Supporting Information for:

# NICKEL PARTITIONING AND TOXICITY IN SEDIMENT DURING AGING: VARIATION IN TOXICITY RELATED TO STABILITY OF METAL PARTITIONING

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## Results

**Ni treatments.** Our spiking procedure created sediment with elevated $Ni_{TOT}$ concentrations and treatments remained distinct from one another during aging (SI Table S1). In Raisin reference sediment, concentrations of $Ni_{TOT}$ increased slightly during aging (from 5–10 mg $kg^{-1}$) likely due to cross-contamination from other treatments; however, $Ni_{TOT}$ in the reference sediment remained lower than all other treatments and below even the most conservative toxicity thresholds.[1] In deep Burntwood sediment, we observed a statistically significant, though small (3%), increase in $Ni_{TOT}$ during the 100 days of aging (SI Table S3), but the change in $Ni_{TOT}$ was within our measurement error (SI Table S2). The increase in $Ni_{TOT}$ during aging is reflected in slightly higher $Ni_{TOT}$ in deep when compared to surface Burntwood sediment (paired t-test, $p = 0.004$).

**Sediment carbon and pH.** Dissolved organic carbon increased substantially as sediment aged, but particulate organic content either did not change or declined slightly (SI Table S3). Organic content of deep Burntwood sediment declined slightly (from 10 to 9.1%) but Burntwood surface sediment and both Raisin sediment horizons had stable organic contents during the entire aging period (SI Table S3). Burntwood surface sediment pH changed non-monotonically, with an initial decline followed by a return to the starting pH (SI Figure S3A). Deep Burntwood sediment had a lower pH than surface sediment ($p < 0.001$). The pH of Raisin surface sediment, which did not change through time, also was greater than deeper sediment (mean difference = 0.36, $p < 0.001$). For both Burntwood and Raisin deep sediment, pH changed through time and the magnitude and direction of change differed among the Ni treatments (SI Table S3). For both sediments, pH increased the greatest amount in sediment with higher $Ni_{TOT}$ (SI Figure S3). Although sediment pH changed as the sediment aged, changes were < 0.3 units over the entire aging period, and no extreme pH conditions were observed.

**Sediment Fe and Mn.** The concentration of $Fe_{TOT}$ in Burntwood deep sediment increased 6% during aging, and $Fe_{TOT}$ in Raisin surface sediment increased 20% during aging (SI Table S3). The increased of $Fe_{TOT}$ in Raisin surface sediment may have resulted from precipitation of oxidized Fe mobilized from deeper sediment. However, Raisin sediment remained much lower in $Fe_{TOT}$ than Burntwood sediment throughout the aging process. The concentrations of amorphous Fe oxides ($Fe_{HFO}$) increased in the surface of both Raisin and Burntwood sediments SI Table S3, Figure S4). $Fe_{HFO}$ in Raisin deep sediment also increased during aging, but Burntwood $Fe_{HFO}$ concentrations remained stable (SI Figure S4). The formation of $Fe_{HFO}$ in all Raisin sediment layers and Burntwood surface sediment increased amorphous Fe oxide concentrations by 20–25% during the aging process. $Fe_{HFO}$ was greater in Burntwood surface than deep sediment ($p = 0.004$) but concentrations in Raisin sediment were similar between depths. Burntwood sediment contained a greater fraction of Fe as crystalline Fe oxide minerals (27–32%) than Raisin sediment (19–27%). Raisin sediment exhibited no change in $Fe_{CFO}$ during aging, but $Fe_{CFO}$ in Burntwood sediment declined by 17–21% during aging (SI Table S3, Figure S4). For both sediments, there were no differences in $Fe_{CFO}$ concentrations between surface and deep sediment ($p > 0.05$). The distribution of Fe oxide minerals also differed among Ni treatments, with lower $Fe_{HFO}$ (Burntwood surface and all deep sediment) and greater $Fe_{CFO}$ (Burntwood surface) in sediment with higher $Ni_{TOT}$ (SI Table S1). This suggests that microbial oxidation of Fe, which produces hydrous oxide minerals, was impaired by elevated sediment Ni.

The total concentration of Mn and the speciation of Mn changed as sediment aged. In both surface sediments and Burntwood deep sediment, $Mn_{TOT}$ concentrations increased as sediment aged (SI Figure S5). The 60–170 mg $kg^{-1}$ increase of $Mn_{TOT}$ may have been due to precipitation of dissolved Mn in either pore or overlying water (similar patterns observed for Cu),[2] but Mn dissolved in flume water was low (< 1 μg $L^{-1}$) during the entire aging period. The fraction of $Mn_{TOT}$ as oxidized Mn was greater in Raisin (76–89%) than Burntwood sediment (60–71%). (SI Table S3, Figure S5). For both sediments, $Mn_{TOT}$ and Mn oxide concentrations were similar

between surface and deep sediment (all $p > 0.05$). Mn in pore waters was positively related to $Ni_{TOT}$ immediately after spiking then declined through time until it reached a similar concentration in all treatments (2 mg $L^{-1}$; SI Table S3). The decline in porewater Mn is not sufficient to account for the increase to $Mn_{TOT}$ as sediment aged. Similar to results for sediment Fe, the changes in Mn speciation (particularly declining porewater Mn) are consistent with sediment that is oxidizing as it ages.

**References**


(1) MacDonald, D. D.; Ingersoll, C. G.; Berger, T. A. Development and evaluation of consensus-based sediment quality guidelines for freshwater ecosystems. *Arch. Environ. Contam. Toxicol.* **2000**, *39*, 20–31.

(2) Costello, D. M.; Hammerschmidt, C. R.; Burton, G. A. Copper sediment toxicity and partitioning during oxidation in a flow-through flume. *Environ. Sci. Technol.* **2015**, *49*, 6926–6933.

