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Interactions of Pb and Cd mixtures in the presence or absence of natural organic matter with the fish gill

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ABSTRACT

Metal gill binding and toxicity can be modeled using the concentration addition model, in which the toxic unit (TU) concept is used to determine if constituent metals are acting in a strictly additive, less than, or greater than additive fashion. To test this hypothesis, rainbow trout (Oncorhynchus mykiss) were exposed to a matrix of Pb plus Cd mixtures (nominal concentrations=0.75, 1.5, 2.25, 3.0 μ mol L⁻¹), in the presence or absence of mainly terrigenous (allochthonous; 10 mg C L⁻¹) natural organic matter (NOM), and metal-gill binding, and toxicity was quantified. Based on its greater affinity for metal-gill binding sites, Cd-gill binding was expected to exceed Pb-gill binding during metal mixture exposure, but this only occurred at the lowest metal concentrations (0.75 μ mol L⁻¹); at higher concentrations Pb-gill binding was greater than Cd-gill binding. These unexpected observations were because Pb and Cd likely bind to different populations of high affinity, low capacity binding sites on the gill, which was borne out in subsequent attempts to mathematically model metal-gill interactions during metal-mixture exposure. The presence of an additional low affinity, high capacity population of Pb-gill binding sites also contributed to higher Pb-gill accumulation. Metal-gill interactions were complicated by NOM, which exacerbated toxicity during Cd-only exposure despite lowering Cd-gill accumulation. NOM also promoted Cd-gill binding in the presence of low-moderate concentrations of Pb (0.75 and 1.50 μ mol L⁻¹). We suggest that direct interactions of Cd–NOM complexes with the gill, and increases in Cd bioavailability due to Pb outcompeting Cd for NOM-metal binding sites due to its greater affinity for such ligands, accounted for greater Cd-gill binding and toxicity. We conclude that interactions of Pb and Cd with the gill cannot be predicted using the concentration addition model, and that NOM is not universally protective against metal-gill binding and toxicity when fish are exposed to metal mixtures.

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1. Introduction

The toxic effects of single metals on fish have been thoroughly studied (e.g. Playle (1998), Paquin et al. (2002), Niyogi and Wood (2004)), but metals rarely appear singly in the environment (Di Toro et al., 2001; Norwood et al., 2003; Playle, 2004). There is therefore a need to understand better and predict how metals in mixtures interact with each other and with aquatic organisms, and how metal bioavailability is influenced by water chemistry variables including natural organic matter (NOM)? A theoretical multiple-metal gill-binding model, developed by Playle (2004), investigated how metals in mixtures might interact with fish. The model combined existing metal–gill interaction models such as the biotic ligand model (BLM;

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Di Toro et al., 2001; Paquin et al., 2002; Niyogi and Wood, 2004) for single metals, and used MINEQL+ to formulate predictions about how these metals would bind to fish gills when present in mixtures. This model was based on the toxic unit concept (Newman and Unger (2003)) and the concentration-addition model (Norwood et al., 2003; Playle, 2004).

While recognizing that metals act at different sites on the fish gill (e.g. Paquin et al. (2002), Marshall (2002), Niyogi and Wood (2004)), the multiple-metal model developed by Playle (2004) worked under the presumption that all the metals reacted the same way with gills and acted as Ca^{2+} analogs. The model also assumed that when half of the metal–gill binding sites were filled with metal, half of the fish in the experiment would die. This was considered equivalent to the LA₅₀ (lethal accumulation that produces 50% mortality), or the metal concentration that would have caused 50% mortality after 96 h (96-h LC50), and considered equivalent to one toxic unit. When the sum of two or more metal concentrations combined was equal to one toxic unit, gill metal

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accumulation was considered additive. When the sum was greater than one toxic unit, metal–gill accumulation was predicted to be less than strictly additive (antagonistic) and when total metal concentration was less than one toxic unit, metal–gill accumulation was predicted to be greater than additive (synergistic).

