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# Ecological Evaluation of Gadolinium Toxicity Compared with Other Heavy Metals Using an Aquatic Microcosm

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Gadolinium (Gd) is a member of a group of rare earth metals known as lanthanides. It has been used for superconductors, magnets, fluorescent materials, electric materials, glass additives and so on (Hirano and Suzuki 1996; Ohmachi 1993). It has been also used as a paramagnetic contrast-enhancing agent in nuclear magnetic resonance imaging (MRI) (Hirano and Suzuki 1996). It is possible that ecosystems will be damaged by the increasing industrial and medical use of gadolinium. However, there are few trials for ecological evaluation of gadolinium toxicity, especially at the community-level. This paper therefore investigated effects of gadolinium on an aquatic microbial microcosm and its pure-culture systems. The aim was: (1) to certify whether some effects observed in the microcosm exposed to gadolinium were community-level responses; and (2) to evaluate ecotoxicity of gadolinium to aquatic microbial communities compared with other heavy metals.

## 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 $NaNO_3$. The microcosm is aseptically constructed by inoculating the organisms into 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 25℃.

The population change of each organism in the microcosm reaches a steady state 50 days after inoculation as a result of interactions between the species (Kawabata et al. 1995). 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 dies out without reaching a steady state, and neither *Eu. gracilis* nor *E. coli* cultured alone can exist so stably as they do in the microcosm. There is good repeatability in these population changes in the microcosm and pure-culture systems, respectively (Matsui et al. 2000).

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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* exists mainly by grazing *E. coli* (Matsui et al. 2000). These suggest that the microcosm contains detritus food chain known as the microbial loop and important process, i.e. photosynthesis, of grazing food chain. The microcosm is therefore considered to mimic essential process in aquatic microbial communities. Actually, a study using this 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). This microcosm is also available for screening community-level toxicity to aquatic microbial communities (Fuma et al. 2000).

The microcosm and its 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 systems were exposed to gadolinium on the 56th day after the beginning of the culture. *Eu. gracilis* pure-culture systems were exposed on the 58th day. *E. coli* pure-culture systems were exposed on the 59th day. As for *T. thermophila* pure-culture systems, *T. thermophila* was inoculated to the microcosm medium to which gadolinium has been added. Gadolinium (atomic weight: 157.25) was added to each system in the form of $GdCl_3$ solution at nominal concentrations of 50, 100, 300 and 1000 μmol total Gd/L (7.9, 15.7, 47.2 and 157 mg/L), respectively. The same volume of distilled water was added to each system for controls. There were three replicates for each treatment. The population density of *Eu. gracilis* and *E. coli* was measured by 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 gadolinium. In controls, the populations of each species remained almost constant for the duration of the experiment. At 50 μmol/L gadolinium, the populations of any species in the microcosm were not affected significantly. However, the populations of *E. coli* showed the tendency to be larger than controls, though this increase was not statistically significant. At 100 μmol/L, the populations of *E. coli* temporarily decreased compared with controls on the 7th day after exposure. This decline was not statistically significant because of variability among replicates. However, it can be regarded as significant in consideration of the trend of the population change during this period. The populations of *E. coli* recovered to the control levels on the 10th day. After the 32nd day, they showed the tendency to be larger than controls, and this population increase became statistically significant on the 122nd day. The populations of the other two species in the microcosm were not affected. At 300 μmol/L, *E. coli* almost died out shortly after exposure. The populations of *T.*

