BEHAVIOR OF LEAD IN A MODEL MICROBIAL PREDATOR–PREY SYSTEM

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Abstract—Predation at the microbial level can affect the fate of toxic trace metals. Metals associated with bacterial prey can be released into the dissolved phase following digestion by a predator, and/or metals can remain in the predator and be transferred potentially to the next level of the food chain. Toxic metal ions in the aqueous phase also are expected to modify the growth and predation rate of a microbial predator. A model predator–prey system was developed to test the effects of Pb on cells and to help elucidate the fate of Pb in this type of interaction. Established methods that have been shown to be suitable for distinguishing dissolved, prey-bound, predator-bound, and ingested Pb were used to establish the pathway of Pb over time. Growth parameters were measured using batch reactors for the protozoan predator Tetrahymena thermophila and the bacterial prey Pseudomonas putida without Pb and at several concentrations of Pb. The effect of prey density on predation and Pb phase distribution also was investigated. Results demonstrate that some kinetic parameters related to prey consumption and growth of T. thermophila are altered by Pb. Upon addition of predator to prey cells in equilibrium with dissolved Pb, dissolved and prey-bound Pb become associated with the predator through ingestion and adsorption. Ingested Pb is excreted later as a bound metal associated with T. thermophila waste matter. A preliminary mathematical model was developed to describe predator–prey dynamics and their influence on the behavior and fate of Pb. Growth data were used to obtain model parameters, and model simulations for Pb fractionation are compared to experimental observations.

Keywords—Predator–prey model Lead Microbial loop Toxic metal fate

INTRODUCTION

A mechanistic understanding of trace-metal movement in the microbial loop of the food web is an important part of being able to predict the fate of toxic trace metals in engineered and natural aquatic systems. The microbial loop refers to the lowest part of the food web. In a pelagic or limnic ecosystem, the microbial loop consists of dissolved organic matter (<0.2-μm diameter), the picoplankton (~0.2–2.0 μm; primarily bacteria), the nanoplankton (2.0–20.0 μm; flagellates), the microplankton (20.0–200 μm; ciliated protozoa, diatoms), and the mesoplankton (>200 μm; zooplankters) [1]. The presence of metal-adsorbent cellular surfaces in the microbial loop likely alters metal speciation and solid/solution phase distribution. Because toxic metals sorb readily to bacterial prey, the potential for metal uptake by predators and their subsequent bioaccumulation exists. Metals also can become associated with bacterivores through direct adsorption (of dissolved metal ions) to predator surfaces and through uptake of dissolved metals (through pinocytosis and facilitated transport) by the predator. Waste materials produced by predators also can act as adsorbents for dissolved metals, and the efflux of intracellular metals by predators may alter the physical/chemical form of the metals from which it originally was ingested. In short, the process of predation of bacteria and cell-bound toxic metals may present a significant opportunity for a change in metal speciation and/or for the bioaccumulation of metals.

Understanding the effects of toxic metals on the bacteria/protozoan part of the microbial food web also is of interest in the context of wastewater treatment plants. In fact, protozoa have been shown to increase the overall efficiency of sewage treatment. Curds showed that activated sludge with protozoa reduced organic matter substantially over protozoa-free sludge [2]. Protozoan community structure can be used as an indicator of the state of wastewater remediation [3]. Toxic metals, including Pb, have been shown to influence species richness and density of ciliate communities in activated sludge [4]. Studies also have shown that heavy metals can block enzymatic functioning in protozoa [5]. In nature, protozoa occur primarily in the benthos, that is, the sediment and detritus at the bottom of lakes. When bacterivorous protozoa prey upon and metabolize bacteria, which tend to assimilate rather than regenerate nutrients, they can change the bioavailability of these nutrients and anything else bound to prey cells.

In this research, we examine metal behavior in a model predator–prey experimental system containing Pseudomonas putida as the bacterial prey and the protozoan Tetrahymena thermophila as the predator. The ciliate protozoan T. thermophila was chosen as a representative predator species because it can grow and survive with P. putida as its only food source [6]. It also is able to grow and survive axenically in a complex medium [7]. Lead adsorption parameters for T. thermophila have been determined previously [6]. Pseudomonas putida G7 was selected as a prey species because of its ability to grow in the minimal mineral salt (MMS) media employed to permit calculation of metal speciation; prior research by the authors on its metal-binding properties; and its ability to serve as prey to our predator species [6].