The present study tested such predictions by exposing rainbow trout (*Oncorhynchus mykiss*) to different mixture combinations of Pb and Cd, two Ca²⁺ analogs considered to be taken up via branchial Ca²⁺ channels (Verbost et al., 1989; Rogers et al., 2003; Rogers and Wood, 2004). Because the relative strength (affinity) with which Cd binds to trout gills (log $K_{Cd-gill} \sim 7.1-8.6$; Hollis et al., 2000; Niyogi et al., 2004, 2008; Playle, 2004; Birceanu et al., 2008) is greater than that of Pb (log $K_{Pb-gill} \sim 6.0-7.0$; Playle, 2004; Birceanu et al., 2008), we predicted that fish would accumulate more Cd on the gills than Pb when simultaneously exposed to equimolar concentrations of these metals. The metal-gill burden was also compared to the toxicity of the metal mixtures by investigating the LT₅₀ (time to 50% mortality) results for fish exposed to Cd and Pb in mixtures for 96 h.

Complexation of metals by inorganic and organic ligands can also influence metal bioavailability and metal-gill binding. Aquatic environments contain natural organic matter (NOM) which complexes metals to reduce metal bioavailability (e.g. Richards et al. (2001), De Schamphelaere et al. (2004), Winter et al. (2007)). The strength (affinity) with which different metals bind to NOM also varies. Although Pb has a higher affinity for NOM ($\log K_{NOM-Pb} = 8.4$) than Cd ($\log K_{NOM-Cd} = 7.4$; Playle, 2004), we know little about how this affects metal bioavailability and toxicity when fish are exposed to metal mixtures. Accordingly, a second goal was to establish how metal bioavailability and metal gill binding were affected when fish were exposed to NOM-Cd-Pb mixtures. Because Pb should outcompete Cd for metal-NOM binding sites, we predicted that the presence of NOM should increase Cd bioavailability, and Cd-gill binding when fish were exposed to Cd-Pb plus NOM mixtures.

A final goal was to use the empirical data derived from our metal–NOM-mixture experiments to validate predictive models using the approaches advocated by Borgmann (Borgmann et al., 2004, 2008), in which the interactions of metals are considered to be competitive or non-competitive.

2. Method and materials

2.1. Fish husbandry

Fingerling rainbow trout (*Oncorhynchus mykiss*: 1–10 g) obtained from Rainbow Springs Trout Hatchery (Thamesford, ON, Canada) were housed in a 50:50 mix of well water and soft (ion-poor) water and fed ground trout chow (Corey Feed Mills, Fredericton, NB) three times a week. After several weeks, the trout were acclimated for at least one week to ion-poor water [Composition (in μ mol L⁻¹): Ca²⁺=53; Na⁺=400, Cl=196, pH~7.0; 13–17 °C], supplied by a reverse osmosis unit (Series E Reverse Osmosis System, Culligan of Canada, Mississauga, Ontario) and supplemented with a Ca²⁺ drip. Trout were starved 24 h prior to experiments to avoid fouling of the experimental water.

2.2. Metal gill accumulation and toxicity during exposure to equimolar Pb plus Cd mixtures

Trout (N=5 per treatment) were exposed to four different equimolar mixtures of Pb plus Cd which included 0.75, 1.5, 2.25 and 3.0 µmol L⁻¹ of each metal (plus nominally metal free controls) in two series of experiments lasting either 3 or 96 h. In each series, gill metal concentrations were determined at the end of the exposure period. However, mortality was also tabulated during the 96 h exposure, and the data used to determine the LT₅₀ for each metal exposure combination. In each series, five trout were placed into 20 L buckets filled with exactly 10 L of ionpoor water to match the chemical composition of the acclimation media. The pH of the exposure media was monitored with a GK2401C combination pH electrode connected to a PHM84 pH meter (Radiometer, Copenhagen, Denmark), and maintained at pH 6.0 through the drop-wise addition of HNO_3 or KOH to each container at regular intervals. We chose to maintain the water at pH 6.0 to not only simulate acidic soft water conditions found in the Canadian Shield, but to also ensure that Pb did not precipitate out of solution due to the formation of PbCO₃ (cerussite), which would have confounded our experiment by decreasing dissolved Pb concentrations. Following pH adjustment, the appropriate amounts of metals were added to the water as chloride salt solutions (CdCl₂ · 5H₂O, Fisher Scientific, Fair Lawn NJ; PbCl₂, Mallinckrodt Specialty Chemicals Company, Phillipsburg, NJ).

