Introduction

It is estimated that three-fourths of the total mass of pyrethroid pesticides used in California in 2005–2006 were used in urban settings.1) Reported use for landscape maintenance, structural pest control, and public health pest control in 2007 was more than 255,000 kg (http://calpip.cdpr.ca.gov/cfdocs/calpip/prod/main.cfm), and pyrethroids are the primary active ingredients in the most commonly purchased residential insecticide products.1) Pyrethroids are hydrophobic compounds that associate with and are transported by particles and organic matter, and the main mode of transport is through stormwater flows.2,3) Because of their association with particles, pyrethroids accumulate in sediment and have been associated with sediment toxicity in a number of studies of both agricultural areas4–6) and urban areas,7,8) and more recently, in urban areas throughout California.9)

The contribution of pyrethroid pesticides to sediment toxicity in four urban creeks in California, USA

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As part of a statewide assessment of pyrethroid pesticides and sediment toxicity in urban creeks, sites throughout California were screened, and thirty were chosen to evaluate the potential of pyrethroids to contribute to biological impacts. Sediment samples from four sites containing varied concentrations of pyrethroids were investigated using toxicity identification evaluations (TIEs) to determine causes of toxicity. Treatments were conducted on both whole sediment and interstitial water to determine the role of pyrethroids in the observed toxicity to the amphipod Hyalella azteca, and to evaluate TIE method performance. Whole sediment treatments included the addition of binding resins for organics and metals, and specific treatments designed to alter pyrethroid toxicity, including the addition of carboxylesterase enzyme, the addition of piperonyl butoxide (a pyrethroid synergist), and the testing of sediments at two temperatures. Interstitial water TIEs included solid-phase extraction (SPE) columns to reduce and return toxicity caused by organics and metals, as well as the treatments specific to pyrethroids. Resin and SPE column treatments characterized the causes of toxicity as organic compounds. Results of pyrethroid-specific treatments in whole sediment were variable, but similar treatments in interstitial water demonstrated pyrethroids were contributing to toxicity. Measured pyrethroid concentrations in whole sediment and interstitial water SPE extracts were high enough to have contributed to toxicity. Using both whole sediment and interstitial water TIEs and chemical analysis provided multiple lines of evidence that pyrethroids contributed to toxicity. © Pesticide Science Society of Japan

Keywords: pyrethroid pesticides, sediment, urban creek, toxicity identification evaluation, Hyalella azteca.
Holmes et al. (2008) conducted a targeted survey of sediments from urban creeks throughout California. Their goal was to screen urban creeks throughout the state and examine the occurrence and biological significance of pyrethroids in urban waterways. Ninety sites were screened for toxicity to the amphipod *Hyalella azteca*, and ultimately thirty toxic sites were chosen for additional toxicity testing and chemical analysis. Of the eight pyrethroids measured, at least one was detected in each of the thirty sediment samples. Measured pyrethroids concentrations were converted to chemical toxic units (CTUs) based on published median lethal concentrations (LC$_{50}$), and corrected for the total organic carbon content of the sediments. One CTU is equivalent to the organic carbon-corrected LC$_{50}$ value for any given pyrethroid, and based on the assumption of chemical additivity, the CTU results were summed. The sum CTU values for the thirty sites ranged from approximately 0.2 to 22, with twenty sites having sediment containing greater than one CTU of total pyrethroids.

Although organic carbon-corrected CTU analysis conducted by Holmes et al. (2008) suggested the cause of most of the observed sediment toxicity was due to pyrethroids, the bioavailability of these pesticides is often regulated by the type of organic carbon present in the sediment, and toxicity identification evaluations (TIE) evidence is necessary to verify pyrethroids as the cause of toxicity. The objective of this study was to determine if current whole sediment and interstitial water TIE methods could provide additional lines of evidence for the contribution of pyrethroids to sediment toxicity. The study evaluated TIE methods that are useful for characterizing broad classes of chemicals responsible for toxicity, and procedures that are specific to identifying toxicity caused by pyrethroids.

**Materials and Methods**

For the initial screening, stations on urban creeks were chosen based on criteria and methods outlined in Holmes et al. (2008). The TIE stations were chosen based on the presence of a strong toxicity signal in the initial screening and on regional representation. Three stations were sampled for TIEs in the densely populated urban regions of southern California, and one station was sampled near the San Francisco Bay area. The watersheds draining to the TIE stations ranged in size from approximately 8 to 168 km$^2$, with developed land use ranging from 5 to 96%. Developed land use in the immediate vicinity of the sampling sites (0.8–1.6 km$^2$) ranged from 54 to 100%.

