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Comparative Toxicity of Thallium(I), Thallium(III), and Cadmium(II) to the Unicellular Alga *Chlorella* Isolated from Lake Erie

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Thallium use is increasing in society (WHO 1996). In the past, Tl found widespread use as a pesticide but it has been banned in North America due to its highly toxic non-selectivity (Nriagu 1998). High technology industries use thallium in semiconductor production, cardiac imaging, alloys, optical glass, and photoelectric cells. Although absolute amounts of thallium use in high technology industries are relatively low, increased incorporation of thallium into this industrial sector will inevitably lead to its unavoidable dispersal and increased mobilization in the environment–hence, concern is growing regarding thallium in the natural environment (Nriagu 1998).

The primary entry of thallium into the environment is through dry deposition of the combustion product Tl_2O (WHO 1996). Coal is the chief source of thallium contamination since coal contains appreciable amounts of this element yet thallium is also released from sulphidic ore combustion, Zn, Cu, and Pb smelting and Cu milling (Smith and Carson 1977). Thus, thallium may be released into the environment from primary mineral extraction and recycling efforts. Upon deposition on water bodies, Tl_2O decomposes readily in water and the resulting Tl(I) cation is thermodynamically stable. However, measurements in Lake Michigan reveal that the concentration of Tl(III) is present in significant amounts, the predominant form being $Tl(OH)_3$ (Lin 1997).

Despite increased thallium use, information of the impact of thallium on aquatic organisms is extremely limited. Moreover, all studies reported to date have examined only Tl(I) toxicity and not the toxicity of Tl(III). What is currently known is that Tl(I) interactions with aquatic organisms is affected by competing inorganic ions but not organic complexing agents. Tl(I) accumulation by the cyanobacterium *Synechocystis* (Avery et al. 1991) and the aquatic higher plant, *Lemna minor* (Kwan and Smith 1991) is inhibited by K^+. Since Tl(I) displays weak complexation with organic ligands, EDTA has no observable effect on Tl(I) uptake by *L. minor* (Kwan and Smith 1991) or Tl(I) toxicity to the marine diatom *Ditylum brightwellii* (Canterford 1980), the cyanobacterium *Anacystis nidulans*, and the chlorophyte *Chlamydomonas reinhardtii* (Lustigman et al. 2000).

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Understanding trace metal toxicity to aquatic organisms requires knowledge of the chemical speciation of the metal of interest. Trace metal speciation is influenced by the variety of ligands, inorganic and organic, that may complex with a metal cation in addition to any changes in redox state the metal may undergo, as is the case for thallium. Biological effects of a trace metal (e.g. toxicity and bioaccumulation) are proportional to the concentration of the free-ion (Campbell 1995). Bioaccumulation is proportional to the first hydrolysis constant of a given trace metal (Fisher 1986); $\log_{10} K_{TlOH} = 2.3$, $\log_{10} K_{CdOH^+} = 3.9$, $\log_{10} K_{TlOH^{2+}} = 11.3$. Therefore, we hypothesized that toxicity of redox species of thallium and cadmium will follow the order Tl(III) > Cd(II) > Tl(I). In this study, we take advantage of refined measurements of thallium hydrolysis constants (Lin and Nriagu 1998) that allowed us to design test media to provide a comparison of the toxicity of these metals based on their respective free-ion concentration.

MATERIALS AND METHODS

Unicellular algae (*Chlorella* sp., cell diameter 3.9 μm; University of Toronto Culture Collection strain UTCC 522) were maintained in Fraquil, an inorganic defined growth medium (Morel et al. 1975). Prior to use, 100 mL of media contained in 250-mL polycarbonate Erlenmeyer flasks were sterilized in a microwave oven (1,000 W): the sterilization protocol was a 55 second microwave exposure, followed by two-22 second periods of microwave exposure, with the contents mixed after each exposure period.

All inoculations and manipulations of media were conducted under laminar flow hoods to avoid contamination. All plasticware were soap washed (Citranox; Fisher Scientific), followed by a de-ionized water (Milli-Q; Millipore Corp.) rinse and ethanol rinse to remove any soap residue, followed by an acid wash involving at least a 48 hour soaking in 3% HCl (Trace Metal Grade; Fisher Scientific), followed by repeated rinsing with Milli-Q water and drying under a laminar flow hood.

