twenty fronds of *L. minor* were placed in each dish. *Lemna* grew in a 15-ml solution containing 10-fold algal nutrient solution (American Public Health Association *et al.*, 1985) as well as in a 150-ml solution containing 2-fold algal nutrient solution (Wang, 1987a). The miniaturization of the test solution gave reproducible results, as evidenced by the frond doubling time of control samples 1.6 ± 0.13 days in a 6-month test period, with a total of 22 tests. This result compared favorably with the doubling time of 1.9 ± 0.36 days as shown in Fig. 1. Taraldsen and Norberg-King (in press) used a 30-ml plastic cup containing 15 ml of test solution.

The advantage of miniaturizing the test solution is obvious. In addition to being more flexible in the experimental design because of its smaller volume, the smaller test volume also minimizes disposal problems after the experiments are completed.

Control Tests

There are two types of control samples: negative controls and positive controls. Almost all toxicity tests use a negative control where no test substance is present. The negative control serves as a quality control in an experiment as well as a reference point to which a test sample is compared. Negative control values vary from time to time as shown in Fig. 1.

Positive controls are less frequently employed, although they are as important as negative controls. A positive control is a sample to which a reference toxicant is added to determine the degree of response over time. Organic and inorganic compounds have been studied as potential reference toxicants, including pentachlorophenol, phenol, sodium dodecyl sulfate, sodium chloride, cadmium, and chromate (Davis and Hoos, 1975; Fogels and Sprague, 1977; Jop *et al.*, 1986; Threader and Houston, 1983). Wang (1987a) found that in an 18-month period, the growth of *L. minor* underwent cyclic changes in the negative control, while the duckweed response to Cr toxicity was nearly constant (Fig. 3). In general, Cr toxicity to *L. minor* was not ameliorated by differences in water quality factors

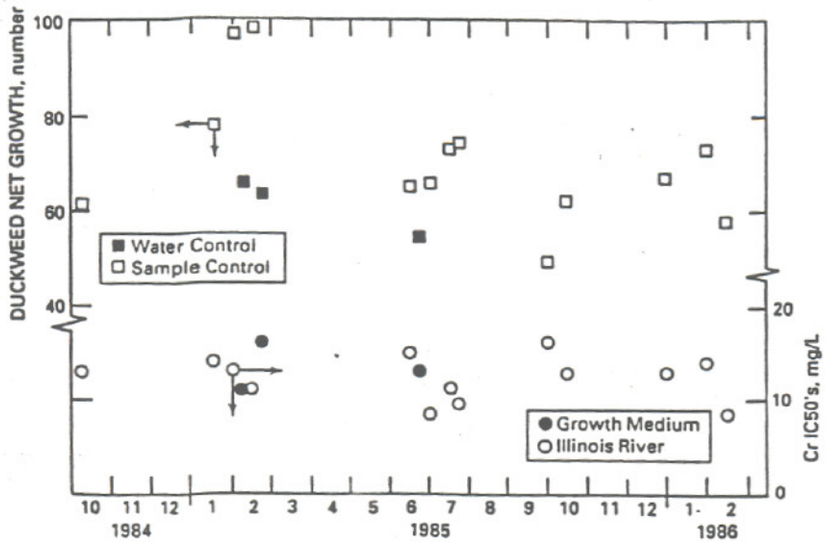

FIG. 3. Duckweed growth in water control (■) and in sample control (□), and Cr toxicity in nutrient medium (●) and in enriched water samples (○), using Illinois River samples. Redrawn from Wang (1987a).

such as suspended solids or dissolved fraction (Wang, 1986b). On the basis of these and other results, Cr (VI) has been recommended as a universal reference toxicant.

Nutrient Media

Duckweed toxicity tests, which generally rely on growth and multiplication as the test end point, require sufficient plant nutrients for optimum conditions. Plant nutrients are uniformly added to control and test samples.

Several nutrient media have been reported. They included the medium used by Ballard (1966), Bristol's medium as given by Eyster (1968); Jacob's medium as given by McLay (1976); Hoagland's, Hunter's, and Bonner-Devirian's media as given by Nasu and Kugimoto (1981); and the medium used by Fekete *et al.* (1976). The composition varied widely among these media.

Nasu and Kugimoto (1981) found that the pH of the nutrient media, the concentration and composition of the nutrients in the medium, and the temperature at which cultures were maintained affected the sensitivity of *L. paucicostata* to heavy metals. They recommended the use of Bonner-Devirian's medium at temperatures above 25°C. Nasu *et al.* (1983) reported that the absorption of Cu and Cd in *L. paucicostata* was suppressed by the addition of EDTA to the medium. Only 30 μM of EDTA was sufficient to prevent Cu absorption at the concentration of 5–10 μM, whereas 400 μM of EDTA was required to prevent Cd absorption at the same concentration as Cu. They found that the growth inhibition of *Lemna* was proportional to the amount of metal absorption.