Bouquet Canyon Creek is located in Los Angeles County and was sampled at its confluence with the Santa Clara River (34.42782, −118.54022, Fig. 1). Cottonwood Creek is located in San Diego County and was sampled approximately 0.75 km upstream of its confluence with the Pacific Ocean (33.04852, −117.29513). Peters Canyon Wash is located in Orange County, and was sampled upstream of its confluence with San Diego Creek (33.69616, −117.81596). Marsh Creek is located in Contra Costa County and was sampled approximately 12.5 km above its confluence with the Sacramento San Joaquin River Delta (37.91832, −121.71415).

Initial *H. azteca* whole sediment exposures followed U.S. EPA methods, but subsequent TIE treatments had the following modifications from the standard test protocol. Initial tests were conducted on a dilution series of 0, 10, 25, 50 and 100% strength sediment. The final concentrations of sediment used in the whole sediment TIEs were chosen based on the initial magnitude of toxicity. Reference sediment was created for the dilutions and all treatment blanks using equal parts sediment from a reference site in the Salinas River (Monterey County, California, USA), and clean, kiln-dried sand (#60, RMC Pacific Materials, Monterey, CA, USA). The sediment was amended with organic peat moss (Uni-Gro, Chino, CA, USA). Prior to use, the toxicity of the reference sediment was assessed with a standard 10-day *H. azteca* survival and growth toxicity test, and the sediment was analyzed for 66 trace organic contaminants including organochlorine pesticides, organophosphate pesticides, and pyrethroids. Only p,p′-DDE was detected, but at a concentration well below the tolerance threshold of the test organism. The average grain size of the formulated sediment was 61.2% sand and 38.8% fines, and the average total organic carbon (TOC) was 0.91%. TIE treatments were conducted in five covered 250 ml beakers containing 50 ml sediment, 200 ml overlying water, and 10 amphipods were used for each TIE treatment. The exposures were conducted under static conditions for 10 days with slow aeration and were fed one ml YCT (yeast, cerophyll and trout chow) per beaker every other day.

Interstitial water was extracted via centrifugation at 2500 g (4°C). The percent moisture varied among sediment samples, but enough sediment was collected to obtain adequate vol-
umes of sample for testing. Interstitial water TIE treatments were conducted in three replicate 20 ml scintillation vials containing 10 ml of water and five amphipods. Interstitial water tests were conducted for up to 10 days using a dilution series of 0, 10, 25, 50 and 100% sample. The sample was diluted with laboratory well water. Overlying water pH, dissolved oxygen and conductivity were measured at the initiation and termination of the exposures using a Hach Senslon Meter with the appropriate electrodes. Ammonia was measured at the initiation and termination of the exposures using the spectrophotometric salicylate method on a Hach 2010 spectrophotometer. All *H. azteca* were obtained from Chesapeake Cultures (Hayes, VA, USA) and were 7–14 d old at the time of test initiation.

TIE characterization treatments were conducted on both whole sediment and interstitial water matrices, and TIE identification treatments were conducted on interstitial water (Fig. 2). TIE treatments are described in detail by Anderson et al.\textsuperscript{15} and U.S. Environmental Protection Agency, but are briefly described here. Whole sediment characterization treatments are designed to determine whether toxicity is caused by organic chemicals or metals. This included addition of 10% (by weight) SIR-300 cation exchange resin (ResinTech, West Berlin, NJ, USA) to reduce the bioavailability of metals, and addition of 10% (by weight) Amberlite XAD4 polymeric adsorbent (Rohm and Haas, Spring House, PA, USA) to reduce the bioavailability of organics. Ammonia was not considered a toxic agent in these tests because unionized ammonia concentrations were well below toxic concentrations in all sediments.

