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The Single and Combined Effects of Wildfire Runoff and Sediment-Bound Copper on the Freshwater Amphipod *Hyalella azteca*

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Abstract: The frequency of wildfire is expected to increase with time as a function of climate change. Recent studies in our laboratory have demonstrated that pyrogenic polycyclic aromatic hydrocarbons can cause greater-than-additive effects in *Hyalella azteca* in the presence of low concentrations of Cu. We hypothesized that freshwater animals inhabiting Cu-contaminated sites, such as those in the vicinity of Cu mines, may be vulnerable to nonadditive toxicity from contaminants released by wildfires. To investigate the interaction between Cu and water conditioned by burnt wood ash (fire extract), we exposed *H. azteca* for 14 d to binary mixtures of 225 mg/kg Cu-enriched artificial sediment (225 mg Cu/kg) and a fire extract dilution series (12.5, 25, 50, 75, and 100%). All binary mixtures of Cu-enriched sediment and fire extract resulted in complete mortality with the exception of Cu-enriched sediment + 12.5% fire extract. The combination of Cu-enriched sediment with 12.5% fire extract had a more-than-additive effect on survival and tissue Cu concentration, but there was no reduction in growth or acetylcholinesterase activity compared to the 225 mg/kg Cu–contaminated sediment or fire extract control, respectively. Acetylcholinesterase activity was significantly reduced in amphipods exposed to fire extract, but the presence of Cu did not exacerbate this effect. The results suggest that Cu-contaminated water bodies that receive runoff from wildfires are at risk of enhanced toxicity. *Environ Toxicol Chem* 2020;39:1988–1997. © 2020 SETAC

Keywords: Wildfire; Copper; Survival; Bioaccumulation; Acetylcholinesterase; *Hyalella azteca*

INTRODUCTION

Wildfires have been increasing in frequency and severity as a result of climate change (Gillett et al. 2004; Westerling et al. 2006). Increased average yearly temperatures and reduced rainfall in combination with an earlier onset of spring all increase wildfire risk (Gillett et al. 2004; Westerling et al. 2006). British Columbia, Canada, experienced the 2 worst fire seasons on record in 2017 and 2018, with 12 161 and 12 985 km^2 burned, respectively (British Columbia Wildfire Services 2019). The trend of worsening wildfire seasons is expected to continue with climate change, so the impact of wildfire runoff on the condition of freshwater is of concern. The loss of trees and groundcover caused by wildfire allows ash and burnt material to wash into freshwater ecosystems, leading to potentially hazardous contamination. Approximately one-third of the 105 largest cities in the world depend on freshwater found in protected forested areas (Dudley and Stolton 2003), which could impact water-treatment facilities in the event of a wildfire (Smith et al. 2011). Research by Bladon et al. (2014) demonstrates how wildfire runoff could cause an increase in operation/maintenance costs for water-treatment facilities and require additional infrastructure to maintain drinking water quality. However, the effects that wildfire runoff could have on freshwater ecosystems are also of concern.

Wildfires can release nitrogen, phosphorus (P), ammonia, dissolved organic carbon (DOC), and pyrogenic polycyclic aromatic hydrocarbons (PAHs) into the aquatic environment via runoff (McEachern et al. 2000; Olivella et al. 2006; Bladon et al. 2008). Increased nutrient levels, particularly P, associated with wildfire have been shown to persist 6 to 7 yr postfire, causing changes in aquatic trophic interactions due to large algae blooms (Silins et al. 2009, 2014). Increased input of DOC into freshwater environments could be beneficial because DOC is well known to ameliorate toxicants, such as metals, by binding toxicants and reducing the toxicant bioavailability to aquatic organisms (Richards et al. 2001; Besser et al. 2003; Niyogi and Wood 2004). The released pyrogenic PAHs persist in the environment, potentially causing long-term effects (Abdel-Shafy and Mansour 2016). Pyrogenic PAHs are organic

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compounds composed of 2 or more benzene rings formed through the incomplete combustion of organic material at high temperatures that usually contain fewer rings (e.g., anthracene, pyrene, phenanthrene) and fewer alkylated substitutions than petrogenic PAHs (Wang et al. 1999), although there is overlap between petrogenic and pyrogenic PAHs. The reduced number of rings allows pyrogenic PAHs to dissolve more easily than compounds with more rings, making them more bioavailable to organisms (Olivella et al. 2006).

The individual components released by wildfire have been frequently studied; however, the study of total post-wildfire runoff toxicity is a recent and developing field. Nunes et al. (2017) exposed mosquitofish (*Gambusia holbrooki*) to postfire surface runoff, which resulted in an increase in oxidative stress but no associated oxidative damage. Silva et al. (2015) exposed *Pseudokirchneriella subcapitata* and *Lemna minor* to an aqueous extract of wood ash, which caused a decrease in density and dry weight, respectively, for the primary producers following acute exposure. However, no deleterious effects were observed for *Daphnia magna* exposed to aqueous extract of wood ash (Silva et al. 2015). Research done exposing the freshwater clam *Corbicula fluminea* to aqueous extract of wood ash resulted in increased mortality and bioaccumulation of PAHs and metals (Silva et al. 2016).

