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Short Communication

TIME COURSE OF METAL LOSS IN *LUMBRICULUS VARIEGATUS* FOLLOWING SEDIMENT EXPOSURE

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Abstract—After exposure for 21 d to sediment spiked with Cd, Pb, Cu, or Zn, oligochaetes (*Lumbriculus variegatus*) held in clean water depurated metal rapidly over the first few hours but much more slowly from 8 h up to 32 h. Results are consistent with previous work suggesting a 6-h depuration period as generally appropriate for sediment bioaccumulation studies with *L. variegatus*.

Keywords—Bioaccumulation test   Depuration   Oligochaete   Sediment   Metal

INTRODUCTION

Many environmental contaminants commonly found in sediments have the potential to accumulate through the aquatic food chain, thus providing a pathway for exposure of higher trophic levels, including humans. Bioaccumulation tests have been developed as one mechanism for assessing the extent of bioaccumulation that may occur from contaminated sediments [1–3], and these procedures have been incorporated into certain regulatory programs [4].

For freshwater sediments, the oligochaete *Lumbriculus variegatus* is an organism commonly used for bioaccumulation testing. Conceptually, this is a simple procedure in which oligochaetes are exposed to test sediments for 28 d, then sieved from the sediments and analyzed for contaminants of interest. The U.S. Environmental Protection Agency and American Society for Testing and Materials protocols [2,3] recommend a holding period in clean water at the end of the sediment exposure to allow organisms to purge their gut of sediment prior to analysis. This purging is included to prevent sediment-bound chemicals in the gut from being measured as a part of the tissue burden. Inclusion of a purging period in the test protocol has been questioned by some because of concerns that tissue-bound chemical will depurate from the organisms during holding in clean water, thereby underrepresenting the steady-state concentration in the organism residing in the sediment [3,5].

In previous work [6], we discussed the influence of different purging periods on predicted concentrations of nonionic organic chemicals in *L. variegatus* and made recommendations relative to those chemicals. This article evaluates the time course of metal loss from *L. variegatus* exposed to metal-contaminated sediment and evaluates those results in light of previous recommendations.

METHODS

Test sediments

Test sediments were prepared from uncontaminated natural sediment collected from West Bearskin Lake (Cook County, MN, USA; 48°3.86′N, 90°24.61′W). Prior to use, sediment was sieved through a 300-μm stainless steel sieve using a small amount of supplemental water (Lake Superior water). After sieving, sediment was allowed to stand for at least 2 d, then excess water was decanted. Sediment grain size was not measured after sieving but prior to sieving was predominately sand and silt (50% > 50μm, 48% between 50μm and 2 μm, 2% between 0.08 and 2 μm). Separate batches of sediment were spiked with one of four metals: Cd or Pb at 8 μmol/g dry weight or Cu or Zn at 12.5 μmol/g dry weight. Each metal was added as a chloride (Cd, Cu, Zn) or nitrate (Pb) salt dissolved in a small amount of deionized water, followed by thorough mixing with a stainless steel impeller mounted on an electric drill. Spiked-metal concentrations were selected to result in substantial metal accumulation without causing adverse effects on the oligochaetes, based on preliminary experiments. After spiking, sediments were stored for 8 to 30 d under refrigeration for equilibration.

Exposure to sediment

Oligochaetes (*L. variegatus*) were exposed to each sediment for 21 d using an intermittent water-renewal system based on the Zumwalt design [7]. One hundred milliliters of test sediment were placed in 300-ml-high form beakers, with an overlying layer of 170 ml of dechlorinated Lake Superior tap water (hardness = 47 mg/L as CaCO₃; alkalinity = 49 mg/L as CaCO₃) and placed into the test system. The following day, approximately 420 adult oligochaetes (>3-cm length) were added to each beaker. Exposure continued for 21 d at 23 ± 1°C with a 16:8-h light:dark photoperiod. Overlying water was automatically renewed at a rate of 3.4 volume additions per day. Twenty-five milligrams per day of Aquatox flake fish food (Ziegler Brothers, Gardners, PA, USA) was added to each beaker 6 d per week. Dissolved oxygen, pH, and temperature

Table 1. Chemical analysis of test sediments, presented as mean ± standard deviation of values measured at 0, 7, 14, and 21 d, except for total organic carbon, which was measured prior to sieving and spiking

Treatment group	Acid volatile sulfide (μmol/g)	Simultaneously extracted metals (μmol/g)	Dry weight (%)	Total organic carbon (%)
Cd	5.9 ± 1.20	6.8 ± 0.44	29.5 ± 0.95	3.76
Pb	2.0 ± 1.37	7.3 ± 0.41	23.8 ± 4.30	2.50
Zn	6.2 ± 1.50	11.2 ± 0.86	29.9 ± 0.42	3.76
Cu	0.17 ± 0.09	12.4 ± 0.41	28.5 ± 0.37	3.76

of overlying water were monitored periodically and remained within acceptable ranges throughout the sediment exposures [2]. Total organic carbon content of the sediment was measured prior to spiking and acid volatile sulfide, simultaneously extracted metals, and sediment dry weight were measured after 0, 7, 14, and 21 d of oligochaete exposure to the sediments (Table 1).