Interstitial water TIE treatments included solid phase extraction (SPE) columns and eluates for metals and organics. A cation column (Supelco Supelclean LC-WCX, 3 ml, St. Louis, MO, USA) was used to reduce metal toxicity, and an HLB column (Oasis Hydrophilic-Lipophilic Balance\textsuperscript{®}, 6 ml, 500 mg, Waters, Milford, MA, USA) was used to reduce organic chemical toxicity. A sequential column treatment was prepared by passing the sample through an HLB column and then a cation column to assess the potential for toxicity caused by mixtures of chemicals. The HLB SPE columns were eluted with acetone and cation SPE columns were eluted with acid. Acetone and acid fractions were added to the same volume of water extracted to create an eluate treatment for testing and chemical analysis. In the results described below, discussion of the eluate toxicity and chemistry refers to the organism response or chemical concentration in the acetone or acid-spiked water.

In addition to these methods, both matrices underwent treatments specific for the characterization and identification of pyrethroid pesticide toxicity. The addition of a carboxylesterase enzyme (lyophilized crude esterase from porcine liver, 21 units/mg solid, Sigma-Aldrich, St. Louis, MO, USA) to sediment overlying water or interstitial water hydrolyzes ester-containing compounds, such as pyrethroid pesticides, to their corresponding acid and alcohol, which are generally not toxic.\textsuperscript{17} A bovine serum albumin (BSA) protein-addition control was included in conjunction with the enzyme treatment to account for reduction of contaminant.

![Fig. 2. Diagrams of whole sediment and interstitial water TIE methods.](image-url)
bioavailability due to complexation by the enzyme addition. Piperonyl butoxide (PBO, Sigma-Aldrich, St. Louis, MO, USA) is a metabolic inhibitor that is used in the TIE process to block the activation of acetylcholinesterase-inhibiting organophosphate pesticides.\(^{18}\) PBO is also a potent synergist of pyrethroid toxicity, because it inhibits metabolic breakdown.\(^{19–21}\) PBO (100 µg/l) was added to sediment overlying water and interstitial water to investigate changes in toxicity. An increase in sample toxicity with PBO addition provides additional evidence of pyrethroids. One additional treatment specific for pyrethroids involves temperature manipulation. The standard U.S. EPA protocol for \(H.\ azteca\) is conducted at 23°C. Research has shown that pyrethroid toxicity increases at lower test temperature because the rate of metabolic breakdown of pyrethroids decreases at lower temperatures.\(^{21,22}\) Where appropriate, tests were conducted at the standard temperature and at a lower test temperature (15°C). An increase in sample toxicity at the lower test temperature was considered additional evidence of pyrethroid toxicity.\(^{23}\) Whole sediment TIEs on the Cottonwood and Peters Canyon samples did not include the latter two treatments because complete mortality was observed in these samples and toxicity could not be increased.

Pyrethroid pesticides were measured in the baseline sediment samples and the HLB SPE column eluates using a gas chromatograph with electron capture device (EPA Method 1660).\(^{24}\) The pyrethroids analyzed included bifenthrin, cyfluthrin, cypermethrin, deltamethrin, esfenvalerate/fenvalerate, fenpropathrin, lambda cyhalothrin and permethrin. These pyrethroids have been detected in previous studies of urban sediments.\(^{7–9}\) Laboratory control standards and matrix spikes were measured for each chemical, and dibromooctafluorobiphenyl was measured as a surrogate. In addition to the pyrethroid analyses, interstitial water was analyzed for the organophosphate pesticides chlorpyrifos and diazinon using enzyme-linked immunosorbant assays (ELISA, Strategic Diagnostics Inc, Newark, DE). ELISA procedures followed those recommended by Sullivan and Goh.\(^{25}\) Accuracy was determined for each batch using external standards, and precision was determined by duplicate measurements. Lowest detectable concentrations for this procedure were 30 ng/l for diazinon and 50 ng/l for chlorpyrifos. Reporting limits were 60 ng/l for diazinon and 100 ng/l for chlorpyrifos. To interpret the contribution of pyrethroids to sediment toxicity, concentrations were converted to CTUs based on published \(LC_{50}\).\(^{10,11}\) It was assumed that toxicity from these pyrethroids would be additive, because pyrethroids share similar modes of action.\(^{21}\) Organic carbon-corrected \(LC_{50}\) were used to account for the reduction of pyrethroid bioavailability by naturally occurring carbon in the sediment.

