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Simple Aquatic Microcosm for Ecotoxicity Screening at the Community Level

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Ecosystems consist of various organisms with different physiological properties and sensitivities to toxic agents one another, and have complex interactions such as competition, predation and association among those organisms. Ecological effects at the community level cannot therefore be deduced from the results of single-species tests as shown in the case of ultraviolet radiation (Bothwell et al. 1994) or some chemicals (Mosser et al. 1972; Hutchinson and Czyrska 1975; Taub 1976; Wilkes 1978; Crow and Taub 1979). For example, after one species has been damaged directly with a toxic agent, another species resistant to the agent may be also affected secondarily by the disappearance of interaction with that damaged species.

Microcosms are experimental ecosystems constructed in the laboratory, and are expected to make it possible to evaluate ecotoxicity at the community level. Microcosm toxicity tests are therefore appearing in the literature in increasing numbers, and the several protocols have been drafted (Giddings 1983). However, most of the microcosms are too complex to screen ecotoxicity of enormous chemicals. The authors therefore try to apply a simple aquatic microcosm to ecotoxicity screening. In this study, the microcosm and its pure-culture systems were exposed to manganese to examine whether the microcosm responds to toxic agents at the community level.

MATERIALS AND METHODS

A microcosm used in this study was developed by Kawabata et al. (1995). It consists of flagellate algae *Euglena gracilis* Z as a producer, ciliate protozoa *Tetrahymena thermophila* B as a consumer and bacteria *Escherichia coli* DH5α as a decomposer. Each organism is axenic. The culture medium is a half strength #36 Taub and Dollar's salt solution (Taub and Dollar 1968) supplemented with 500 mg/L proteose peptone (Difco Laboratories, Detroit, MI, USA) instead of $NaNO_3$. This medium contains only 0.27 mg/L manganese. The microcosm is aseptically constructed by inoculating the organisms from stock cultures to the sterilized medium, and then statically cultured in an incubator with fluorescent lamps under a 2500 lx and a 12 h light-dark cycle at

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25℃.

In the microcosm the population change of each organism reaches a steady state after 50 days as a result of interspecies interactions. All species can co-exist in the microcosm for as long as one year. Each organism can be cultured alone in the same medium and conditions as the microcosm. However, *T. thermophila* cultured alone can exist for only 20 days without reaching a steady state, and neither *Eu. gracilis* nor *E. coli* cultured alone can exist so stably as they do in the microcosm (Matsui et al. 2000).

The microcosm is maintained with energy of proteose peptone in the early stage of culture. After exhaustion of proteose peptone, it is maintained with energy which *Eu. gracilis* fixes by photosynthesis. Each species is supported with metabolites or the breakdown products of the other two species. *T. thermophila* cannot exist without *E. coli*, because *T. thermophila* grazes *E. coli* as its staple food (Matsui et al. 2000). These suggest that the microcosm simulates detritus food chain known as the microbial loop and important process, i.e., photosynthesis, of grazing food chain. The microcosm is therefore considered to simulate basic process in aquatic microbial communities. Actually, a study using the microcosm could propose a hypothesis that dissolved DNA was produced by predation of bacteria by ciliates (Kawabata et al. 1998), which was validated by a field study in a hypereutrophic pond (Ishii et al. 1998).

The microcosm, *E. coli*, *Eu. gracilis* and *T. thermophila* pure-culture systems were constructed in 250 mL polypropylene bottles with screw caps (Nalge Nunc International, Rochester, NY, USA) containing 150 mL culture medium, respectively. The microcosm and *E. coli* pure-culture systems were exposed to manganese on the 102nd day after the beginning of the culture, and *Eu. gracilis* pure-culture systems were exposed on the 105th day. As for *T. thermophila* pure-culture systems, *T. thermophila* was inoculated to the microcosm medium to which manganese has been added, because *T. thermophila* cultured alone can exist for only 20 days without reaching a steady state. Manganese was added to each system in the form of $MnCl_2$ solution at nominal concentrations of 5.5, 27, 55, and 550 mg total Mn/L. The same volume of distilled water was added to each system for controls. There were three replicates for each treatment. The population density of each organism was measured after exposure to manganese. The population density of *Eu. gracilis* and *E. coli* was measured by the colony counting method of Nair and Netrawali (1979) and of Kawabata et al. (1995), respectively. The population density of *T. thermophila* was measured microscopically.

