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Effects of Dysprosium on the Species-Defined Microbial Microcosm

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Dysprosium (Dy) is a member of a group of rare earth metals known as lanthanides. It has been used as magnets, magneto-optical materials and other industrial materials (Hirano and Suzuki 1996). It has been also used as an activable tracer in environmental studies (Knaus 1991), a contrast agent for nuclear magnetic resonance imaging (MRI) in the medical field (Lai and Jamieson 1993), and a control agent of nuclear reactions (Evans 1990). It is therefore possible that disposal of the products and wastes containing dysprosium will result in environmental pollution. However, there are few studies on ecotoxicity of dysprosium, especially at the community level. This paper therefore investigated effects of dysprosium 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 dysprosium were community-level responses; and (2) to evaluate ecotoxicity of dysprosium to the microcosm compared with other heavy metals.

MATERIALS AND METHODS

A microcosm used in this study was developed by Kawabata et al. (1995). 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, USA) instead of $NaNO_3$. The microcosm 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. In this microcosm, one species is supported with metabolites or the breakdown products of the other two species. However, *T. thermophila* exists mainly by grazing *E. coli*. The microcosm can be therefore considered to mimic essential process in aquatic microbial communities (Matsui et al. 2000).

The microcosm and its pure-culture systems were constructed in 250 mL polypropylene bottles with screw caps (Nalge Nunc International, USA) containing 150 mL culture medium. They were cultured in an incubator with cool white fluorescent lamps under a photosynthesis photon flux density of 28 μmol/m^2/s and a 12 h light-dark cycle at 25 ℃. The microcosm, *Eu. gracilis* and *E. coli* pure-culture systems were exposed to dysprosium in the steady state, i.e.,

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on day 72, 70 and 72 after the beginning of the culture, respectively. The *T. thermophila* pure-culture system was exposed to dysprosium in the beginning of the culture, because *T. thermophila* cultured alone died out without reaching a steady state. Dysprosium (atomic weight: 162.50) was added to each system in the form of $DyCl_3$ solution at nominal concentrations of 50, 100, 180, 300, 560 and 1000 μmol total Dy/L, respectively. There were three replicates for each treatment. After exposure to dysprosium, cell densities, Dy concentrations and pH were measured.

Cell densities of *Eu. gracilis* and *E. coli* were measured by colony counting methods of Nair and Netrawali (1979) and of Kawabata et al. (1995), respectively. Cell densities of *T. thermophila* were measured microscopically. Significant differences in the cell densities between the exposed and control systems were tested by Dunnett's test, which was performed using the StatView software version 5.0 (SAS Institute Inc., USA) at the significance level of 0.05, after the cell densities were log-transformed ($\log_{10}$ (N+1)).

For determination of Dy concentrations, the cultures were filtered with 0.2 μm pore-sized filters (Toyo Roshi, Japan). The resulting filtrate (a <0.2 μm fraction; defined as a dissolved fraction) was applied to ultrafiltration membranes to obtain a <10 kDa fraction. Dy concentrations in the <0.2 μm and <10 kDa fractions were determined by inductively coupled plasma-mass spectrometry (ICP-MS). Concentrations of Dy in the >0.2 μm and >10 kDa fractions were calculated by subtracting <0.2 μm concentrations from nominal concentrations and <10 kDa concentrations from <0.2 μm concentrations, respectively.

RESULTS AND DISCUSSION

Figure 1 shows effects of dysprosium on cell densities in the microcosm. In non-exposed controls, cell densities of each species remained almost constant for the duration of the experiment. At 50 or 100 μM dysprosium, cell densities of any species in the microcosm were not affected significantly. At 180 μM, cell densities of *E. coli* significantly decreased compared with controls, but they recovered to the control levels in the end of experiment. The other two species were not affected significantly. At 300 μM, *E. coli* died out. Cell densities of *T. thermophila* slightly decreased, but they recovered to control levels in the end of experiment. Significant effects on *Eu. gracilis* were not observed. At 560 μM, *T. thermophila* and *E. coli* died out. Cell densities of *Eu. gracilis* slightly increased. At 1000 μM, all species died out.