Treatment blanks were tested concurrently with all TIE treatments. Whole sediment blanks consisted of the amendment (e.g., Amberlite) added to reference sediment, and interstitial water blanks consisted of the treatment administered to clean laboratory dilution water. Whole sediment dilution blanks were also created to account for the dilution of the sediment by the amendments. Dilution blanks were created by adding 10% (by weight) reference sediment to the test sediment. Whole sediment and interstitial water TIE treatment blanks were first evaluated to determine if sample TIE manipulations negatively influenced toxicity. Individual whole sediment TIE treatment results were then compared to the baseline result using a separate variance \(t\)-test \((\alpha=0.05)\). If the dilution blank demonstrated a reduction of toxicity, amendment treatments were also statistically compared to the dilution blank. Interstitial water treatments were compared to the baseline using dilution toxic units (DTUs) calculated by dividing 100 by the percent interstitial water median lethal concentration \((LC_{50})\) calculated from each treatment dilution series. Significant differences between interstitial water treatments were determined by comparing the 95% confidence limits from the \(LC_{50}\) calculations. Treatments with confidence limits that did not overlap were considered significantly different.

### Table 1. Sediment concentrations of pyrethroids corrected for organic carbon (OC) content (µg/g OC)

<table>
<thead>
<tr>
<th>Pyrethroids</th>
<th>(LC_{50})^(b)</th>
<th>Sedimentation Concentration (µg/g OC)^(c)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bouquet Canyon Creek</td>
<td>Cottonwood Creek</td>
</tr>
<tr>
<td>Bifenthrin</td>
<td>0.52</td>
<td>7.65</td>
</tr>
<tr>
<td>Cyfluthrin</td>
<td>1.08</td>
<td>2.98</td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>0.38(^{b})</td>
<td>1.35</td>
</tr>
<tr>
<td>Lambda-cyhalothrin</td>
<td>0.45</td>
<td>0.43</td>
</tr>
<tr>
<td>Permethrin</td>
<td>10.8</td>
<td>2.35</td>
</tr>
</tbody>
</table>

\(^{a}\) Total organic carbon (OC) and chemical toxic unit (CTU) are 2.26% and 22.2 for Bouquet Canyon Creek; 3.85% and 10.9 for Cottonwood Creek; 2.47% and 10.0 for Marsh Creek and 3.13% and 1.43 for Peters Canyon Wash. \(^{b}\) Unless noted from Ref. 10. \(^{c}\) Not detected. \(^{d}\) From Ref. 11.
Results and Discussion

1. Sediment chemistry

Analytical quality assurance parameters were all within acceptable limits with surrogate recoveries ranging from 81% to 118%, laboratory control standard recoveries ranging from 74 to 115%, and matrix spike recoveries ranging from 73 to 117%. ELISA laboratory control standard recoveries ranged from 100 to 140%, and relative percent differences between duplicates were less than 5%. Concentrations of chlorpyrifos and diazinon were not detected or below reporting limits in all interstitial water samples.

Up to five pyrethroids were detected in the four sediment samples (Table 1). Summation of the pyrethroid CTUs suggested that there were ample concentrations of pyrethroids in three of the four samples to account for the observed toxicity. Peters Canyon sediment contained 1.43 organic carbon-corrected CTUs and correspondingly demonstrated the weakest toxicity signal. Weston et al.\(^8\) determined that toxic unit analysis was a fair predictor of sediment toxicity to \(H. \text{azteca}\) in urban creek sediment from residential areas of northern California. These authors also noted that there was a difference between observed and expected mortality in samples that contained one to four toxic units of pyrethroids. These differences could be attributed to variations in sediment LC\(_{50}\) among different sediment types.\(^8\)

Although the toxic unit approach has been used to evaluate the contribution of pyrethroids to sediment toxicity,\(^8\) some researchers have demonstrated the organic carbon-corrected concentrations of pyrethroids in sediments do not correlate well with the bioavailable concentrations as measured with solid-phase microextraction or Tenax extraction.\(^12,13,26-28\) The bioavailability of sediment contaminants can be controlled by the type of organic carbon in the sediment. Black carbon can strongly adsorb some chemicals, but organic matter derived from plant material, while not as strong a sorbent, could provide a route of exposure for some sediment-dwelling organisms through ingestion.\(^29\) Additional evidence through the TIE process verified if pyrethroids contributed to toxicity.

