

Supporting Information for:

Hitting reset on sediment toxicity: Sediment homogenization alters the toxicity of metal-amended sediments

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Appendix S1. Detailed results on sediment and water physicochemistry

Cu experiment – physicochemistry

During the test chamber incubation, Cu was released from the sediments into the overlying water and the flux of Cu decreased rapidly during equilibration (Figure S1). For Ocoee sediments, freshly spiked sediments released Cu about 1.5× faster than the aged sediments ($p < 0.001$). For the high total Cu concentration (310 and 760 mg kg⁻¹ treatments) Dow sediments, Cu flux was much faster from freshly spiked sediments than aged ones (10–30× greater flux), but low Cu_{TOT} sediments (52 and 140 mg kg⁻¹) had a similar Cu flux regardless of sediment preparation ($p < 0.001$). Across all treatments, Cu fluxes exhibited a negative power-law relationship with time, which indicates that fluxes from sediment declined very rapidly during equilibration.

During the *Hyalella azteca* exposure, we observed many differences in sediment physicochemistry between the two sediment preparations and some differences between Dow and Ocoee sediments. At the beginning of the exposure period, pH of freshly spiked sediments was less than aged sediment, and the pH of freshly spiked sediments was negatively correlated to Cu_{TOT} (Figure S2). Hydrolysis of CuCl₂ added during the spiking procedure reduced the sediment pH (Hutchins et al. 2009) at the beginning of exposure, but the pH returned to pre-spike pH and was similar across all Cu treatments by the end of the exposure period (Figure S2). As expected, AVS was negatively related to Cu_{TOT} in both freshly spiked and aged Ocoee and Dow sediments, and the sulfide-rich Ocoee sediments had greater AVS concentrations in aged sediments when compared to those freshly spiked (Cu × Preparation, $p = 0.016$). For both aged and freshly spiked Ocoee sediments, AVS was lower in surficial sediments (Cu × Depth, $p = 0.002$) and at the end of the exposure period compared to the start of the bioassay (Cu × Time, $p = 0.012$). These results are consistent with observations that oxidation of surface sediment (and lower pH) decreases concentrations of AVS and presumably metal sulfides (De Jonge et al. 2012, Simpson et al. 2012, Costello et al. 2015, 2016). For the relatively low-sulfide Dow sediment (AVS < 1 μmol g⁻¹), we observed no differences of AVS among sediment preparations, depths, or times ($p > 0.05$).

Sediment Fe and Mn concentrations were relatively similar between sediment preparations, but changes were observed during the exposure period. Total Fe was stable in Dow sediments but declined through time slightly in all Ocoee sediments (–10%; $p = 0.003$); the decline in total Fe was coupled with a similar 10% decline in concentration of amorphous Fe oxides ($p = 0.028$). Amorphous Fe oxides in Dow sediments were stable but crystalline Fe oxides declined through time in surface sediments only (–15%; $p = 0.045$). Amorphous Fe

oxides accounted for a larger fraction of Fe_{TOT} in Dow than Ocoee sediments (19 and 7%, respectively), but Ocoee sediments had a greater fraction of Fe as crystalline oxide minerals than Dow (47 and 25%, respectively). Total and oxidized Mn declined slightly through time in Dow sediments (-11% and 7%, respectively; $p = 0.04$). Although total Mn was stable in Ocoee sediments, oxidized Mn declined through time (-11%; $p = 0.025$) and was in lower concentration in freshly spiked than aged sediments (14% less; $p = 0.008$).

The two metal amendment approaches produced sediments with statistically similar Cu_{TOT} concentrations ($p > 0.05$), but the partitioning of Cu differed between freshly spiked and aged sediments. For all Cu-amended sediments, porewater Cu concentrations increased with Cu_{TOT} , but the partitioning of Cu between aqueous and particulate fractions (i.e., K_D) differed between Ocoee and Dow sediments. For Ocoee sediments, K_D did not differ ($p = 0.70$) between the freshly spiked treatment ($\log K_D = 5.14 \pm 0.23 \text{ L kg}^{-1}$) and the aged sediment ($\log K_D = 5.05 \pm 0.59 \text{ L kg}^{-1}$, Figure S3). For Dow sediment, aged sediment had a K_D ($\log K_D = 4.35 \pm 0.64 \text{ L kg}^{-1}$) that was slightly greater than freshly spiked sediments ($\log K_D = 3.62 \pm 0.84 \text{ L kg}^{-1}$, Figure S3), but the difference was not statistically significant ($p = 0.07$). For both Ocoee and Dow sediment, there was no statistically significant change in partitioning between the start and end of the bioassay ($p = 0.50$ and 0.11 , respectively).