The green alga *Selenastrum capricornutum* and the common duckweed *L. mi-*

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End Points

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nor are frequently used for phytotoxicity testing. It would be highly desirable to share the same nutrient stock solutions for these two tests. Wang (1986c) used double-strength algal nutrient solution, which is recommended by *Standard Methods* (American Public Health Association *et al.*, 1985). Instead of seven stock solutions recommended by *Standard Methods*, Wang prepared three solutions. The same approach has been adopted by the American Society for Testing and Materials (in press), who recommend 20-fold (20×) strength. This same approach will also be recommended in *Standard Methods* (American Public Health Association *et al.*, in press) where 10-fold (10×) strength as shown in Table 3 will be suggested.

End Points

Many end points have been used to express duckweed test results. These end points are generally based on the population of duckweed plants: frond number, plant number, root number, dry or fresh biomass, root length, frond diameter, C-14 uptake, chlorophyll, and the like (Bishop and Perry, 1981; Said *et al.*, 1979; Culley *et al.*, 1981; Lockhart *et al.*, 1983; Bishop and Perry, 1981; Glandon and McNabb, 1978; Sahai *et al.*, 1977; Fekete *et al.*, 1976).

The most commonly used test end point is frond number. Any visible, protruding bud is included in order to avoid individual bias. The frond count can be made repeatedly until accurate results are obtained. This determination is simple, rapid, and nondestructive. These advantages, however, are weighted against disadvantages.

The first disadvantage is that frond count is irrelevant to frond size or biomass. It has been observed frequently that under toxic stress small buds may protrude

TABLE 3
PREPARATION OF DUCKWEED NUTRIENT SOLUTION

Stock	Compound	Concentration (mg/liter)
A	$NaNO_3$	22,500
	$NaHCO_3$	15,000
	K_2HPO_4	1,040
B	$MgCl_2 \cdot 6H_2O$	12,164
	$CaCl_2 \cdot 2H_2O$	4,410
	$FeCl_3$	96
	$Na_2EDTA \cdot 2H_2O$	300
	$MnCl_2$	264
C	$MgSO_4 \cdot 7H_2O$	14,700
	H_3BO_3	185.5
	$ZnCl_2$	3.270
	$CoCl_2$	0.780
	$NaMoO_4 \cdot 2H_2O$	7.260
	$CuCl_2$	0.009

Note. Ten milliliters each of three stock solutions are diluted into 1 liter to make 10-fold strength (10×) of algal nutrient solution. (American Public Health Association *et al.*, in press).

and be counted as individual fronds. A small bud may be less than 5% of the biomass of a healthy frond in a control group, yet these two fronds are considered equal. The result of using frond count as the end point is to grossly underestimate the toxic effect to duckweed. In comparison, algal response to toxicity is unlikely to exhibit such a distortion. This is a reason why results of duckweed and algal toxicity tests should be interpreted with caution.

The other and more important disadvantage is that frond count does not reflect whether the plant is alive or dead. This problem was clearly demonstrated in an experiment using industrial effluents (Wang and Williams, 1988). In the effluent, new fronds developed and died during 96 hr exposure. Live and dead fronds could not be distinguished by using the frond count method.

Cowgill and Milazzo (1989) indicated that determinations of dry biomass (constant weight at 60°C) were the least time-consuming and least subject to human error. This method, although an improvement by taking into account change in biomass as a toxic response, does not distinguish between live and dead plants.

Duckweed plants can also exhibit many symptoms when they are under stress. These symptoms include chlorosis (loss of pigment), necrosis (localized dead tissue), colony breakup, root destruction, loss of buoyancy, and gibbosity (humpback or swelling). For example, Cd is known to cause chlorosis (Seto *et al.*, 1979).

Potentially the most sensitive and accurate test end points include viable biomass and physiological activity. Examples are tests of adenosine triphosphate content, fluorescence emission (Caux *et al.*, 1988), C-14 uptake (Lockhart *et al.*, 1983), and oxygen production (Huber *et al.*, 1982). More studies are needed in this area.

Test Results

Numerous duckweed toxicity tests have been conducted. Table 4 gives test results for single compounds, including organic and inorganic substances. Table 5 shows the test results for complex mixtures.