2. Whole sediment TIEs

Whole sediment toxicity test water quality parameters were all within acceptable limits for \(H. \text{azteca}\). Survival in all whole sediment TIE controls and blanks was greater than 90% with the exception of the SIR-300 blank tested with the Peters Canyon sample (Table 2). The SIR-300 treatment did not reduce toxicity in any of the samples. There was some reduction of toxicity in the dilution controls for the Cottonwood and Marsh whole sediment TIEs. Reduction of toxicity with the addition of clean reference material can be indicative of dilution or sorption of contaminants onto the material used to dilute the sediment.

Addition of Amberlite significantly reduced the toxicity of Bouquet, Cottonwood, and Peters Canyon sediments (Table 2). Toxicity was also reduced in the Marsh sediment, but the reduction was not statistically significant (\(p=0.053\)). Toxicity was also reduced in the dilution controls for Cottonwood and Marsh, but in the case of Cottonwood, the reduction of toxicity by Amberlite was significantly greater than the dilution. Because toxicity was not reduced by the addition of SIR-300, the cause of toxicity can be characterized as an organic contaminant in three of the four samples.

The results of the pyrethroid-specific treatments applied to whole sediment were variable. Although there were significant concentrations of pyrethroids in three of the sediments, pyrethroid-specific whole sediment TIE treatments did not provide conclusive evidence across the board that pyrethroids were causing toxicity. The addition of enzyme to the overlying water significantly reduced toxicity in all of the samples, but only the results of the Bouquet addition were significantly different from the addition of BSA, indicating that reduction of toxicity in the other three samples could have been caused by binding with the organic matter in the protein-based enzyme. Weston and Amweg (2007) evaluated this whole sedi-

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### Table 2. Percent survival of amphipods in whole sediment TIE treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bouquet Canyon</th>
<th>Cottonwood</th>
<th>Marsh</th>
<th>Peters Canyon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sediment Baseline</td>
<td>4 (5)</td>
<td>16 (15)</td>
<td>6 (9)</td>
<td>16 (5)</td>
</tr>
<tr>
<td>Sediment Control</td>
<td>94 (9)</td>
<td>98 (4)</td>
<td>96 (5)</td>
<td>94 (9)</td>
</tr>
<tr>
<td>Dilution Control</td>
<td>2 (4)</td>
<td>54 (18)</td>
<td>54 (29)</td>
<td>16 (5)</td>
</tr>
<tr>
<td>Sediment w/ Amberlite</td>
<td>36 (25)*</td>
<td>76 (18)*</td>
<td>20 (14)</td>
<td>62 (8)i</td>
</tr>
<tr>
<td>Amberlite Blank</td>
<td>100 (0)</td>
<td>98 (4)</td>
<td>100 (0)</td>
<td>98 (4)</td>
</tr>
<tr>
<td>Sediment w/ SIR-300</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>8 (13)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>SIR-300 Blank</td>
<td>100 (0)</td>
<td>94 (5)</td>
<td>96 (5)</td>
<td>78 (8)</td>
</tr>
<tr>
<td>Sediment w/ Enzyme</td>
<td>68 (16)**</td>
<td>94 (9)</td>
<td>72 (15)</td>
<td>80 (12)</td>
</tr>
<tr>
<td>Enzyme Blank</td>
<td>100 (0)</td>
<td>96 (9)</td>
<td>94 (9)</td>
<td>94 (9)</td>
</tr>
<tr>
<td>Sediment w/ BSA*</td>
<td>40 (12)</td>
<td>96 (9)</td>
<td>66 (11)</td>
<td>86 (13)</td>
</tr>
<tr>
<td>BSA Blank</td>
<td>98 (4)</td>
<td>98 (4)</td>
<td>96 (9)</td>
<td>98 (4)</td>
</tr>
<tr>
<td>Sediment w/PBO*</td>
<td>NA(^g)</td>
<td>0 (0)***</td>
<td>NA</td>
<td>2 (4)***</td>
</tr>
<tr>
<td>PBO Blank</td>
<td>NA</td>
<td>96 (5)</td>
<td>NA</td>
<td>94 (9)</td>
</tr>
<tr>
<td>Sediment at 15°C</td>
<td>NA</td>
<td>8 (11)</td>
<td>NA</td>
<td>2 (4)***</td>
</tr>
<tr>
<td>15°C Blank</td>
<td>NA</td>
<td>96 (5)</td>
<td>NA</td>
<td>92 (11)</td>
</tr>
</tbody>
</table>

\(^a\) Values are means and the standard deviation in parenthesis.  
\(^*\) significant reduction of toxicity and significantly different from the dilution control.  
\(^**\) significant reduction of toxicity and significantly different from BSA.  
\(^***\) significant increase of toxicity.  
\(^*\) Concentration of sediment tested was 10%.  
\(^**\) Concentration of sediment tested was 25%.  
\(^*\) Concentration of sediment tested was 50%.  
\(^*\) BSA=bovine serum albumin.  
\(^*\) PBO=piperonyl butoxide.  
\(^g\) Not analyzed.