Within the solid-phase fractions, the partitioning of Cu differed between aged and freshly spiked sediments. Among all Cu fractions in both sediments and preparation techniques, the concentration of Cu from each selective extraction was related positively to Cu_{TOT} ($p < 0.001$ for all), thus all differences discussed are relative to Cu_{TOT} . Copper extracted during the AVS procedure (i.e., Cu_{SEM}) was similar among all treatments and times for freshly spiked and aged Ocoee sediment ($p = 0.09$), but freshly spiked Dow sediment had a greater fraction of Cu_{SEM} than aged Dow sediment ($p < 0.001$). In Dow sediments, a greater proportion of Cu was bound to amorphous Fe oxides (0.5–1.4%) and crystalline Fe oxides (3–11%) than in Ocoee sediments ($\text{Cu}_{\text{HFO}}/\text{Cu}_{\text{TOT}} = 0.06\text{--}0.2\%$ and $\text{Cu}_{\text{CFO}}/\text{Cu}_{\text{TOT}} = 0.8\text{--}2.4\%$). For both Dow and Ocoee sediments, freshly spiked sediments had greater Cu_{HFO} concentrations than aged sediments ($p < 0.001$ and 0.04 , respectively). In Dow sediments, Cu_{HFO} concentrations were lower at the end of the experiment than beginning ($p = 0.02$), but Cu_{HFO} in Ocoee sediments remained stable through time ($p = 0.28$). For both Dow and Ocoee, Cu_{CFO} was greater in freshly spiked sediments than aged sediments ($p < 0.03$), which was similar to patterns observed for Cu_{HFO} . Cu_{CFO} declined in freshly spiked Ocoee surface sediments, but Cu_{CFO} concentrations in all aged sediments were stable through time (Time \times Depth \times Treatment, $p = 0.002$). In Dow aged

sediments, Cu_{CFO} increased through time in deep sediments, but Cu_{CFO} in freshly spiked sediments did not change through time (Time \times Depth \times Treatment, $p = 0.01$).

Ni experiment – physicochemistry

During the equilibration and exposure period, Ni was released into the water column, and the flux of Ni was related positively to the sediment Ni concentration ($p < 0.001$) (Figure S4). Through time, the flux of Ni from all Raisin sediments declined logarithmically ($p = 0.025$), but Ni flux from Burntwood sediment did not change through time ($p = 0.25$) (Figure S4). For both Raisin and Burntwood sediments, there was no difference in Ni flux between freshly spiked and aged sediments ($p > 0.13$). The rapid rate of overlying water exchange (8 times daily) led to filtered Ni concentrations that were low during the exposure period (95% of observations $< 60 \mu\text{g L}^{-1}$) and below the toxicity threshold for *H. azteca* (Besser et al. 2013).

Sediment physicochemical characteristics differed between the two sediment preparations (i.e., freshly spiked vs. aged), with Raisin and Burntwood sediments having different responses to preparation treatments. Sediment pH was higher in surface than deep sediment across all preparation techniques, sediment types, and sampling periods (Figure S5). In Burntwood sediments, pH ranged 6.9–7.4 and was weakly related to Ni_{TOT} ; the pH of the control sediment differed from that of the highest Ni treatment by < 0.1 pH units. At the start of the *H. azteca* toxicity assay, pH was lower in the freshly spiked treatments than in the aged sediments ($p < 0.001$) (Figure S5). In the aged sediments, pH declined during the exposure period, but we were unable to measure pH in the freshly spiked sediments due to electrode malfunction. In Raisin sediment, aged sediments had a higher pH than freshly spiked sediments across all time periods and Ni_{TOT} treatments ($p < 0.001$) (Figure S5). There was a slight positive relationship between pH and Ni_{TOT} , likely due to NaOH added as a hydrolysis buffer during Ni amendments, and the pH increase with Ni_{TOT} was strongest in freshly spiked sediments at the end of the exposure period (Figure S5). Although pH in Raisin sediment varied more than that in Burntwood sediment, the pH remained circumneutral (range 6.8–7.5) and would likely not drastically change either Ni speciation or toxicity. In Raisin sediment, AVS was negatively related to Ni_{TOT} ($p < 0.001$), likely due to formation of insoluble NiS (Simpson et al. 1998, U.S. EPA 2005). Similar to patterns observed for Ocoee sediment in the Cu experiment, AVS in Raisin sediment did not differ either between depths ($p = 0.20$) or through time ($p = 0.78$), but AVS concentrations were greater in aged treatments than freshly-spiked ($p = 0.04$). As expected, AVS in metal-amended Burntwood sediments was very low ($< 0.3 \mu\text{mol g}^{-1}$) in all sediment preparation treatments.

