Table 1: Total Cu concentrations in wipes passed along weathered or as- purchased wood, copper concentrations released into water or SSF, and soluble copper concentrations in the 10 kDa filtrate after exposure to water or SSF. (a) As- purchased lumber exposed to water (N=2) or SSF (N=3) for 1 hour. (b) Weathered lumber exposed to SSF for 1 hour (N=3).

a. As-Purchased

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Wood Type | Total copper on wipes in µg/wipe (whole wipe microwave digests) | WATER | | SSF | |
| Copper extracted in water in µg/wipe (Percent) | Copper solubilized in water in µg/wipe (Percent) (10 kDa centrifugation) | Copper extracted in SSF in µg/wipe (Percent) | Total copper solubilized in SSF in µg/wipe (Percent) (10 kDa centrifugation) |
| Untreated | 0.3 ± 0.1 | ND | ND | ND | ND |
| ACA | 133 ± 90 | 52.28 ± 0.05 (39.19 ± 0.03) | 44 ± 4 (33 ± 3) | 57 ± 11 ( 43 ± 8) | 58 ± 11 (43 ± 8) |
| MCA-1 | 672 ± 387 | 142 ± 75 (21 ± 10) | 42 ± 8 (6 ± 1) | 300 ± 41 (45 ± 6) | 304 ± 43 (45 ± 6) |
| MCA-2 | 119 ± 25 | 32 ± 5 ( 27 ± 4) | 24 ± 3 (20 ± 2) | 159 ± 29 (100 ± 24) | 158 ± 11 (100 ± 9) |

b. Weathered

|  |  |  |  |
| --- | --- | --- | --- |
| Wood Type | Total copper on wipes in µg/wipe (whole wipe microwave digests) | Copper extracted in SSF in µg/wipe (Percent) | Copper solubilized in SSF in µg/wipe (Percent) (10 kDa centrifugation) |
| Untreated | 0.03 | 0.03 ± 0.2 ( 4.0 ± 2.0) | 0.004 ± 0.002 (0.4 ± 0.2) |
| ACA | 0.023 ± 0.003 | 0.03 ± 0.01 (100 ± 44) | 0.04 ± 0.02 (100 ± 66) |
| MCA-1 | 0.9 ± 0.3 | 0.9 ± 0.2 (100 ± 22) | 0.9 ± 0.1 (100 ± 15)) |
| MCA-2 | 0.10 ± 0.01 | 0.10 ± 0.01 (100 ± 13) | 0.10 ± 0.01 ( 100 ± 12) |

Supplementary Information:

Table S1: Summary of quality control results for ICP-MS and ICP-OES. ND indicates copper amounts were non-detectable.

|  |  |  |  |
| --- | --- | --- | --- |
| **QC Parameter** | **Average, ppb** | **Range, ppb** | **% Recovery Range** |
| Microwave Blank | ND | ND | <LLCV |
| Microwave Blank Spike, 100 ppb | 98 | 88-110 | 88-108 |
| Microwave Blank Spike, 500 ppb | 552 | 468-625 | 85-125 |
| Initial Calibration Verification, 50 ppb | 48 | 48.4-48.6 | 94-109 |
| Initial Calibration Blank | ND | ND | <LLCV |
| Continuing calibration verification, 100 ppb | 105 | 97.1-110 | 97-110 |
| Low Level Calibration Verification, 1 ppb | 1.1 | 0.98-1.28 | 97-128 |
| Low Level Calibration Verification, 2.5 ppb | 2.6 | 2.1-3.2 | 84-128 |
| High Level Calibration Verification, 2 ppm | 2.0 | 1.87-2.11 | 94-106 |
| Laboratory Spike, 100 ppb | 106.8 | 92-114 | 92-114 |
| Laboratory Spike, 30 ppm | 29.0 | 28.3-29.7 | 94-99 |