RESULTS AND DISCUSSION

Figure 1 shows the changes in the population densities of the three species in the microcosm after exposure to manganese. In controls, the populations of each species remained almost constant for the duration of the experiment. At 5.5 mg/L manganese, the populations of *E. coli* temporarily decreased just after

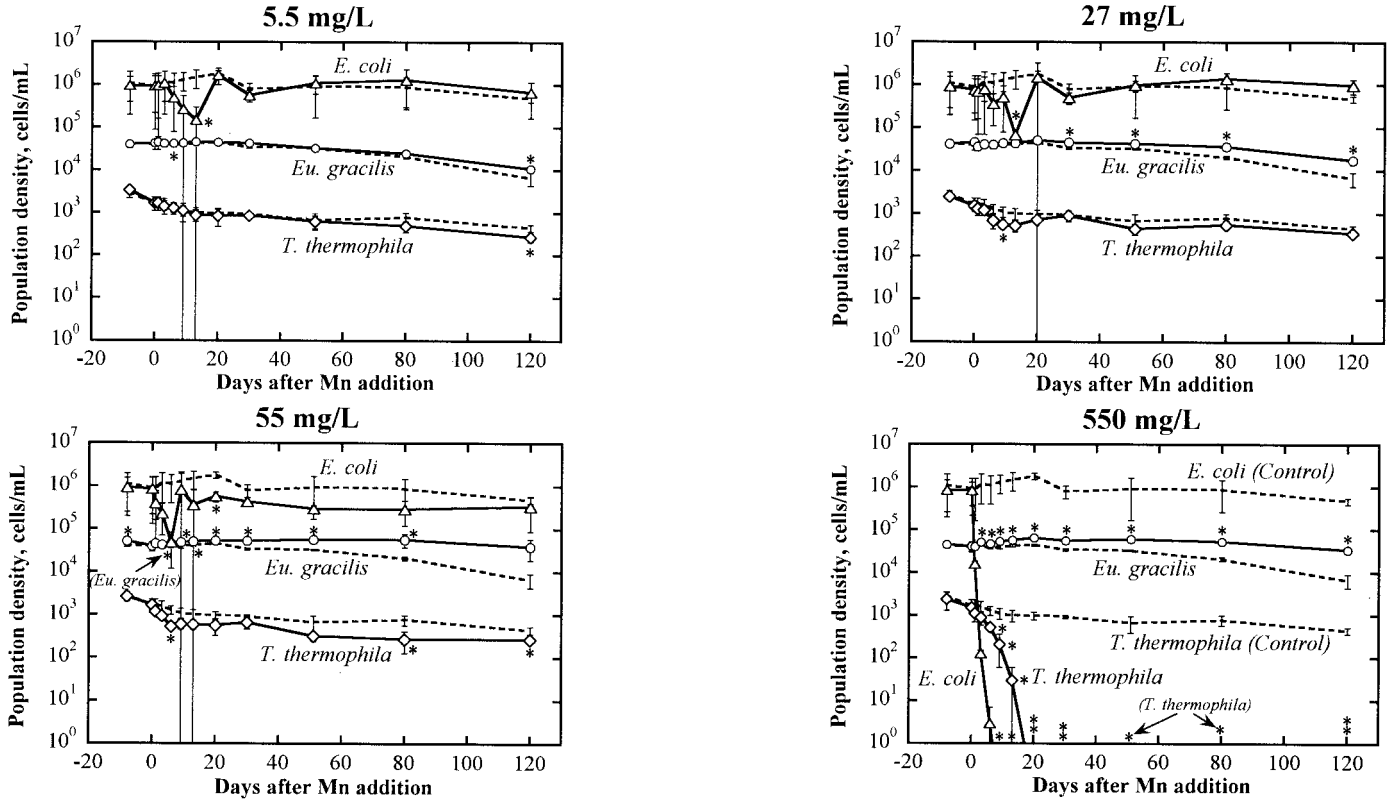

Figure 1. Effects of manganese on the populations in the microcosm. Solid lines represent results of the microcosm exposed to manganese. Broken lines represent results of controls. Values are the mean of three replicates. Error bars are standard deviations. Asterisks indicate statistically significant differences from controls ($p<0.05$, Student's t-test).