Although studying the toxicity of isolated wildfire runoff is of great importance, the interaction of wildfire runoff with other potential toxicants remains largely unknown. Contamination that occurs in natural environments often interacts with preexisting contaminants to form mixtures that can potentially exacerbate or ameliorate the contaminants' individual effects. Cotoxicity has been studied in metals, with findings of less-than-additive, additive, and more-than-additive results for metal cotoxicity (Norwood et al. 2003). Cotoxicity, therefore, is an important consideration when attempting to determine the ecological risk of contamination events. Previous research in our laboratory demonstrated that Cu toxicity is enhanced in the presence of certain hydrocarbon contaminants which can be constituents of wildfire runoff, such as the pyrogenic PAHs phenanthrene (PHE) and phenanthrene quinone (PHQ), in *Hyalella azteca* (Gauthier et al. 2015). *Hyalella azteca* exposed to Cu–PHE and Cu–PHQ mixtures experienced 26.9 ± 1.7 and 73.3 ± 13.3% more mortality, respectively, than could be explained by the toxicity of the individual toxicants alone (Gauthier et al. 2015). In addition, Gauthier et al. (2016) examined amphipod acetylcholinesterase (AChE) activity following exposure to Cu and PHE individually and in combination. Acetylcholinesterase is an important enzyme that regulates acetylcholine concentrations at the synapse by hydrolyzing and inactivating acetylcholine (Soreq and Seidman 2001). Exposure to Cu (Brown et al. 2004; Frasco et al. 2005; Haverroth et al. 2015) and PAHs (Kang and Fang 1997; Kim et al. 1999) reduce AChE activity individually in aquatic organisms. When animals were exposed to PHE, Gauthier et al. (2016) did observe a reduction in AChE activity in *H. azteca*, but exposure to Cu did not differ from control or influence the toxic effects of PHE. Although there are studies on single PAH and metal interactions, there is a gap in our understanding of how total wildfire runoff affects Cu toxicity.

The combination of Cu and wildfire runoff could occur in a natural system. The 2014 Mount Polley Mining Corporation Cu and gold mine experienced a large tailings impoundment breach that released approximately 17 M m^3 of Cu-rich water and 8 M m^3 of solid tailings into Polley Lake, down Hazeltine Creek and into Quesnel Lake (Petticrew et al. 2015). Quesnel Lake is a large (surface area 266 km^2), deep (maximum 610 m), oligotrophic fjord lake located in the Cariboo region of British Columbia, Canada (Petticrew et al. 2015). Copper was identified as a potential contaminant of concern following the breach, with waterborne concentrations as high as approximately 100 μg/L that dropped to below 6.6 μg/L by May of 2015 (British Columbia Ministry of Environment and Climate Change Strategy 2015). Following the breach, the solid tailings Cu concentration in Quesnel Lake was as high as 1200 mg Cu/kg (Hatam et al. 2019). Although restoration efforts have taken place, solid tailings from the breach remain in Polley and Quesnel Lakes. In 2017, the Elephant Hill wildfire swept through the Cariboo region, where the mine is located. The Cu-rich solid tailings released from the Mount Polley Mining Corporation tailing impoundment breach in combination with the runoff from the 2017 Elephant Hill wildfire could interact similarly to the results found by Gauthier et al. (2015), increasing Cu toxicity to freshwater organisms. An opposing hypothesis is one of Cu amelioration where DOC from wildfire runoff could reduce Cu toxicity via competitive binding to the site of toxic action (Niyogi and Wood 2004).

The objective of the present study was to determine the extent to which wildfire runoff exacerbates or ameliorates Cu sediment toxicity. To accomplish this objective, *H. azteca* were exposed for 14 d under controlled laboratory conditions to a Cu-enriched sediment in combination with increasing concentrations of an artificially created wildfire runoff. Endpoints measured were survival, growth, whole-body Cu tissue concentration, and AChE activity.

METHODS

Culturing method

Hyalella azteca were obtained in June of 2017 from Environment and Climate Change Canada, Pacific and Yukon Laboratory for Environmental Testing. They were cultured in the Alberta Water and Environmental Science Building at the University of Lethbridge in 40-L tanks with 30 L of standard artificial medium (Borgmann 1996). Tanks were kept at 23 °C using heated water baths. An 80% water change was conducted once a week. Animals were fed 120 mg of finely ground Tetramin® tropical fish flake food (Tetra; catalog no. 77101) 3 times a week on nonconsecutive days. To provide a habitat substrate for cultured *H. azteca*, 3 × 3 cm cotton gauze squares were placed on the bottom of each culture tank. Tanks were provided with a 16:8-h light:dark photoperiod and lit with full-spectrum fluorescent bulbs emitting light at approximately 645 lux. Juveniles were separated from adults using a mesh filter stack with 600-μm mesh to collect adults and 215-μm mesh to collect juveniles. Isolated juveniles were transferred to a separate tank for at least 24 h prior to being used in subsequent experiments.