Fe and Mn concentrations were relatively consistent though time, between depths, and between freshly spiked and aged treatments, but there were large differences between Raisin and Burntwood sediments. Mean Fe_{TOT} was $45 \pm 1.3 \text{ g kg}^{-1}$ in the metal amended Burntwood sediments, whereas mean Fe_{TOT} in Raisin sediments was approximately 4× lower ($13 \pm 1.6 \text{ g kg}^{-1}$). Both Burntwood and Raisin sediments had greater Fe_{TOT} and crystalline Fe oxide concentrations in freshly spiked than aged surface sediments at the start of the exposure period, but these differences were small (<10%) and did not persist to the end of the exposure period ($Ni \times Time \times Depth$, $p < 0.005$). Amorphous Fe oxide concentrations did not differ either through time or between sediment preparations for both Raisin and Burntwood, but Burntwood sediment ($7 \pm 0.7\%$ of Fe_{TOT}) had a much lower fraction of Fe_{TOT} as amorphous Fe oxides than Raisin sediments ($48 \pm 7\%$ of Fe_{TOT}). Relative concentrations of crystalline Fe oxides were similar between Burntwood and Raisin sediments (25% of Fe_{TOT}). Burntwood sediment ($940 \pm 23 \text{ mg kg}^{-1}$) had significantly greater Mn_{TOT} in surface sediments at the end of the exposure period than either of the other sampling times and depths ($Time \times Depth$, $p = 0.003$) but the difference was <5%. Oxidized Mn did not differ among either treatments, depths, or sampling times in Burntwood sediment and was, on average, 67% of Mn_{TOT} . Total Mn in Raisin sediment ($760 \pm 65 \text{ mg kg}^{-1}$) was greater in freshly spiked than aged sediments ($p = 0.013$) and increased through time ($p = 0.02$), but these differences were relatively minor (<10% of Mn_{TOT}). Oxidized Mn concentrations were similar in all freshly spiked Raisin sediments and enriched significantly in the surface of aged sediments at the start of the experiment ($Time \times Depth \times Preparation$, $p = 0.004$). Regardless of differences among treatments, oxidized Mn was a large fraction of Mn_{TOT} in all Raisin sediments ($96 \pm 11\%$ of Mn_{TOT}). Overall, differences in Fe and Mn concentrations and species were greater between Raisin and Burntwood sediment than the differences among sediment preparation methods, depths, or sampling times.

The two sediment preparation approaches produced Ni_{TOT} treatments that were indistinguishable for Raisin sediment, but for Burntwood sediment, Ni_{TOT} was on average 10% greater in the freshly spiked treatments when compared to the aged sediments ($Preparation$, $p = 0.005$). Total Ni concentrations were consistent between depths and stable through time for Raisin and Burntwood sediments, regardless of preparation method. Partitioning of Ni to porewater in Raisin sediment did not differ between aged and freshly spiked preparations and was stable through time (Figure S6). However, partitioning of porewater Ni in Burntwood sediment differed at the start of the experiment (freshly spiked had a lower K_D than aged), but the two sediment preparation treatments were very similar at the final sampling point (Figure

S6). Nickel partitioned differently among solid-phase fractions as a function of sediment preparation method and depth, and more so in Burntwood sediments than Raisin.

Similar to our observations for Cu, all Ni fractions from laboratory prepared sediments were related to concentrations of Ni_{TOT} ($p < 0.001$ for all) and all differences discussed here are expressed relative to Ni_{TOT} . In Burntwood sediments, a substantial fraction of Ni_{TOT} was associated with amorphous (25–54%) and crystalline Fe oxides (32–38%). Ni_{SEM} was lower in surface than deep sediments ($p = 0.02$) and Ni bound to crystalline Fe oxides (Ni_{CFO}) was greater in surface than in deep sediments ($p = 0.02$). Concentrations of Ni_{SEM} and Ni_{HFO} in freshly spiked Burntwood sediment declined over time (all $p < 0.04$), whereas solid-phase associations of Ni in aged Burntwood sediments were stable through time. Similar to Burntwood sediments, a substantial fraction of Ni_{TOT} in Raisin sediment partitioned to Fe oxide minerals, but with a greater fraction associated with amorphous (34–74%) than crystalline (2–12%) Fe oxides. Solid-phase Ni partitioning in Raisin sediments was stable through time and between sediment preparation techniques. Ni partitioning to crystalline Fe oxides in Raisin sediment was slightly greater in surface than deep sediments of both sediment preparations ($p = 0.04$).

Zn experiment – physicochemistry

Only minor differences in sediment geochemistry were observed between freshly spiked and aged Raisin sediments. AVS concentrations were relatively high in Raisin sediment, positively related to Zn_{TOT} ($p < 0.001$) and did not differ between sediment preparation methods ($p = 0.32$). Sediment pH was circumneutral in all treatments and sampling periods (7.3–7.9 pH units), positively related to Zn_{TOT} ($p = 0.01$), and lower in aged deep sediments than any other treatment or depth (Depth \times Preparation, $p = 0.001$). Mean Fe_{TOT} was slightly higher in freshly spiked ($16.1 \pm 2.0 \text{ g kg}^{-1}$) than aged sediments ($14.8 \pm 1.5 \text{ g kg}^{-1}$; $p = 0.04$), but the relative speciation of Fe oxides did not differ among sediment preparations ($p > 0.05$). For both aged and freshly spiked Raisin sediments, amorphous Fe oxides declined slightly through time ($p = 0.02$); 7% of Fe_{TOT} was as Fe_{HFO} at the beginning of the experiment and 6% at the end of the exposure period. Crystalline Fe oxide concentrations did not differ among sediment preparation methods, depths, or sampling periods and were similar fractions of Fe_{TOT} across all sediments ($42 \pm 8\%$). Sediment Mn_{TOT} was similar among all sediments ($870 \pm 110 \text{ mg kg}^{-1}$), but Mn oxides were slightly higher in freshly spiked ($600 \pm 55 \text{ mg kg}^{-1}$) when compared to aged sediments ($550 \pm 53 \text{ mg kg}^{-1}$; $p = 0.008$).