The fate of prey-bound and dissolved toxic metals after ingestion by a predator such as T. thermophila is uncertain. Tetrahymena digests its food within food vacuoles. Once full, food vacuoles leave the oral region of the cell and fuse with the cell membrane; what remains inside (the fecal material) is egested. It is conceivable that fecal material is the ultimate fate of metal in this microbial food web. Ingested metals also
Behavior of lead in a model microbial predator–prey system

may accumulate within the predator and be passed up the trophic levels of the microbial loop. It also is possible that some metal desorbs from food particles during digestion and then is egested in the dissolved form. This phenomenon has been shown in protozoa ingesting iron colloids [8]. In stressed Tetrahymena cells, electron-rich cytoplasmic granules form and have been shown to sequester heavy metals (Cd, Zn, Cu, Pb) as a detoxification mechanism [9]. Tetrahymena species also are known to produce metallothioneins in response to the presence of heavy metals. These proteins have been shown to increase cellular tolerance to Cd [10].

Dissolved and predator-bound metals can alter the behavior and growth of predators. For example, in Tetrahymena pyriformis, Cd inhibits motility [11]. Toxic metals also have resulted in both inhibitory and stimulatory effects on prey ingestion by phagocytosis, as well as changes in the growth rate of bacterial predators [12]. Copper at 0.001 M was shown to stimulate the grazing rate of T. pyriformis, but then inhibited it at concentrations above 0.002 M [13].

Experimental bioreactor systems have been developed by the authors to provide a controlled environment where predator–prey interactions can be measured in the context of well-defined metal speciation [6]. The objectives of the present study were to evaluate the fate of Pb in this model predator–prey system, to assess the effect of Pb on growth and predation rates, and to use experimental results to obtain model parameters that permit predictions regarding metal–microbe interactions.

**MATERIALS AND METHODS**

*Pseudomonas putida growth parameters*

The MMS medium described in Patton et al. [6] was used to provide a solution matrix in which the speciation of test metals could be defined. The MMS medium consists of (per liter of solution): 30 mg of CaCl2·2H2O, 35 mg of MgSO4·7H2O, 120 mg of (NH4)2SO4, 15 mg of KNO3, 0.84 mg of NaHCO3, 3,800 mg of NaNO3, 0.015 mg of FeSO4·7H2O, 3,800 mg of pyruvate, and 0.002 mg of vitamin B-12. The components of the MMS medium are restricted to those with known metal stability constants. This medium was designed to eliminate competing trace metals and to prevent metal precipitation or the formation of solid phases that could adsorb added metals. The ionic strength of MMS was 0.05 M and the pH was six. Calculations with MINEQL+ (Ver 4 for Windows; Environmental Research Software, Hallowell, ME, USA) show that Pb is present primarily (92%) as the divalent cation (Pb2+) and approximately 7% as the aqueous sulfate complex, PbSO4·2H2O, in MMS at all Pb levels used in this research.

*Pseudomonas putida G7* was obtained from ATCC (Manassas, VA, USA) and was grown in MMS on a shaker table at 150 rpm at room temperature. The concentration of pyruvate and the density of bacteria cells were recorded over time. Bacterial cell density was measured as the absorbance at 600 nm. The depletion of pyruvate by bacteria was modeled as bacterial growth divided by the yield of bacteria growing on pyruvate (Yg: g bacteria/g pyruvate)

\[
\frac{dS}{dt} = \frac{1}{Y_g} \frac{\mu_{\text{max}} S - B}{K_{Sg} + S}
\]

(2)

The Yg was calculated by plotting pyruvate concentrations against the corresponding P. putida concentration during exponential growth phase. The K_{Sg} was estimated by fitting the batch growth results for P. putida with the calculated growth parameters and minimizing the residual error.