Artificial wildfire runoff preparation

Balsam fir and Fraser fir trees were burned to create ash and burnt material. Coniferous tree species were selected to be relevant to the subalpine fir- and Engelmann spruce–dominant forest found around the Mount Polley Mining Corporation breach area. A total of 52.80 kg of balsam fir and 8.07 kg of Fraser fir were burned in an outdoor fire pit until the fire extinguished itself approximately 6 h after ignition. The ash and burnt material was then combined with culture water at a 1:4 v/v ratio (Silva et al. 2015). The mixture was stirred daily for 1 wk prior to use and continuously aerated. The aged mixture was filtered through aquarium filter wool to remove large fragments of debris. The resulting fire extract test water was then diluted with culture water to create a fire extract concentration gradient of 0, 12.5, 25, 50, 75, and 100%. The concentrations of fire extract were selected based on a worst-case scenario, exposure to undiluted runoff (100%), and diluting from that scenario to a more likely 12.5% fire extract which might occur in a small water body.

Cu-enriched sediment preparation

Copper-bound sediment at a concentration of 225 mg Cu/kg was used in this experiment because a preliminary experiment revealed that this concentration did not reduce survival but did reduce growth in *H. azteca*. By selecting a Cu-exposure concentration that induces sublethal toxicity, we could determine if coexposure with fire extract exacerbates or ameliorates any toxicity associated with Cu-enriched sediment. The Cu-enriched sediment was created by making a 2.21-mg/L solution of $CuSO_4$ $5H_2O$ (purity >98.5%; VWR International; catalog no. BDH3312-2) in double distilled water (ddH_2O). The Cu solution was combined with bentonite clay at a ratio of 2 mL of solution to 5 g of bentonite. The mixture was then manually stirred until all clay was saturated with solution and thoroughly combined. The mixture then was left overnight before being used in the toxicity test. Sediment Cu concentration was confirmed via a graphite furnace atomic absorption spectrophotometer (GF-AAS) to be 225 ± 1.5 mg/kg (Agilent Technologies).

Toxicity test

To investigate the possible interaction of Cu-enriched sediment and fire extract, a chronic 14-d sediment toxicity test was performed using *H. azteca* following Canadian Environmental Protection Service Biological Test Method protocols with some adjustments (Government of Canada 2012). All tests were run with mixed-age *H. azteca* to represent wild populations (50% neonates, 50% adults). All tests were run at 23 ± 1 °C with a 16:8-h light:dark photoperiod and lit with full-spectrum fluorescent bulbs emitting light at 500 lux. All tests were carried out in 300-mL tall-form glass beakers filled with 50 mL of Cu-enriched sediment and 200 mL of test water. Animals were exposed to varying concentrations of fire extract and a single concentration of 225 mg Cu/kg bound sediment. There was a total of 11 treatments: laboratory control, bentonite control, 12.5% fire extract, 50.0% fire extract, 100.0% fire extract, 225 mg Cu/kg, 225 mg Cu/kg + 12.5% fire extract, 225 mg Cu/kg + 25.0% fire extract, 225 mg Cu/kg + 50.0% fire extract, 225 mg Cu/kg + 75.0% fire extract, and 225 mg Cu/kg + 100.0% fire extract. Laboratory control consisted of culture water with a 3 × 3 cm square of 70-µm mesh to serve as a substrate. The bentonite control consisted of culture water with bentonite clay prepared as described for the Cu-enriched sediment but without the addition of Cu salt. Each replicate had 12 mixed-aged *H. azteca* randomly assigned. Half of the animals were juveniles (<9 d old), and half were adults (>4 wk). Potential bias in animal size assignment to each replicate was controlled by randomly sorting exposure vessels prior to the addition of animals and only adding 2 animals at a time to each replicate. In addition, randomly placed extra vessels were among the test vessels during animal vessel assignment which had amphipods added as if they were part of the experiment. The animals in the extra test vessels were then immediately dried and weighed to ensure that replicate weight between test vessels did not vary significantly ($t_4 = 0.29$, $p = 0.78$). Animals were fed ad libitum with finely ground Tetramin tropical fish food flakes 6 times throughout the experiment on nonconsecutive days. An 80% water renewal was conducted on day 7 to control ammonia levels. Following the toxicity test, animals were transferred to clean culture water and left for 24 h to depurate the contents of their guts. After depuration, the *H. azteca* were rinsed for 1 min in a 50-µM ethylenediaminetetraacetic acid (EDTA) solution made using culture water to remove bound metals from the body surface of the amphipods (Norwood et al. 2007). Animals were then transferred to preweighed aluminum weigh boats and rinsed with culture water for 1 min to remove any remaining EDTA, and then the culture water was removed using a pipette. Animals were then dried for 48 h at 60 °C and weighed.

Sediment Cu and whole-body amphipod Cu analysis

To determine the concentration of Cu in the test sediment and tissue of the exposed *H. azteca*, samples were analyzed with a GF-AAS. Prior to analysis, sediment and tissue were digested in 67 to 70% nitric acid (trace metal grade; Fisher Chemical; catalog no. A509-P212) at a ratio of 1:10 w/v at 80 °C for 6 and 3 h, respectively. Samples were then cooled to room temperature and diluted with ddH_2O at a ratio of 1 mg sample to 2 mL ddH_2O. Digests were then cooled overnight at 4 °C, and the supernatant was analyzed.