Our two sediment preparation methods resulted in Zn concentrations that were, on average, 10% higher in the freshly spiked than aged sediments ($p = 0.02$). Both preparation methods produced metal-amended sediments that were consistent between depths and stable

through time (all $p > 0.50$). There was a positive relationship between Zn_{TOT} and concentrations of Zn in filtered overlying water ($p = 0.046$), and Zn in overlying water differed among our preparations through time (Preparation \times Time, $p = 0.01$). Specifically, Zn concentrations in surface water above aged sediments were lower at the beginning of the experiment (median = $70 \mu\text{g L}^{-1}$) than at the end of the exposure and lower than surface waters above freshly spiked sediments throughout the test (median = $190 \mu\text{g L}^{-1}$). Porewater Zn was positively related to Zn_{TOT} ($p = 0.02$), but there were no differences in porewater Zn between sediment preparation methods ($p = 0.73$). Partitioning coefficients for Zn were similar between freshly spiked ($\log K_D = 4.8 \pm 0.7 \text{ L kg}^{-1}$) and aged sediments ($\log K_D = 4.6 \pm 0.7 \text{ L kg}^{-1}$). In both sediment preparations, Zn_{SEM} was positively related to Zn_{TOT} , but in freshly spiked sediments, Zn_{SEM} was 33% greater in surface than deep sediments at the same Zn_{TOT} concentration (Zn \times Preparation \times Depth, $p = 0.02$). Zn_{SEM} concentrations (molar) exceeded AVS concentrations only at the highest two treatment concentrations of both sediment preparations (i.e., $Zn_{TOT} > 1400 \text{ mg kg}^{-1}$). Zinc associated with Fe oxides increased with increasing concentrations of Zn_{TOT} ($p < 0.001$). A greater fraction of Zn_{TOT} was bound to crystalline ($38 \pm 8\%$) than amorphous ($1.3 \pm 2.0\%$) Fe oxides. The proportion of Zn_{TOT} bound to amorphous Fe oxides averaged 5% in sediments with the greatest Zn_{TOT} concentrations (aged = 2600 mg kg^{-1} , freshly spiked = 3300 mg kg^{-1}), but $Zn_{HFO}:Zn_{TOT}$ was $< 1\%$ in all other treatments.

References

- Besser, J. M., W. G. Brumbaugh, C. G. Ingersoll, C. D. Ivey, J. L. Kunz, N. E. Kemble, C. E. Schlegel, and E. R. Garman. 2013. Chronic toxicity of nickel-spiked freshwater sediments: Variation in toxicity among eight invertebrate taxa and eight sediments. *Environmental Toxicology and Chemistry* 32:2495–2506.
- Costello, D. M., C. R. Hammerschmidt, and G. A. Burton. 2015. Copper sediment toxicity and partitioning during oxidation in a flow-through flume. *Environmental Science & Technology* 49:6926–6933.
- Costello, D. M., C. R. Hammerschmidt, and G. A. Burton. 2016. Nickel partitioning and toxicity in sediment during aging: Variation in toxicity related to stability of metal partitioning. *Environmental Science & Technology* 50:11337–11345.
- Hutchins, C., P. Teasdale, S. Lee, and S. Simpson. 2009. The effect of sediment type and pH-adjustment on the porewater chemistry of copper- and zinc-spiked sediments. *Soil & Sediment Contamination* 18:55–73.
- De Jonge, M., J. Teuchies, P. Meire, R. Blust, and L. Bervoets. 2012. The impact of increased

- oxygen conditions on metal-contaminated sediments part I: effects on redox status, sediment geochemistry and metal bioavailability. *Water Research* 46:2205–2214.
- Simpson, S. L., S. C. Apte, and G. E. Batley. 1998. Effect of short-term resuspension events on trace metal speciation in polluted anoxic sediments. *Environmental Science & Technology* 32:620–625.
- Simpson, S. L., D. Ward, D. Strom, and D. F. Jolley. 2012. Oxidation of acid-volatile sulfide in surface sediments increases the release and toxicity of copper to the benthic amphipod *Melita plumulosa*. *Chemosphere* 88:953–961.
- U.S. EPA. 2005. Procedures for the derivation of equilibrium partitioning sediment benchmarks (ESBs) for the protection of benthic organisms: metal mixtures (cadmium, copper, lead, nickel, silver and zinc). US Environmental Protection Agency, Washington, DC.