Toxicity tests were conducted in trace metal-free Fraquil that was prepared by omitting all EDTA and trace metal nutrients from the medium. By utilizing intracellular trace metal stores, cells were able to grow for 6-8 cell divisions in the absence of essential trace metals in the test medium. Trace metals were added aseptically to the sterilized growth medium and these media were the allowed to equilibrate for 24 hours prior to inoculating with algae. Metals were added as inorganic salts: $Tl(NO_3)_3$ (Fluka Chemicals); $Cd(NO_3)_2$ (Fisher Scientific), and $TlNO_3$ (Fluka Chemicals). Metal stocks were prepared in dilute HCl. Stocks were diluted aseptically in Mill-Q water prior to spiking media. No change in pH occurred from the metal additions; media acidity remained at pH 7.3. Preliminary toxicity tests (data not shown) were conducted to establish the range of toxicity thresholds. Results from one experiment that illustrates the toxic threshold of Tl(II), Cd(II) and Tl(I) are presented here. Metals were added singly to the test

media; a control treatment received no metal additions. Each treatment was conducted in triplicate. Tl(III) was added at total dissolved concentrations 10^{-7} *M* and 10^{-6} *M* Tl(III); Cd(II) and Tl(I) were added at total dissolved concentrations of 10^{-8} *M*, 10^{-9} *M*, and 10^{-10} *M*. The concentration of free metal ions in the toxicity test media were calculated using MINEQL+ ver. 4.06 (Environmental Research Software, Hallowell, ME), with formation constants for the hydrolysis of Tl(III) and Tl(I) provided by Lin and Nriagu (1998), and for Cd(II) provided from Martel et al. (1998).

Chlorella in the exponential growth phase were harvested by centrifugation (5 min at 5,000 x g, followed by a rinse with trace metal-free Fraquil, re-centrifugation, and re-suspension in trace metal-free Fraquil). This cell wash procedure was designed to concentrate the cells and remove any trace of EDTA and other trace metals from the growth medium. Cells were inoculated into the equilibrated test media to achieve an initial cell density of 2 x 10^4 cells·mL^{-1}; this low concentration of cells was chosen so as to not disturb the chemical speciation of the test media by a high concentration of cells (that scavenge metal from solution and produce organic ligands that might complex Tl(III) and Cd(II) chemical species). The inoculated test media were placed in a random order on a gyratory shaker (100 rpm), and provided continuous lighting (150 µmole photons·m^{-2}·s^{-1}) at 20-23°C. At daily intervals over a 4 day period, 5-7 mL samples from each flask were removed by pouring, thus avoiding the insertion of any item that might contaminate the treatment. Specific growth rates (µ) of algae in the test media were calculated as follows:

$$\mu = \delta \log_{10} \text{cells·mL}^{-1} / \delta t \qquad (1)$$

where, cell density (cells·mL^{-1}) in culture samples was enumerated using a hemocytometer on samples fixed with Lugol's Iodine (1% vol:vol), and t = time (hours). In practice, µ was determined by a linear least squares regression through the sampling points representing the exponential growth phase in each treatment replicate. The toxicological response was evaluated by comparing specific growth rates in each treatment relative to the specific growth rate of the control. Specific growth rate is considered to be highly sensitive endpoint for examining toxicological effects in monocultures of microalgae (Nyholm 1985) since it is the sum effect of the toxicant expressed at the population level.

Tl(I) is capable of oxidation to Tl(III). An experiment was conducted to determine if *Chlorella* could oxidize Tl(I) to Tl(III) during the toxicity test. Algae were grown in 1-L of test medium containing 10^{-8} *M* Tl(I). After 4 days, the medium was collected by filtering algae onto a 0.4-µm pore size polycarbonate filter (90 mm Durapore filter) contained in a Teflon™ filtration apparatus (Savillex, Minnetonka, MN). Inorganic dissolved redox forms of thallium can be separated using a resin exchange technique (Lin and Nriagu 1999). A sample (500 mL) of the filtrate was acidified to pH 1 using nitric acid (Seastar Chemicals) and passed through a column containing 3 mL of Chelex-100 (H-form; Bio-Rad); any Tl(III) present in the filtrate will be captured by the resin at pH 1 whereas Tl(I) will pass through the resin bed. The total dissolved thallium

concentration in the filtrate before and after the treatment with the resin was determined by anodic stripping voltammetry (Metrohm 663 sampling stand, Ecochimie MicroAUTOLAB II voltammeter).