*Tetrahymena thermophila growth parameters*

The ciliated protozoan T. thermophila was used as the predator in experiments and was obtained from P. Bruns (Department of Microbiology, Cornell University, Ithaca, NY, USA; current affiliation: Howard Hughes Medical Institute, Chevy Chase, MD, USA). Tetrahymena thermophila initially was grown in Neff media (5.0 g/L dextrose, 2.5 g/L yeast extract, and 2.5 g/L proteose peptone [Difco, Franklin Lakes, NJ, USA]). Cultures grew in an incubator at 30°C without shaking. Before predation experiments, P. putida cultures were grown to stationary phase, centrifuged, rinsed three times, and resuspended in MMS without the pyruvate, KH2PO4, vitamin B-12, and FeSO4 (referred to here as MMS-2). In a comparable manner, T. thermophila cells were rinsed three times and resuspended in MMS-2 containing stationary phase P. putida as the only carbon/energy source. Axenic control bioreactors with P. putida or T. thermophila only were run simultaneously in duplicate. The effect of initial prey density on T. thermophila growth parameters was investigated using three different initial concentrations of P. putida: One base concentration (0.01 g/L dry wt) and suspensions with 3 and 10× the base concentration.

Populations of predator and prey were enumerated over time by different methods. Axenic P. putida cultures were enumerated by absorbance at 600 nm. Tetrahymena thermophila in both axenic and xenic cultures were enumerated using a Coulter Multisizer II (Beckman Coulter, Fullerton, CA, USA) by counting particles in the >8.0 μm size range. Pseudomonas putida cells from xenic cultures were enumerated using plate counts because bacterial cells could not be distinguished easily from T. thermophila waste material with the Coulter counter or spectrophotometer. The predator’s net growth rate was modeled as

\[
\frac{dP}{dt} = \frac{\mu_{\text{max}} B}{K_{Sp} + B - k_{de}} P
\]

(3)

where \(\mu_{\text{max}}\) (g/L) is the maximum specific growth rate of the predator (P) when \(B \gg K_{Sp}\), \(K_{Sp}\) is the P. putida concentration (g/L) when the specific growth rate is one-half the maximum,
and $k_p$ is the intrinsic predator death rate or maintenance coefficient (h).

The double saturation model is an alternative approach to Monod kinetics for describing predator growth [15]. Using the double saturation model, net predator growth is described as

$$\frac{dP}{dt} = \left( \frac{P_{\text{max}}, B^2}{K_p + B^2} - k_p \right) P$$

(4)

The double saturation model is said to better account for the dependence on substrate of the bacterial prey and the threshold prey concentration necessary for predation to occur [15]. Predator growth data were fit to the double saturation model and the Monod model, and the resulting fits were compared.

The *T. thermophila* population was sampled for a time interval sufficient to obtain a maximum growth rate ($P_{\text{max}}$) and intrinsic death rate constant ($k_p$), as described above for *P. putida*. The $K_p$ was estimated by comparing the specific growth of *T. thermophila* ($P_{\text{max}} B P / (1 + P)$) to both models and minimizing the residual error. To facilitate analysis, in some cases, variations in *T. thermophila* data were smoothed by fitting cell concentration over time to a second-order polynomial ($y = at^2 + bt + c$), and growth rates were taken as the slope of that curve.

The rate of *P. putida* consumption via predation ($r_{\text{pred}}$) was modeled as the protozoan growth rate divided by the yield coefficient of *T. thermophila* growing on *P. putida* ($Y_P$, g/g)

$$r_{\text{pred}} = \frac{1}{Y_P} \frac{P_{\text{max}} B P}{K_p + B}$$

(5)

and $Y_P$ was estimated by fitting the model to the data for net predator growth.

**Effect of Pb and prey density on *T. thermophila* growth and predation**

Predation experiments were conducted in 500-ml jacketed glass beakers pretreated with dimethyldichlorosilane to reduce Pb adsorption to glass surfaces. Before each use, the beakers were cleaned by washing in 10% trace metal-grade HNO$_3$, and rinsed with distilled, deionized water. A constant temperature controller was used to circulate water through the reactor and rinsed with distilled, deionized water. A constant temperature controller was used to circulate water through the reactor and was maintained. Axenic control reactors also were maintained. Initial *T. thermophila* counts were made with a Coulter counter, and both predator and prey species were enumerated over the course of the next 30 h.