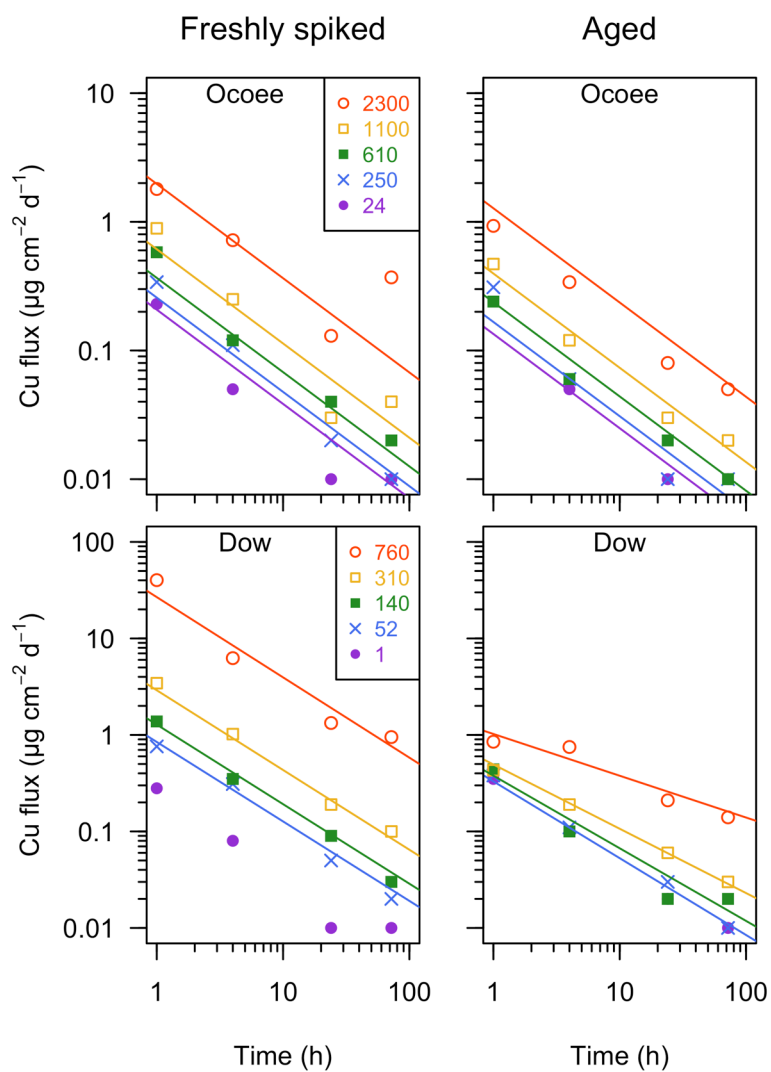


Figure S1. Cu flux to overlying water during the first 72-h of the test chamber equilibration period. Fluxes were measured in two sediment types that were either aged or freshly spiked. Warmer colors indicate sediments with higher total Cu (mean concentrations in legend). Solid lines indicate best-fit lines determined by ANCOVA. Note the log scale on both axes and the different axes for Dow and Ocoee sediments.

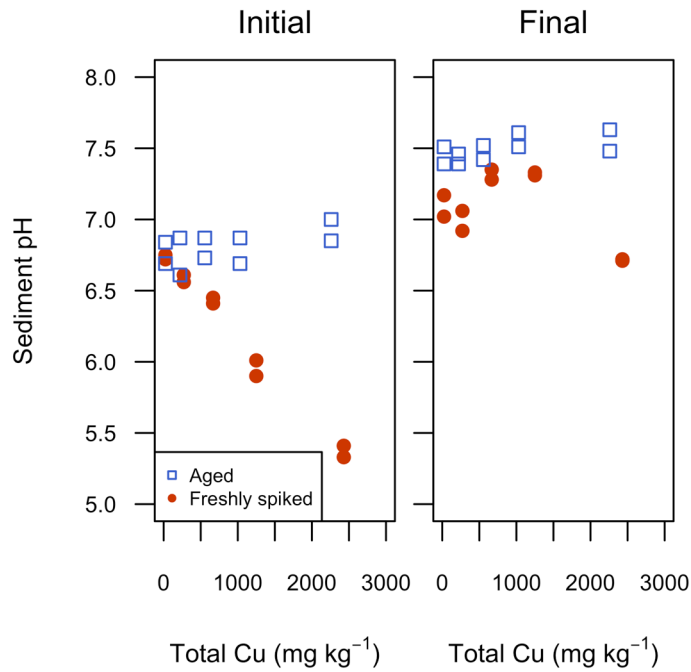


Figure S2. Sediment pH in aged and freshly spiked Ocoee sediments measured at the start and end of a 28-d *Hyalella azteca* bioassay. Sediment pH was negatively related to sediment total Cu only in the freshly spiked treatments at the start of the bioassay ($\text{Cu} \times \text{Time} \times \text{Preparation}$, $p = 0.018$). Dow sediments show similar patterns for pH.

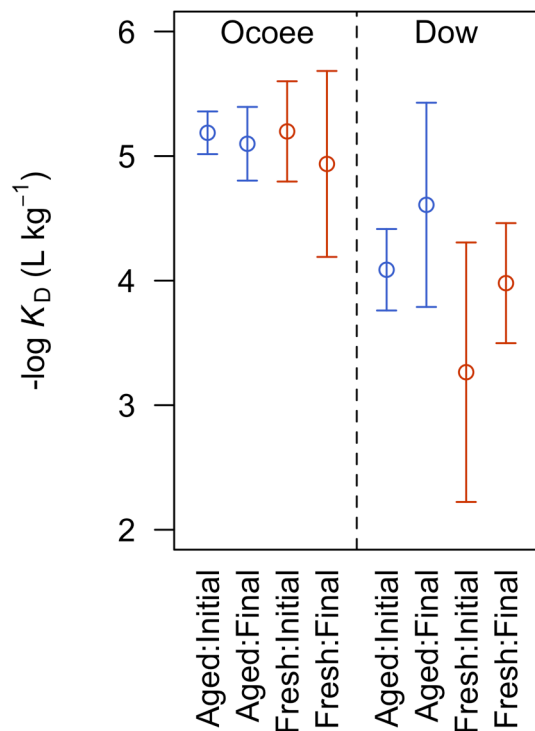


Figure S3. Mean Cu partitioning (K_D) in aged and freshly spiked Ocoee and Dow sediments measure at both the start and end of a 28-d *H. azteca* bioassay. Cu partitioning to porewater did not change through time ($p > 0.1$), and differences in partitioning between aged (blue) and freshly spiked (red) sediment did not differ for Ocoee and were marginally different ($p = 0.07$) for Dow sediment. Error bars represent ± 1 standard deviation.