Figure 2A shows effects of dysprosium on cell densities in the *Eu. gracilis* pure-culture system. In controls, cell densities almost remained constant for the duration of the experiment. At 50 or 100 μM, adverse effects were not observed. At 180-1000 μM, *Eu. gracilis* died out.

Figure 2B shows effects of dysprosium on cell densities in the *T. thermophila*

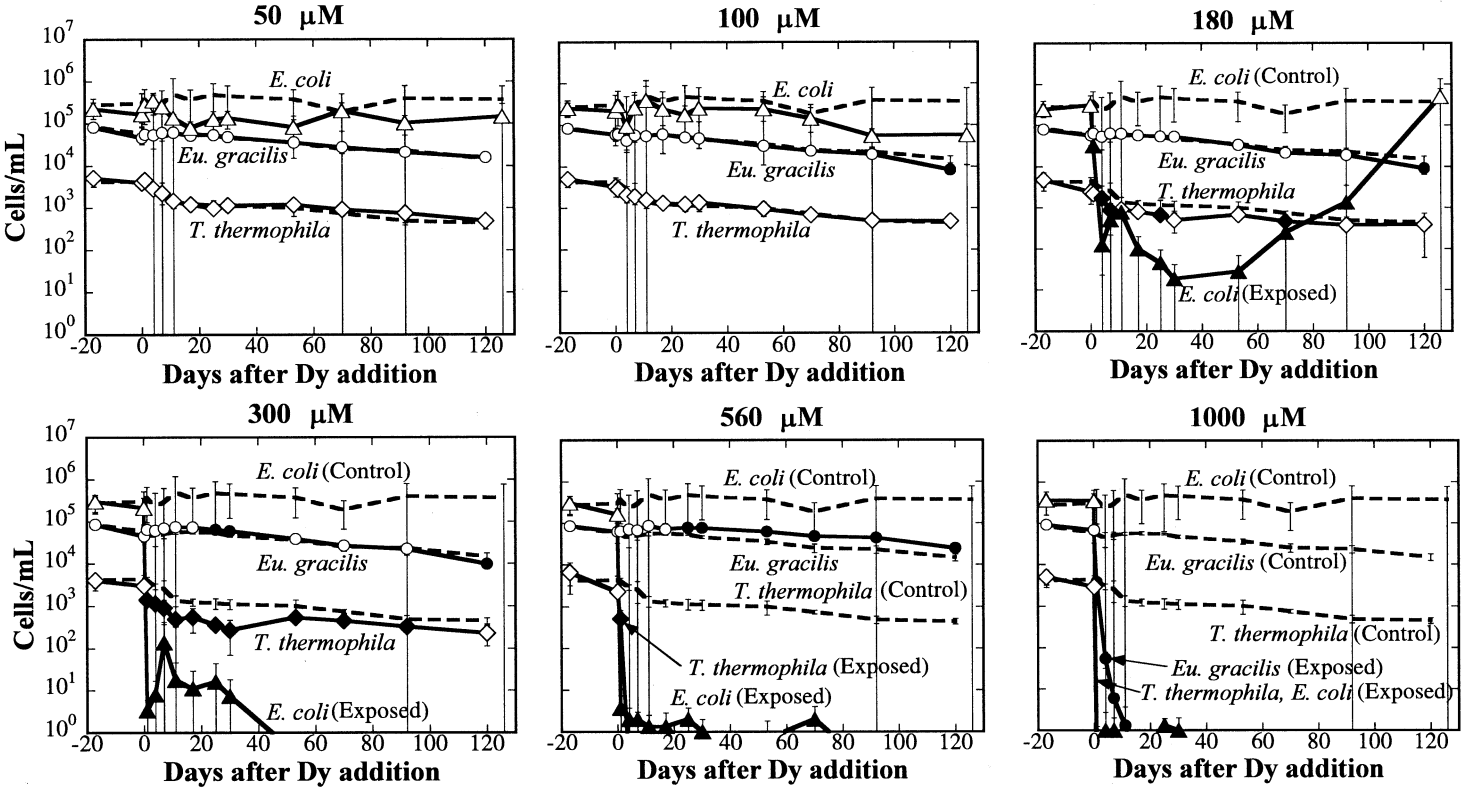

Figure 1. Effects of Dy on the microcosm. Solid lines represent cell densities in the microcosm exposed to Dy. Broken lines represent cell densities of controls. Error bars are standard deviations (n=3). Closed symbols represent statistically significant differences from controls ($p<0.05$).