**Glossary of abbreviations**

| | |
|---|---|
| AVS | Acid volatile sulfides |
| $DO_{PEN}$ | Depth of dissolved oxygen penetration in sediment |
| DOC | Dissolved organic carbon |
| EC10 | Chemical concentration that elicits a 10% reduction in growth |
| $f_{OC}$ | Fraction of organic carbon in sediment |
| $Fe_{CFO}$ | Crystalline iron oxides |
| $Fe_{HFO}$ | Amorphous iron oxides |
| $Fe_{TOT}$ | Total sediment iron |
| ICP-MS | Inductively coupled plasma mass spectroscopy |
| ICP-OES | Inductively coupled plasma optical emission spectroscopy |
| $K_D$ | Sediment–pore water partitioning coefficient |
| $Mn_{TOT}$ | Total sediment Mn |
| $Ni_{CFO}$ | Nickel associated with crystalline iron oxides |
| $Ni_{HFO}$ | Nickel associated with amorphous/hydrous iron oxides |
| $Ni_{SEM}$ | Nickel simultaneously extracted during measurement of AVS |
| $Ni_{TOT}$ | Total sediment nickel |
| OM | Sediment organic matter content |
| PES | Polyether sulfone membrane filter |
| QA/QC | Quality assurance/quality control |
| RGR | Relative growth rate |
| SD | Standard deviation of a mean |
| TC | Total sediment carbon content |

**Table S1. Sediment Ni treatments**

Mean (n = 11) total sediment Ni concentrations of deep sediment averaged over the entire experiment. Values in parenthesis are standard deviations of the mean.

| Burntwood Total Ni ($mg\ kg^{-1}$ dw) | Raisin Total Ni ($mg\ kg^{-1}$ dw) |
|---|---|
| 55 (1.0) | 6.0 (1.7) |
| 140 (3.8) | 260 (25) |
| 270 (9.5) | 620 (74) |
| 550 (20) | 1300 (86) |
| 1200 (27) | 3000 (180) |

**Table S2. Summary of QA/QC from Ni flume experiment**
Quality assurance/quality control (QA/QC) for analytical chemistry samples from the Ni flume aging experiment. Values in parenthesis are the number of QA/QC standards analyzed for each analyte.

| Analyte | Procedural reproducibility (% relative difference) | Standard recovery (%) |
|---|---|---|
| AVS | 14 ± 10 (7) | 101 ± 23 (6) |
| Total carbon | 10 ± 8 (30) | |
| DOC | | 102 ± 31 (8) |
| Total Ni | 5 ± 4 (15) | 93 ± 2 (11)[b] |
| SEM Ni | 19 ± 14 (8) | |
| Ascorbate Ni[a] | 9 ± 9 (14) | |
| Dithionite Ni[a] | 6 ± 6 (18) | |
| Total Fe | 5 ± 4 (15) | 88 ± 2 (11)[b] |
| Ascorbate Fe[a] | 9 ± 7 (14) | |
| Dithionite Fe[a] | 5 ± 5 (18) | |
| Total Mn | 4 ± 3 (15) | 103 ± 2 (11)[b] |
| Dithionite Mn[a] | 5 ± 4 (18) | |

[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 S3. Summary of statistical results**

Summary of statistical results from multiple regressions predicting Burntwood sediment physicochemistry from sediment aging time and total Ni concentration.

| Response variable | Depth | Predictor variables | | |
|---|---|---|---|---|
| | | Time | $Ni_{TOT}$ | Time x $Ni_{TOT}$ |
| $DO_{PEN}$ | | < 0.001* | 0.08 | 0.31 |
| pH | surface[#] | 0.001 | < 0.001* | 0.66 |
| | deep | 0.04* | 0.18* | 0.006 |
| OM | surface | 0.85* | 0.17 | 0.84 |
| | deep | 0.004* | 0.63 | 0.74 |
| DOC | deep | < 0.001* | 0.12* | 0.09 |
| AVS | surface | -- | -- | -- |
| | deep | -- | -- | -- |
| $Fe_{TOT}$ | surface | 0.06* | 0.13 | 0.22 |
| | deep | < 0.001* | 0.11* | 0.07 |
| $Fe_{HFO}$ | surface | < 0.001* | < 0.001 | 0.89 |
| | deep | 0.33* | < 0.001 | 0.65 |
| $Fe_{CFO}$* | surface | < 0.001* | < 0.001 | 0.59 |
| | deep | < 0.001* | 0.02 | 0.49 |
| $Mn_{TOT}$ | surface | < 0.001* | 0.80 | 0.16 |
| | deep | < 0.001* | 0.11* | 0.27 |
| Mn oxides | surface | < 0.001* | 0.03 | 0.55 |
| | deep | < 0.001* | 0.32 | 0.90 |
| Porewater Mn | deep | < 0.001* | < 0.001 | 0.008 |
| $Ni_{TOT}$ | surface | 0.99* | <0.001* | 0.96 |
| | deep | < 0.001* | < 0.001* | 0.25 |
| $Ni_{SEM}$* | surface | < 0.001 | < 0.001* | 0.56 |
| | deep | 0.78* | < 0.001* | 0.81 |
| $Ni_{HFO}$ | surface | 0.64* | < 0.001* | 0.62 |
| | deep | 0.47* | < 0.001* | 0.96 |
| $Ni_{CFO}$* | surface | < 0.001* | < 0.001* | 0.34 |
| | deep | < 0.001* | < 0.001* | 0.19 |
| Porewater Ni | deep | < 0.001* | < 0.001 | < 0.001 |

Table S3 (cont.). Summary of statistics for Raisin sediment.