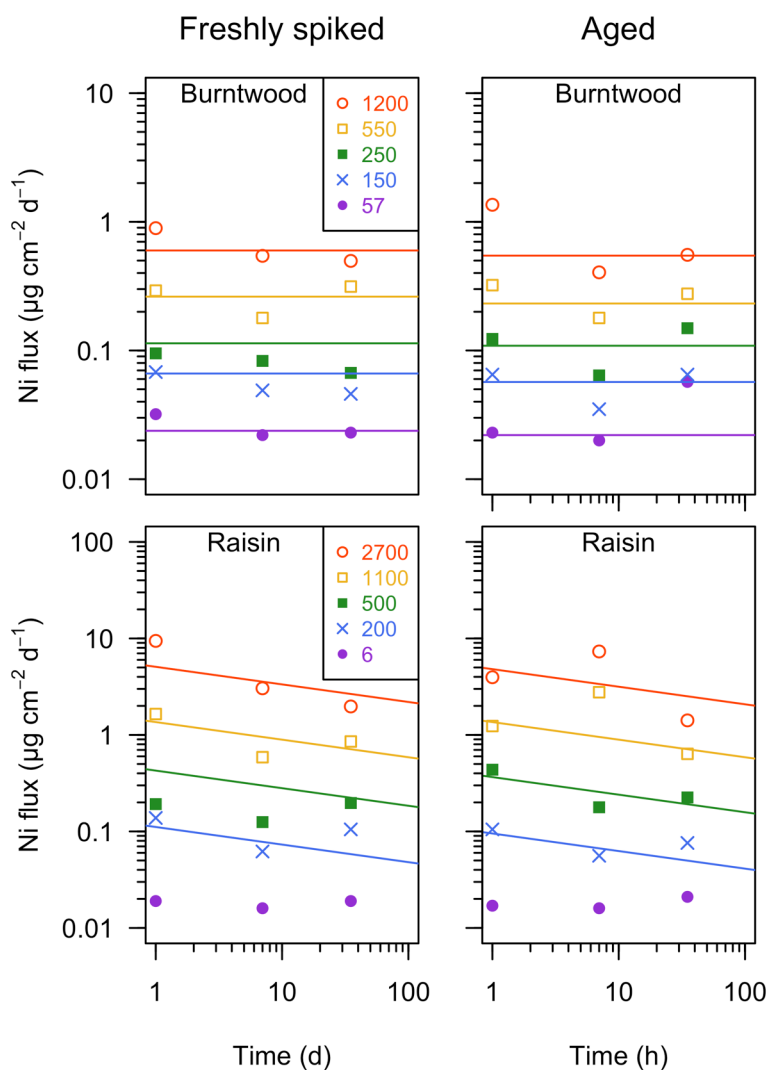


Figure S4. Ni flux to overlying water during the 35-d experimental period (7-d equilibration and 28-d chronic *H. azteca* test). Fluxes were measured in two sediment types that were either aged or freshly spiked. Warmer colors indicate sediments with higher total Ni (mean concentrations in legend). Solid lines indicate best-fit lines determined by ANCOVA. Note the log scale on both axes and the different axes for Burntwood and Raisin sediments.

