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Note

Malathion-Tolerance and Degrading Abilities of Brackish Zooplankton, *Sinocalanus tenellus* and *Oithona davisae*

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INTRODUCTION

Pesticides applied to paddy field have frequently been detected from environmental water.[1] Their influence to aquatic organisms, especially freshwater phytoplankton and zooplankton as the primary and secondary producer, respectively, has been investigated from the view point of toxicity,[2] growth inhibition,[3,4] absorption[5] and impact on plankton community.[6-10] On the other hand, Nakamura & Mochida[11] paid attention to the function of phytoplankton as a scavenger decomposing pollution chemicals and evaluated kinetically the pesticide-degrading abilities of phytoplankton. Although Fukushima[12] has described that the pesticide-degrading ability of brackish and marine zooplankton should not be neglected, little information on the tolerance of zooplankton to pesticides and their pesticide-degrading ability is available.

In the present study, these abilities of copepoda calanoida, *Sinocalanus tenellus* and copepoda cyclopoida, *Oithona davisae*, both of which were dominant zooplankton and the vital secondary producer in brackish lakes Shinjiko and Nakanoumi, respectively, were evaluated by the 50% lethal concentration (LC_{50}) for malathion, the rate constant of malathion-disappearance caused by the zooplankton and bioconcentration factor (BCF) of malathion for the zooplankton.

MATERIALS AND METHODS

1. Zooplankton

S. tenellus and *O. davisae* are dominant zooplankton in brackish lakes Shinjiko and Nakanoumi, respectively. Zooplankton samples collected by slowly towing a NXX 15 plankton net in each lake were carefully transferred to a polychloroethylene tank. The adults were collected by filtering the samples on nylon net (98 μm opening). The collected zooplankton was rinsed with each lake water sterilized by being put through a 0.2 μm membrane filter (Toyo Roshi Kaisha, Ltd.) and immediately tested.

2. Pesticide

Malathion (*S*-1,2-bis(ethoxycarbonyl)ethyl *O,O*-dimethyl phosphorodithioate, technical grade, 97.5%), was donated by Sumitomo Chemical Co., Ltd. and used without purification. Other reagents were purchased from Wako Pure Chemicals.

3. Measurement of 50% Lethal Concentration of Malathion for Zooplankton

An acetone solution containing malathion (530 to 4910 ppm) was prepared and 0.1 ml of the solution was added to a 200 ml-Erlenmeyer flask. After drying up the acetone, *S. tenellus* or *O. davisae* (43–56 and 25–30 individuals, respectively) with 100 ml of each sterilized lake water was added to the flask. After the zooplankton was exposed to various concentrations of malathion for 24 hr, the dead zooplankton was counted. The zooplankton that remained motionless when touched gently with a glass rod was regarded as dead. The LC_{50} values of malathion for the zooplankton were evaluated from their mortalities by probit analysis.[13]

4. Measurement of Disappearance Rate of Malathion

The disappearance rates of malathion were determined in the presence of the zooplankton. An acetone solution (0.1 ml) of malathion (less than 50 ppm) was added to a 200-ml Erlenmeyer flask. After drying up the acetone, *S. tenellus* or *O. davisae* (2430 and 4900 individuals, respectively) with 100 ml of each sterilized lake water was added to the flask. The flask stoppered with a silicone-gum plug was incubated at 20°C under continuous illumination at 770 lux reciprocal shaking. As a control, the zooplankton-free sterilized lake water containing the pesticide was incubated under the same conditions. An 8.0-ml portion of each incubating solution was pipetted out at an appropriate time interval to

count the number of zooplankton and to determine the residual amount of malathion.