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ment TIE treatment with pyrethroid-spiked natural sediments and sediments contaminated with pyrethroids. The toxicity of their samples was generally reduced with the addition of BSA and further reduced with the enzyme in 75% of their samples, whereas only one sample in four in the current study had toxicity further reduced by the enzyme.

Addition of PBO to sediment overlying water significantly increased toxicity in Cottonwood and Peters Canyon sediment. The PBO treatment was not used with Bouquet and Marsh because the tested concentrations of sediment had strong toxicity signals that could not be increased. Amweg and Weston (2007) evaluated this method for use in whole sediment TIEs and found that the addition of 25 μg/l PBO increased toxicity of pyrethroid-spiked sediments and pyrethroid-contaminated sediments two-fold. These authors compared a dilution series of sediments treated with PBO to an untreated dilution series. By calculating LC₅₀ they could compare the effectiveness of the treatment.³⁰ While this allows for the use of the PBO treatment with sediments that produce strong toxicity signals, the procedure was considered too resource-intensive to be included in the current study.

Testing at 15°C increased the toxicity of both Cottonwood and Peters Canyon sediment, and the Peters Canyon temperature treatment was significantly different from that of the baseline. Although the response in the Cottonwood temperature treatment was not significantly different from that of the baseline, the toxicity signal was doubled. A recent study has demonstrated that a temperature reduction of 5°C increases the toxicity of pyrethroids to H. azteca two-fold, and a reduction of 10°C increases the toxicity three-fold.²³ These increases would be dependent on the concentrations of pyrethroids in the sediment.

### 3. Interstitial water TIEs

Interstitial water toxicity test water quality parameters were all within acceptable limits for H. azteca, and survival in all interstitial water TIE controls and blanks was greater than 70%. The TIE results for the interstitial water samples were more consistent among sites than the whole sediment results. Based on the percent interstitial water LC₅₀, the DTUs in the baseline samples ranged from 3.1 for Marsh to 18.5 for Bouquet (Table 3). The cation column treatments significantly reduced toxicity in two samples, but none of the cation column eluates were toxic. Reduction of toxicity with the cation column would normally indicate the toxicity was caused by metal contaminants, but lack of toxicity in the eluate treatment suggests that non-metal contaminants were bound to the column. It is not part of the standard cation procedure to elute the column with solvents such as acetone, but this could be attempted in future studies to determine the role of the cation column in binding organic compounds, and therefore reducing toxicity. The HLB column significantly reduced toxicity in three samples and there was significant toxicity observed in the HLB eluates, indicating toxicity caused by organic contaminants. Similar reduction and return of toxicity was observed in the sequential column treatments, also indicating organic-related toxicity.

The toxicity of all interstitial water samples was significantly reduced by the addition of enzyme, but the response in the Marsh enzyme treatment was not significantly different from the Marsh BSA treatment, indicating the reduction of toxicity could have been caused by binding with the protein-based enzyme. This result was similar to that observed in the whole sediment treatment for the Marsh sample. Toxicity was significantly increased by the addition of PBO and by testing at 15°C in the Cottonwood, Marsh and Peters Canyon samples. Increase of toxicity with these treatments is indicative of pyrethroid toxicity. Bouquet had the strongest interstitial water toxicity signal, and the calculated number of DTUs in the baseline was near the maximum value possible. The addition of PBO slightly increased the toxicity, but toxicity was decreased by the temperature treatment. Both of these responses were not significantly different from the baseline and could be attributed to variability of the organisms. Interstitial water chemical concentrations were not measured in these samples, but toxic concentrations of several pyrethroids were measured in the HLB column eluate (Table 4). Concentrations