2.3. Effects of NOM on Pb-gill and Cd-gill accumulation

These series of experiments were similar to the initial 3 h Pb plus Cd exposure experiments (Section 2.2), but in this case the fish were exposed to a greater variety (a matrix) of Pb plus Cd mixtures (24 metal mixture combinations plus controls: N=5 per treatment) in the absence or presence of NOM. Nominal metal concentrations included mixtures comprised of different combinations of 0.75, 1.5, 2.25 and 3.0 $\mu mol \ L^{-1}$ of Pb or Cd. Two sets of experiments were performed. The first quantified metal-gill binding when the fish were exposed to Pb+Cd mixtures alone, while the second examined metal-gill binding in the presence of added (exogenous) NOM (10 mg L⁻¹). The metal addition and pH monitoring protocols were also identical to the 3 h experiment (Section 2.2). Fish (N=5) were randomly added to each exposure container, and the container location was randomized to prevent "tank effects" (position effects). Water temperature was maintained at 12 °C by immersing the containers in a water bath which was continually replenished with well water at the same temperature. Water samples (35 mL) were collected at 1 and 3 h, and two sub-samples (7 mL each) filtered using a 0.45 µm Millex-HV syringe filter (Millipore, Bedford, MA). Both the unfiltered and filtered water samples were acidified with one drop of concentrated HNO₃ and subsequently analyzed for total and dissolved Cd and Pb, total Ca²⁺, Na⁺, and Cl⁻ An additional sub-sample of unacidified water was collected in borosilicate vials for later determination of total dissolved organic carbon (DOC) concentration. At the conclusion of the 3 h metal exposure, fish were killed by a blow to the head, the gill baskets removed and rinsed for 10 s in deionized water to remove loosely bound metal, and transferred to individual 1.5 mL centrifuge tubes and frozen at -40° C until processed for metal quantification.

The exposure water and gill sampling protocol was identical for the 3 h metal plus NOM exposure. The NOM was collected from Luther Marsh (Grand Valley, Ontario; 43° 54.3' N; 80° 24.5' W) in June 2005 and concentrated using a portable reverse osmosis unit (RO; Limnological Research Corporation, Kelowna B.C.) in the field. The approximately 40-fold (200–5 L) NOM concentrate was then treated in the laboratory with a H⁺ cation exchange resin at pH 2.0 (USF C-211H cation resin, U.S. Filter Corporation, Rockford IL) to remove metals and other bound constituents (Schwartz et al., 2004; Gheorghiu et al., 2010).

To compare the toxicity of Pb and Cd in mixtures with and without NOM, fish were exposed to the same combination of Pb+Cd concentrations used in the 3 h experiments, but in the presence or absence of NOM over 96 h. In addition to the metal mixtures and NOM, the Ca²⁺ concentration and pH were maintained as described for the 3 h experiments, and filtered and unfiltered water samples were taken daily. Gills were sampled for Pb and/or Cd accumulation after 96 h.

2.4. Analytical methods

Gills were processed for metal quantification by determining their wet mass and digesting them in 5 times their mass in 1 N trace-metal grade HNO_3 for 3 h at 80 °C. The digests were then vortexed and the supernatant diluted 10 times with ultrapure water for Pb analysis and 20 times for Cd analysis. Metal concentrations were quantified in gill and water using a graphite furnace atomic absorption spectrophotometer (Varian SpectrAA 880 with GTA 100 atomizer; Varian Canada Inc., Mississauga, ON) against standards prepared using certified reference solutions (Fisher Scientific, Nepean ON). Total and dissolved organic carbon (DOC) was measured in undiluted water samples using a Schimadzu 5050A Total Organic Carbon Analyzer (Mandel Scientific). Water Ca^{2+} and Na^+ were measured using flame AAS (SpectrAA 880, Varian Canada Inc., Mississauga ON) and water CI⁻ measured using a commercial kit (Biopacific Diagnostics, North Vancouver, B.C.).

2.5. Modeling of Pb and Cd-gill interactions in the presence or absence of NOM

Modeling of experimental results was performed using Systat to determine if Pb and Cd were behaving in a competitive or non-competitive manner (cf. Borgmann et al. (2008)). Competitive interactions are defined as competition by two or more metals for the same ligand, resulting in increases in the half-saturation constant ($K_{0.5}$) or decreases in the apparent binding constant ($1/K_{0.5}$) for the metal of interest, while non-competitive interactions generally lead to reductions in only the maximal number of binding sites for the metal (Borgmann et al., 2008). To model Pb and Cd interactions, the measured water metal concentrations were converted to free ion activity in WHAM (Windermere Humic Aqueous Model), and the system was set open to the atmosphere.

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