Table S2: Detailed data for total copper extracted from wipes upon digestion in the microwave system and for wipes passed along weathered wood and exposed to SSF.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | | | | | | | | | | | |
|  | **Replicate** | **Total (µg/wipe)** | **Average Total (µg/wipe)** | **Total SD** | **Percent released** | **Average Percent Released** | **Released SD** | **Percent 10 kDa** | **Average Percent 10 kDa** | **10 kDa SD** | |
| ACA | 1 | 0.021 | 0.02 | 0 | 82 | 100\* | 44 | 82 | 100\* | 66 | |
| 2 | 0.025 | 164 | 171 |
| 3 | NS | 96 | 211 |
| MCA-1 | 1 | 0.68 | 0.9 | 0.2 | 126 | 100\* | 22 | 117 | 100\* | 15 | |
| 2 | 1.03 | 88 | 87 |
| 3 | NS | 88 | 101 |
| MCA-2 | 1 | 0.68 | 0.09 | 0.03 | 113 | 100\* | 12 | 114 | 100\* | 11 | |
| 2 | 1.03 | 111 | 115 |
| 3 | NS | 90 | 94 |
| Untreated\*\* | 1 | 0.026 | 0.03 | NS | ND | ND | ND | ND | ND | ND | |
| 2 | NS | ND | ND |
| 3 | NS | ND | ND |
|  |  | \* For values exceeding 100 percent after averaging, the values were reported as 100%.  \*\*All of the values for copper extracted from wipes passed along untreated woods are below detectable reporting limits. Measured values are estimates. (ND = not detectable) | | | | | | | | | |
|  |  |
|  |  | SD=standard deviation  NS=Second and (if relevant) third replicate were not sampled. | | | | | | | | |  |

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Table S3: Detailed data for total copper extracted from wipes upon digestion in the microwave system and for wipes passed along as-purchased wood and exposed to water. | | | | | | | | | | | |  |
| **Treatment** | **Replicate** | **Total (µg/wipe)** | **Average Total (µg/wipe)** | **Total SD** | **Percent released** | **Average Percent Released** | **Released SD** | **Percent 10 kDa** | **Average Percent 10 kDa** | **10 kDa**  **SD** | |
| ACA | 1 | 57 | 133 | 90 | 39.22 | 39.19 | 0.03 | 36 | 33 | 3 | |
| 2 | 232 | 39.17 | 31 |
| 3 | 111 | NS | NS |
| MCA-1 | 1 | 282 | 672 | 387 | 29 | 21 | 11 | 7 | 6 | 1 | |
| 2 | 678 | 13 | 5 |
| 3 | 1056 | NS | NS |
| MCA-2 | 1 | 120 | 119 | 25 | 24 | 27 | 4 | 18 | 20 | 2 | |
| 2 | 94 | 30 | 21 |
| 3 | 144 | NS | NS |
| Untreated \*\* | 1 | 0.4 | 0.3 | 0.1 | ND | ND | ND | ND | ND | ND | |
| 2 | 0.3 | ND | ND |
| 3 | 0.2 | ND | ND |
|  |  | \*\* All of the values for copper extracted from wipes passed along untreated woods are below detectable reporting limits. Measured values are estimates. (ND = not detectable)  SD= standard deviation | | | | | | | | | |
|  | |
|  |  | NS=Third replicate was not sampled. | | | | | | | | |  |
| Table S4: Detailed data for total copper extracted from wipes upon digestion in the microwave system and for wipes passed along as-purchased wood and exposed to SSF. | | | | | | | | | | | |
| **Treatment** | **Replicate** | **Total (µg/wipe)** | **Average Total (µg/wipe)** | **Total SD** | **Percent released** | **Average Percent Released** | **Released SD** | **Percent 10 kDa** | **Average Percent 10 kDa** | | **10 kDa SD** |
| ACA | 1 | 57 | 133 | 90 | 43 | 43 | 8 | 44 | 43 | | 8 |
| 2 | 232 | 51 | 51 |
| 3 | 111 | 34 | 35 |
| MCA-1 | 1 | 282 | 672 | 387 | 51 | 45 | 6 | 53 | 45 | | 6 |
| 2 | 678 | 39 | 41 |
| 3 | 1056 | 44 | 42 |
| MCA-2 | 1 | 120 | 119 | 25 | 1611 | 100\* | 24 | 142 | 100\* | | 9 |
| 2 | 94 | 114 | 124 |
| 3 | 144 | 126 | 131 |
| Untreated -1\* | 1 | 0.4 | 0.3 | 0.1 | ND | ND | ND | ND | ND | | ND |
| 2 | 0.3 | ND | ND |
| 3 | 0.2 | ND | ND |
|  |  | \* Note: All the values for copper extracted from wipes passed along untreated woods are below detectable reporting limits. Measured values are estimates. (ND = not detectable)  \*\* For values exceeding 100 percent after averaging, the values were reported as 100%.  SD=standard deviation | | | | | | | | | |
|  |  |  | | | | | | | | | |
|  |  |  | | | | | | | | |
|  |  |  |
|  |  |  | |  |  |  |  |  |  | |  |

