Bull. Environ. Contam. Toxicol. (2002) 68:420–427
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DOI: 10.1007/s00128-001-0271-z

Bulletin of Environmental Contamination and Toxicology

Toxicity of Gadolinium to Some Aquatic Microbes

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Received: 1 June 2001/Accepted: 26 September 2001

The toxicity of gadolinium to algae and bacteria was determined as part of an effort to develop a biological process to purify drums containing spent nuclear reactor heavy water moderator (D_2O). This water was contaminated with high concentrations of gadolinium nitrate, a chemical used as a neutron poison during former nuclear reactor operations at the Savannah River Site (SRS) near Aiken, SC. Nuclear reactors were operated for approximately 30 years at the SRS to produce nuclear weapons materials for national defense. Throughout this period, a heavy water solution of gadolinium nitrate was utilized in a standby emergency shutdown system that could inject this chemical into the reactor moderator coolant water. The chemical was used for this purpose because the high neutron absorption cross sections of some gadolinium isotopes make gadolinium salts such as $GdNO_3$ effective in controlling nuclear activity in aqueous systems (Gilbert et al. 1985; Rodenas et al. 1990). The use of this practice resulted in a large inventory of this degraded heavy water containing gadolinium nitrate.

Microbiological and chemical studies were initiated to evaluate the potential use of bacteria and algae for water purification of the drums. Since metals are often toxic to microbes when present at concentrations substantially higher than natural environmental levels, it was hypothesized that Gd may be toxic to selected microorganisms (algae and bacteria) at the very high concentrations (average 80,000 mg/L, maximum 259,000 mg/L) present in most of the drums. Two principal components of the study included: (1) chemical and microbiological characterization of representative drums, and (2) an evaluation of the toxicity of gadolinium to selected species of algae. The relative toxicity of nitrate salts composed of Gd, Na, K, and NH_4 were compared because a proposed cleanup method involves precipitation of the gadolinium in the drums as a phosphate (either sodium, potassium or ammonium) with subsequent removal of the nitrate salt using biological means.

In addition to wastewater from nuclear production reactor operations, gadolinium waste is also generated from medical applications, especially MRI, and various electronic components including CD disks. Despite growing and widespread usage of this rare element, there is a paucity of information on the toxicity of gadolinium.

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MATERIALS AND METHODS

Eight drums of the wastewater representing a wide range of Gd concentrations and other chemical conditions (based on prior sampling) were selected for the present study. Conditions included relatively high and low values for gadolinium, pH, conductivity and % D_2O, relative to H_2O. One-liter samples were collected from each drum. Gadolinium (mg/L) was measured by inductively coupled argon plasma spectroscopy, pH (units) by a combination electrode, D_2O (mole %) by infrared spectroscopy, conductivity (μmhos/cm) by the electrolytic method and NO_3 by ion chromatography.

Microbiological processing and analysis techniques included plate counts and microscopical counts. Duplicate spread plates were prepared using 10 μL and 100 μL inoculums taken from each drum sample to determine colony forming units (Balkwell, 1989). The plates were prepared and incubated at room temperature prior to enumeration. Total direct bacteria counts of cells were performed by spotting 50 μL of well-mixed water onto toxoplasmosis slides (Santo Domingo, et al. 1998). Nutrient additions were made to the samples by first transferring 7.0 mL of each drum sample into sterile 15-mL centrifuge tubes. Then, 250 uL of a glucose solution was added to each vial to yield a final glucose concentration of 0.34%. Glucose was selected as a substrate because it was readily available and is usable by many bacteria as a carbon source. Capped vials were placed on a rotating shaker for 72 hours prior to being analyzed by the direct count method.

Three experiments were conducted to evaluate the toxicity of gadolinium to algae. Samples were prepared in triplicate. In the initial experiment, four species of algae were inoculated into standard culture media with and without $GdNO_3$ substituted for conventional nitrate (e.g. $NaNO_3$) to provide $GdNO_3$ at concentrations resulting in Gd concentrations of 0, 10, 100, 1,000, 10,000, 80,000 and 260,000 mg/L (thus, bracketing the quantities in all of the drums requiring treatment). Gd and NO_3 concentrations of the media were measured at the start of the experiment and after one and two weeks. Algal cultures included *Chlorella pyrenoidosa, Scenedesmus quadricauda, Closterium* sp. and *Cyanidium caldarum.* All four strains were obtained from the Carolina Biological Supply Company, Burlington, NC. *Chlorella* and *Scenedesmus* were grown in modified Bold Basal (BB) medium (Nichols and Bold 1965). Closterium was grown in Alga Gro Freshwater Medium and *Cyanidium* in Doemel's *Cyanidium* medium (Carolina Biological Supply Co., 1978.)