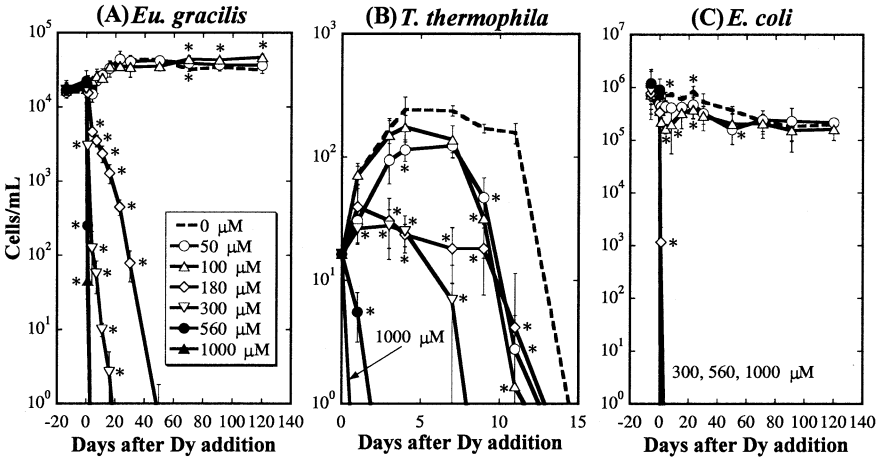

Figure 2. Effects of Dy on the pure-culture systems. Error bars are standard deviations (n=3). Asterisks represent statistically significant differences from controls ($p<0.05$).

pure-culture system. In controls, the cell densities increased until day 4, remained almost constant until day 11, and decreased to extinction on day 15. At 50 μM, the pattern of population change was similar to that of controls, but the cell densities were lower than controls and the extinction was earlier than controls. At 100 μM, no effects were observed until day 7. However, after that, the cell densities were lower than controls and the extinction was earlier than controls. At 180 or 300 μM, *T. thermophila* died out earlier than controls without significant growth. At 560 or 1000 μM, *T. thermophila* did not grow at all, and died out shortly after beginning of the culture.

Figure 2C shows effects of dysprosium on cell densities in the *E. coli* pure-culture system. In controls, the cell densities were almost constant for the duration of the experiment. At 50 or 100 μM, significant effects were not observed except for slight and temporal decrease shortly after exposure at 100 μM. At 180-1000 μM, *E. coli* died out.

In the microcosm, 96-100 % of added Dy was insoluble (>0.2 μm) on days 1-92 after addition of 50-560 μM Dy (data not shown). This means that added dysprosium, which was expected to be Dy^{3+}, was transformed to insoluble forms shortly after the addition to the microcosm.

In the *Eu. gracilis* pure-culture system, 97-100 % of added Dy was insoluble at 50 or 100 μM (data not shown). Shortly after 180 μM exposure, 24 % of added Dy was a soluble form (<0.2 μm), which decreased with time, and almost disappeared on day 91. Molecular weight of soluble Dy was mostly <10 kDa, and

only a few portions were >10 kDa (Fig. 3A). At 300 or 560 μM, approximately a half or three fourths of added Dy were soluble, respectively, and molecular weight of the soluble Dy was mostly <10 kDa (Fig. 3A).

In the *T. thermophila* pure-culture system, 98-100 % of added Dy was insoluble at 50-180 μM (data not shown). At 300 or 560 μM, soluble Dy was detected, though its portion was small. Molecular weight of the soluble Dy was mostly <10 kDa (Fig. 3B).

In the *E. coli* pure-culture system, 99-100 % of added Dy was insoluble at 50 or 100 μM (data not shown). Shortly after 180-560 μM exposure, soluble Dy was detected. The portions of the soluble Dy increased with concentrations of added Dy, and they were 60 % at 560 μM. Molecular weight of the soluble Dy was largely <10 kDa. On day 30, the soluble Dy mostly disappeared (Fig. 3C).