Purging/depuration

After 21 d of exposure, oligochaetes were sieved from the sediment with a 300-μm stainless steel sieve and placed in a shallow tray containing clean water. Two groups of 15 organisms each were removed immediately from a single beaker for determination of total body burden of metal; 16 additional groups of 15 organisms were isolated and placed in separate, 300-ml-high form beakers without sediment, which were subsequently placed in the intermittent water-renewal system. Groups of two beakers were removed for tissue analysis at 1, 2, 4, 6, 8, 12, 24, and 32 h after the initial sieving. Conditions during this purging/depuration period were identical to the sediment exposure except that the beakers contained no sediment and the organisms were not fed. It has been suggested that reingestion of purged sediment by benthic invertebrates might decrease the effectiveness of the purging period [8], so at 2, 4, and 6 h, clean pipettes were used to remove any visible fecal material from the beakers.

Chemical analyses

Acid-volatile sulfide and simultaneously extracted metals were analyzed using procedures described by Leonard et al. [9]. Tissue samples were analyzed by atomic absorption spectrophotometry according to published methods [10].

RESULTS AND DISCUSSION

For all four metals, measured concentrations in oligochaetes showed a rapid decline during the first few hours of depuration, followed by much slower losses (Fig. 1). While it is not possible to create a mass balance of metal from these data, this is consistent with the expectation that metal associated with gut contents is lost quickly, followed by slower depuration from other compartments within the organism. The rate of initial metal loss is generally consistent with the rate of sediment purging by *L. variegatus* measured previously (purging 98% complete after 6 h [6]). At first glance, it seems as though there was little depuration of Zn. This appearance may be due in part to the high background Zn in oligochaetes. Although measurement variability was high, net loss of Zn during the first 6 h appeared to be roughly 1 μmol/g dry weight, which is comparable with results for Pb and Cu. Separate analyses of Zn in *L. variegatus* from our culture unit have shown background Zn of about 4 μmol/g dry weight (data not shown), suggesting that relatively little zinc was accumulated from the sediment.

Total metal body burdens at time 0 were elevated 4.3-, 2.4-, 2.2-, and 1.2-fold above the 24-h body burdens for Cd, Pb, Cu, and Zn, respectively, highlighting the importance of the clean-water holding period if tissue-bound metal is the parameter of interest, as also noted by other authors [8,11–13]. While we presume the initial loss of metal is primarily due to metal loss associated with gut contents, if there was a subset of tissue-associated metal that was rapidly lost during the first hours of clean-water holding, it would be difficult to distinguish this from metal in gut contents. To evaluate this possibility, we made a rough calculation of total metal mass lost from the organisms during gut purging by subtracting the average measured concentration between 8 and 32 h from the concentration at 0 h. If one assumes that the gut contents had the same composition as bulk sediment, the amount of test sediment that would have to be purged to account for the observed metal loss can be calculated. These estimates were 3.5, 18.0, 4.9, and 8.6% (sediment mass as a percent of total dry wt) for Cd, Pb, Cu, and Zn, respectively. These estimated gut contents are within the range of those commonly observed for *L. variegatus* [6,14–17], reinforcing the presumption that purging of sediment from the gut is the primary cause of the initial rapid decline in body burden rather than loss from some other internal metal pool. Variability of total metal body burdens generally decreased after gut contents were eliminated, as previously noted by Cain [18].

In studies where the intent of sediment bioaccumulation testing is to measure uptake of chemical into organism tissues but not chemical present in the gut contents, the test methodology must balance the competing biases introduced by incomplete gut purging when purging time is too short versus depuration from tissues when purging time is too long. In a previous evaluation of nonionic organic compounds [6], we proposed a 6-h purging period as a reasonable balance between these factors. Figure 1e shows the loss curves for the present experiments with metals, normalizing the metal loss to the total loss observed between 0 and 32 h. In general, metal concentrations were relatively stable after 6 h of purging, suggesting that any length of purging between 6 and 32 h would produce similar results. There is some suggestion that copper continued to be lost from the organisms after 6 h of purging (Fig. 1d and e), though this might be an artifact of variability in the data at 6, 8, and 32 h.