Culture media was prepared in 25 mL batches in 50 mL flasks prior to being autoclave sterilized. Flasks were subsequently inoculated with 600 μL of algal culture. The flasks were then placed in a Pschrotherm shaker/incubator (New Brunswick Scientific) for two weeks at 20°C, with a 12 h light (200 μE^{-2} s^{-1}):12 h dark illumination regime and 100 rpm rotation. Control flasks, not inoculated

with algae but otherwise treated identically with all treatment conditions, were examined for nitrate and gadolinium concentrations at the start and conclusion of all three experiments.

Densities of live algae were determined by examining aliquots from the flasks with an inverted microscope using fluorescence microscopy which allows the differentiation of live and dead cells by the detection of chlorophyll fluorescence when cells are subjected to excitation by blue or green light (Wilde and Fliermans,1979; Wood et al. 1985). Density estimates were made by randomly counting fields until minimums of 400 cells per sample were tabulated. Counts were performed after incubation periods of one- and two-weeks. Aliquots (250 µl) were collected from the flasks for microscopical observations.

A second experiment was conducted to further elucidate the toxicity of $GdNO_3$ to algae. Based on the results of Experiment 1, the range of Gadolinium that the algae were exposed to was decreased and two additional algal strains were evaluated. These were isolates of the species, *Mastigocladus laminosus*, a thermophilic blue green alga derived from SRS reactor effluent streams and cultured in Medium ND (Castenholz 1982). Three different pH levels, 3.5, 4.5, and 5.5 were also compared. Algae were enumerated at the start of the experiment and at one- and two-week intervals.

A third experiment was conducted to evaluate the comparative toxicity of $GdNO_3$, $NaNO_3$, KNO_3, and NH_4NO_3 to algae. Modified BB Medium was formulated to contain 10, 100, 1,000, and 10,000 mg/L of Gd as $GdNO_3$, Na as $NaNO_3$, K as KNO_3, and NH_4 as NH_4NO_3.. Triplicate samples containing each medium were inoculated with 600 µl of a *Chlorella vulgaris* suspension and algal concentrations were determined immediately and after one week of incubation under Pschrotherm incubator conditions previously described.

RESULTS AND DISCUSSION

Complete results of chemical and microbiological analyses of the drum samples are shown in Table 1. The samples with the highest gadolinium concentrations generally displayed proportionally higher concentrations of NO_3, and conductivity.

Although only three of the drum samples produced culturable bacteria on 1% PTYG agar, total direct counts showed that some bacteria were present in all drums. This indicates that at least some bacteria are capable of tolerating > 99% D_2O with nitrate and gadolinium concentrations greater than 200,000 mg/L (Table 1).
The microbial density increased in six of the eight drum samples, as measured using the direct count method, when glucose was added to the drum samples. Bacteria densities increased more than two orders of magnitude with nutrient

Table 1. Characterization Data: Drums of Spent Moderator Water

Drum #	18156	15208	15817	4600	15793	15861	15435	13749
Microbiological data								
Direct Count Cells/ml	1.24E+05	6.14E+05	4.74E+04	1.29E+05	5.57E+04	8.25E+03	1.22E+06	9.28E+03
Spread Plate CFU/ml	ND	2.00E+01	ND	ND	ND	ND	6.35E+03	6.20E+02
Direct Count Cells/ml after 72 hr. nutrient addition	3.44E+05	2.03E+03	3.80E+04	2.08E+05	1.43E+06	1.38E+06	>1.0E+08	2.44E+04
Chemical data								
Gd (mg/L)	202500	203800	0.09	142	97	0.5	0.26	6118
Nitrate (mg/L) (Measured)	183936	279985	<1	179	63	<1	<1	5954
pH	1.63	5.36	6.14	3.62	6.79	6.34	5.59	6.66
D_2 0%	78.17	99.59	98.73	98.54	99.65	99.47	99.17	2.99
Conductivity (μmhos)	84400	97500	4.07	394	199	1.4	10.8	9020
Nitrate (mg/L) (Calculated**)	239963	241503	0.1	168	115	0.6	0.3	7250

* ND = Not Detected

**Based on molecular weight ratios for Gd and NO_3 in $Gd(NO_3)_3 \cdot 6H_2O$

addition in three of the samples. All of these contained less than 100 mg/L gadolinium. Cells in many of these samples were larger and/or in the process of dividing when viewed under the microscope indicating the presence of actively growing cells. The largest increase in cellular density occurred with the sample from drum #15435, which had a very low gadolinium concentration. This sample also had the largest number of colony forming units as determined by the spread plate method. The addition of an organic carbon source, glucose, appeared to stimulate the growth of the bacteria that were already present in the samples.