Toxicity of dysprosium was mitigated in the microcosm compared with its pure-culture systems. For example, at 180-560 μM, *Eu. gracilis* was not affected adversely in the microcosm, while it died out in the pure-culture system. At 50-300 μM, *T. thermophila* was not affected significantly in the microcosm except for slight decrease at 300 μM, while it died out earlier than controls in the pure-culture system. At 180 μM, *E. coli* only temporarily decreased in the microcosm, while it died out in the pure-culture system.

One possible mechanism of this mitigation of dysprosium toxicity in the microcosm except for *T. thermophila* exposed at 50-180 μM is a lower concentration of Dy^{3+} in the medium of the microcosm compared with the pure-culture systems. That is, the most toxic forms of metals are generally inorganic non-chelated ions, which are available for uptake into organisms from medium. It is therefore thought that Dy^{3+} is the most toxic form. The <10 kDa Dy, which included Dy^{3+}, did not almost exist in the microcosm, while significant concentrations of <10 kDa Dy existed in the *Eu. gracilis* pure-culture system at 180-560 μM, in the *T. thermophila* pure-culture system at 300 μM and in the *E. coli* pure-culture system at 180 μM as described above. It is thought that the following two mechanisms account for the lower concentrations of <10 kDa Dy in the microcosm. One possible mechanism is a higher concentration of metabolites and breakdown products that adsorb Dy and thus make it insoluble in the microcosm. There is no evidence directly supporting this hypothesis, but the higher concentrations of metabolites and breakdown products in the microcosm are deduced from the fact that the sum of cell densities of all species in the microcosm was higher than those in each pure-culture system before exposure to dysprosium (Figs. 1 and 2). Adsorption of dysprosium by metabolites and breakdown products is deduced from the fact that lanthanides are bound to organic ligands such as organic acids (Sun et al. 1997), proteins (Evans 1990) and so on.

It is thought that the other mechanism for the lower concentrations of <10 kDa Dy in the microcosm was higher pH in the microcosm. Except for 1000 μM