Copper analysis was performed as described by Lindh et al. (2019). Supernatant was analyzed on an Agilent 240 FS atomic absorption spectrometer with a GTA 120 graphite tube atomizer (Agilent Technologies). Samples were measured at a wavelength of 324.8 nm and a slit width of 0.5 nm using a Cu hollow cathode lamp (Agilent Technologies; catalog no. 5610101400). An additional step was included to the

furnace burn profile (step 9, temperature 2300 °C, time 4.0 s, flow 0.3 L/min), to diminish carryover of the previous sample; otherwise, samples were run in accordance with the manufacturer's guidelines. All samples were diluted with ddH_2O as necessary to fall within the calibration range (10–90 µg/L Cu). The certified reference material SLRS-6 (National Research Council Canada) was run every 10 samples to gauge the accuracy of the analysis (mean percentage of recovery for sediment and tissue 92.7 and 94.1%, respectively) along with a blank to ensure minimum carryover (mean blank for sediment and tissue 0.09 and 0.55 µg/L, respectively). Analytical duplicates were run approximately every 20 samples (mean difference for sediment and tissue 1.1 and 1.0%, respectively). Measured Cu concentrations were adjusted by weight of the amphipod, dilution, and blank concentration.

AChE analysis

Acetylcholinesterase activity and protein content were measured using methods described by Bartlett et al. (2016). A minimum of 5 amphipods were used in each replicate. Amphipods were homogenized in 500 µL homogenizing buffer (Tris buffer; Invitrogen; catalog no. 15504020) at pH 8 with 1% v/v Triton X-100 (Alfa Aesar; catalog no. J62289) and centrifuged at 10 000 g for 10 min at 4 °C. The resulting supernatant was used in the assay. Triplicate additions to the microplate were as follows: 40 µL of homogenizing buffer (blank), electric eel cholinesterase (enzyme standard; Sigma-Aldrich; catalog no. C3389) or supernatant, 250 µL Ellman's reagent (5,5′-dithiobis[2-nitrobenzoic acid]; Thermo Scientific; catalog no. 22582), and 10 µL of acetylthiocholine iodide (Sigma-Aldrich; catalog no. A5751). Absorbance was measured on a microplate reader (Varioskan Flash; Thermo Scientific) at 405 nm in 2-min intervals for 30 min.

Protein quantification of the amphipod supernatant was measured using a bicinchoninic acid assay as described by Bartlett et al. (2016). Triplicate additions to the microplate were as follows: 25 µL of homogenizing buffer, bovine albumin protein standards (0, 100, 250, 500, and 1000 µg/mL; Sigma-Aldrich; catalog no. A7906), electric eel cholinesterase, or homogenate supernatant were added with 200 mL of the bicinchoninic acid working reagent. The plate was incubated at room temperature for 2 h, and absorbance was measured on a microplate reader (Varioskan Flash; Thermo Scientific) at 562 nm. Protein concentrations were calculated using the standard curve made from the bovine serum standards.

Acetylcholinesterase activity was calculated using the equation

$$\text{Specific Activity} = \frac{A \times R_{Vol} \times 1000}{E \times PL \times H_{Vol} \times PR}$$

where specific activity is in µmol/min/g protein, A is the change in absorbance per minute, R_{Vol} is the reaction volume (300 mL), 1000 is a unit conversion factor (grams to milligrams), E is the extinction coefficient for 5,5-dithiobis[2-nitrobenzoic acid] ($1.36 \times 10^4\ M^{-1}\ cm^{-1}$), PL is the pathlength (0.875 cm), H_{vol} is the homogenate volume (500 mL), and PR is protein in the homogenate (mg/mL).

Water quality

Water quality was measured at the beginning (day 0), the halfway point (day 7), and the end (day 14) of the experiment in one replicate of each treatment. Dissolved oxygen and temperature were measured every other day using a dissolved oxygen probe (YSI Pro20 dissolved oxygen meter; YSI). The pH was measured using a pH meter (accumet™ Excel XL15 pH meter; Fisher Scientific). Conductivity was measured using a Traceable conductivity meter (VWR; catalog no. 89094-762). Hardness and alkalinity were measured using the titration method described by the American Public Health Association (Clesceri et al. 1989). Dissolved organic carbon (DOC) samples were filtered, stored at –20 °C, and analyzed by the Aquatic Toxicology Research Centre Environmental Laboratory at Lakehead University.

Statistical analysis

A one-way analysis of variance followed by a post hoc Tukey's test was used to determine statistically significant differences among treatments. The total Cu tissue data set was log-transformed to meet assumptions of homogeneity. In addition, the more-than-additive accumulation of Cu in tissue of Cu + fire extract was analyzed using a one-sample single-tailed *t* test where the means of the Cu and the fire extract exposures were combined and compared with the Cu + 12.5% fire extract exposure. All of the data were analyzed using R, Ver 3.5.2 (R Development Core Team 2018). Mean differences were considered to be statistically significant when $p \le 0.05$.

RESULTS

Survival

Survival in both laboratory and bentonite controls was not affected by the experimental conditions (Figure 1). All exposures that contained fire extract concentrations >12.5%, including those with and without Cu-enriched bentonite, experienced 100% mortality by the end of the 14-d exposure. Survival in the 12.5% fire extract and the 225 mg/kg Cu–enriched sediment exposures did not differ from control. However, when 12.5% fire extract was combined with the 225 mg/kg Cu–enriched sediment, there was a 41% reduction in survival ($F_{4,31} = 45.89$, $p < 0.001$; Figure 1).