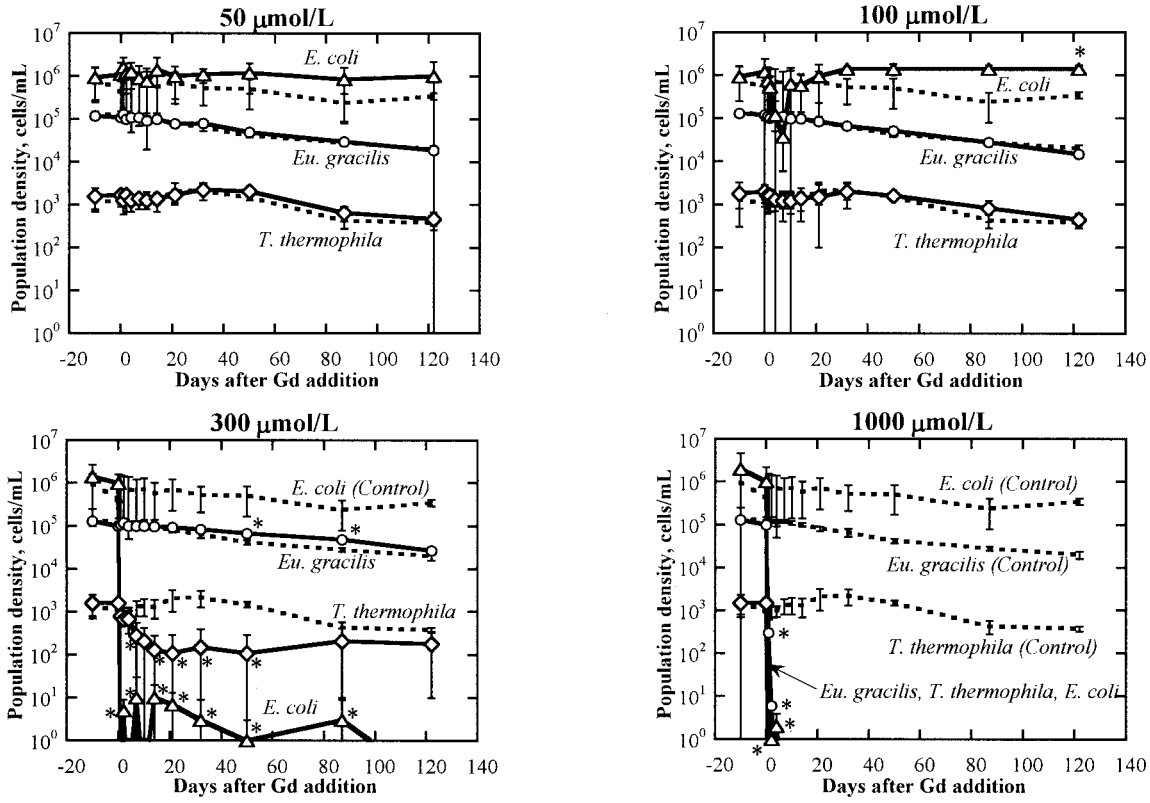


**Figure 1.** Effects of gadolinium on the populations in the microcosm. Solid lines represent results of the microcosm exposed to gadolinium. Broken lines represent results of controls. Values are the mean of three replicates. Error bars are standard deviations. Asterisks indicate statistically significant differences from controls ($p<0.05$, Dunnett's test).