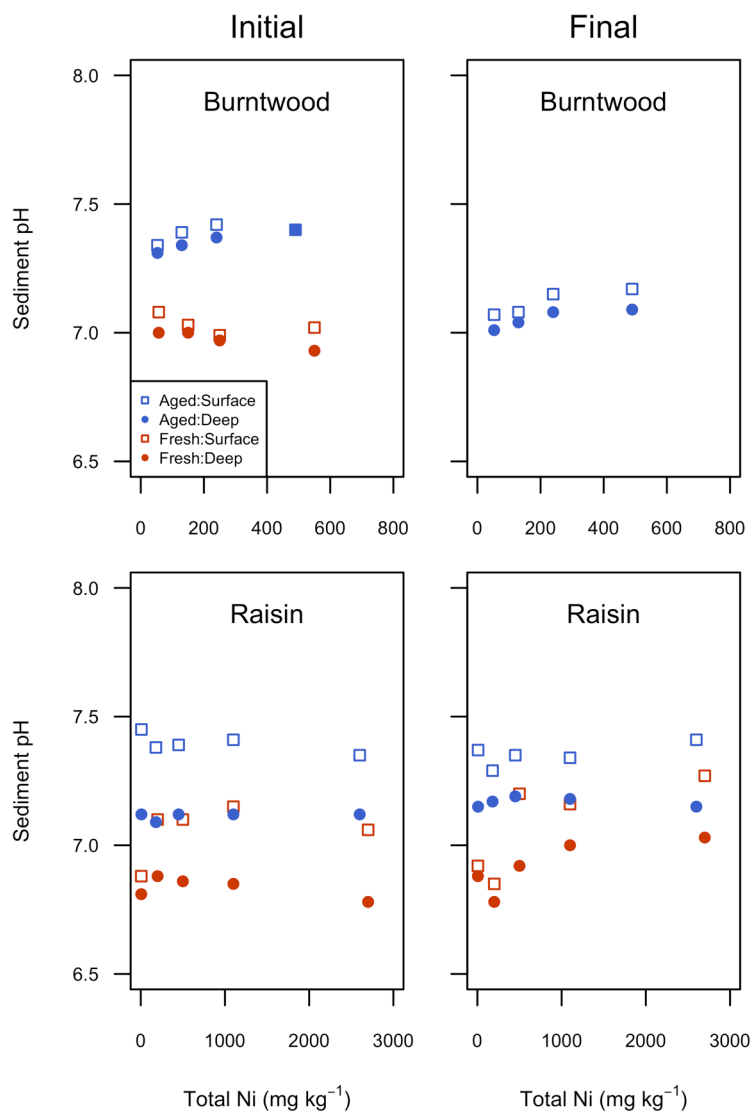


Figure S5. Sediment pH measured in surface (0–1 cm) and deep (1–3 cm) sediments at the start (“Initial”) and end (“Final”) of a 28-d *H. azteca* bioassay. In Burntwood sediments, pH was lower in deep sediments and decreased during the exposure period (freshly spiked treatments were not measured at end of experiment). In Raisin sediments, pH was positively related to Ni_{TOT} at the end of the exposure period and the slope was greater for the freshly spiked sediments.

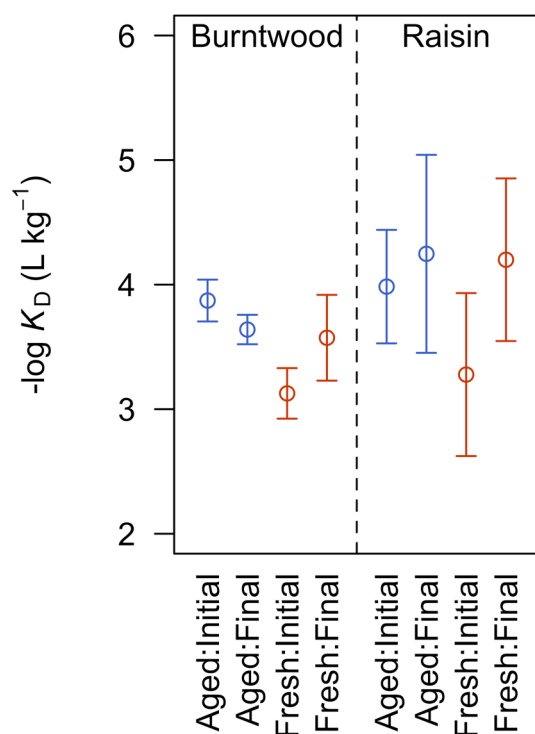


Figure S6. Mean Ni partitioning (K_D) in aged and freshly spiked Burntwood and Raisin sediments measure at both the start and end of a 28-d *H. azteca* bioassay. Ni partitioning was consistent across treatment and sampling time for Raisin sediment (all $p > 0.09$), but Burntwood sediment K_D was different for aged and freshly spiked treatments at the initiation of the experiment (Time \times Treatment, $p = 0.01$). Error bars represent ± 1 standard deviation.