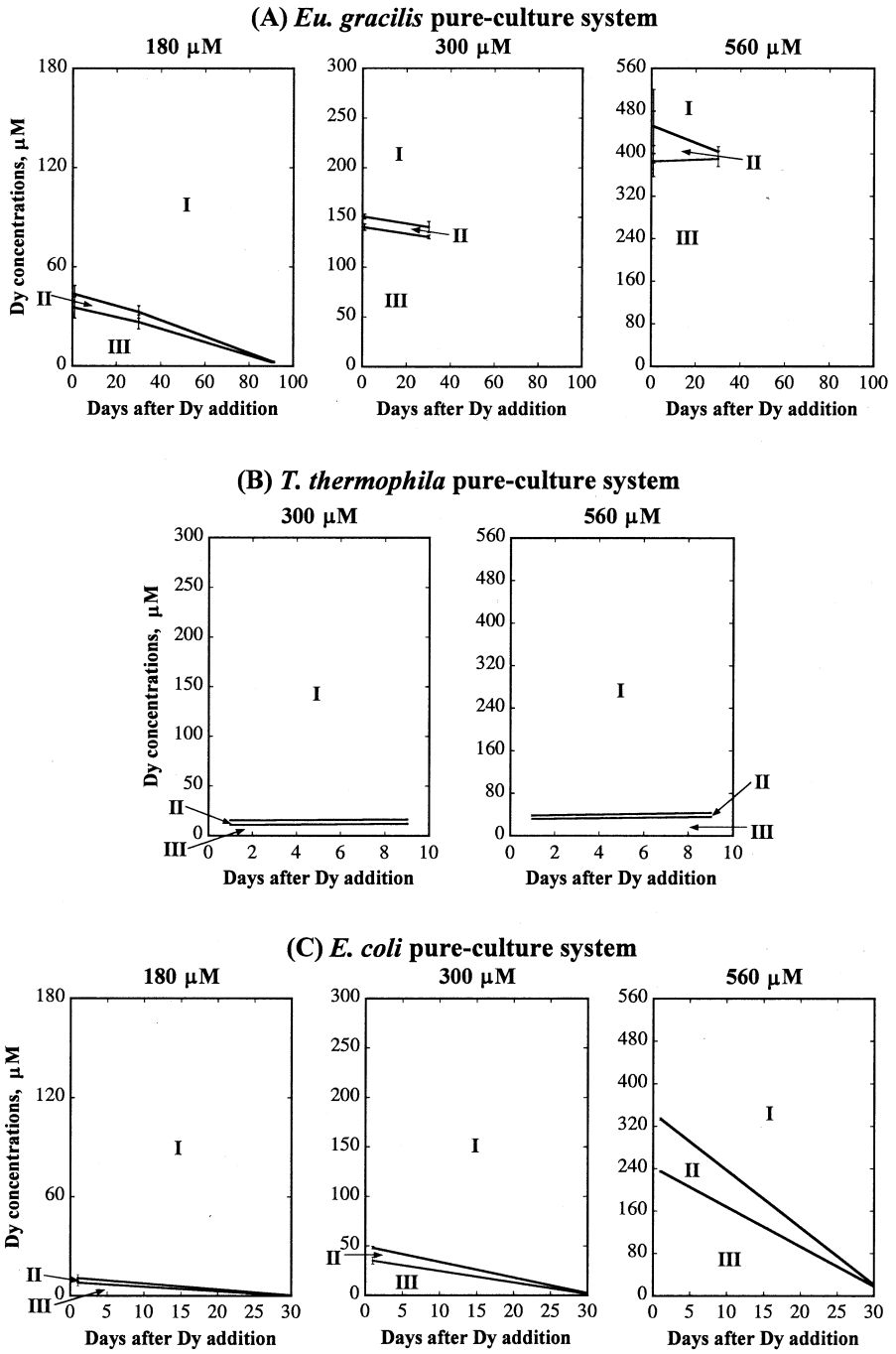

Figure 3. Concentrations of each size of Dy in the pure-culture systems. I: > 0.2 μm; II: < 0.2 μm and > 10 kDa; III: < 10 kDa. Error bars are standard deviations (n=3).

Table 1. pH in the microcosm and pure-culture systems after Dy addition.

Dy concentrations	Microcosm	Pure cultures		
		Eu. gracilis	*T. thermophila*	*E. coli*
0 μM	8.5±0.1	7.5±0.1	7.3±0.1	7.7±0.1
50 μM	8.4±0.2	7.2±0.2	7.1±0.1	7.6±0.1
100 μM	8.4±0.2	7.1±0.2	6.9±0.1	7.6±0.1
180 μM	8.2±0.2	6.4±0.1	6.5±0.1	7.4±0.1
300 μM	8.1±0.2	6.2±0.1	6.3±0.1	7.0±0.1
560 μM	7.4±0.2	6.1±0.1	6.2±0.1	6.5±0.0
1000 μM	6.4±0.1	5.9±0.1	6.1±0.1	6.3±0.1

Mean±Standard deviation

exposure, the microcosm showed alkaline or neutral pH, while the pure-culture systems showed neutral or acidic pH (Table 1). At alkaline pH, dysprosium is precipitated in the form of hydroxide (Nakamura et al. 1997). This suggests that larger amounts of Dy hydroxide were precipitated in the microcosm compared with the pure-culture systems. It is thought that the higher pH in the microcosm arose from photosynthesis by *Eu. gracilis*, which did not exist in the *T. thermophila* and *E. coli* pure-culture systems. In controls, photosynthesis activities in the microcosm were higher than those in the *Eu. gracilis* pure-culture system (unpublished data).

The other possible mechanism of the mitigation of dysprosium toxicity in the microcosm is the higher cell densities in the microcosm compared with the pure-culture systems as mentioned above. Under this situation, an amount of Dy absorbed by a cell in the microcosm would be smaller than that in the pure-culture systems when the concentrations of absorbable Dy in the medium were the same one another. The hypotheses described above suggest that toxicity of dysprosium to aquatic microbial communities may be mitigated in eutrophic systems compared with oligotrophic systems, because concentrations of organic matters that may adsorb Dy^{3+}, pH and densities of organisms are higher in eutrophic lakes and ponds.

The mitigation of toxicity to *T. thermophila* in the microcosm exposed to 50-180 μM dysprosium might arise from higher robustness of *T. thermophila* in the microcosm compared with the pure-culture system. There is no evidence directly supporting this hypothesis, but it is partly supported with the fact that *T. thermophila* cultured with *Eu. gracilis* and/or *E. coli* had higher cell densities and existed for longer time than that cultured alone (Matsui et al. 2000).

