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Note

Comparative effects of different families of herbicides on recovery potentials in *Lemna* sp.

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The effects of 9 herbicides in 5 different families on the growth of *Lemna* sp. were studied through 7-day exposure. The treated plant was transferred to fresh medium to observe recovery from inhibition. Inhibition and the recovery of growth were estimated on the basis of frond number and area, and expressed as the relative growth rate (RGR) compared with the untreated control. Patterns of RGR in exposure and recovery periods showed a tendency corresponding to the different families of herbicides. Considering the recovery potential of *Lemna* sp. from inhibition by chemicals and the large difference in RGRs between exposure and recovery periods, it is appropriate to take them into account for ecotoxicological risk assessment of chemicals.

Keywords: *Lemna* sp., herbicide, toxicity, recovery.

Introduction

Herbicides play an important role in agricultural practices, and there are more than 300 herbicides. Throughout the world, most agricultural fields are widely treated with herbicides to control weeds, and the chemicals could pollute the aquatic environments by spray drift, leaching, runoff, or accidental spills,[1,2] and herbicide residues are commonly found in surface waters although at a very low level.[3] Thus, herbicides might be major nonpoint pollutant of the land. Aquatic plant toxicity tests have been frequently conducted to determine the potential impact of contaminants on primary producers and to assess their environmental risks.[4–6] *Lemna* spp. are recommended for aquatic phytotoxicity assessment, because it grows quickly and reproduces faster than other vascular plants.[7,8]

Among studies conducted to determine the effect of pesticides on *Lemna* sp., determining EC_{50} was used to evaluate the risk of toxicants on the organism. Considering the actual situation, however, the recovery of proliferating ability after exposure to chemicals is one of the most important factors in addition to acute toxicity, but few studies have been conducted on the recovery potential.[9] Our previous study with *Lemna* sp., exposed for 7 days to sulfonylurea herbicides, showed that the EC_{50} of the plant for some sulfonylureas was lower than their Expected Environmental Concentrations (EEC) of 3–20 ppb, but recovery was possible after removal of the chemicals.[10] As there are several modes of actions by other herbicides, such as inhibition of photosystems and multiple biosyntheses, they might cause a different influence on the inhibition and recovery potentials. The main objective of this study was to assess the recovery of *Lemna* sp. after exposure to different classes of herbicides with different modes of action.

Materials and Methods

1. Chemicals and reagents

All of the herbicides tested were chosen to represent chemicals in current major agricultural use across a wide range of general classes and modes of action, and are listed in Table 1. The chemicals (99.9%, analytical standard) were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan), or Kanto Chemical Co. Inc. (Tokyo, Japan). Stock solutions (1000 ppm) were prepared in either acetone or water, and different concentrations of test solution were prepared by mixing with 20X-APP growth medium based on OECD guidelines.[8] All stock solutions were prepared just before the experiments.

2. Test organism

Fronds of *Lemna* sp. were collected from the pond in front of Shinjiko Nature Museum, Izumo, Japan. The procedures for collection and purification of *Lemna* sp. were described previously.[11] After purification, sufficient colonies were transferred aseptically from the stock culture into fresh sterile medium, and cultured for 10 days under the test condition before starting the test.

3. Exposure and recovery experiments

Lemna sp. was tested according to the draft OECD guidelines for the testing of chemicals.[8,11] The experiment was conducted for all chemicals as 7-day exposure at 0, 1, 10, 100 and 1000 ppb followed by a 7–10 day recovery in fresh medium. The tests were performed under static conditions using 9 fronds initially in each 100 ml test beaker containing 50 ml of growth medium. The beakers were covered with transparent wrapping paper with pores for aeration. After each exposure period, the 9 mother fronds were collected, washed in sterilized distilled water, and transferred to fresh medium for the recovery test. Frond numbers were counted on the third, fifth, and seventh days of the exposure and recovery periods, and on the tenth day when the recovery was slow. Inhibition and recovery of growth were estimated on the basis of frond number, which was calculated on the basis of frond

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Table 1. Herbicides used in this study