| Response variable | Depth | Predictor variables | | |
|---|---|---|---|---|
| | | Time | $Ni_{TOT}$ | Time x $Ni_{TOT}$ |
| $DO_{PEN}$ | | < 0.001 | 0.01 | 0.40 |
| pH | surface | 0.89* | 0.15* | 0.76 |
| | deep | 0.06* | 0.86 | 0.001* |
| OM | surface | 0.48* | 0.26 | 0.57 |
| | deep | 0.83* | 0.98 | 0.67 |
| DOC | deep | < 0.001* | 0.48* | 0.06 |
| AVS | surface* | 0.001* | < 0.001$^{\phi}$ | 0.22 |
| | deep | 0.35* | < 0.001$^{\phi}$ | 0.73 |
| $Fe_{TOT}$ | surface | < 0.001* | 0.82* | 0.94 |
| | deep | 0.27* | 0.006* | 0.67 |
| $Fe_{HFO}$ | surface | < 0.001* | 0.12 | 0.99 |
| | deep | 0.01* | 0.002* | 0.89 |
| $Fe_{CFO}$* | surface | 0.74* | 0.21 | 0.55 |
| | deep | 0.31* | 0.16 | 0.37 |
| $Mn_{TOT}$ | surface | < 0.001 | 0.24 | 0.63 |
| | deep | 0.10 | 0.004 | 0.81 |
| Mn oxides | surface | 0.43* | 0.29 | 0.11 |
| | deep | 0.02* | 0.61 | 0.22 |
| Porewater Mn* | deep | < 0.001* | < 0.001 | < 0.001 |
| $Ni_{TOT}$ | surface | 0.07* | < 0.001* | < 0.001 |
| | deep | 0.77* | < 0.001* | 0.99 |
| $Ni_{SEM}^{\phi}$ | surface | 0.002* | < 0.001$^{\phi}$ | 0.002 |
| | deep | 0.07* | < 0.001$^{\phi}$ | 0.04 |
| $Ni_{HFO}^{\phi}$ | surface | 0.26* | < 0.001$^{\phi}$ | 0.69 |
| | deep | 0.51* | < 0.001$^{\phi}$ | 0.25 |
| $Ni_{CFO}^{\phi}$ | surface | 0.73* | < 0.001$^{\phi}$ | 0.76 |
| | deep | < 0.001* | < 0.001$^{\phi}$ | 0.05 |
| Porewater $Ni^{\phi}$ | deep | < 0.001* | < 0.001$^{\phi}$ | 0.02 |

* variable was log transformed for analysis; # non-monotonic through time, therefore time was considered a discrete variable and analyzed with ANCOVA; $^{\phi}$ variable was square root transformed for analysis; see glossary of abbreviations for acronym definitions.

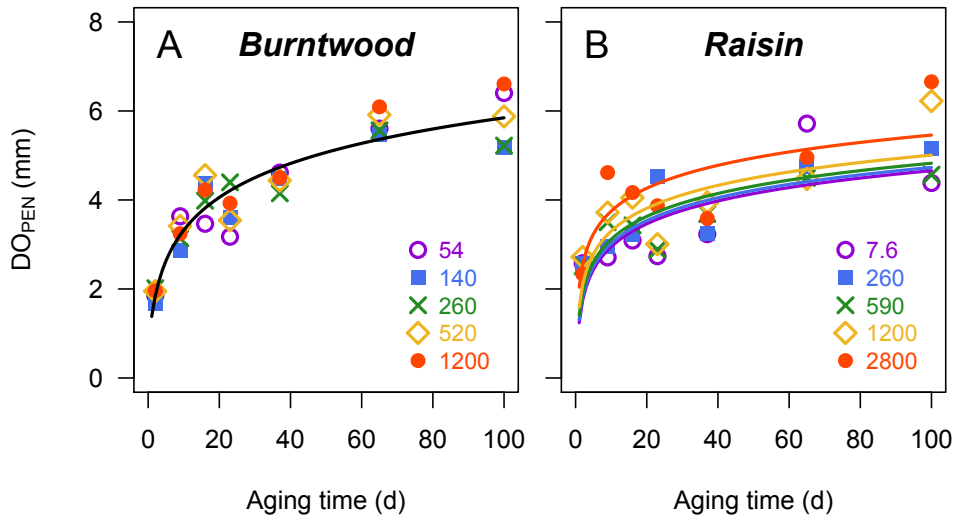


**Figure S1. Dissolved oxygen penetration**
Sediment dissolved oxygen penetration depth ($DO_{PEN}$) through time in Burntwood (A) and Raisin (B) sediments as they aged in a flow-through flume. Warmer colors (orange > yellow > green > blue > violet) indicate higher sediment $Ni_{TOT}$ (mean $Ni_{TOT}$ in surface sediment reported in legend). For Burntwood sediment, $DO_{PEN}$ did not differ among Cu treatments and the solid line indicates the best-fit line from linear regression. For Raisin sediment, the depth of $DO_{PEN}$ was greater in sediment with more Ni, and solid lines indicate best-fit lines for specific treatments.

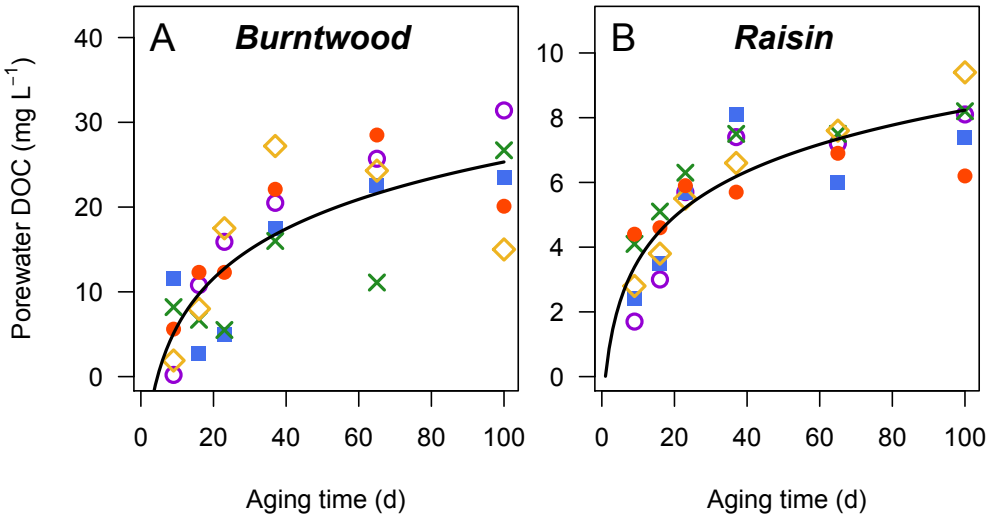


**Figure S2. Porewater dissolved organic carbon**
Sediment porewater dissolved organic carbon (DOC) through time in Burntwood (A) and Raisin (B) sediment. Warmer colors indicate higher sediment $Ni_{TOT}$. DOC did not differ among Ni treatments and solid lines indicate best-fit lines from linear regression. Note the difference in y-axis scales between panels.