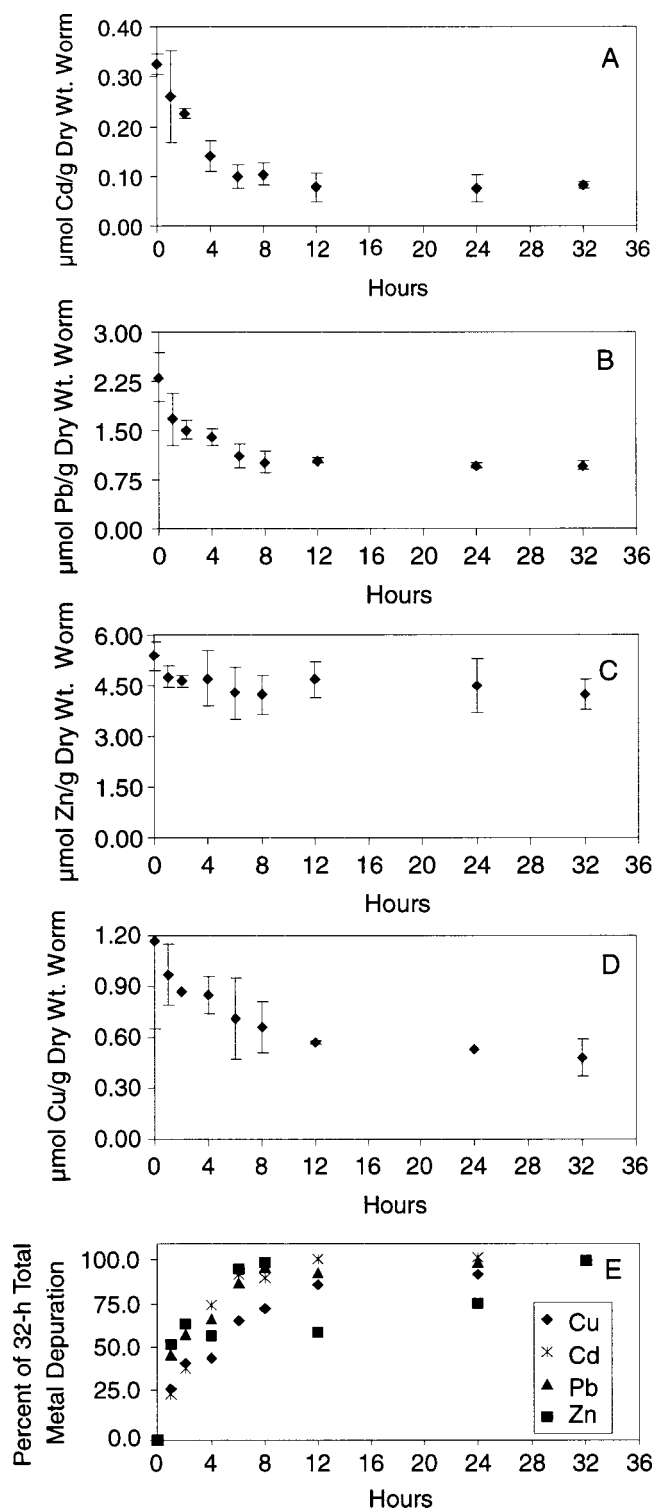We should note that the 21-d sediment exposure used in these experiments is shorter than the 28-d exposure common

Fig. 1. Depuration of Cd (**A**), Pb (**B**), Zn (**C**), and Cu (**D**) from *Lumbriculus variegatus* following exposure to metal-spiked sediment. Error bars are ± 2 standard deviations; note changes in scaling of *y*-axis among panels. (**E**) Rate of depuration for all four metals, normalized to the total metal loss observed between 0 and 32 h.

to the American Society for Testing and Materials and the U.S. Environmental Protection Agency bioaccumulation test methods [2,3]. This was done because the shorter exposure had relevance to separate research being conducted in our labo-

ratory. While not identical to standard guidance, we see no reason to believe that the results of these experiments would not be applicable to 28-d exposures as well.

Overall, these data indicate that the 6-h purging period suggested previously for nonionic organic chemicals is also likely to be appropriate for cationic metals, allowing samples for both groups of analytes to be collected using the same purging procedure. While longer purging periods (e.g., 24 h) may be suitable for high K_{ow} chemicals and perhaps metals, uncertainty about the loss rates for at least some metals may make use of a 6-h purging period for *L. variegatus* advisable, at least until the kinetics of metal loss are better understood.

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