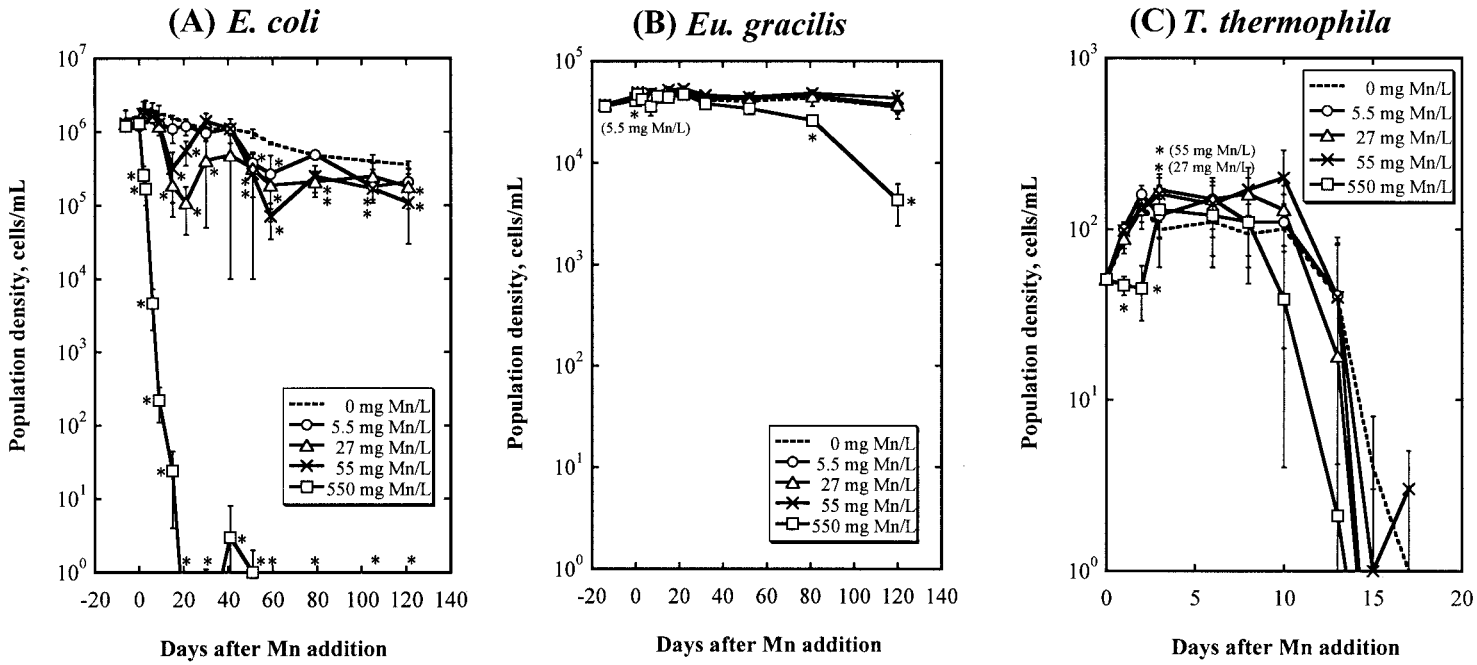

Figure 2. Effects of manganese on the populations of pure-cultured E. coli (A), Eu. gracilis (B) and T. thermophila (C). Values are the mean of three replicates. Error bars are standard deviations. Asterisks indicate statistically significant differences from controls ($p<0.05$, Student's t-test).

manganese addition. The populations of *Eu. gracilis* and *T. thermophila* were not affected. At 27 and 55 mg/L, the populations of *E. coli* temporarily decreased just after manganese addition. The populations of *Eu. gracilis* were slightly larger than controls. The populations of *T. thermophila* were not affected significantly. At 550 mg/L, *E. coli* first died out, and then *T. thermophila* also died out. The populations of *Eu. gracilis* were slightly larger than controls.

Our previous study (Fuma et al. 1998b) certified that the microcosm exposed to various toxic agents generally showed the following dose-response patterns: (1) no damage, i.e., no population decrease in any species; (2) temporary damage, i.e., temporary population decrease in some species; (3) severe damage, i.e., extinction of one or two species; and (4) complete destruction, i.e., extinction of all species. According to this classification, the results described above indicate that up to 55 mg/L manganese temporarily damaged the microcosm, and 550 mg/L manganese severely damaged it.

The population change of each organism in the microcosm reaches a steady state 50 days after beginning of the culture (Kawabata et al. 1995). This means that at least 50 days are required as pre-exposure time when toxicity to the steady-state microcosm is evaluated. However, this pre-exposure time can be shortened if the microcosm is continuously synthesized in the laboratory. If so, the steady-state microcosm can be chosen among various stage of the microcosm whenever toxicity tests are conducted. It is thought that continuous synthesis of the microcosm is not so inefficient in the laboratory where toxicity of enormous chemicals is routinely tested, because the microcosm can be readily synthesized and cultured (Kawabata et al. 1995; Matsui et al. 2000). In this study, the population density of each organism in the microcosm was measured over 120 days after exposure to manganese. However, shorter observation time may be sufficient for actual toxicity screening, because almost all the effects could be observed within 30 days after exposure to each concentration of manganese (Fig. 1).