2.0 Materials and Methods:

2.1 Surface Wipe Sampling

Four wood types (one liquid copper-treated [ACA], two micronized copper-treated [MCA], and one untreated [southern yellow pine]) intended for above-ground use were purchased from a national chain home improvement store. Boards belonging to each wood type were weathered as previously described for 399 days (Platten et al., 2016). Surface wipe sampling of the as-purchased and weathered boards for each wood type were performed according to protocols developed by the Consumer Product Safety Commission (CPSC) (Thomas et al., 2004). Individual polyester cloth wipes were dampened to two times their original weight with 0.9% saline solution. The wipe was attached to a 1.1 kg weight and pulled back and forth along a 450 cm² area on the board 5 times (10 passes). Each cloth wipe was passed over a different location on a single board. The area sampled on each board was 450 cm2 and the sampling surface area on each wipe was 50 cm2. After sampling, each wipe was stored in an individual 50 ml centrifuge tube.

2.2 Bioaccessibility Assay

All glassware and vessels were acid-washed in 20% nitric acid for 24 hours and rinsed three times with deionized water (DI, 18 MΩ∙cm, ASTM Type I trace element quality, Millipore, Bedford, MA). DI water was used to prepare all solutions. Synthetic stomach fluid (SSF) with a pH of 1.5 to mimic the highly acidic environment in the stomach under normal fasting conditions was prepared as previously described (Bradham et al., 2011) using 0.42 M HCl (32-35% analytical grade), 0.40 M glycine (certified ACS grade) obtained from Fisher Scientific, Inc. (Pittsburgh, PA) and DI water. Thirty ml of SSF or DI water was added to each tube containing a wipe sample or control. After attaching screw caps, the tops of the tubes were wrapped with parafilm to prevent leakage and the tubes were shaken continuously at room temperature for 1 hour at 140 rpm on an orbital shaker.

MCA (34.54% copper, 0.62% azole) served as a positive control and was used to identify whether surface wipes affected the release of copper into solution. 300 µL of MCA (350 ppm) was applied to individual wipes. Wipes were suspended during MCA application to ensure no loss of MCA sample. The surface wipes were allowed to dry overnight. Next, MCA-treated wipes or 300 µL of MCA solution were transferred to 50 ml centrifuge tubes containing 30 ml of SSF or DI water and allowed to shake at 140 rpm for 1 hour at room temperature.

To collect the total copper (soluble + insoluble) fraction released from the wipes, a Buchner funnel system attached to a vacuum was set up and a 50 ml centrifuge tube was placed into the receiving flask to collect the solution. The wipe and solution were transferred to the funnel and vacuum was applied to drain the solution from the wipes. An aliquot of the solution was collected and designated as the whole fraction. A separate aliquot of the solution was transferred to a 10 kDa centrifuge filter unit (Amicon Ultra-15, 10K, Millipore, Bedford, MA) and centrifuged at 5000 x g for 10 minutes. An aliquot of the filtrate was designated as the soluble fraction. The two collected fractions (solution and filtrate) were acidified in 2% nitric acid (67-70% Optima™, Fisher Scientific, Inc., Pittsburgh, PA) and stored at 4C until further analysis via Inductively Coupled Plasma - Mass Spectrometry (ICP-MS). The Buchner funnel system was washed with 20 ml of 20% nitric acid and thoroughly rinsed with DI water between each sample (Fig. 1). Matrix (SSF only) and matrix spiked with 30 parts per billion (ppb) of copper standard were included in the bioaccessibility assay. Extractions for all wipes passed along wood boards were performed in duplicate for water and triplicate for SSF. Extractions for wipes treated with copper azole technical material were performed in duplicate for water and SSF. Extractions for copper azole technical material alone were performed in duplicate for water and SSF.