5. Measurement of Bioconcentration Factor of Malathion for Zooplankton

Measurement of bioconcentration factor (BCF) of malathion was carried out using the zooplankton exposed to the pesticide for three days. The lake water in the presence of zooplankton, used for measurement of disappearance rates of the pesticide, was incubated under the same conditions for three days. A 50.0-ml portion and a 5.0-ml portion of the solution were pipetted out and the concentrations of malathion within the zooplankton and in the solution were determined, respectively. Three 1.0-ml portions of incubating solution were also pipetted out to count the number of the zooplankton. BCF was evaluated as the ratio of the concentration in the zooplankton and the medium.

6. Determination of Malathion

The sampled solution (5.0 ml) was centrifuged at 4000 rpm (Kubota Corporation KR/702) for 10 min. To 4 ml of the supernatant was added 10 ml of 10% NaCl aqueous solution and it was extracted with three 15-ml portions of CH_2Cl_2. The CH_2Cl_2 layers were combined, dried over anhydrous Na_2SO_4 and evaporated to dryness. To the residue, 1.0 ml of acetone solution containing propaphos (0.18 ppm) was added as an internal standard.

The another sampled solution (50.0 ml) containing the zooplankton was filtered through a 1.0 μm membrane filter (Toyo Roshi Kaisha Ltd.) and rinsed with 10 ml of each sterilized lake water. The zooplankton on filter was ground in a mortar, to which Al_2O_3 was added, with a pestle and extracted with five 3-ml portions of CH_2Cl_2. The CH_2Cl_2 layers were combined, dried over anhydrous Na_2SO_4 and evaporated to dryness. The internal standard solution (3.0-ml) was added to the residue.

Two microliters of these solutions were injected into GLC (Shimadzu Corporation GC-7A) equipped with a FTD (Rb) and the amount of malathion was determined by the peak height ratio method. The GLC conditions: Column; 2% Silicone DC QF-1 on 80/100 mesh Gaschrom Q, 3 mmϕ × 1 m, glass column. Temperature; column 210°C, injection and detector 250°C. Gas flow; He (carrier gas) 30, H_2 2.4, air 185 ml/min.

7. Kinetic Analysis of Disappearance Rate of Malathion

First-order kinetic analysis[11] was used to evaluate the disappearance rate of malathion caused by zooplankton.

The disappearance rate of malathion in the brackish-water was calculated by the following equations:

$$-d[S]/dt=(k_0+k_Z\cdot[Z])\cdot[S] \quad (1)$$

$$\ln([S]/[S]_0)=-k_0\cdot t-k_Z\cdot[Z]\cdot t \quad (2)$$

where the concentration of malathion in medium, concentration of zooplankton, the rate constant of disappearance of malathion caused by zooplankton and that of control are expressed by $[S]$, $[Z]$, k_Z and k_0, respectively.

All analyses were carried out by using a computer program on NEC · PC9801 RX.

RESULTS AND DISCUSSION

The mortalities of *S. tenellus* and *O. davisae* at malathion concentrations less than 1.0 ppm were very low (Fig. 1). The survival rates of *S. tenellus* and *O. davisae* even at 0.64 ppm, which is *ca.* 10 times higher than the LC_{50} (0.065 ppm)[2] of malathion for *Moina macrocopa* used frequently in toxicity test of chemicals, were 92.9 and 100.0%, respectively. Thus, the brackish zooplankton showed much higher tolerance abilities to malathion than freshwater zooplankton *M. macrocopa*. This also suggests that the former zooplankton may have a high malathion-degrading ability. The LC_{50} values of malathion for *S. tenellus* and *O. davisae* were 1.95 ppm and >4.91 ppm, respectively.