Applications

Duckweed toxicity tests are highly versatile in the aquatic environment. The tests are applicable to lake, river, or ground water; single chemical compounds or complex effluents from industrial or municipal sources; organic or inorganic compounds; rain samples; and sediment samples (Wang, 1986a–d, 1987a, b; Wang and Williams, 1988, 1990; Hartman and Martin, 1984; Fekete *et al.*, 1976). Wang and Williams (1988, 1990) reported that duckweed was more sensitive to industrial effluents than higher plants such as cabbage and millet.

Sediment toxicity testing using duckweed is especially noteworthy because sediment samples can be tested either as aqueous extract or as sediment slurry (Wang, 1986d). Some compounds remained biologically active in the presence of soil particles. Birmingham and Colman (1983) found that both *Lemna* species and an alga, *Anabaena flos-aquae*, detected significant phytotoxicity of a herbicide, Reglone A-R, in a soil–water mixture at a 33-ppm concentration level. Hartman and Martin (1985) also reported that the presence of suspended sediment had a minor effect on the toxicity of alachlor and atrazine to *L. minor*.

Duckx

Test substances	Spe
Ba	*Lemna m*
Ba	*L. minor*
B	*L. minor*
Cd	*L. minor*
Cd	*L. valdiv*
Cd	*L. valdiv*
Cd	*L. valdiv*
$CdCl_2$	*L. polyrr*
$CdSO_4$	*L. polyrr*
$CdCl_2$	*L. pauci*
Cl	*L. minor*
Cr(VI)	*L. minor*
Cr(VI)	*L. minor*
Cr(VI)	*Spirodel*
Cu	*L. mino*
Cu	*L. valdi*
Cu	*L. mino*
Cu	*L. mino*
$CuSO_4$	*L. gibba*
$CuSO_4$	*L. gibba*
Pb	*L. mino*
Mn	*L. mino*
Ni	*L. mino*
Ni	*L. mino*
Se	*L. mino*
SO_4	*L. mino*
Zn	*L. mino*
AE	*L. mino*
Alachlor	*L. mino*
Atrazine	*L. mino*
Carbofuran	*L. mino*
O-Cresol	*L. gibb*
O-Cresol	*L. gibb*
CTAC	*L. min*
DEP	*L. gibb*
EG	*L. gibb*
Glyphosate	*L. min*
LAS	*L. min*
Pentachlorophenol	*L. min*
Phenol	*L. min*
Salicylic acid	*L. min*
SDS	*L. min*
2,4,6-TCP	*L. gibb*
2,4,6-TCP	*L. gibb*

Note. All IC_{50}'s (50% inhibit increase, except as noted. AE (2-ethylhexyl)phthlate; EG. Eth 2,4,6-TCP, 2,4,6-trichlorophen

[a] Flow-through test.
[b] Oxygen production as test
[c] Growth rate as test end p

The use of herbici 1981 (Nielsen and L environment. Herbic been detected in fog weed tests are an ine

TABLE 4
DUCKWEED TOXICITY TESTS OF SINGLE COMPOUNDS

Test substances	Species	Duration	IC_{50}'s	References
Ba	*Lemna minor*	4 days	24 mg/liter	Wang (1988)
Ba	*L. minor*	4	26	Wang (1986a)
B	*L. minor*	4	>60	Wang (1986a)
Cd	*L. minor*	4	0.2	Wang (1986a)
Cd	*L. valdiviana*	7	0.15	Hutchinson and Czyrska (1975)
Cd	*L. valdiviana*	14	0.3	Hutchinson and Czyrska (1975)
Cd	*L. valdiviana*	21	0.3	Hutchinson and Czyrska (1975)
$CdCl_2$	*L. polyrrhiza*	14	0.9	Charpentier *et al.* (1987)
$CdSO_4$	*L. polyrrhiza*	14	0.6	Charpentier *et al.* (1987)
$CdCl_2$	*L. paucicostata*	7	10	Nasu *et al.* (1984)
Cl	*L. minor*	4	930	Wang (1986a)
Cr(VI)	*L. minor*	4	35	Wang (1986a)
Cr(VI)	*L. minor*	14	6	Mangi *et al.* (1978)
Cr(VI)	*Spirodela polyrhiza*	14	>10	Mangi *et al.* (1978)
Cu	*L. minor*	4	1.1	Wang (1986a)
Cu	*L. valdiviana*	21	0.14	Hutchinson and Czyrska (1975)
Cu	*L. minor*	7[a]	0.119	Walbridge (1977)
Cu	*L. minor*	7[a]	0.8	Bishop and Perry (1981)
$CuSO_4$	*L. gibba*	7[c]	2.21	Davis (1981)
$CuSO_4$	*L. gibba*	7[a,c]	3.51	Davis (1981)
Pb	*L. minor*	4	8	Wang (1986a)
Mn	*L. minor*	4	31	Wang (1986a)
Ni	*L. minor*	4	0.45	Wang (1986a)
Ni	*L. minor*	4	0.30	Wang (1987b)
Se	*L. minor*	4	2.4	Wang (1986a)
SO_4	*L. minor*	4	>1,000	Wang (1986a)
Zn	*L. minor*	4	10	Wang (1986a)
AE	*L. minor*	7[a]	21	Bishop and Perry (1981)
Alachlor	*L. minor*	14	0.01	Hartman and Martin (1985)
Atrazine	*L. minor*	14	>0.1	Hartman and Martin (1985)
Carbofuran	*L. minor*	14	>10	Hartman and Martin (1985)
O-Cresol	*L. gibba*	7[a,c]	152	Davis (1981)
O-Cresol	*L. gibba*	7[c]	246	Davis (1981)
CTAC	*L. minor*	7[a]	0.1	Bishop and Perry (1981)
DEP	*L. gibba*	7[c]	2,060	Davis (1981)
EG	*L. gibba*	7[c]	17,159	Davis (1981)
Glyphosate	*L. minor*	7	2	Hartman and Martin (1984)
LAS	*L. minor*	7[a]	2.7	Bishop and Perry (1981)
Pentachlorophenol	*L. minor*	60 hr	3.2[b]	Huber *et al.* (1982)
Phenol	*L. minor*	4	>12	Wang (1986a)
Salicylic acid	*L. minor*	7	107	Wang and Lay (1989)
SDS	*L. minor*	7[a]	43	Bishop and Perry (1981)
2,4,6-TCP	*L. gibba*	7[a,c]	0.03	Davis (1981)
2,4,6-TCP	*L. gibba*	7[c]	0.13	Davis (1981)