It is expected that Pb uptake by a predator will increase (at the same total Pb level) if the prey concentration is higher, assuming that any toxic effects to growth caused by the increased metal uptake are outweighed by the benefits derived from an increase in the predator’s food supply. In a separate experiment, the effect of initial prey densities on predation and Pb fate was studied. The initial *P. putida* concentrations were 0.01, 0.03, and 0.13 g/L, hereafter referred to as $1\times$, $3\times$, and $10\times$, respectively. The initial Pb$_{\text{total}}$ concentration was 0.5 μM in all reactors except control reactors, which contained no Pb.

Total, dissolved, and predator-bound Pb were measured over time. The procedures for determining Pb speciation are described in detail by Patton et al. [6]. Briefly, at each time point, an aliquot for the measurement of total Pb was removed and acidified with trace metal–grade HNO$_3$, to a final concentration of 2.0% before analysis. Another aliquot of the cell culture was filter centrifuged with Centricon Biomax filters (Millipore, Billerica, MA, USA; <5,000 nominal mol wt limit), and the supernatant, which contained only dissolved Pb (i.e., Pb$_{\text{equilibrium}}$), was acidified with trace metal–grade nitric acid for analysis. The Pb adsorption by *P. putida* in axenic bioreactors was measured as the difference between total and dissolved Pb. In xenic reactors, Pb associated with the predator (Pb$_{\text{predator}}$ = Pb$_{\text{ingested}}$ + Pb$_{\text{adsorbed}}$) was measured directly by digesting membranes (5.0-μm pore size) with filtered cells in 10% trace metal–grade HNO$_3$. Filtrate from 5.0-μm filters was not analyzed, because the loss of Pb to the postfilter nalgene hardware was significant. All glassware was coated with dimethyldichlorosilane to minimize loss of Pb to reactor walls. Wall loss was determined with a reactor containing Pb and no cells. The Pb concentration was measured at the beginning and end of experiments and the difference, attributable to wall loss, consistently was <10%. All samples were analyzed for Pb by graphite furnace atomic absorption spectrometry (no matrix modifier; 20.0-μL analyte sampled; replicate analysis of Pb standards gave a coefficient of variation of <5%).

Ingested Pb was determined as the difference between Pb$_{\text{adsorbed}}$ and the Pb$_{\text{adsorbed}}$, which was calculated using the Langmuir isotherm previously established for Pb on *T. thermophila* [6]

$$\text{Pb}_{\text{adsorbed}} = \frac{K_p \Gamma_{\text{max}} \text{Pb}_{\text{equilibrium}}}{1 + K_p \text{Pb}_{\text{equilibrium}}}$$

(6)

where $K_p$ = 17.0 L/μmol and $\Gamma_{\text{max}}$ = 1.3 μmol/g, and Pb$_{\text{equilibrium}}$ is the concentration of dissolved Pb in μM.

The uptake of Pb by adsorption to prey was calculated using a linear Pb isotherm for *P. putida* previously established in this laboratory [6]

$$\text{Pb}_{\text{adsorbed}} = K_p \text{Pb}_{\text{equilibrium}}$$

(7)

where $K_p$ was measured to be 25.755 ml/g.

The remainder of adsorbed Pb, calculated as Pb$_{\text{total}}$ – (Pb$_{\text{equilibrium}}$ + Pb$_{\text{adsorbed}}$ + Pb$_{\text{predator}}$), was assumed bound to waste matter produced by *T. thermophila*.

The concentration of Pb ingested by predation was modeled as the product of the predation rate and the specific sorbed metal concentration associated with *P. putida* minus Pb excreted by *T. thermophila*.
where $k_w$ was the excretion coefficient of Pb from *T. thermophila*. The $k_w$ was determined from the slope of the natural logarithm of Pb associated with *T. thermophila* versus time after the prey was consumed.