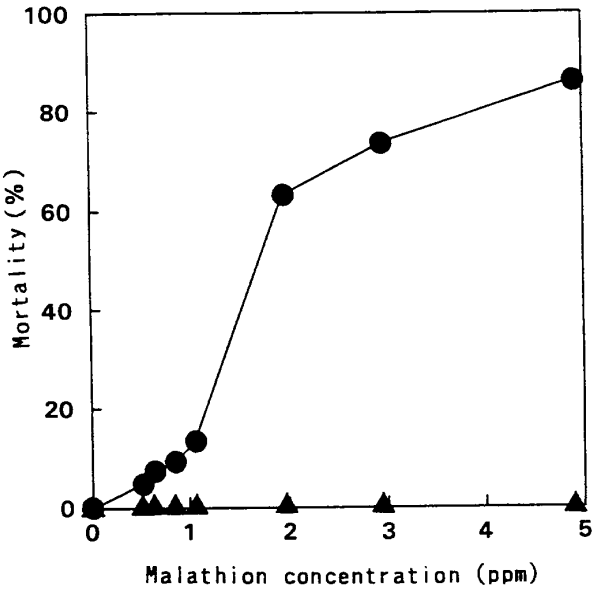

Fig. 1 Toxicity of malathion to *Sinocalanus tenellus* and *Oithona davisae*.

●: *S. tenellus*, ▲: *O. davisae*.

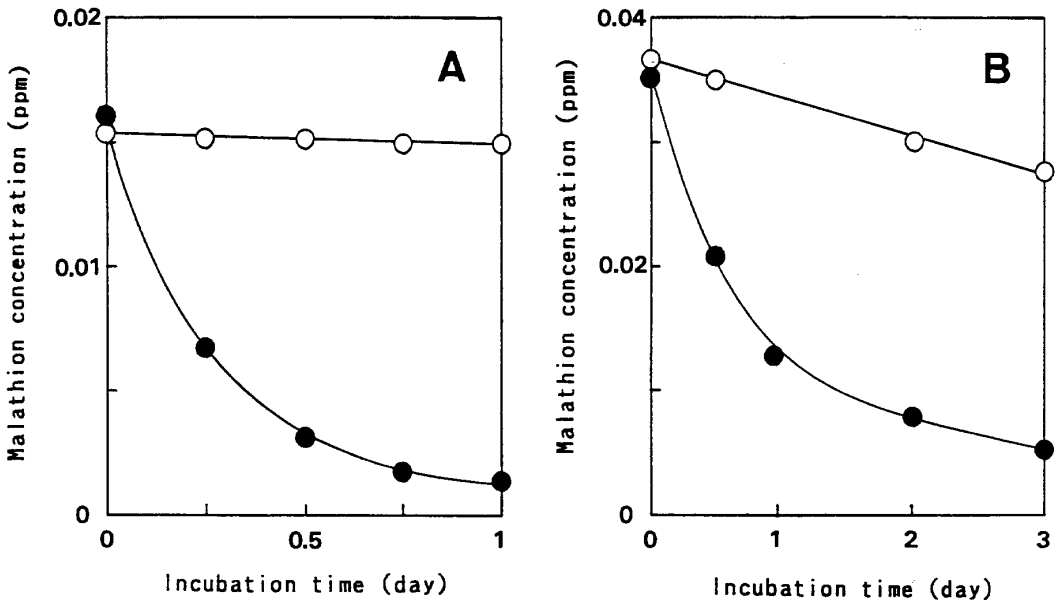

Fig. 2 Disappearance of malathion in the presence (●) and absence (○) of *Sinocalanus tenellus* (A) and *Oithona davisae* (B).

Table 1 Rate constants for the disappearance of malathion in the presence of *Sinocalanus tenellus* and *Oithona davisae*.

Zooplankton	k_0(SD)[a)]	k_Z(SD)[b)]
S. tenellus	6.7 (0.8)	8.5 (0.8)
O. davisae	26.8 (0.6)	3.7 (0.0)

a) 10^{-2} day^{-1}.
b) 10^{-2} (individual/ml)$^{-1}$ day^{-1}.