As described above, it is thought that some effects of dysprosium on one species in the microcosm were modified by other co-existing species. This means that this microcosm test detected community-level effects of dysprosium as in the case of to γ-rays (Fuma et al. 1998), acids (Miyamoto et al. 1998), manganese (Fuma et al. 2000), copper (Fuma et al. 2003a) and gadolinium (Fuma et al. 2001). However, effects of these toxic agents other than dysprosium were not always

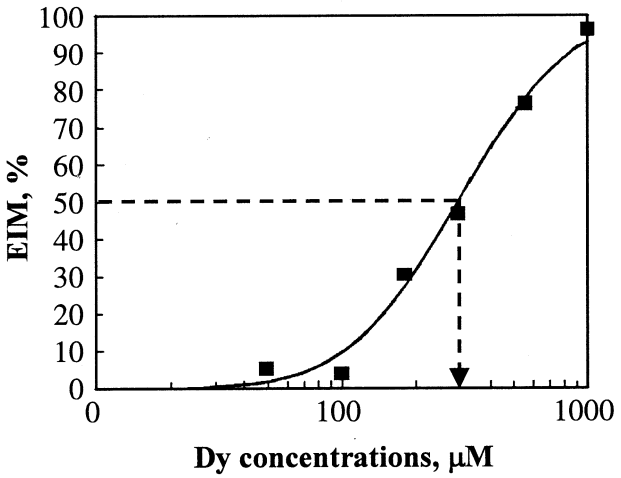

Figure 4. The Dy concentration-EIM relationship.

mitigated in the microcosm compared with the pure-culture systems. Some effects were enhanced in the microcosm.

For comparison of effects of dysprosium on the microcosm with those of some other heavy metals, the authors used an index holistically representing differences in cell densities between exposed and control microcosms by the Euclidean distance function, which was proposed previously (Fuma et al. 2003b). The effect index for the microcosm (EIM) was defined as follows:

$$EIM = \frac{100}{T}\int_0^T \sqrt{\frac{1}{3}\Delta N_{Eu}(t) + \frac{1}{3}\Delta N_T(t) + \frac{1}{3}\Delta N_E(t)}\ dt \quad [\%]$$

where

$$\Delta N_X(t) = \left\{\frac{N_{X,Con}(t) - N_{X,Exp}(t)}{N_{X,Con}(t)}\right\}^2$$

X = Eu (*Eu. gracilis*), T (*T. thermophila*) or E (*E. coli*).

$N_{X,Con}(t)$ = The log-transformed ($\log_{10}$ (N+1)) cell density of species X in the control microcosm on day t

$N_{X,Exp}(t)$ = The log-transformed ($\log_{10}$ (N+1)) cell density of species X in the exposed microcosm on day t

T = Experimental periods.

The EIM of dysprosium was calculated by substituting the present experimental data for parameters in the above equation. The resulting EIM was positively correlated with nominal Dy concentration, and the relationship between them could be fitted by a sigmoid curve (Fig. 4). A 50 % effect concentration for the microcosm (EC_{M50}), a concentration at which the EIM became 50 %, was

calculated by a probit method using the computer program Ecotox-Statics version 2.2 (developed by Prof. Yoshioka and distributed through the Japanese Society of Environmental Toxicology). The EC_{M50} of dysprosium was evaluated to be 300 μM, while the EC_{M50}s of manganese, nickel, copper and gadolinium reported elsewhere (Fuma et al. 2003b) were 4100 μM, 45 μM, 110 μM and 250 μM, respectively. It is therefore concluded that toxicity of dysprosium to the microcosm was almost the same as that of gadolinium, a member of a group of lanthanides. This agrees with the fact that lanthanides generally have similar characteristics one another. For example, a median lethal dose (LD_{50}) of dysprosium was almost the same as that of gadolinium when chlorides of these metals were intraperitoneally injected to male mouse (Haley 1965). Comparison of the EC_{M50}s indicates that dysprosium had significantly higher toxicity to the microcosm than manganese, and had significantly lower toxicity than nickel. Since toxicity of dysprosium to the microcosm was only slightly lower than that of copper, which has been regarded as a toxic group (Alexander and Fairbridge 1999), it is thought that further studies are required concerning dysprosium toxicity to aquatic microbial communities.

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