Figure 2(A) shows the population changes of *E. coli* in the pure-culture systems after exposure to manganese. In controls, the populations continued to slightly decrease for the duration of the experiment. At 5.5, 27 and 55 mg/L, the populations slightly fluctuated, and sometimes became significantly smaller than controls. The degrees of these declines in the populations did not depend on the concentrations of added manganese. In contrast, as described above, the same concentrations of manganese temporarily decreased the populations of *E. coli* in the microcosm just once after manganese addition (Fig. 1). It is considered that these differences in responses of *E. coli* to manganese between the microcosm and pure-culture systems arose from community-level responses in the microcosm, though the mechanism is unknown. At 550 mg/L, pure-cultured *E. coli* decreased drastically after exposure, and almost died out on the 21st day after exposure (Fig. 2(A)).

Figure 2(B) shows the population changes of *Eu. gracilis* in the pure-culture systems after exposure to manganese. In controls, the populations remained constant for the duration of the experiment. At 5.5, 27 and 55 mg/L, they were not affected. At 550 mg/L, they were the same as controls until the 52nd day after exposure, but after that, they became smaller than controls. In contrast, at 27, 55 and 550 mg/L, the populations of *Eu. gracilis* in the microcosm slightly increased compared with controls (Fig. 1). This population increase of *Eu. gracilis* in the microcosm might be indicative of some change in available resources or nutrients within the system, though there is no evidence for this hypothesis. It is also presumed that in the microcosm exposed to 550 mg/L manganese, the possible decrease of *Eu. gracilis*, which was expected from the results of the pure-culture system, might be prevented by community-level detoxification such as follows: (1) *T. thermophila* or *E. coli* decreased manganese concentrations in the medium by absorption or adsorption of manganese prior to their extinction; and (2) A chemical form of added manganese was transformed to less toxic one. That is, Mn^{2+} was chelated with metabolites or breakdown products of *T. thermophila* or *E. coli*.

Figure 2(C) shows the population changes of *T. thermophila* in the pure-culture systems exposed to manganese. In controls, the populations increased until the 2nd day after inoculation, remained constant from 2nd to 10th day, decreased after 10th day, and died out on the 17th day. At 5.5, 27 and 55 mg/L, the populations were not affected significantly. At 550 mg/L, the growth of *T. thermophila* was inhibited in the beginning of the culture. However, the populations became the same as the controls on the 3rd day, and were maintained at the control levels until the 8th day. After that, they tended to be smaller than controls, though this decline was not statistically significant.

In the microcosm exposed to 550 mg/L manganese, *E. coli* first died out, and then *T. thermophila* also died out, as described above (Fig. 1). This extinction of *E. coli* can be considered to be direct effects of manganese, because *E. coli* cultured alone also died out at the same concentration of manganese (Fig. 2(A)). On the other hand, the extinction of *T. thermophila* in the microcosm can be considered to be secondary effects of manganese, because the populations of *T. thermophila* cultured alone were not significantly affected by 550 mg/L manganese (Fig. 2(C)). That is, it is thought that 550 mg/L manganese extinguished *T. thermophila* in the microcosm by extinguishing *E. coli*, because *T. thermophila* in the microcosm grazes *E. coli* as its staple food, and *T. thermophila* cannot exist in the microcosm medium without *E. coli* for more than 20 days (Matsui et al. 2000). The same mechanism accounted for decrease or extinction of *T. thermophila* in the microcosm exposed to 500 or 1000 Gy γ-rays (Fuma et al. 1998a), pH4 acidification (Fuma et al. 1998b) and 6.4 mg/L copper (Fuma et al. *unpublished data*), respectively. This suggests that external perturbations to natural aquatic ecosystems may damage some protozoa not only directly but also secondarily by damaging bacteria on which the protozoa feed.

The microcosm used in this study has interspecies interactions which support the population of each species and the structure of the whole system (Matsui et al. 2000). As described above, some species in the microcosm responded to manganese differently from those in pure-culture systems due to secondary effects and community-level detoxification in the microcosm. This suggests that the microcosm can detect community-level effects as well as direct effects of toxic agents despite its simplicity. Additionally, the microcosm can be readily synthesized only by inoculating the organisms from stock cultures to the medium (Kawabata et al. 1995; Matsui et al. 2000). It has good stability (Kawabata et al. 1995) and repeatability (Matsui et al. 2000). It simulates basic process in aquatic microbial communities. Organisms constituting the microcosm are genetically and physiologically studied well (Kawabata et al. 1995), which makes it possible to evaluate genetic and physiological effects in the future. These characteristics indicate that the microcosm is useful for screening toxicity to aquatic microbial communities.

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