The disappearance rates of malathion were investigated by using *S. tenellus* and *O. davisae* at a concentration much lower than their LC_{50} values. The disappearance of malathion in the presence of *S. tenellus* or *O. davisae* was more rapid than that in the absence of the zooplankton (Fig. 2). The rapid disappearance is probably due to their high pesticide-uptake abilities and pesticide-degrading abilities. The disappearance can be analyzed by first-order kinetics. The rate constants (k_Z's) for the disappearance on individual basis caused by *S. tenellus* and *O. davisae* were calculated by using Eq. (2). k_Z's for *S. tenellus* and *O. davisae* were 8.5×10^{-2} and 3.7×10^{-2} (individual/ml)$^{-1}$ day^{-1}, respectively (Table 1).

BCF (4530) of malathion for *S. tenellus* was slightly higher than that (3900) for *O. davisae*. The ratios of accumulation amounts within these zooplankton species to total disappearance amounts were evaluated to be 0.5 and 0.2%, respectively. It was considered that 81.3 and 70.5% of the total disappearance amounts must have been caused by the *S. tenellus* and *O. davisae*, respectively. The metabolites of malathion were not detected from the zooplankton nor from the solution on FTD-GLC.

Differences in pesticide-tolerance and degrading abilities may be explained by evaluating activities of pesticide-degrading enzyme within the zooplankton. On the other hand, there were great differences in the k_0 values of malathion between the lake water of Shinjiko and Nakanoumi (Table 1). The degradation of malathion was 4 times faster in the water of lake Nakanoumi than in the water of lake Shinjiko. This difference must have been caused by difference of pH in water. The pH of water in lake Nakanoumi (7.5) was higher than that in lake Shinjiko (6.6).

Our study demonstrated that two brackish copepods *S. tenellus* and *O. davisae* showed higher tolerance to malathion than that of freshwater cladoceran *M. macrocopa* and that the former zooplankton had the abilities to remove malathion from the medium. Therefore, the zooplankton might play an important role as a scavenger for released pollution chemicals by using their uptake and degrading abilities. It is necessary to make clear to what extent the zooplankton contributes as a scavenger for pollution chemicals in the ecosystem. More experiments with other pesticides, along with population research of zooplankton in lakes Shinjiko and Nakanoumi, are necessary to address this question.

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要　約

汽水産動物プランクトン *Sinocalanus tenellus* および *Oithona davisae* のマラチオン耐性と分解能

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淡水産動物プランクトンの薬物耐性・消失能は化合物の安全性評価試験の結果から一般に低いと考えられるが，汽水・海産の動物プラクトンについては明確ではない．本研究では，汽水湖である宍道湖および中海から優占種としてそれぞれ分離したカイアシ類 *Sinocalanus tenellus* および *Oithona davisae* の，有機リン系殺虫剤マラチオン耐性・消失能を検討した．耐性能として薬物の半数致死濃度（LC_{50} 値）を求める一方，消失能として動物プランクトンに起因するマラチオン消失速度定数（k_z）を他の要因（光および湖水自身）に起因するそれ（k_0）と区別して動力学的に算出するとともに，動物プランクトンのマラチオン生物濃縮係数（BCF）を測定した．得られた LC_{50} 値は *S. tenellus* および *O. davisae* に対してそれぞれ 1.95 および >4.91 ppm であり，すでに知られている淡水産枝角類 *Moina macrocopa*（0.065 ppm）よりいずれも高い耐性能を示した．これら動物プランクトン存在下のマラチオンの消失は速やかで *S. tenellus* および *O. davisae* に起因する k_z はそれぞれ 8.5×10^{-2} および 3.7×10^{-2} (individual/ml)$^{-1}$day^{-1} と評価された．また BCF はそれぞれ 4530 および 3900 であり，動物プランクトンの体内蓄積マラチオン量はマラチオン全消失量のそれぞれ 0.5% および 0.2% であり，全消失量のそれぞれ 81.3% および 70.5% が代謝・分解により消失していると考えられた．この結果は，環境水中に流入した微量の農薬に対しては動物プランクトンが分解・消失要因として寄与していることを示唆するものと考えられた．