Weight

Weight could only be compared among treatments that did not result in complete mortality. The controls and 12.5% fire extract–exposed amphipods had 24.1 to 37.1 and 37.9 to 48.6% greater per organism weight than the 225 mg Cu/kg and the 225 mg Cu/kg + 12.5% fire extract exposures, respectively ($F_{4,31} = 23.7$, $p < 0.001$; Figure 2). However, the weight per organism in the surviving exposures that contained Cu-enriched sediment did not differ (Figure 2).

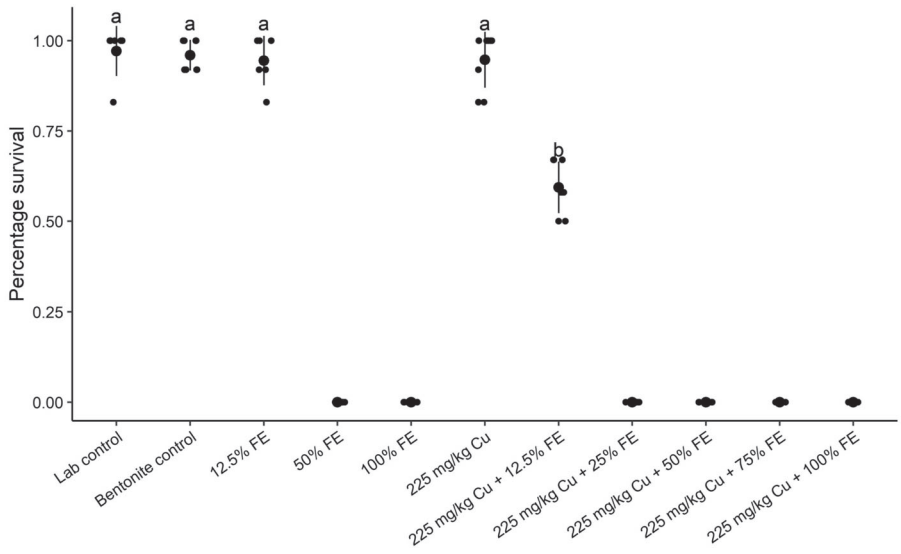

FIGURE 1: Mean of proportional survival ± standard error for surviving *Hyalella azteca* following exposure to various percentage concentrations of fire extract (FE) and 225 mg Cu/kg sediment (Cu). Replication of laboratory control and fire extract exposures was $n = 6$. Replication of bentonite, Cu, and Cu + FE exposures was $n = 8$. Bars sharing the same letter designation are not statistically different from one another ($p \leq 0.05$).

Amphipod whole-body Cu tissue concentration

Results from the Cu GF-AAS analysis revealed differences in whole-body amphipod Cu tissue concentrations between treatments ($F_{4,12} = 110.8$, $p < 0.001$). All non-control exposures had increased concentrations of Cu in their whole-body tissue (Figure 3). This increased Cu was greatest in amphipods exposed to Cu + fire extract, which was also greater than the concentration of Cu in animals exposed to Cu or fire extract individually by 84.2 and 44.4%, respectively (Figure 3). In fact, a more-than-additive increase in tissue Cu concentrations was found for animals exposed to Cu + fire extract than to animals exposed to Cu or fire extract individually ($t_3 = 2.30$, $p = 0.05$).

AChE assay

Acetylcholinesterase activity was found to differ among treatments ($F_{4,10} = 32.44$, $p < 0.001$). Mean measured amphipod AChE activity did not differ between laboratory and bentonite controls (Figure 4). Exposure to either fire extract or Cu + fire extract resulted in a similar reduction in AChE activity of 58.2 and 49.4%, respectively, compared to laboratory control and 64.8 and 57.4%, respectively, compared to bentonite control; however, the activity between animals exposed to fire extract and those exposed to Cu + fire extract did not differ (Figure 4). There was also an increase in AChE activity found for amphipods exposed to 225 m/kg Cu–enriched sediment of