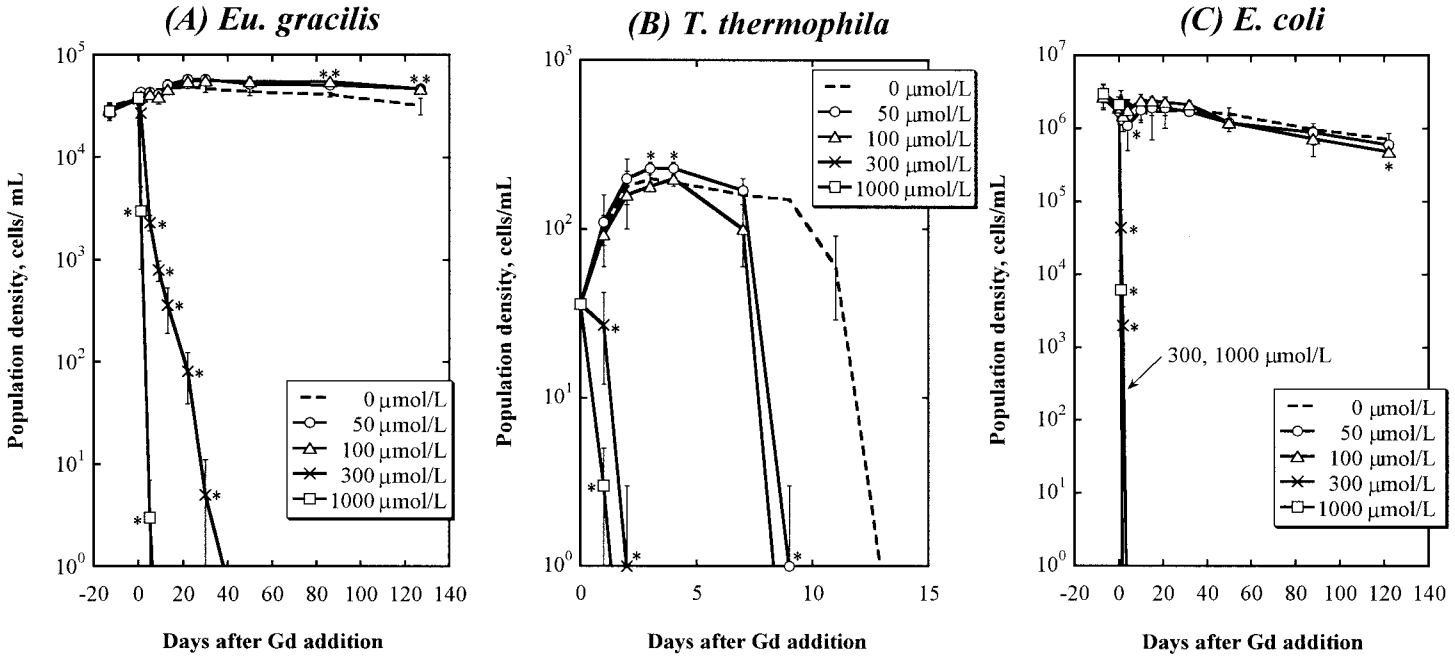


**Figure 2.** Effects of gadolinium on the populations of pure-cultured Eu. gracilis (A), T. thermophila (B) and E. coli (C). Values are the mean of three replicates. Error bars are standard deviations. Asterisks indicate statistically significant differences from controls ($p<0.05$, Dunnett's test).

*thermophila* decreased, and became smaller than controls on the 7th day. This decline was maintained until the 50th day. However, the populations of *T. thermophila* recovered to control levels on the 87th day. The populations of *Eu. gracilis* were not affected significantly. At 1000 μmol/L, all species died out shortly after exposure.

Figure 2A shows the population changes of *Eu. gracilis* in the pure-culture systems after exposure to gadolinium. In controls, the populations remained constant for the duration of the experiment. At 50 and 100 μmol/L, they were not affected until the 50th day after exposure, but after 86th day, they were maintained at higher levels than controls. This increase was slight, but statistically significant. At 300 and 1000 μmol/L, *Eu. gracilis* decreased after exposure, and died out on the 50th day and 9th day, respectively.

Figure 2B shows the population changes of *T. thermophila* in the pure-culture systems exposed to gadolinium. In controls, the populations increased until the 2nd day after inoculation, remained almost constant from 2nd to 9th day, decreased after 9th day, and died out on the 14th day. At 50 and 100 μmol/L, the populations were not affected until the 7th day. However, after that, *T. thermophila* drastically decreased, and died out earlier than controls, that is, on the 11th and 9th day, respectively. At 300 and 1000 μmol/L, *T. thermophila* did not grow at all, and died out on the 3rd and 2nd day, respectively.

Figure 2C shows the population changes of *E. coli* in the pure-culture systems after exposure to gadolinium. In controls, the populations of *E. coli* continued to slightly decrease for the duration of the experiment. At 50 and 100 μmol/L, the population change of *E. coli* was not different from controls. At 300 and 1000 μmol/L, *E. coli* decreased drastically after exposure, and died out on the 4th and 2nd day, respectively. This suggests that *E.coli* is resistant bacteria to gadolinium, because Muroma (1959) reported that 10 μmol/L gadolinium had a bactericidal effect on bacteria *Micrococcus pyogenes* var. *aureus* and *Shigella dysenteriae*.

It is not clear whether microorganisms have different sensitivities to gadolinium from mammalian cells, because there are few studies on gadolinium toxicity to microorganisms. Cytotoxicity of gadolinium is therefore compared between mammals and microorganisms constituting the microcosm. Mammalian cells seem to have a wide range of sensitivities to gadolinium. That is, it was reported that the viability of rat alveolar macrophages was significantly decreased by exposure to 3 μmol/L (Kubota et al. 2000) or 27 μmol/L (Mizgerd et al. 1996) gadolinium. Behra-Miellet et al. (1996) reported that the viability of human neutrophils was not significantly affected by exposure to 100 μmol/L gadolinium, and was significantly decreased at 1000 μmol/L. The viability of mouse cells was not affected by exposure to 190 μmol/L gadolinium as for peritoneal macrophages (Naito et al. 1996) and 1000 μmol/L as for alveolar macrophages (Kubota et al. 2000). In this study, 100 μmol/L gadolinium did not significantly decrease populations of pure-cultured algae *Eu. gracilis*, protozoa *T. thermophila*

and bacteria *E. coli* except for earlier extinction of *T. thermophila* than controls, and each species was extinguished at 300 μmol/L (Fig. 2). It is deduced from these results that microorganisms such as these taxonomic groups may be more resistant to gadolinium than sensitive mammalian cells such as rat, and may be more sensitive than resistant mammalian cells such as human and mouse.