**RESULTS AND DISCUSSION**

**Pseudomonas putida growth parameters**

The maximum growth rate of the bacteria ($\mu_{\text{max}}$) was 0.4/h ($r^2 = 0.86$), and the intrinsic death rate ($k_d$) was 0.002/h ($r^2 = 0.79$). The observed yield of bacteria growing on pyruvate ($Y_p$) was 0.27 g/g ($r^2 = 0.91$). The saturation constant ($K_S$) was found by fitting data to Equation 1. The best-fit value of $K_S$ was 0.05 g/L ($r^2 = 0.83$). Because stationary phase *P. putida* cells were used in predation experiments, these growth parameters (other than $k_w$) are not required for modeling of the batch experimental obtained data for *T. thermophila* and Pb; however, the parameters are reported here because they are of use in model simulations of conditions where *P. putida* growth occurs.

**Tetrahymena thermophila growth parameters**

The growth curve for *T. thermophila* feeding on *P. putida* is shown in Figure 1. The experimental control (*T. thermophila* with no prey) usually did not show significant growth. In the cases where there was some growth in the control reactor, predation-related growth was corrected for the control. The maximum growth rate of *T. thermophila* ($\mu_{\text{max}}$) growing on *P. putida* was 0.16/h. This result is consistent with that reported in studies by other investigators of *T. thermophila* growing on *P. putida* in a batch culture, where the maximum observed growth rates ranged from 0.16/h (Hauptmann University 2000; http://bibd.uni-giessen.de/ghtm/2000/uni/ d000050b.htm) to approximately 0.22/h [7]. The intrinsic death rate of the xenic population ($k_d$) was 0.005/h ($r^2 = 0.85$). The saturation constant $K_S$ was found to be 0.028 g/L ($r^2 = 0.79$) using the Monod model and 0.001 g/L ($r^2 = 0.86$) with the double saturation model. Fits of the Monod and double saturation model to the data for the specific growth rate of the predator are shown in Figure 2. Although both models were satisfactory, the double saturation model was selected as a model for *T. thermophila* growth in subsequent experiments because it better minimized the residual squared error between the data and model.

Measurements of the true yield of *T. thermophila* growing on *P. putida* ($Y_p$) were confounded by an apparent use of substrate for something other than growth. Aside from growth, cells spend a significant amount of energy on maintenance, such as repairing cells, intracellular nutrient transport, motility, etc. *Tetrahymena thermophila* cells also appeared to re-ingest excreted material or their own dead cell material. The value for $Y_p$ was found to be 0.9 g/g ($r^2 = 0.73$) by minimizing error between the data and the logistic growth curve. A model simulation using the measured or fitted parameters is shown in Figure 2 along with the experimental observations.

**Effects of Pb on *T. thermophila* growth parameters**

The effects of the three concentrations of Pb on the growth parameters of *T. thermophila* are summarized in Table 1. For each initial dissolved Pb concentration and for the control containing no Pb, *T. thermophila* growth was better fit by the double saturation model than the Monod model (e.g., $r^2 = 0.88$ vs. 0.94 for the Monod vs. double saturation model fit for the lowest Pb concentration). Lead had a dose-dependent effect on the maximum growth rate, with *T. thermophila* growing more slowly at higher Pb concentrations: $\mu_{\text{max}}$ decreased linearly as the initial concentration of dissolved Pb increased, so that $\mu_{\text{max}} = -0.09[\text{Pb}]_\text{initial dissolved} + 0.16$ ($r^2 = 0.60$). At the highest Pb concentration, there was no observed growth. The Pb slightly altered the saturation coefficient ($K_S$), as described by the relationship: $K_S = -0.0015[\text{Pb}]_\text{initial dissolved} + 0.001$ ($r^2 = 0.82$).