2.3 Total copper extraction from surface wipes

An additional set of wipes (three wipes per copper-treated wood type and two wipes per untreated wood type) were placed onto a cutting mat using acid-washed plastic forceps. An acrylic ruler and a rotary cutter were used to measure and cut, respectively, a 3 in.2 square from the sample area of each wipe. The square was cut into 9-1 inch squares and each piece was weighed and the masses recorded. The average mass for all wipes was 1.9 ± 0.1 mg for wipes sampled from new boards and 1.81 ± 0.09 mg for wipes sampled from weathered boards. Each 1 inch square was transferred to a Teflon™ digestion vessel containing 10 ml of concentrated nitric acid (67-70% Optima™, Fisher Scientific, Inc., Pittsburgh, PA) and allowed to pre-digest for 15 minutes under a fume hood. After 15 minutes, the vessels were properly sealed and the samples digested using MARS-5 or MARS-6 microwave systems (CEM Corporation, Matthews, NC). All samples underwent microwave-assisted digestion for 15 minutes at an operating temperature of 200C at 1200 W (100% power) with a maximum pressure of 800 psi under standard control settings. The method concluded with a 5-minute cooling period in the oven and an additional 1 hour cooling period in the fume hood. The samples were diluted to 2% nitric acid concentration prior to analysis by ICP-MS (Fig. 1). After ICP-MS analysis, the measured copper concentrations for all 9 squares for each wipe were compiled to obtain total copper concentration per wipe. For each digestion set, reagent blanks and 30 ppm copper-spiked reagent blanks were also prepared and analyzed.

2.4 Instrumental Analysis

Backscatter electron - scanning electron microscopy (BSE-SEM) imaging (JEOL JSM-

7600F, Tokyo, Japan) was used to confirm the presence of copper (Cu) particles on wipes passed along 1 month old MCA-1 and MCA-2-treated woods. Elemental analysis was performed using energy dispersive X-ray spectroscopy (EDS) mapping (Fig. 2). The quantification of Cu concentrations from surface wipe samples were performed according to USEPA Method 6020A using inductively coupled plasma-mass spectrometry (ICP-MS) (USEPA, 2007b) or USEPA Method 6010D using inductively coupled plasma-optical emission spectrometry (ICP-OES, Thermo Scientific, Waltham, MA, USA) (USEPA, 2014). Use of ICP-MS or ICP-OES was dependent on availability of the instrument and detection limits. Procedures for calibration, calibration verification, quality assurance and quality control as detailed in Methods 6010D or 6020A were also performed. Commercially available reference standards (VHG Labs, Manchester, NH) were used for analysis. Scandium was used as an internal standard (spectral line 335.5 nm) for ICP-OES and 45Sc and 89Y for ICP-MS. The method reporting limit for copper was 1.0 ppb (ICP-MS) or 2.5 ppb (ICP-OES). The linear dynamic range of 0.001 (ICP-MS) or 0.0025 (ICP-OES), to 2.0 ppm was verified based on successful recoveries of low and high level calibration verification solutions. Any solutions with Cu concentrations below the method reporting limit were reported as “below detectable reporting limits” and designated as “ND” for not-detectable. Duplicates, matrix spikes, and blanks were included in the analysis. The range of blanks were below the 1.0 ppb or 2.5 ppb lowest level calibration verification (LLCV) recovery limit. Duplicates were within 75-125% of the expected value. Matrix spiked samples were within 80-120% of the expected value. Dilution check samples were within 90-110% of the expected values (Supplementary 1).

To obtain the corrected copper concentration, the measured copper concentration (µg/L) was multiplied by the sample solution volume (L). Next, this value was divided by the average mass of the wipe (g.). The total insoluble and soluble fractions that were released were expressed as percent in vitro bioaccessibility (IVBA) and were calculated using the formula:

% IVBA = (((in vitro extractable µg Cu)/wipe) )/(((Total µg Cu)/wipe) ) × 100%

2.5 Statistical Analysis

Two-way ANOVA with significance levels at 0.05 or lower was used to examine the relationship between wood type and in vitro bioaccessibility. In cases where significance levels were below 0.05, post hoc Tukey’s tests were performed to identify significant differences between each treatment. For any data that failed tests for normality and variance, square root transformations were undertaken to achieve normality and equal variance prior to analysis. All analyses were performed using SigmaPlot 13.0 (Systat Software Inc., San Jose, CA).