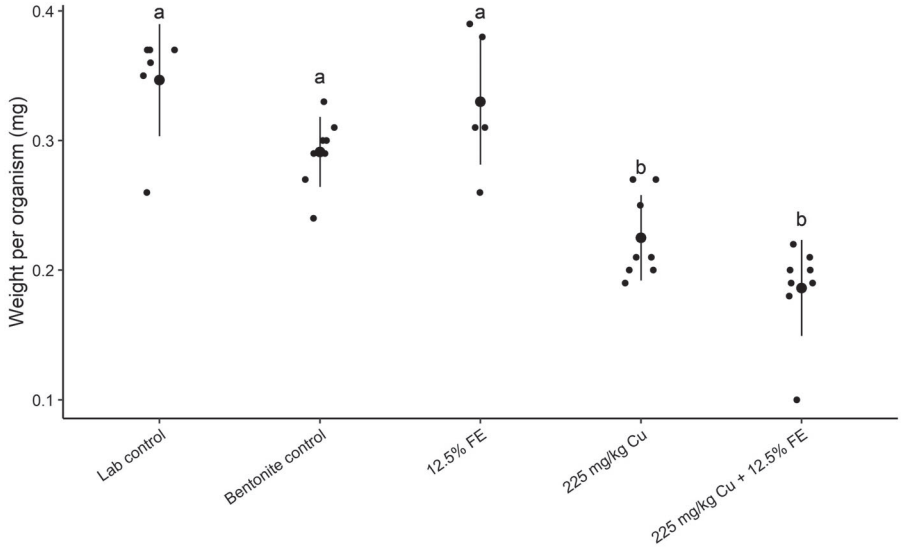

FIGURE 2: Mean weight per organism ± standard error for *Hyalella azteca* following exposure to 12.5% fire extract (FE), 225 mg/kg sediment bound Cu, and 225 mg/kg sediment-bound Cu-12.5% FE mixture. Replication of laboratory control and FE was $n = 6$. Replication of bentonite, Cu, and Cu + FE was $n = 8$. Bars sharing the same letter designation are not statistically different from one another ($p \leq 0.05$).

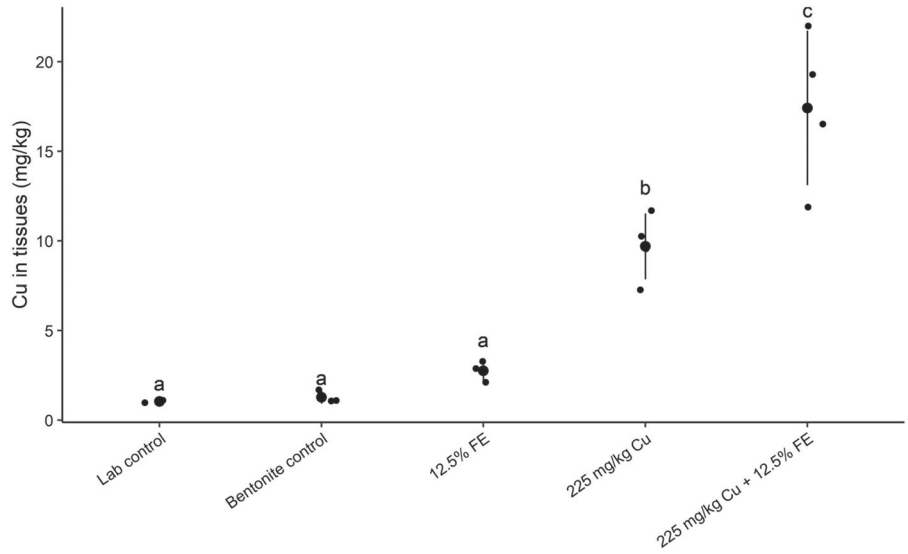

FIGURE 3: Mean tissue Cu concentration ± standard error for *Hyalella azteca* following exposure to 12.5% fire extract (FE), 225 mg/kg sediment bound Cu, and 225 mg/kg sediment-bound Cu + 12.5% FE mixture. Bars sharing the same letter designation are not statistically different from one another ($p \leq 0.05$). For laboratory control, bentonite control, and 12.5% fire extract, $n = 3$. For Cu and Cu + FE, $n = 4$.

38.8 and 27.4% compared to laboratory and bentonite controls, respectively (Figure 4).

Water quality

Dissolved organic carbon, pH, and alkalinity were observed to increase with increasing fire extract percentage concentration both with and without the addition of Cu-enriched sediment (Table 1). Hardness decreased in exposures with Cu-enriched sediment with increasing fire extract percentage concentrations, but this was not observed in exposures that did not contain Cu-enriched sediment (Table 1). All other water quality parameters were within acceptable deviations of average values described in the Canadian Environmental Protection Service Biological Test Method (Government of Canada 2012).

DISCUSSION

Survival, growth, and bioaccumulation

We found that Cu-enriched sediment and fire extract interact to cause more-than-additive toxic effects on *H. azteca*. The Cu + fire extract combination induced a decrease in survival following 14-d exposure to 225 mg Cu/kg and 12.5% fire extract, whereas no significant mortality was observed in the 225 mg Cu/kg sediment or 12.5% fire extract alone. These