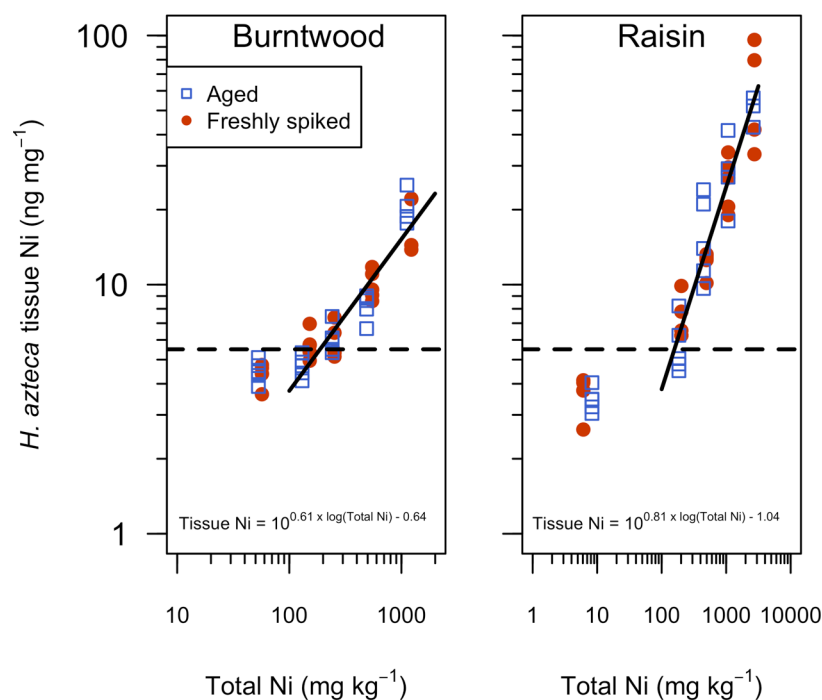


Figure S7 *Hyalella azteca* tissue Ni concentrations relative to Burntwood and Raisin sediment total Ni concentrations. For each of the sediments, *H. azteca* exposed to aged and freshly spiked sediments had similar accumulation of Ni in tissues, but amphipods exposed to Raisin sediments accumulated more Ni (relative to Ni_{TOT}) than those exposed to Burntwood sediments (coefficients in best-fit line equations). The dotted horizontal line denotes the median tissue Ni concentration of neonates at the beginning of the exposure period (5.5 ng mg⁻¹). Control sediment (i.e., no added Ni) are shown on plots but were not included in regression models. Note the log scale on both axes and the difference in x-axis scale between panels.

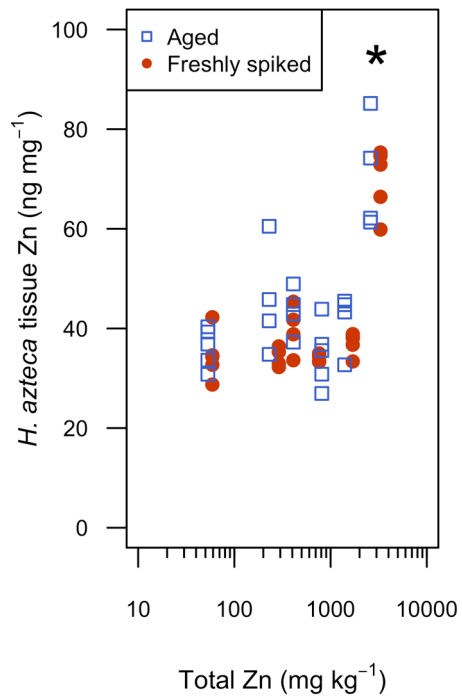


Figure S8. *Hyalella azteca* tissue Zn concentrations relative to sediment total Zn concentrations for aged and freshly spiked Raisin sediment. The asterisk indicates that *H. azteca* growing on the highest Zn_{TOT} treatment had significantly greater tissue Zn than all other treatments ($p < 0.001$). Note the log scale on the x-axis.

Table S1. Summary of QA/QC for sediment and overlying water chemistry.

Analyte	Procedural reproducibility (% relative difference)	Standard recovery (%)
AVS	19 ± 24 (9)	101 ± 22 (8)
DOC		100 ± 16 (15)
Total Cu	6 ± 4 (6)	101 ± 2 (7) ^b
SEM Cu	12 ± 7 (8)	
Ascorbate Cu ^a	27 ± 24 (11)	
Dithionite Cu ^a	26 ± 25 (11)	
Total Ni	4 ± 4 (16)	
SEM Ni	21 ± 11 (4)	
Ascorbate Ni ^a	6 ± 4 (6)	
Dithionite Ni ^a	10 ± 7 (6)	
Total Zn	2 ± 2 (3)	
Ascorbate Zn ^a	16 ± 9 (3)	
Dithionite Zn ^a	12 ± 16 (3)	
Total Fe	6 ± 5 (23)	89 ± 1 (7) ^b
Ascorbate Fe ^a	9 ± 7 (20)	
Dithionite Fe ^a	8 ± 7 (20)	
Total Mn	4 ± 3 (24)	105 ± 2 (7) ^b
Dithionite Mn ^a	7 ± 6 (20)	

^a ascorbate and dithionite metals are from select extraction of amorphous (ascorbate) and total metals oxides (dithionite); ^b recovery of NIST standard reference material 2781

Table S2. Total metal (mg kg⁻¹) toxicological thresholds (± 95% confidence intervals) from *Hyalella azteca* survival and growth concentration–response curves. Standard 28-d bioassays were used to compare metal amended sediments that were either freshly spiked or aged in a mesocosm for >100 d.

Metal	Sediment	Preparation	LC50	EC50 (growth)
Cu	Dow	Aged	435 (341–590)	263 (214–328)
	Dow	Freshly spiked	395 (339–465)	259 (222–314)
	Ocoee	Aged	2520 (1800–4410)	1030 (891–1200)
	Ocoee	Freshly spiked	1590 (1360–1900)	994 (863–1180)
Ni	Burntwood	Aged	>1120 ^a	1790 (1300–4440)
	Burntwood	Freshly spiked	1720 (1090–3800)	1660 (1340–3300)
	Raisin	Aged	504 (355–702)	1340 (1070–1790)
	Raisin	Freshly spiked	803 (364–2390)	1510 (1260–1840)
Zn	Raisin	Aged	>2600 ^a	>2600 ^a
	Raisin	Freshly spiked	>3300 ^a	>3300 ^a

^a no significant concentration–response curve could be calculated and threshold is reported as greater than the maximum concentration tested; ^b upper confidence interval was unbounded (i.e., positive infinity).