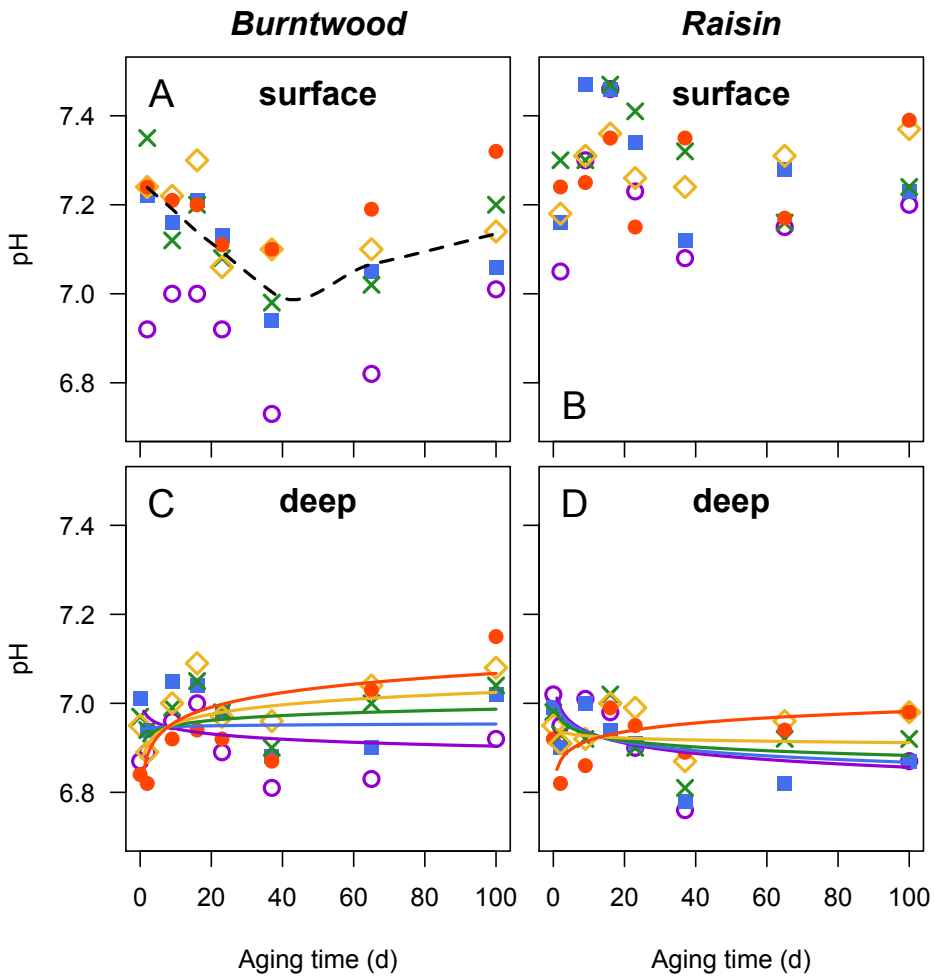


**Figure S3. Sediment pH**
Sediment pH through time in Burntwood (A & C) and Raisin (B & D) sediment. Warmer colors indicate higher sediment $Ni_{TOT}$. pH in surface sediment did not differ among Ni treatments and Raisin surface sediment was stable through time (B). Burntwood surface pH changed non-monotonically though time (A), and dashed lines indicate a LOESS smoother fitted to the data. The change in pH in deep sediment during aging differed among Ni treatments, and solid lines are best-fit lines for specific treatments as determined by multiple regression.