<table>
<thead>
<tr>
<th>Initial dissolved Pb (g/L)</th>
<th>$\mu_{\text{max}}$ (g/L)</th>
<th>$K_S$ (g/L)</th>
<th>$Y_p$ (g/g)</th>
<th>$k_d$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.16</td>
<td>0.001</td>
<td>0.9</td>
<td>0.005</td>
</tr>
<tr>
<td>0.3</td>
<td>0.23</td>
<td>0.0003</td>
<td>0.81</td>
<td>0.034</td>
</tr>
<tr>
<td>1.8</td>
<td>0.03</td>
<td>0.0001</td>
<td>0.69</td>
<td>0.035</td>
</tr>
</tbody>
</table>

* NA = not applicable, no growth observed.

Table 1. The effect of Pb on *Tetrahymena thermophila* growth parameters.
Although there was an indication that the yield coefficient ($Y_p$) decreased with Pb concentration so that $Y_p = -0.47\times[Pb]_{dissolved} + 0.9$, this change was confounded by the experimental difficulty associated with the measurement of yield (see preceding paragraph) and may not be significant. The death rate ($k_{dP}$) increased with initial dissolved Pb concentration. Intrinsic death rate without Pb was 0.005/h, and with Pb, even at the lowest dissolved Pb concentration of 0.3 μM, $k_{dP}$ increased to 0.035/h. The coefficient describing Pb excretion from *T. thermophila* ($k_e$) also showed a linear relationship to initial dissolved Pb concentration, so that $k_e = -0.025\times[Pb]_{initial\ dissolved} + 0.11$.

The above relationships between model parameters and dissolved Pb concentration are approximations, because dissolved Pb varied somewhat from the initial concentration during each batch experiment. Conducting future experiments in chemostats, where steady state conditions in Pb concentration could be achieved, could refine these relationships.

**Effect of prey concentration on predation and fate of Pb**

Figure 3 shows the growth of *T. thermophila* at the three different initial prey densities. All experiments had the same initial Pb level (0.5 μM). The rate and amount of growth increased with starting prey density. Therefore, any toxic effect resulting from the increased uptake of cell-bound Pb was not as important as the positive effect on growth of the increased prey concentration.

The initial concentration of prey did not impact the temporal pattern of Pb fractionation. For example, Figure 4 shows the change over time in the fraction of ingested Pb, for different initial prey densities. A lower percentage of Pb was ingested by the end of the experiment for the highest prey density. If the predator were accumulating Pb in proportion to the number of prey cells it ate, we would expect to see the opposite trend. Instead, more Pb was adsorbed to predator cells at the highest prey density because their concentration was higher as a result of growth on the prey. Initially, *P. putida* cells were in equilibrium with dissolved Pb. Upon addition of predator (immediately following the initial observation), the fraction of Pb associated with *P. putida* decreased as the cells were removed by predation (compared to the amount of Pb ingested, the adsorption of Pb to *T. thermophila* had a negligible effect on the fraction of dissolved Pb). Lead associated with *T. thermophila* increased with ingestion of *P. putida* and then decreased, presumably as ingested Pb was excreted. It should be noted that the Pb adsorbed to and internalized by the *P. putida* prey was not differentiated. Although this distinction should not make a difference in the total concentration of Pb ingested via predation, it possibly could affect the excretion and assimilation of the metal by the predator. For example, the process of digestion in the predator gut could result in a different fate for prey-bound metals versus prey-assimilated metals. Furthermore, the ratio of adsorbed to internal Pb could change with the external Pb concentration and/or time. It would be of interest in future experiments to distinguish these two pools of prey-associated Pb to extend our understanding of the factors regulating Pb excretion in this model system.

Exposure of *Tetrahymena* to sublethal concentrations of Pb has been shown to postpone growth phase, increase doubling time, and decrease or stop endocytosis (the formation of food vacuoles) [16]. The severity of such effects depends on factors other than the total concentration of Pb, notably Pb speciation and phase distribution. For example, pH strongly affects toxicity by changing the fraction of total Pb that is in the form of the free ion, Pb$^{2+}$, which (based on the preponderance of research related to microbial response to metal ions) controls the metal uptake rate. Nilsson [16] reported an initial lag time in the growth of *Tetrahymena* exposed to 2.6 mM Pb at a pH of 6.8. At 3.9 and 5.2 mM Pb, the generation time was prolonged by a factor of 1.2 and 1.5, respectively. All other factors being equal, Pb toxicity increases as pH decreases, and more of Pb total exists as Pb$^{2+}$. However, at high pH, *Tetrahymena* actually may receive an increased dose of Pb, because it feeds on the Pb adsorbed to prey and, at very high lead concentrations, the Pb dose may be increased by feeding on precipitated Pb minerals. Once inside *Tetrahymena* cells, in cases where normal cell functioning continues, Pb is found sequestered in dense granules, small vesicles, and mitochondria [9].