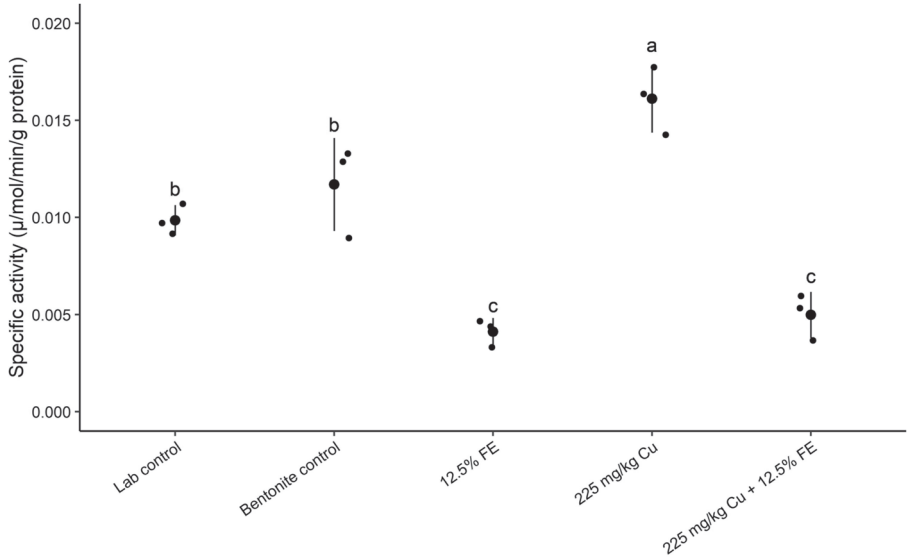

FIGURE 4: Specific activity of acetylcholinesterase ± standard error for *Hyalella azteca* following exposure to 12.5% fire extract (FE), 225 mg/kg sediment bound Cu, and 225 mg/kg sediment bound Cu + 12.5% FE mixture. Bars sharing the same letter designation are not statistically different from one another ($p \leq 0.05$). $n = 3$.

TABLE 1: Average water quality parameters (±standard deviation) in water samples for toxicity test using *Hyalella azteca* exposed to a Cu-enriched sediment and artificially created fire extract[a]

Treatment	Temperature (°C)	DO (% O_2)	pH[b]	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)	Conductivity (µS/cm)	DOC (mg/L)
Laboratory control	22.6 ± 0.5	96.3 ± 4.3	7.88 (7.83–7.91)	57 ± 7.8	182 ± 19	456 ± 39	3.3
Bentonite control	23.7 ± 0.1	92.8 ± 0.7	8.42 (7.91–8.57)	71 ± 18	152 ± 42	819 ± 68	14.7
Cu	23.2 ± 0.5	91.5 ± 1.9	8.43 (7.90–8.56)	100 ± 31	124 ± 66	856 ± 102	15.0
12.5% FE	22.2 ± 0.2	88.9 ± 1.5	8.46 (8.41–8.51)	259 ± 18	87 ± 19	1022 ± 177	10.1
50.0% FE	22.3 ± 0.2	90.6 ± 1.7	8.78 (8.61–8.91)	634 ± 51	107 ± 30	2443 ± 133	41.6
100.0% FE	22.3 ± 0.2	91.4 ± 2.9	9.16 (8.88–9.18)	1234 ± 12	133 ± 42	4013 ± 90	80.3
Cu + 12.5% FE	23.5 ± 0.3	91.5 ± 4.7	8.64 (8.41–8.68)	278 ± 3.6	85 ± 30	1190 ± 322	23.2
Cu + 25.0% FE	23.4 ± 0.5	91.9 ± 6.9	8.81 (8.51–8.86)	349 ± 56	71 ± 15	1627 ± 304	36.9
Cu + 50.0% FE	23.1 ± 0.8	92.6 ± 5.7	9.02 (8.70–9.03)	691 ± 70	72 ± 15	2563 ± 237	58.5
Cu + 75.0% FE	22.9 ± 0.5	91.3 ± 1.1	9.07 (8.88–9.07)	927 ± 23	67 ± 18	3257 ± 32	74.4
Cu + 100% FE	23.7 ± 0.0	88.3 ± 0.9	9.17 (8.92–9.20)	1260 ± 99	66 ± 7.0	3953 ± 38	96.3

[a]The data given for pH, alkalinity, hardness, and conductivity had $n = 3$, temperature and dissolved oxygen had $n = 7$, dissolved organic carbon had $n = 2$.
[b]pH water quality reported as median (range).
DO = dissolved oxygen; DOC = dissolved organic carbon; FE = fire extract.

results are in agreement with research done by Gauthier et al. (2015), who performed a study with *H. azteca* exposed to combinations of waterborne Cu with pyrogenic PAHs, which partially comprise fire extract. Amelioration of Cu toxicity by DOC present in the fire extract was not observed, which is incongruent with decreased toxicity found for *Ceriodaphnia dubia* exposed to Cu and DOC by Kim et al. (1999). This inability to ameliorate Cu toxicity may be due to exposure route because *C. dubia* are filter-feeders and were exposed to Cu via water, although the present study exposed epibenthic *H. azteca* to a Cu-enriched sediment. The benthic amphipods very likely consume Cu that is bound to DOC but when the molecule enters the acid environment of the digestive system, it releases Cu in its ionic form, which is bioavailable to the organism.

Growth was inhibited in amphipods exposed to Cu-enriched sediment. The addition of fire extract to the exposure yielded no more significant effect on growth in the exposed animals. Consequently, growth inhibition was likely due to the presence of the Cu-enriched sediment and not the fire extract present in the exposure. Marsden (2002) demonstrated that the amphipod *Paracorophium excavatum* experienced reduced growth in animals exposed to Cu sediment at 46 mg/kg. Borgmann and Norwood (1997) exposed *H. azteca* to 5.7 µmol Cu/g of sediment, which demonstrated impaired growth but over a longer exposure period of 4 wk compared to the present study's 14-d exposure. The metabolic cost of detoxifying and excreting excess Cu likely reduces resources available in exposed animals, leading to growth inhibition (Marsden and Rainbow 2004). It has also been theorized that amphipods exposed to Cu may experience feeding impairment, which could lead to reduced growth; but this theory requires further research (Borgmann and Norwood 1997).