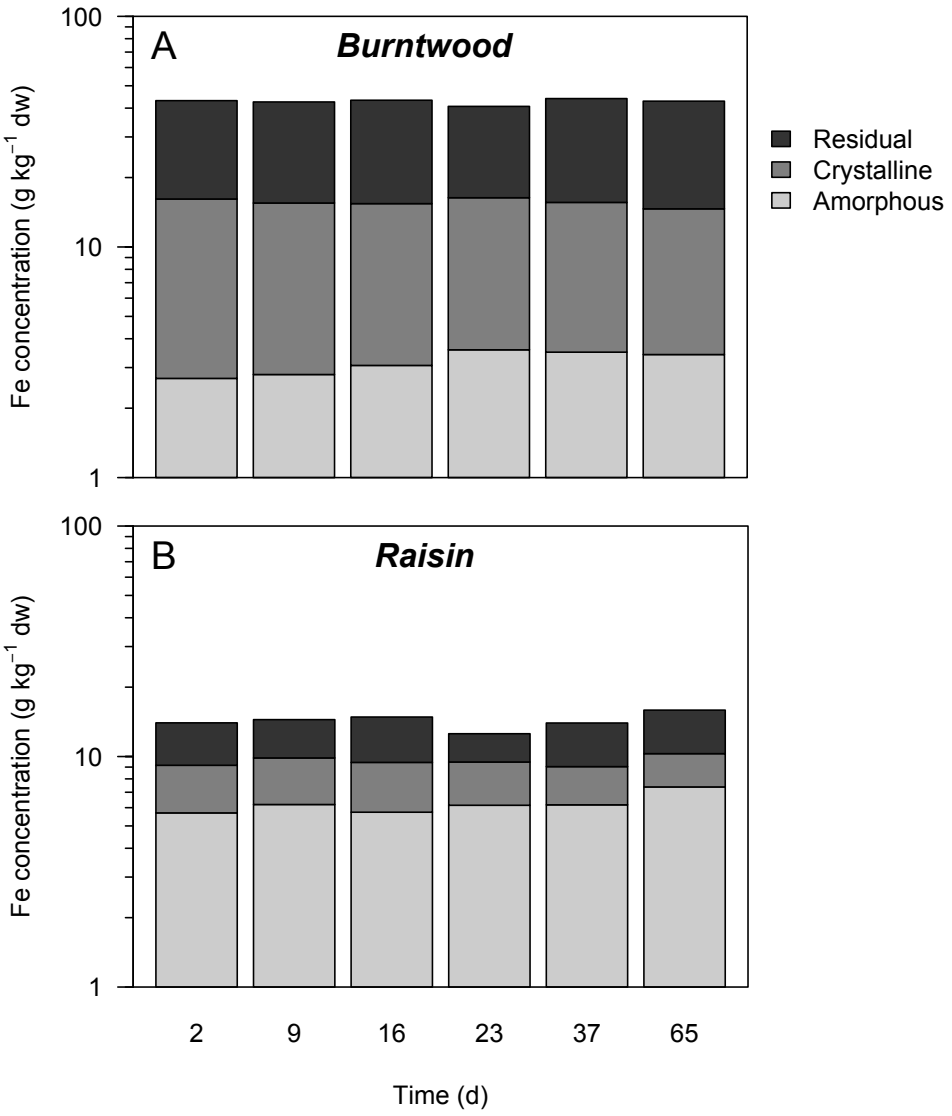


**Figure S4. Fe speciation in surface sediment**
Speciation of Fe in Burntwood (A) and Raisin (B) surface sediments as they aged in a flow-through flume. The full height of the bars is the average $Fe_{TOT}$ for a given sample day. For both sediments, the amorphous Fe fraction increased through time (logarithmic growth), and the fraction of Fe as amorphous oxidized Fe was greater in Raisin (43–46%) than Burntwood (6–8%). Crystalline Fe oxides declined through time only in Burntwood sediment. Note that the y-axis differs between panels and is on a log scale.

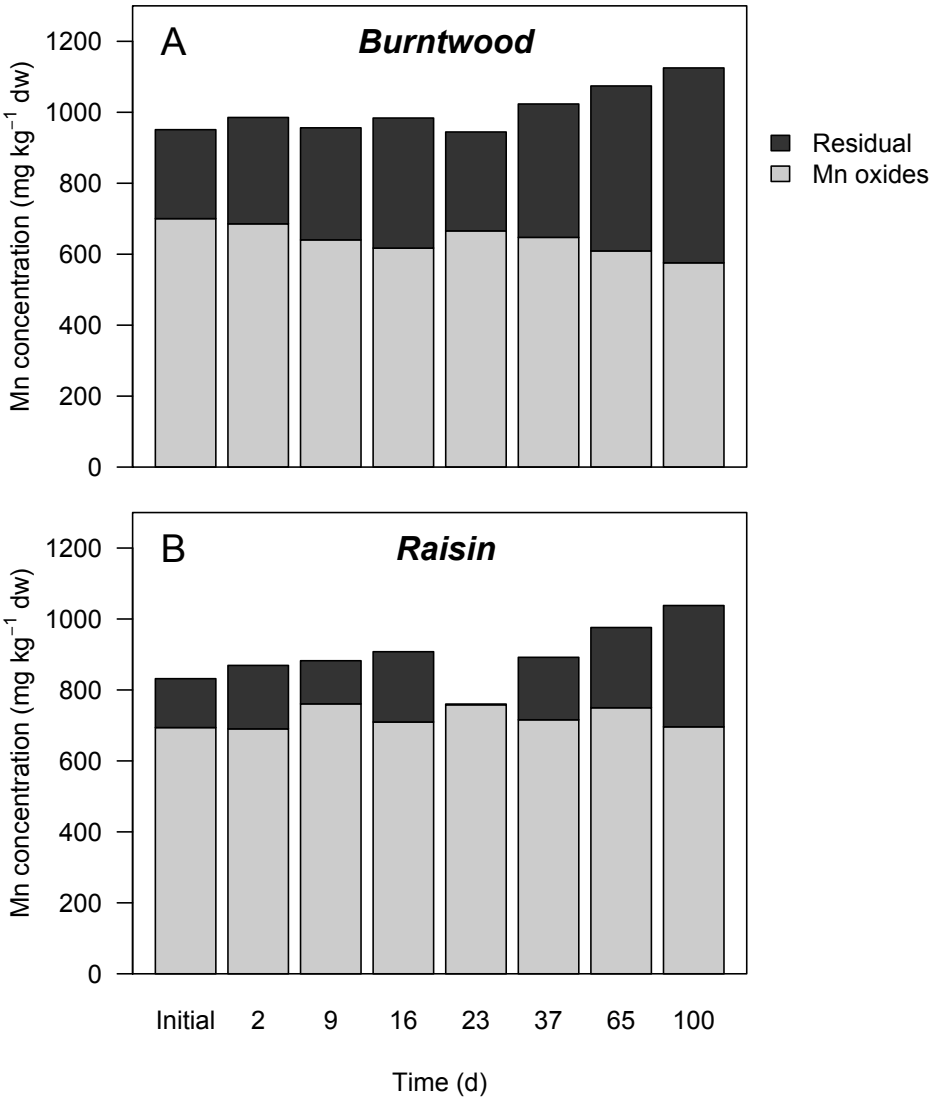


**Figure S5. Mn speciation in surface sediment**
Speciation of Mn in Burntwood (A) and Raisin (B) surface sediments as they aged in a flow-through flume. The full height of the bars is the average $Mn_{TOT}$ for a given sample day. For both sediments, total Mn increased through time (linear in Burntwood, and logarithmic in Raisin sediment) and Mn oxide minerals declined in Burntwood sediment only (logarithmic decay). The fraction of Mn as oxidized minerals was greater in Raisin (~80%) than Burntwood (~65%).