RESULTS AND DISCUSSION

This is the first study to compare the aquatic toxicity of Tl(III) and Tl(I) to phytoplankton using controlled chemical speciation. No oxidation of Tl(I) to Tl(III) occurred over 5 days in the media containing 10^{-8} *M* Tl(I) and the test algae. Although the algae could not oxidize Tl(I) to Tl(III) no measure of Tl(III) reduction to Tl(I) by the algae was attempted. Given the highly hydrolyzed state of Tl(III) at pH 7.3 (see below) very little free Tl^{3+} would be available for reduction to Tl^{+}. Thus, the thermodynamic predictions of Tl(I) and Tl(III) speciation were most likely not confounded by changes in redox state that may have occurred in the media and a direct comparison of Tl(I) toxicity to Tl(III) toxicity is therefore possible. The results of the toxicity tests show that Tl(III) is much more toxic than Tl(I) (Fig.1). Although the highest total dissolved Tl(III) concentration tested was 100-fold greater than the highest concentration of Tl(I) assayed, the strong hydrolysis of Tl(III) to predominantly the $Tl(OH)_3$ species at pH 7.3 reduced the free Tl^{3+} ion to approximately 2 x 10^{-12} *M*. In contrast, Tl(I) is weakly hydrolyzed and the free Tl^{+} ion at the highest Tl(I) concentration was essentially equivalent to the total dissolved Tl(I) concentration, 1 x 10^{-8} *M*. A 21% growth rate inhibition in *Chlorella*, relative to control, was observed at 2 x 10^{-13} *M* Tl^{3+}, which was not significantly different ($P = 0.05$) than the 1 x 10^{-8} *M* Tl^{+} treatment that caused a similar depression (13% inhibition). Thus, Tl^{3+} was approximately 50,000-fold more toxic than Tl^{+}. However, since the rates of reduction from Tl(III) to Tl(I) in this system are not known it is possible that some Tl(III) from the large pool of Tl(III) (10^{-7} M) may have been converted to Tl(I) and thus contributed to the toxicity in the Tl(III) treatments. Further study is required to establish thallium reduction kinetics as they relate to thallium toxicity. One constraint to detecting a reduction of Tl(III) to Tl(I) is that the current method for measuring Tl(III) in the presence of Tl(I) has a precision of 5-10%, thus small changes (e.g. 1-5%) in the redox state of Tl(III) to Tl(I) would not be detectable using the current analytical technique (Lin and Nriagu 1998).

A Cd^{2+} concentration of 8.7 x 10^{-9} *M* caused a 50% inhibition of the specific growth rate of *Chlorella* (Fig. 1). Given that the inhibition at 8.7 x 10^{-9} *M* Cd^{2+} was greater than the observed inhibition caused by Tl^{+} at 1 x 10^{-8} *M*, and the comparison of Tl^{3+} and Tl^{+} toxicity presented above, it follows that the toxicity to *Chlorella* (UTCC 522) follows the order: Tl(III) > Cd(II) > Tl(I), as predicted on the basis of the first hydrolysis constant. This conclusion is supported by a study of the freshwater crustacean *Daphnia magna* that revealed the aquatic toxicity of Cd(II) > Tl(I) (Borgmann et al. 1998).

No morphological changes in *Chlorella*, detectable using light microscopy (400X) in were evident in any of the thallium and cadmium treatments in this study.

There exists a significant gap in knowledge that prevents confident risk assessment of thallium in the aquatic environment. For example, the state of New York established an ambient water quality standard of 4 x 10^{-8} *M* thallium (NYSDEC 1987) but provide no rationale or toxicity data to support this limit. On the other hand, the Province of Ontario has established an interim water quality objective of 1.5 x 10^{-9} *M* that is based on the lowest concentration of thallium known (3.5 x 10^{-8} *M*) to harm aquatic life (*L. minor* shows chronic toxicity at 3.5 x 10^{-8} *M* Tl) divided by an uncertainty factor of 24 (OMEE 1995), illustrating a chronic lack of fundamental information regarding this element. This is also reflected in the USEPA not establishing water quality criteria for thallium because of insufficient data (USEPA 1980).