Name	Chemical Family[16]	CAS number	Mode of Action[16]
Alachlor	Chloroacetamide	15972-60-8	Inhibition of very-long-chain fatty acid biosynthesis
Atrazine	Triazine	1912-24-9	Inhibition of photosynthesis at photosystem II
Bensulfuron-methyl	Sulfonylurea	83055-99-6	Inhibition of acetolactate synthase
Cyclosulfamuron	Sulfonylurea	136849-15-5	Inhibition of acetolactate synthase
Cyhalofop-butyl	Aryloxyphenoxypropionate	122008-85-9	Inhibition of acetyl CoA carboxylase
Diuron	Urea	330-54-1	Inhibition of photosynthesis at photosystem II
Paraquat	Bipyridylium	1910-42-5	Photosystem-I-electron diversion
Simetryn	Triazine	1014-70-6	Inhibition of photosynthesis at photosystem II
Thiobencarb	Thiocarbamate	28249-77-6	Inhibition of very-long-chain fatty acid biosynthesis

area with a fraction of 0.2 compared with the standard mother frond. Each concentration was tested in triplicate.

To evaluate the ability of mother fronds to produce daughter fronds, the relative growth rate (RGR) was determined on the seventh day compared with the untreated control according to the equation below.

$$\mathrm{RGR}\,(\%) = \frac{\left(\begin{array}{c}\text{Number of daughter fronds in}\\ \text{the test vessel on 7th day}\end{array}\right)}{\left(\begin{array}{c}\text{Number of daughter fronds in the}\\ \text{control vessel on 7th day}\end{array}\right)} \times 100$$

Results and Discussion

Lemna is a vascular monocot plant, and its growth is expected to be inhibited by herbicides, which affect photosynthesis (triazine, urea and bipyridylium), fatty acid biosynthesis (aryloxyphenoxypropionate and thiocarbamate), amino acid biosynthesis (sulfonylura), or other processes, which are universally important in all types of plants.

The representative growth curve of *Lemna* sp. during the exposure and recovery periods is presented in Fig. 1. The frond number of *Lemna* sp. in the control cultures increased almost exponentially during both exposure and recovery periods, and the fronds remained green and healthy throughout the experiment. When herbicides were added, growth was affected depending on the type and concentration of the chemicals. Although growth was inhibited, no visible changes in appearance and no lethal effects were observed at any concentrations of any chemicals, except for paraquat. Higher concentrations of paraquat (100 and 1000 ppb) caused plant death with a bleaching effect. After 7-day exposure, when the fronds were transferred to fresh medium for recovery, *Lemna* sp. started to grow again even in plots where they did not grow during the exposure period. RGRs of *Lemna* sp. during exposure to herbicides and the subsequent recovery are summarized in Fig. 2.

Five typical patterns were observed as follows: (1) Cyhalofop-butyl and thiobencarb inhibited growth moderately even at 1000 ppb, and growth recovered to more than 70% RGR. (2) Atrazine inhibited growth completely at 1000 ppb, but 76% RGR was observed in recovery. (3) Simetryn, alachlor and diuron inhibited growth less than 16% RGR at 100 ppb, and slight improvement was observed in recovery with 29–40% RGR. (4) Paraquat with 86% RGR in exposure at 10 ppb caused death at 100 ppb, as mentioned above. (5) Bensulfuron-methyl and cyclosulfamuron showed higher toxicity with 24% RGR at 10 ppb and 48% RGR at 1 ppb, respectively, but recovery was possible even at 1000 ppb with 57% RGR for bensulfuron-methyl and with 71% RGR at 10 days during the recovery period (data not shown) for cyclosulfamuron.

Patterns of RGRs in the exposure and recovery periods showed a tendency corresponding to the mode of action of the herbicides. Cyhalofop-butyl and thiobencarb (fatty acid biosynthesis inhibitor) were relatively nontoxic, and *Lemna* sp. exhibited rapid recovery as well as the untreated control even at 1000 ppb. Triazine and urea herbicides (inhibitor of photosynthesis at photosystem II) showed moderate toxicity among the herbicides used, and moderate recovery was observed. Paraquat (electron flow modulator at photosystem I) caused death above the critical concentration, and no recovery was observed. Sulfonylureas (acetolactate synthase inhibitor) showed the highest toxicity, but the recovery potential of *Lemna* sp. from inhibition by the herbicides was greater than with other types of herbicides. Although alachlor has the same mode of action as thiobencarb (Table 1), their patterns were different. It was suggested that other factors than the mode of action also determine toxicity with exposure and the recovery potential.