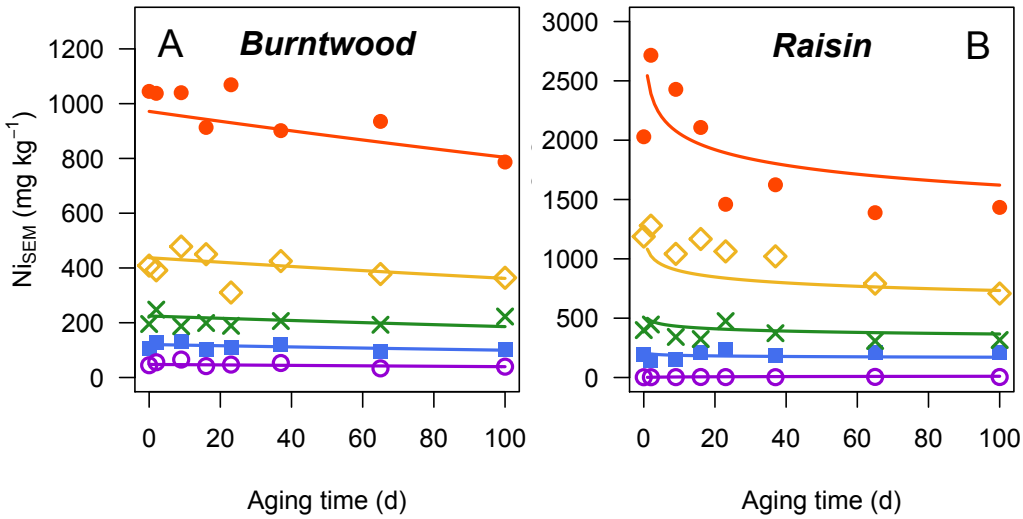


**Figure S6. $Ni_{SEM}$ in surface sediment**
Ni simultaneously extracted from the AVS procedure in Burntwood (A) and Raisin (B) surface sediment as sediment aged in a flow-through flume. Warmer colors indicate higher sediment $Ni_{TOT}$. Solid lines indicate best-fit lines for specific $Ni_{TOT}$ treatments as determined by multiple regression. Note the y-axis scale changes between panels.

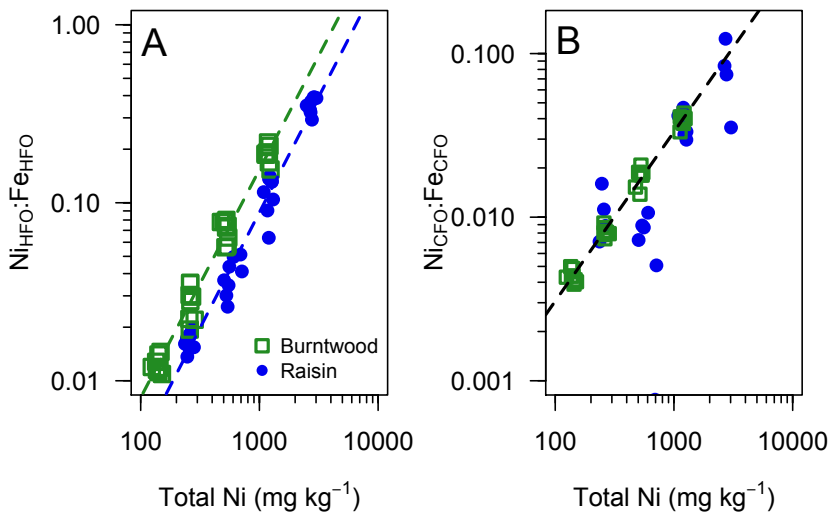


**Figure S7. Ni:Fe in surface sediment**
Ratios of Ni:Fe in amorphous (A) and crystalline (B) Fe oxide extractions for both Burntwood and Raisin surface sediment (patterns for deep sediment were similar). Dashed lines indicate best-fit linear regressions. Reference sediment with no amended Ni have been excluded from these plots due to very low ratios.

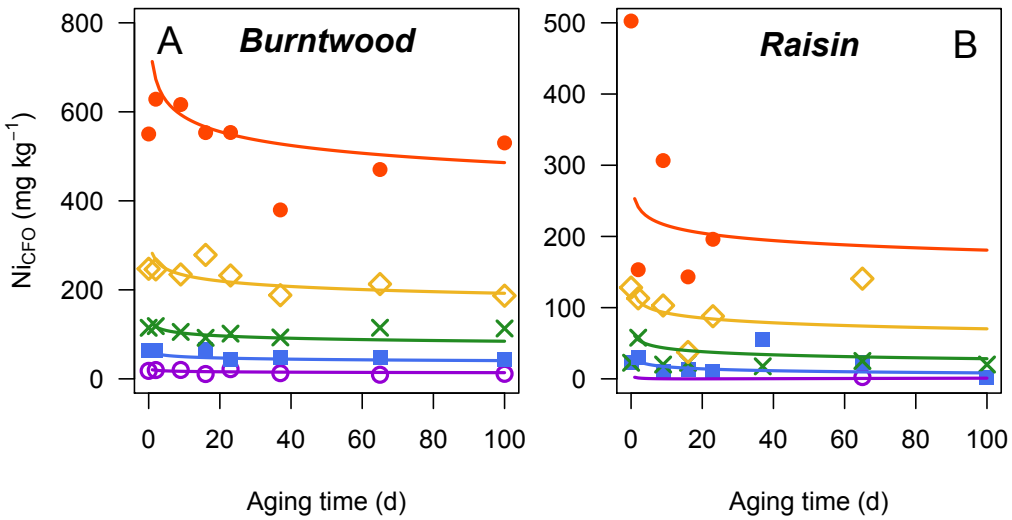


**Figure S8. $Ni_{CFO}$ in deep sediment**
Ni extracted from crystalline metal oxides in Burntwood (A) and Raisin (B) deep sediment as it aged in a flow-through flume. Warmer colors indicate higher sediment $Ni_{TOT}$. Solid lines indicate best-fit lines for specific $Ni_{TOT}$ treatments as determined by multiple regression. In many Raisin sediment, $Ni_{CFO}$ was below detection limit and points are omitted (n = 13). Note the y-axis scale changes between panels.