Each species in the microcosm did not necessarily respond to gadolinium in the same manner as its corresponding species in pure-culture systems. For example, at 300 μmol/L gadolinium, *Eu. gracilis* was not affected in the microcosm (Fig. 1), while it died out in the pure-culture systems (Fig. 2A). At 50 and 100 μmol/L, *T. thermophila* was not affected in the microcosm (Fig. 1), while it died out earlier than controls in the pure-culture systems (Fig. 2B). At 300 μmol/L, *T. thermophila* did not die out in the microcosm, though it temporarily decreased compared with controls (Fig. 1). On the other hand, at the same concentration, pure-cultured *T. thermophila* did not grow at all, and died out much earlier than controls (Fig. 2B). This mitigation of gadolinium toxicity to *Eu. gracilis* or *T. thermophila* in the microcosm might arise from co-existence of other species. That is, there is the following possibility: (1) Co-existing species decreased gadolinium concentrations in the medium by absorption or adsorption of gadolinium. (2) Co-existing species transformed a chemical form of added gadolinium to less toxic one. For example, $Gd^{3+}$ might be chelated with metabolites or breakdown products of co-existing species. This is supported with the fact that adding organic ligands which can form gadolinium-organic species complex led to a great reduction of the gadolinium bioconcentration in algae (Sun et al. 1997), which is expected to reduce gadolinium toxicity to the algae.

Gadolinium at 50 or 100 μmol/L did not affect the populations of *E. coli* cultured alone (Fig. 2C). In contrast, at the same concentrations, the populations of *E. coli* in the microcosm showed the tendency to be larger than controls, though they temporarily decreased shortly after addition of 100 μmol/L gadolinium (Fig. 1). This initial temporary decrease of *E. coli* in the microcosm might arise from transformation of $Gd^{3+}$ to more toxic chemical forms by co-existing *Eu. gracilis* or *T. thermophila.* The later increase tendency of *E. coli* in the microcosm might arise from the following mechanism: Gadolinium affected metabolic activities of *Eu. gracilis* or *T. thermophila*. This caused some positive change in available resources or nutrients within the system. As a result, the populations of *E. coli* were increased. However, there is no evidence for this hypothesis. The different responses between the microcosm and its pure-culture systems described above suggest that this microcosm test could detect community-level effects as well as direct effects of gadolinium as shown in γ-rays (Fuma et al. 1998a), acidification (Fuma et al. 1998b), copper (Fuma et al. *unpublished data*) and manganese (Fuma et al. 2000), respectively.

It is considered that the steady-state microcosm exposed to gadolinium showed the following concentration-response patterns: (1) No damage at 50 μmol/L (2) Temporary damage at 100 μmol/L, i.e., temporary population decrease of some

**Table 1.** Effect levels of Gd, Cu, Ni and Mn on the steady-state aquatic microcosm.

| | Gd[1] (μmol/L) | Cu[2] (μmol/L) | Ni[2] (μmol/L) | Mn[3] (μmol/L) |
|---|---|---|---|---|
| No damage | 50 | 10 | 10 | — |
| Temporary population decrease of some species | 100 | — | — | 100–1000 |
| Extinction of some species | 300 | 100 | 100 | 10000 |
| Extinction of all species | 1000 | — | 1000 | — |

[1]This study [2]Fuma et al. (1998b) [3]Fuma et al. (2000)
— : Not examined

species (3) Severe damage at 300 μmol/L, i.e., extinction of some species (4) Complete destruction at 1000 μmol/L, i.e., extinction of all species. Similar concentration-response patterns were observed in the steady-state microcosm exposed to other heavy metals such as copper, nickel (Fuma et al. 1998b) and manganese (Fuma et al. 2000), which are widely used in various industries and whose ecotoxicity has been investigated well. Table 1 shows the concentration-response patterns in the steady-state microcosm exposed to gadolinium compared with those metals. Gadolinium requires 300 μmol/L for severe damage to the microcosm, while copper, nickel and manganese requires 100 μmol/L, 100 μmol/L and 10000 μmol/L, respectively. Therefore, it can be preliminarily evaluated that toxicity of gadolinium to aquatic microbial communities is almost the same as copper and nickel, and is by two orders of magnitude greater than manganese. Since copper and nickel have been regarded as toxic groups (Alexander and Fairbridge, 1999), gadolinium toxicity to aquatic microbial communities should be evaluated more precisely and realistically.

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