Toxicity also is affected by media composition. For the same Pb total, a medium low in metal-binding ligands (such as dissolved organic matter) will have a higher concentration of Pb$^{2+}$ and, hence, higher metal toxicity. The rate of *Tetrahymena* phagocytosis was observed to decrease by 52, 37, and 0% when the concentration of proteose peptone/liver extract media was 0.5, 1, and 2%, respectively [17]. In another study, the 20-h dose resulting in 50% mortality to *Tetrahymena* exposed to PbNO$_3$ increased significantly with the addition of calcium carbonate [18]. This likely was the result of higher
levels of inorganic ligands (HCO$_3^-$, CO$_3^{2-}$) and/or cation competition between Ca$^{2+}$ and Pb$^{2+}$.

In the experiment with $10^4$ P. putida, T. thermophila was exposed to a lower level of Pb$^{2+}$, because more of the added Pb was adsorbed to prey cells (relative to the $1 \times$ and $3 \times$ prey levels). However, if Pb is desorbed from ingested prey cells inside the acid environment of the food vacuoles, it is conceivable that adverse affects of Pb on Tetrahymena would appear after digestion of prey. Indeed, during excretion, the cytoproct, the region of the cell from which digested food is expelled, is reported to be high in Pb [17].

The experimental Pb concentrations used in this research are in the same range as soluble Pb in the mixed liquor of activated sludge plants, where levels as high as 500 μg Pb/L ($\sim$2.4 μM: [4]) are reported. However, the experimental Pb concentrations are high compared with those found in natural waters. For example, Benoit [19] reports total Pb levels of approximately 2 ppb in the urbanized reach of the Quinnipiac River, Connecticut, USA, or roughly a factor of 40 below the lowest total Pb level used in this research. The Pb levels selected for use in our experiments permitted precise and accurate graphite furnace atomic absorption spectrometry analysis of Pb in samples without requiring use of ultraclean techniques. The experimental Pb levels also did not exceed the solubility product of any Pb solid phases and, as noted above, the solution speciation was dominated by the free ion. Lead also was not toxic to the prey at the levels used. Because adsorption of Pb to the prey obeyed a linear isotherm and adsorption to the predator was negligible, it is reasonable to expect that the results obtained can be extrapolated to lower Pb levels. Under conditions where Pb binding to prey follows a linear isotherm, the experimental use of elevated Pb levels has the net effect of accelerating the time course of Pb uptake that otherwise would be observed for predators in the field.

Model predictions

Using kinetic parameters related to growth of T. thermophila on P. putida obtained in these laboratory experiments and previously determined Pb adsorption isotherms for the predator and prey, a computer model for Pb behavior was developed using Stella 8.0 modeling software (High Performance Systems, Lebanon, NH, USA). Figure 5 shows the observed effect of Pb on T. thermophila cell growth over time and the model predictions. Growth model predictions were based on the effects of Pb on growth parameters summarized in Table 1. The model effectively captures the temporal trends of the observations. Using these growth parameters and previously reported Pb adsorption isotherms [6], independent predictions (with no adjustments to model parameters) can be made for the behavior of Pb over time in the model predator–prey system at different Pb levels. The results of model predictions are shown in Figure 6 for the experiment at low Pb (initial dissolved Pb of 0.3 μM). The model predicts that the dissolved Pb and prey-bound Pb concentrations decrease upon addition of predator cells, because predators are consuming the bacterial prey and Pb is adsorbing to predator cells. After approximately 10 h, Pb begins to be excreted by T. thermophila. The predictions from the model effectively capture the observed trends in Pb adsorbed to cell surfaces and Pb associated with waste material, but underestimate Pb ingested and, consequently, overestimate dissolved Pb. The model predicts that the concentration of ingested Pb peaks at around 15 h and then decreases again as it is excreted. This prediction did not match the data. Instead, ingested Pb increased immediately (at the first measurement taken at 2.5 h) and began to decrease again soon after. Model predictions and observations of Pb behavior in the high lead experiment (in which cells were exposed to an initial dissolved Pb concentration = 1.8 μM) are shown in Figure 7. Lead toxicity is much more evident under these conditions (see cell growth in Fig. 5). The model predicts that Pb primarily remained bound to prey cells or in the dissolved state, because there was little T. thermophila growth or predation. Model predictions that negligible Pb is adsorbed to T. thermophila or associated with waste material were consistent with observations; however, there is a discrepancy again between the model simulation for ingested Pb and dissolved Pb versus observations.