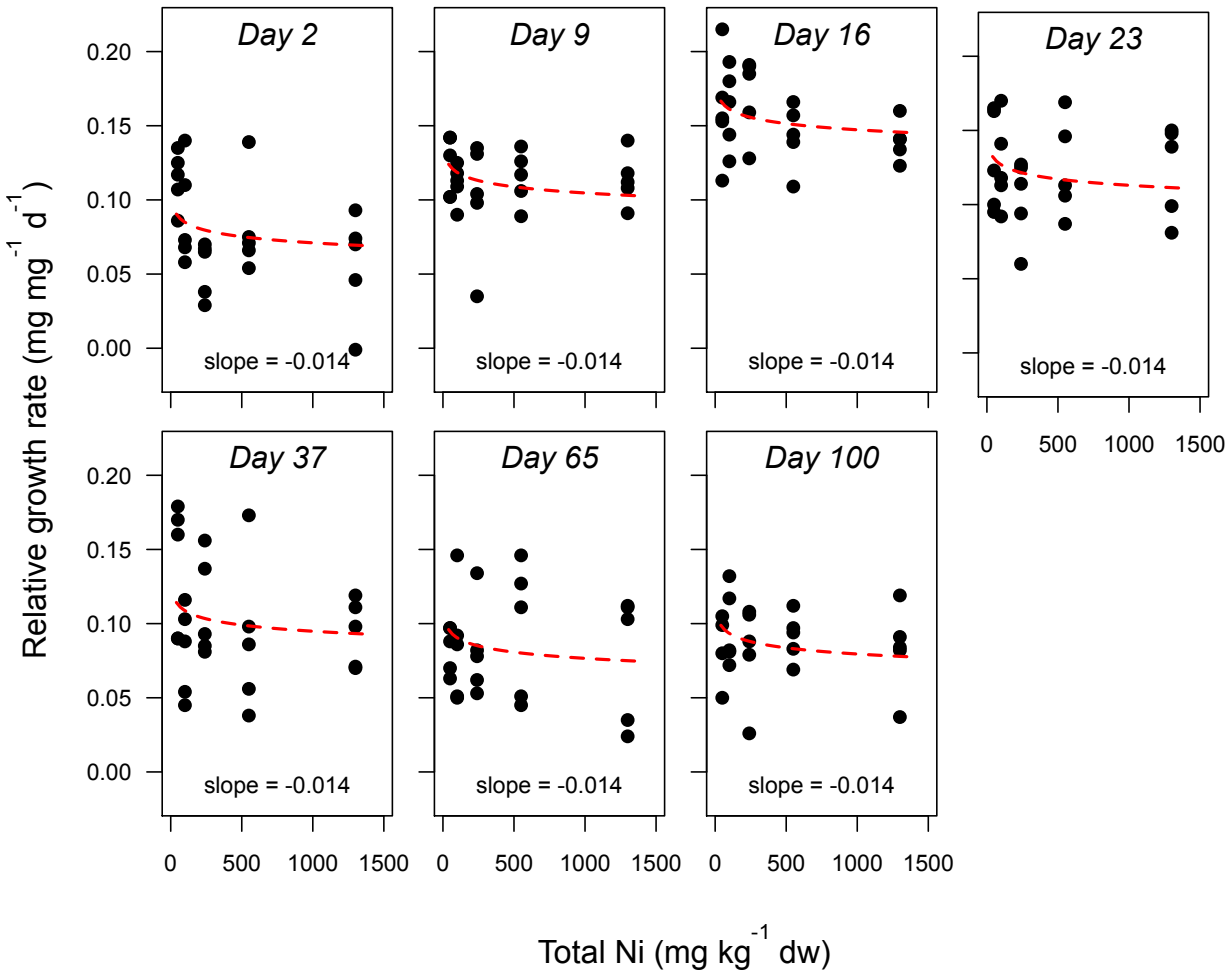


**Figure S9. *Hyalella azteca* dose–response through time**
Relative growth rates (RGR) for *Hyalella azteca* growing on spiked and aged Burntwood sediment. Each symbol represents the RGR of surviving *H. azteca* placed on an individual sediment cup and dashed lines (and reported slopes) represent best-fit lines for the dose-response relationship on that particular day. A multiple regression model determine that RGR was reduced at higher sediment Ni and the dose–response did not change as sediment aged.