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**Table 3.** Dilution toxic units (DTUs) calculated from the dilution series of each interstitial water TIE treatment (100 divided by the percent interstitial water LC₅₀).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bouquet Creek</th>
<th>Cottonwood Creek</th>
<th>Marsh Creek</th>
<th>Peters Canyon Wash</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>18.5</td>
<td>5.4</td>
<td>3.1</td>
<td>7.3</td>
</tr>
<tr>
<td>Cation PCS</td>
<td>18.5</td>
<td>1.6</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>Cation Eluate</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>HLB PCS</td>
<td>7.4</td>
<td>&lt;1</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>HLB Eluate</td>
<td>15.9</td>
<td>6.0</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>Seq. Cation HLB PCS</td>
<td>6.6</td>
<td>&lt;1</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>Sequential HLB Eluate</td>
<td>16.7</td>
<td>4.8</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>Sequential Cation Eluate</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>Enzyme</td>
<td>3.4</td>
<td>&lt;1</td>
<td>1.4</td>
<td>1.1</td>
</tr>
<tr>
<td>BSA</td>
<td>15.9</td>
<td>1.8</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>PBO</td>
<td>18.9</td>
<td>12.7</td>
<td>20.0</td>
<td></td>
</tr>
<tr>
<td>15°C</td>
<td>14.7</td>
<td>9.9</td>
<td>7.5</td>
<td>18.2</td>
</tr>
</tbody>
</table>

³°HLB=hydrophilic lipophilic balance solid-Phase extraction column. PCS=post column sample. BSA=bovine serum albumin. PBO=piperonyl butoxide. ³¹=significant reduction of toxicity. ³²=significant reduction of toxicity and significantly different from BSA. ³³=significant increase of toxicity.
of bifenthrin and cypermethrin exceeded water-only LC50s in all HLB eluates and the concentration of permethrin in the Marsh HLB eluate exceeded the LC50. The concentrations of pyrethroids in the HLB eluates were not corrected for organic carbon content because dissolved organic carbon was not measured in the eluate treatments.

Pyrethroid-specific TIE treatments for whole sediment have been thoroughly evaluated and their use is becoming more routine. In the current study, the results of the whole sediment treatments provided valuable TIE evidence, but were variable in some samples. Samples which had a high magnitude of toxicity such as Bouquet and Marsh Creeks were not analyzed using TIE treatments that increase toxicity, but could have been evaluated by treating a dilution series of the sediments. This technique has been shown to provide additional lines of evidence of pyrethroid toxicity using PBO addition and temperature manipulation. This approach can be labor intensive, but is recommended for sediments showing variable TIE response signatures.

The ability to apply these procedures to a dilution series is simplified in interstitial water TIEs because the water matrix is easier to manipulate. In the current TIEs, the use of all TIE treatments applied to a dilution series of each sample provided additional evidence to identify pyrethroids as the likely cause of toxicity.

**Conclusions**

The success of a TIE depends on using multiple lines of evidence to determine the cause of toxicity. The overall weight of evidence suggests that pyrethroids contributed to the toxicity of all four sediments. The concentrations of pyrethroids in the sediments were greater than published sediment LC50 concentrations for *H. azteca*. In all cases, an organic chemical was characterized as the cause of toxicity, as evidenced by the addition of Amberlite to the whole sediment and the use of the HLB column for the interstitial water. In the whole sediment samples, pyrethroid-specific treatments provided evidence of pyrethroid toxicity in three of the four samples. In the interstitial water tests, all three treatments indicated pyrethroid toxicity for the Cottonwood and Peters Canyon samples. Results of the enzyme treatment indicated pyrethroid toxicity in the Bouquet sample and results of the PBO and temperature treatments indicated pyrethroid toxicity in the Marsh Creek sample. The enzyme treatment provided the main line of evidence for pyrethroid toxicity in the Bouquet sample. Because the Bouquet toxicity signal was so strong in both test matrices, the effectiveness of the PBO and temperature treatments was reduced by not testing with a dilution series in the whole sediment tests, and by not having a broad enough dilution series in the interstitial water tests. Testing the interstitial water samples with the enzyme proved to be an efficient method of determining pyrethroid toxicity. Lastly, the contribution of pyrethroids was positively identified through the testing and analysis of the column eluate treatments. The eluate treatments were all toxic, and all contained concentrations of pyrethroids greater than published water-only LC50 concentrations for *H. azteca*.

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**References**

5. J. W. Hunt, B. S. Anderson, B. M. Phillips, B. Largay, R. S.