The present study found that amphipods exposed to Cu + 12.5% fire extract had a more-than-additive accumulation of Cu in their whole-body tissues compared with either contaminant individually. Metals are not easily transported across membranes, but PAHs present in the fire extract can disrupt membrane fluidity, leading to increased ion uptake (Sikkema et al. 1994). Once in the cell, the metals produce reactive oxygen species, leading to cell damage and enzyme alteration including cytochrome p450 (CYP) (Gauthier et al. 2014). Inactivation of CYP reduces the ability of the cell to break down and detoxify PAHs (Gauthier et al. 2014). In this way, Cu and PAHs present in the fire extract might facilitate each other to increase their individual toxicological effects, leading to the more-than-additive effect on mortality observed in the present study.

AChE activity

One possible mechanism of toxicity studied was AChE activity. Acetylcholinesterase activity in *H. azteca* was found to be greatly diminished when exposed to fire extract, which is likely due to the presence of PAHs in the fire extract. Gauthier et al. (2016) found similar results for *H. azteca* exposed to PHE with AChE activity at 77 ± 7% of control. Because of their nonpolar nature, PAHs enter the cell through passive diffusion across membranes. Once in the cell, PAHs can inactivate AChE when nitroaromatic compounds bind to the α-anionic site of AChE (Pohanka 2011). A reduction in AChE activity has been shown to have a corresponding effect on amphipod activity, causing a 53.1% decrease in time spent swimming (Gauthier et al. 2016). However, other studies have reported decreased AChE activity in animals exposed to non-nitro-containing PAHs, which could be due to in vivo transformation of PAHs to nitro-PAHs (Shailaja et al. 2006; Gauthier et al. 2016). Similar toxic mechanisms could explain the results found in the present study, but further investigation into pyrogenic PAH toxicity is warranted. Because a variety of pyrogenic PAHs are found in fire extract, it is likely that there are several toxic mechanisms at work that could potentially reduce AChE activity in amphipods.

Contrary to the results found in the present study, previous research has shown that aquatic invertebrates exposed to metals generally demonstrate a decrease in AChE activity (Forget et al. 1999) or no difference from controls (Gauthier et al. 2016). However, the exposure route could contribute to

the difference among studies because these studies all used waterborne concentrations of metals whereas the present study employed sediment-bound Cu. Some research does mention AChE activation phenomena. Abou-Donia et al. (2002) studied the effects of uranyl acetate on the central nervous system in rats, where a significant increase in AChE activity was observed in the cortex. The authors suggested that an increase in AChE could deplete acetylcholine at the synapse, leading to abnormal neural transmission. Similar effects were observed by Dethloff et al. (1999) and Romani et al. (2003) in rainbow trout (*Oncorhynchus mykiss*) and sea bream (*Sparus aurata*), respectively, in the brain when exposed to Cu. Romani et al. (2003) theorized that Cu ions improve the catalytic efficiency of AChE by enhancing the anionic moiety of the active site, therefore increasing the formation of the enzyme–substrate complex with choline molecules. A similar effect could have occurred in the amphipods of the present study; however, whole-body AChE was used in the present study, not isolated brain tissue.

Implications for risk assessment

Climate change has increased the frequency and severity of wildfires worldwide, and this trend is expected to continue with rising global temperatures (Stocks et al. 1998; Dale et al. 2001). It is not unreasonable to predict that these wildfires will interact with metal-contaminated environments such as metal mining sites that are located in forested areas. Cocontamination of a metal and wildfire runoff could be the case for locations which have been impacted by Cu contamination and wildfire runoff. In such locations, the combined toxicity could negatively affect freshwater invertebrate species, resulting in reduced population numbers. Freshwater invertebrates act as a valuable food resource for many vertebrates, such as fish and amphibians, so a reduction in invertebrate numbers could result in reduced vertebrate numbers. It is also possible that the co-contaminants could directly affect vertebrate species, but further research needs to be done. It is important to have effective risk assessment to understand how cotoxic events, such as the combination of Cu contamination and wildfire runoff, will impact aquatic ecosystems. Results from the present study strongly suggest that Cu-contaminated water bodies that receive runoff from nearby wildfires may be at risk of enhanced toxicological effects.

CONCLUSION

The present study revealed that fire extract is highly toxic to amphipods, resulting in 100% mortality in every experimental treatment >12.5% fire extract, regardless of Cu concentration. However, when fire extract was present at sublethal concentrations, it showed a greater-than-additive effect on survival when amphipods were coexposed to Cu and fire extract. Copper-contaminated sediment inhibited growth in amphipods. However, sublethal fire extract concentrations had no additional effect on growth. The combination of Cu and fire extract resulted in more-than-additive whole-body Cu uptake in amphipods, which likely contributed to the more-than-additive effects on survival. The presence of a Cu-enriched sediment increased AChE activity, whereas fire extract significantly reduced it. The increased AChE activity resulting from Cu exposure was insufficient for overcoming the fire extract-induced inhibition. Further research is required to test the toxicity of more representative wildfire runoff, such as including undergrowth foliage and soil in combination with a Cu-enriched sediment to determine if more-than-additive toxicity is still observed in a more realistic scenario. In conclusion, the cotoxic effect of Cu-enriched sediment and wildfire runoff is a concern that warrants further investigation to determine how the combination interacts and affects aquatic ecosystems.

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