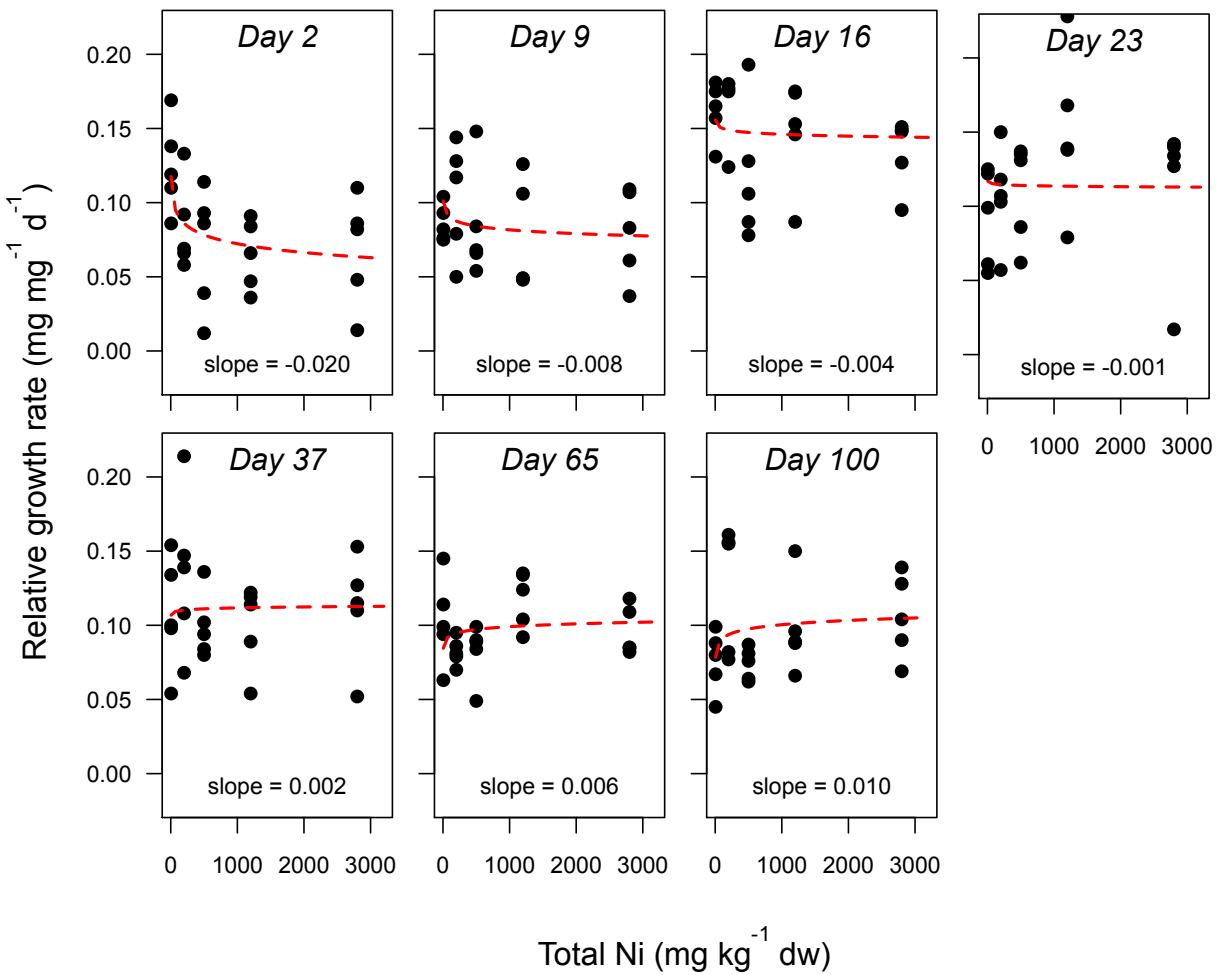


Figure S9 (cont.) Relative growth rates for *Hyalella azteca* growing on spiked and aged Raisin sediment. For Raisin sediment, multiple regression determined that the dose–response relationship between RGR and sediment Ni changed as this sediment aged.

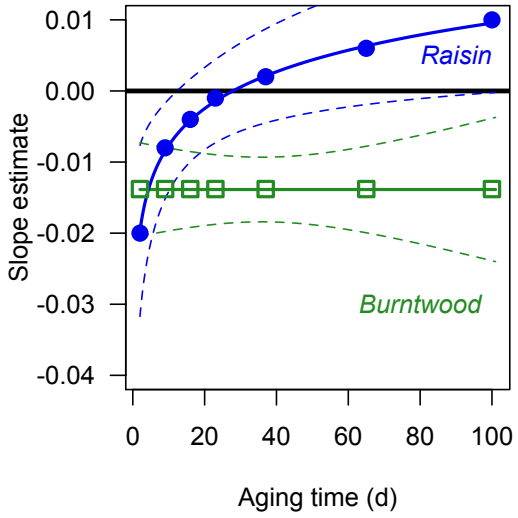


**Figure S10. Summary of *H. azteca* dose–response statistical models**
Summary of results from multiple regression describing the response of *H. azteca* relative growth rates to sediment total Ni ($Ni_{TOT}$) and aging time. Points designate dose–response estimates on sampling dates and solid lines show the interpolated trend through time. Larger negative values indicate reduced growth at lower Ni concentrations and values close to zero indicate that all concentrations tested were non-toxic. Dashed lines indicate 95% confidence intervals of the slope estimates, and the point at which confidence intervals for Raisin sediment overlap 0 (solid horizontal line) indicates that all sediment concentrations tested were statistically non-toxic (12 days).

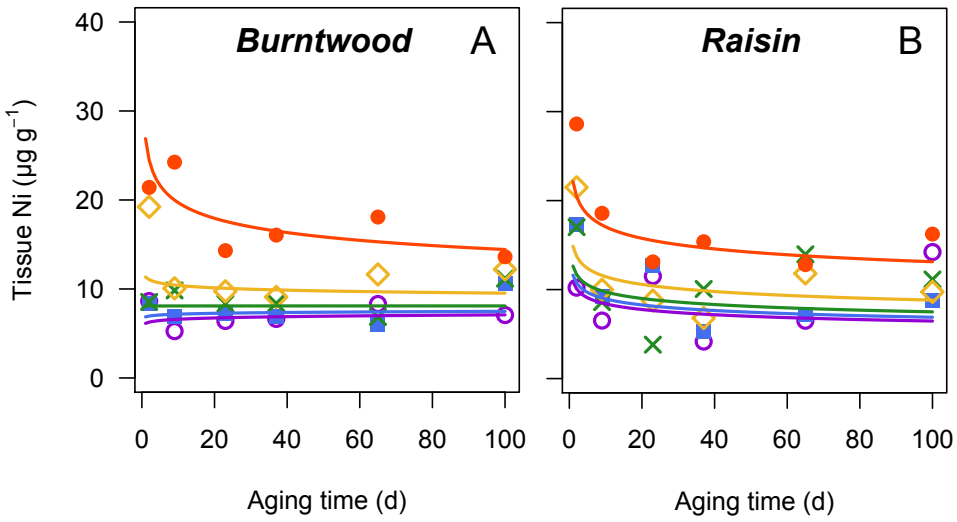


**Figure S11. *Hyalella azteca* tissue Ni**
*Hyalella azteca* tissue Ni concentrations as spiked Burntwood (A) and Raisin (B) sediment aged in a flow-through flume. Each symbol denotes the mean tissue concentrations of all surviving *H. azteca* from a single treatment on a give date. Warmer colors indicate organisms that were exposed to higher $Ni_{TOT}$ sediment. Solid lines denote best-fit lines determined from multiple regression.

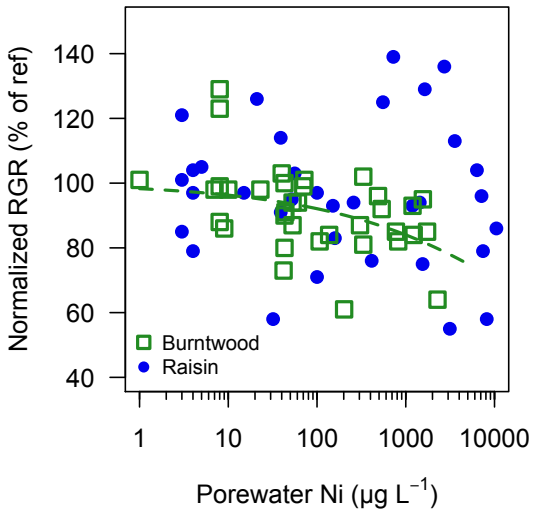


**Figure S12. *Hyalella azteca* dose–response to porewater Ni**
*Hyalella azteca* growth in response to porewater Ni. Each symbol represents the mean growth rate of replicate chambers from a single treatment on a given sampling date (n = 5) normalized relative to the predicted growth on reference sediment. Dashed lines indicate best-fit lines from non-linear least squares logistic regression (Raisin porewater Ni model was not significant). Note the log scale on the x-axis.