The last row in Table 1 shows the expected (calculated) levels of nitrate based on the gadolinium measurements and the molecular weights of the elements in the $Gd(NO_3)_3 \cdot 6H_2O$ compound used in the reactor process where the water in the drums originated. Measured concentrations of Gd, NO_3, and conductivity were strongly correlated with each other. Expected (calculated) and measured values of nitrate were generally similar. The only case where measured NO_3 was greater than calculated NO_3 was for Drum # 4600 (179 vs. 168mg/L) (Table 1).

Chlorella vulgaris was more tolerant to gadolinium nitrate than *Scenedesmus quadricauda* or *Cyanidium caldarum* (Figure 1). The microscopical examinations also revealed some viable algal cells at gadolinium concentrations up to 260,000 mg/L. However, growth was greatly impeded at gadolinium concentrations between 100 mg/L and 1,000 mg/L. The growth data in Figure 1 represent the change in algal populations between the first and second week of inoculation in the media based on a single quantitative count of algae at each condition shown..

Figure 2 shows the amount of *Chlorella* growth in each of two consecutive 1-week periods when exposed to various concentrations of Gd and pH with BB media. At all pH conditions tested, algal growth decreased dramatically between 100 mg/L and 1,000 mg/L. These data support the conclusion that algal growth is inhibited at concentrations in this range.

At pH 4.5 and 5.5, *Chlorella* grew much better when gadolinium concentrations were 0 and 100 mg/L compared to a gadolinium concentration of 10 mg/L (Figure 2) (This was also evident in Figure 1). This result, which may appear as a data anomaly at first glance, can be explained by the methodology. The 0 mg/L gadolinium samples were prepared with the standard modified BB media formulation which contains 182.5 mg/L of nitrate as $NaNO_3$; whereas, the formulations for 10 to 10,000 mg/L gadolinium samples were prepared by substituting Gd/NO_3 in place of sodium nitrate. Thus, the 0, 10, and 100 mg/L gadolinium formulations contained 182.5, 11.9, and 118.5 mg/L nitrate, respectively and the lower growth at 10 mg/L Gd, relative to the 0 mg/L and 100 mg/L concentrations was most likely due to nitrate limitation rather than gadolinium toxicity.

Growth was substantially higher in the second week of incubation than in the first week with the exception of the 10 mg/L Gd exposures at pH 4.5 and pH 5.5. Once again, the lower growth at 10 mg/L Gd is thought to be due to the depletion