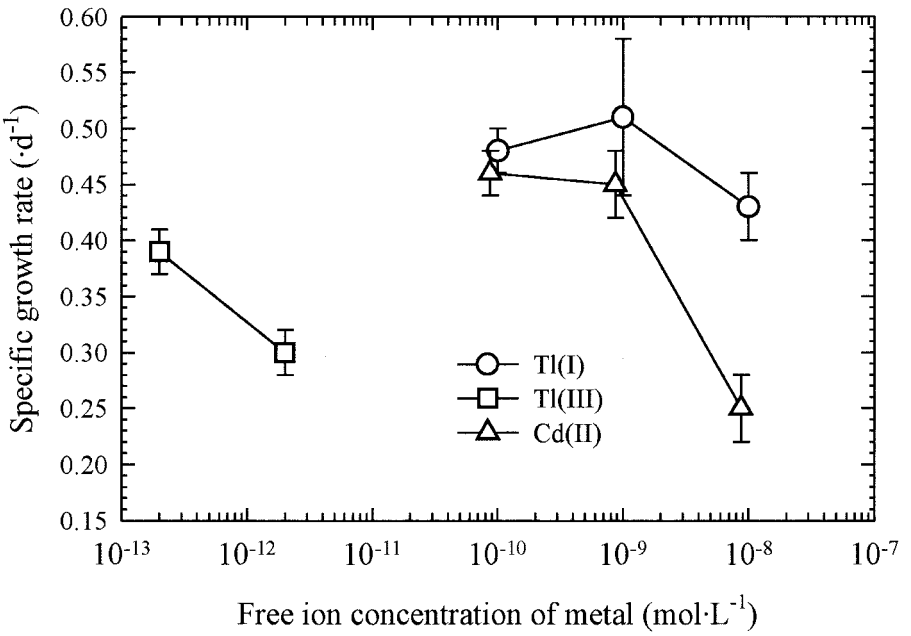

Figure 1. Comparative toxicity of thallium and cadmium to the unicellular alga, *Chlorella* sp. (UTCC 522). Free metal ions (Tl^{+}, Tl^{3+}, Cd^{2+}) were calculated using thermodynamic principles (*see* Materials and Methods). Specific growth rate of the control (no added metal) was 0.50 ± 0.03·d^{-1}). Values are mean ± SD, $n = 3$.

Previous study has shown that the percentage of Tl(III) ranges from 43-73% (average 66 ± 9%) in the Raisin and Huron Rivers (Michigan), which is essentially the same as the reported proportion for Tl(III) in Lake Michigan (68 ± 6%). If we consider that 67% of total dissolved thallium is Tl(III) then impairment of phytoplankton growth may occur since approximately 1.3 x 10^{-8} *M*

Tl(I) would be present at the 4 x 10^{-8} *M* NYSDEC guideline. Our results (Fig.1) show a 13% inhibition of specific growth rate due to 10^{-8} *M* Tl(I). Fig. 1).

The concentration of total dissolved thallium in the open waters of the lower Great Lakes is low (4.5 x 10^{-11} *M* in Lake Erie; 2.8 x 10^{-11} *M* in Lake Ontario) although thallium levels are elevated in polluted harbors, e.g. Hamilton Harbour, Lake Ontario 1.30 x 10^{-10} *M* (Cheam et al. 1995). The organism used here to assay the toxicity of thallium was isolated from central Lake Erie, thus, this organisms may be more sensitive to the elevated thallium levels used in the toxicity tests than organisms isolated from more thallium polluted areas.

For comparison, the Great Lakes Water Quality (GLWQ) Objective for Cd is 1.8 x 10^{-9} *M* total Cd in unfiltered water (IJC 1989). Based on our results (Fig. 1), the GLWQ Objective for Cd would not cause acute toxicity to *Chlorella* (UTCC 522). There are no GLWQ Objectives for thallium.

Our results support the notion that any water quality objectives for thallium should take into account the redox species of thallium present in the water. Although the aquatic toxicity of Tl^{3+} is orders of magnitude greater than that of Tl^{+}, total dissolved Tl(III) must be extremely elevated (e.g. 10^{-6} *M*), relative to levels found even in polluted environments, for toxicity due to Tl^{3+} to occur. In comparison, Tl^{+} is less toxic but the bioavailable fraction of Tl^{+} as a proportion of the total dissolved Tl(I) concentration is near unity. Thus, from an acute toxicity perspective, water quality objectives should focus on Tl(I). However, important caveats are that mechanisms of *in situ* conversion of Tl(III) to Tl(I), possibly by grazing activity, or the trophic transfer of Tl(III) and Tl(I) accumulated by prey into higher organisms (Twiss et al. 1996; Reinfelder and Fisher 1991) are unknown. In addition, the biotic production of dimethylthallium in the ocean has recently been reported (Shedlbauer and Heumann 1999). No measurement of dimethylthallium in fresh water has been made, nor is the aquatic toxicity of this thallium species known.

In summary, this study shows that the two redox forms of thallium do not display the same toxicity to the unicellular chlorophyte, *Chlorella*: Tl(III) is approximately 50,000-fold more toxic than Tl(I), on the basis of the respective free ion concentration of each thallium redox state. Although Tl^{3+} is more toxic, the chemical speciation of Tl(III) is dominated by $Tl(OH)_3$ at circumneutral pH, thus the bioavailable concentration of Tl^{3+} is much lower than that of Tl(I), the less toxic redox form of thallium. Since Tl(III) chemical species comprise a significant (ca. 70%) proportion of the total dissolved thallium it follows that water quality guidelines for the protection of aquatic organisms should focus on the Tl(I) species.

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