The differences between RGRs during exposure and recovery periods may be due to different abilities of the plant to metabolize and exclude individual chemicals. Differences in the diffusion of herbicides across the cell membrane by passive transport may also influence the recovery potential. In addition, differences in the mode of action of herbicides may influence physiological properties in the recovery of *Lemna* sp., but the mechanisms have not been examined yet.

Although algae are often used for ecotoxicological studies as a test organism, it is reasonable to include different organisms with different sensitivity for risk assessment. In relation to our study, Saenz *et al.* examined the toxicity of paraquat to *Scenedesmus*

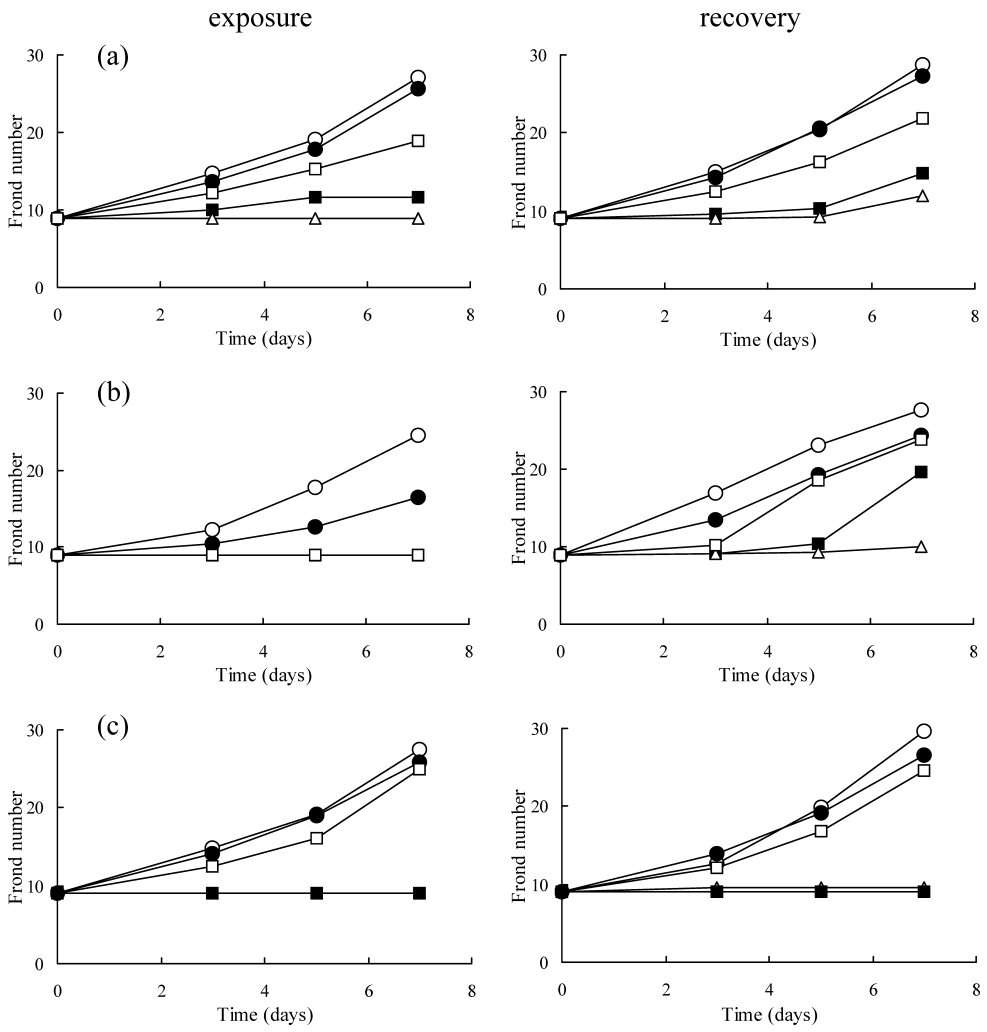

Fig. 1. Frond growth of *Lemna* sp. with 7-days exposure and recovery period in fresh medium after exposure to (**A**) alachlor, (**B**) cyclosulfamron, and (**C**) paraquat at 0 (○), 1 (●), 10 (□), 100 (■), and 1000 ppb (△).