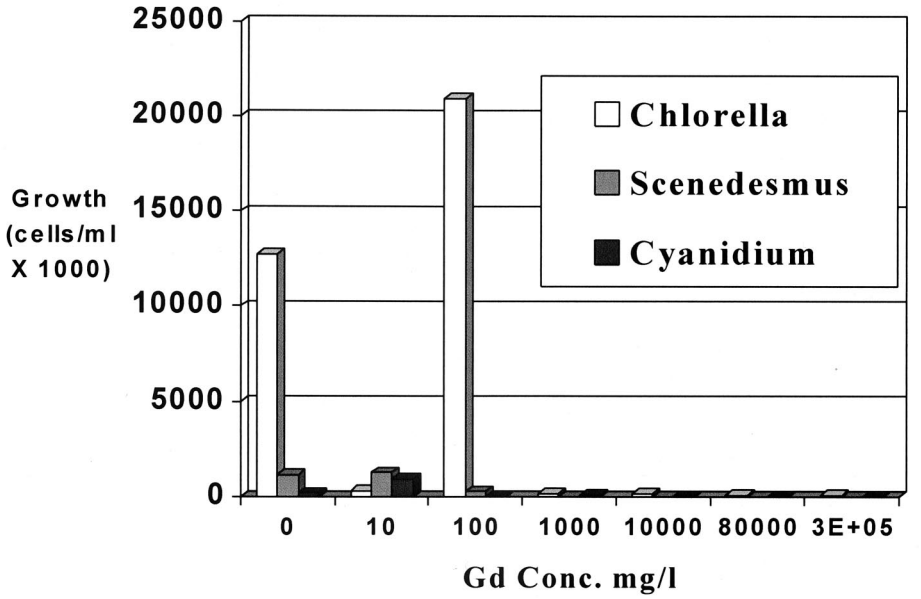

Figure 1. Growth of three species of algae at various Gd concentrations

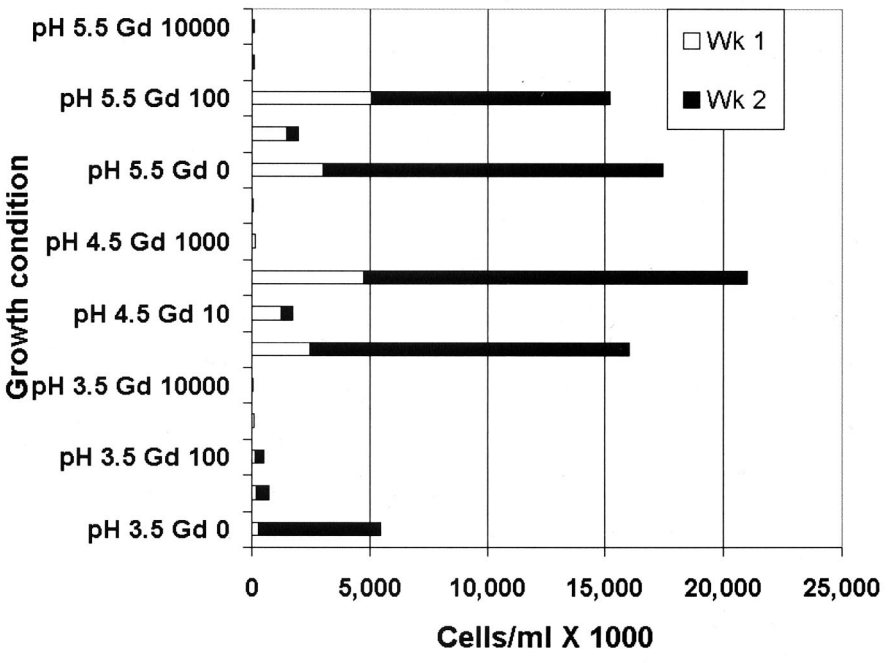

Figure 2. Growth of Chlorella at various pH and Gd Concentrations

of nitrate after the first week. The higher growth in week #2 compared to week #1 in the other samples may represent adaptation to the gadolinium in the medium.

Table 2. shows the growth of *Chlorella* in modified BB media containing nitrate salts prepared with gadolinium, sodium, potassium and ammonium at concentrations of 10, 100, 1,000 and 10,000 of the cationic portion of each compound. Gadolinium appears to stimulate growth at 10 mg/L but results in reduced growth at the higher concentrations. The highest growth occurred with sodium at 100mg/L. However, at the highest concentration tested (1000,000 mg/L) potassium resulted in the best growth. Table 2 shows the relationship of algal growth in relation to both metal concentrations and nitrate ion concentrations. It is evident that the toxic effects observed were caused by metal toxicity rather than by nitrate toxicity since substantial growth occurred with NO_3 concentrations as high as 26,950 mg/L and 15,890 mg/L with $NaNO_3$, and KNO_3, respectively, while no significant growth was observed with $Gd(NO_3)_3$ when the nitrate concentration was 1,180 mg/L.

Table 2. *Chlorella* growth at various nitrate concentrations in media containing metal nitrate salt solutions

Salt	Metal Conc. (mg/L)	Nitrate Conc. (mg/L)	Growth (cells/ml) in 1 week[a]
$Gd(NO_3)_3$	10	11.82	7.1E+5 ±1.6E+5
	100	118.2	5.7E+5 ±8.5E+4
	1000	1182.8	1.4E+4 ±1.5E+4
	10000	11828.2	0 ±0
$NaNO_3$	10	26.95	3.2E+5 ±2.8E+4
	100	269.5	2.4E+6[b]
	1000	2695.6	5.3E+5 ±1.3E+5
	10000	26956.5	2.3E+5 ±1.0E+5
KNO_3	10	15.5	4.5E+5 ±5.4E+4
	100	155	1.5E+6 ±1.1E+5
	1000	1550	4.1E+5 ±7.0E+4
	10000	15500	2.7E+5 ±4.6E+4
NH_4NO_3	10	44.28	2.3E+5 ±2.4E+5
	100	442.8	1.5E+5 ±1.8E+5
	1000	4428.6	1.4E+5 ±6.4E+4
	10000	44285.7	0 ±0

[a]Means of duplicate counts ± 1SD
[b]Only one replicate suitable for counting

Overall, it was concluded that although some microbes can survive at extremely

high Gd concentrations, gadolinium is generally toxic to algae at concentrations between 100 and 1,000 mg/L. It was also demonstrated that gadolinium is more toxic to algae than potassium or sodium.

Acknowledgments. We thank Marilyn Franck, Mary Anne Johnson, Fatina Washburn, Sherold Johnson, Mike Polochko, Robert Ray and Charlie Varner for providing technical assistance on this project. Funding for the work was provided under Contract DE-AC09-89SR18035 with the U.S. Department of Energy.

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