Note. All IC_{50}'s (50% inhibition concentrations) are obtained with static tests and the test end point is frond count increase, except as noted. AE, $C_{14.5}$ alcohol ethoxylate; CATC, Cetyl trimethyl ammonium chloride; DEP, Di-(2-ethylhexyl)phthlate; EG, Ethylene glycol; LAS, $C_{11.8}$ linear alkylbenzene sulfonate; SDS, Sodium dodecyl sulfate; 2,4,6-TCP, 2,4,6-trichlorophenol.

[a] Flow-through test.
[b] Oxygen production as test end point.
[c] Growth rate as test end point.

The use of herbicides in the United States increased 280% between 1966 and 1981 (Nielsen and Lee, 1987) and resulted in their widespread distribution in the environment. Herbicides such as alachlor, atrazine, butylate, and cyanazine have been detected in fog and rain (Richards *et al.*, 1987; Glotfelty *et al.*, 1987). Duckweed tests are an inexpensive and sensitive biomonitoring tool for detecting phy-

TABLE 5
DUCKWEED TOXICITY TESTS OF COMPLEX SAMPLES

Sample	Species	Duration	IC_{50}	References
SRC-11 fuel oil	*L. perpusilla*	8 days	3.4%	King and Coley (1985)
SRC-11 fuel oil	*L. minor*	8	4	King and Coley (1985)
SRC-11 fuel oil	*L. gibba*	8	4.2	King and Coley (1985)
Raw coal distillate	*L. minor*	8	3.5	King and Coley (1985)
Raw coal distillate	*L. perpusilla*	8	3.8	King and Coley (1985)
Raw coal distillate	*L. gibba*	8	4.2	King and Coley (1985)
Coal ash leachate	*S. oligorhiza*	NA	76	Klaine (1985)
PIE 1	*L. minor*	5	20	Wang and Williams (1988)
2	*L. minor*	5	<2	Wang and Williams (1988)
PIE 1	*L. minor*	4	38	Wang and Williams (1990)
2	*L. minor*	4	49	Wang and Williams (1990)
3	*L. minor*	4	49	Wang and Williams (1990)
4	*L. minor*	4	22	Wang and Williams (1990)
5	*L. minor*	4	29	Wang and Williams (1990)
6	*L. minor*	4	91	Wang and Williams (1990)
7	*L. minor*	4	43	Wang and Williams (1990)
8	*L. minor*	4	<1.6	Wang and Williams (1990)
9	*L. minor*	4	<1.6	Wang and Williams (1990)
PIE 1	*L. minor*	5	73	Wang (unpublished)
2	*L. minor*	5	47	Wang (unpublished)

Note. All IC_{50}'s (50% inhibition concentration, %) are obtained with static tests and the test end point is frond count increase. NA, not available; PIE, pretreated industrial effluent.

totoxicity in fog and rain samples. Effects of acid rain can also be measured by using duckweed (Schindler, 1988).

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