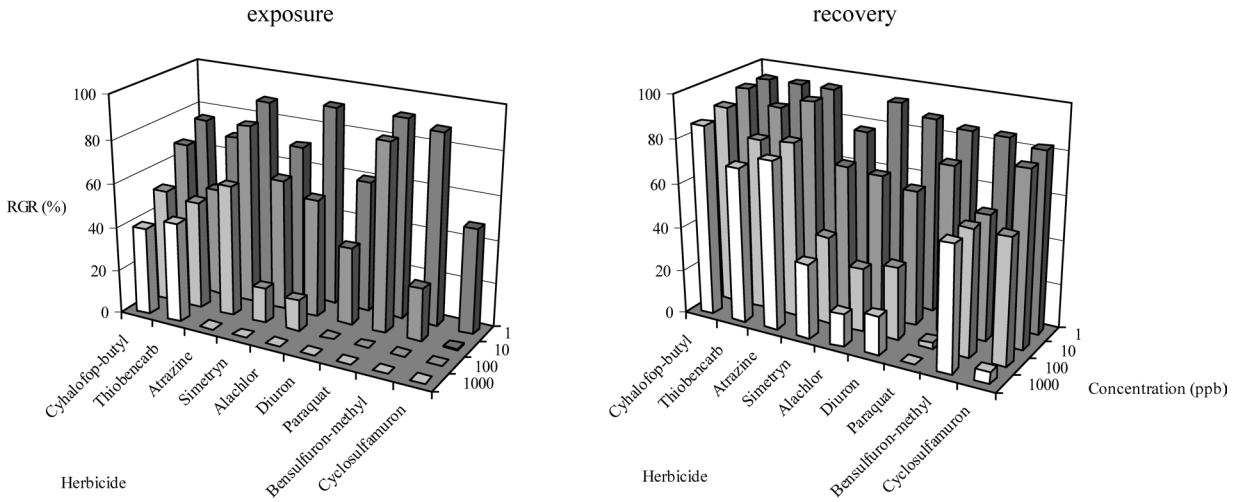

Fig. 2. Relative growth rate (RGR) of *Lemna* sp. with 7-day exposure and recovery in fresh medium after exposure to nine herbicides at 0, 1, 10, 100, and 1000 ppb.

quadricauda and its recovery.[12] In their study, EC_{50} for *S. quadricauda* was 220 ppb, and recovery of growth after exposure to 3.2 ppm of paraquat was possible. In the case of *Lemna* sp., EC_{50} of paraquat was between 10 and 100 ppb in our study and 51 ppb in Fairchild *et al*.,[4] but no growth was observed after exposure to 100 ppb. A similar result was obtained from another study with *Lemna* sp. at 100 ppb of paraquat.[13]

Recovery potential should be taken into account for ecotoxicological risk assessment of chemicals, considering the large difference in RGR between exposure and recovery periods in some chemicals. We also demonstrated previously that growth of *Lemna* sp. was possible after exposure to some sulfonylureas at concentrations below their EC_{50}.[10] In addition, the exposure period to chemicals should also be considered to determine their potential impact. Recent studies demonstrated that a longer period of exposure caused more serious adverse effects on *Lemna* sp.[14–15] The exposure period could affect both toxicity and recovery. In our previous study, it was concluded that sulfonylurea would not pose a significant risk to *Lemna* sp. for up to two weeks of exposure at EEC, which is larger than their EC_{50}.[10] Therefore, considering EC_{50} and recovery after different exposure periods would likely provide adequate information about the environmental risk assessment of pesticides.

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