It should be noted that the concentration of ingested Pb was measured as the difference in total Pb associated with T. thermophila cells (as determined by filtration through 5-μm filters) and the concentration adsorbed to the outside of the cells (based on the Pb isotherm for T. thermophila). However, the model calculations of ingested Pb are related to the number of prey cells eaten by predators and the Pb adsorption isotherm of the prey. Thus, one explanation for the discrepancy between the model simulation and experimental observation is that...
measurements of ingested Pb include a form or forms of particulate Pb different from that calculated by the model. This could occur if some waste material or lysed cells present in the original inoculum of T. thermophila were captured by the 5.0-μm filters used to separate T. thermophila from the waste or bacteria-associated Pb. Filter capture of particles other than the predator would inflate the concentration of Pb considered to be ingested. The plausibility of this hypothesis was tested through a model simulation in which it was assumed that lysed cells and waste matter (called debris in the model) were added in addition to the T. thermophila inoculum. These particulates would adsorb Pb, but not consume prey. Lead adsorbed by the debris would be measured along with Pb associated with the predator, and the sum (determined in the simulation) can be compared to the experimental observations in which we speculate this occurred. Because debris likely would have a greater specific surface area than the predator cells, it would be expected to have a greater affinity (per unit mass) for Pb, and it was assigned an adsorption constant of 100 L/g (this value is arbitrary and is selected for purposes of illustration). Also, it was assumed that the ingestion of Pb via debris was first order with respect to the concentration of debris and was assigned a rate constant of 0.05/h.

Figure 8 shows the revised simulation for the low-Pb case (initial dissolved Pb = 0.3 μM) and demonstrates that the presence of debris under these conditions could account for some of the discrepancy between experimental observations and the initial model simulation (in which no inert particles are assumed to be present).

An alternative explanation for the difference between the experimental data and the initial model simulations is that the model description is missing one or more Pb interactions that affect the observations. For example, we do not consider how the ingestion of dead predator cells by T. thermophila will affect the fate of Pb in this system. Although the model readily could be modified to include this possibility and to assess its importance, this and other scenarios with a modified model would be best performed after the needed parameters were obtained from additional experiments. In cases where differences in observations and model simulations occur, observations can guide the evolution of a model toward a set of constitutive equations that are adequate to render predictions consistent with observations. Changes in the model, in turn, stimulate new experiments to obtain the needed additional model parameters and to verify predictions of the modified model.

CONCLUSION

In summary, these results demonstrated that some kinetic parameters related to prey consumption and growth of T. thermophila are altered by Pb concentration. Upon addition of predator to prey cells in equilibrium with dissolved Pb, dissolved and prey-bound Pb became associated with the predator through ingestion and adsorption. Ingested Pb later was excreted as a bound metal associated with T. thermophila waste matter. A preliminary mathematical model was developed to describe predator–prey dynamics and their influence on the behavior and fate of Pb. Differences in model predictions and observations suggest possibilities for model alterations that can result in a closer approximation to experimental observations. Additional experiments are required to resolve whether differences in the model and observations result from measurement artifacts, missing processes in the model, or both.

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