The use of sediment toxicity identification evaluation methods to evaluate clean-up targets in an urban estuary

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Abstract

The Ballona Creek Estuary (BCE) in Los Angeles, California, is in a highly urbanized watershed and is contaminated by a variety of chemicals and has prevalent sediment toxicity. Sediment cleanup targets for BCE have been established for copper, cadmium, lead, zinc, chlordane, DDTs, PCBs and PAHs, based on sediment quality guidelines. A sediment toxicity identification evaluation (TIE) was conducted to examine how these targets corresponded to toxicity observed with the estuarine amphipod *Eohaustorius estuarius*. Whole sediment and pore water TIEs were used to identify the cause of toxicity. Passive samplers were deployed to determine the bioavailable fraction of contaminants. Spiked sediment tests were conducted to determine the thresholds of toxicity for selected constituents. Toxicity was found to be widespread, but temporally and spatially variable. Whole sediment and pore water TIEs both indicated pyrethroid pesticides were the most likely contaminant group contributing to the toxicity. Concentrations of the chemicals listed for cleanup were found to often exceed target values, but were not observed at concentrations likely to cause toxicity. Bioavailable fractions of the target chemicals quantified using passive samplers did not exceed toxicity thresholds. Spiked sediment tests established 10 day LC50s for 4,4’ DDE, 4,4’ DDT, alpha-chlordane and cyfluthrin at >3050, 266, >2120 and 0.33 µg/g organic carbon, respectively. The cyfluthrin LC50 was within the range of concentrations observed in the estuary sediments, but LC50s for the other three chemicals were orders of magnitude greater than observed levels. The combination of TIE, sediment chemistry and the results from spiked sediment exposures indicate pyrethroid pesticides are more likely the cause of the observed toxicity than any of the contaminants targeted for cleanup. The results of this study indicate the importance of using a TIE approach to determine chemicals of concern and dose-response information to set cleanup targets, rather than using sediment quality guidelines.

Introduction

Like many urban bays and estuaries, sediments in the Ballona Creek Estuary (BCE) in Los Angeles, California are contaminated with a variety of trace metals and organic compounds (Bay *et al.* 1997). The watershed for BCE is 61% residential, 17% commercial, 15% open space, 7% industrial, and 0% agriculture (McPherson *et al.* 2002). Previous data demonstrating the presence of sediment toxicity and elevated contaminants led to BCE being included on US Environmental Protection Agency’s 303(d) list as an impaired water body. The subsequent Toxics Total Maximum Daily Load (TMDL) that was developed listed specific chemicals (copper, cadmium, lead, zinc, chlordane, DDTs, PCBs and PAHs), as the cause of impaired sediment quality. This list of chemicals and cleanup target concentrations (Table 1) established by the regulatory agency were based on National Oceanic and Atmospheric Administration Effects Range-Low (ERL) sediment quality guidelines (Long *et al.* 1995).

Sediment quality guidelines (SQG) such as the ERL are based on toxicity and chemical data from nationwide databases (Long *et al.* 1995). Chemicals

Table 1. List of chemicals and cleanup target concentrations for sediments in the Ballona Creek Estuary.

Chemical	Target Concentration
Cadmium	1.2 mg/kg
Copper	34 mg/kg
Lead	46.7 mg/kg
Silver	1.0 mg/kg
Zinc	150 mg/kg
Total DDT's	1.6 µg/kg
Total Chlordanes	0.5 µg/kg
Total PCBs	22.7 µg/kg
Total PAHs	4022 µg/kg

on the target list have been measured in BCE at concentrations exceeding the ERL (Bay *et al.* 2005). However, the ERL was not developed to determine a cause of toxicity or for use as cleanup targets. For the target compounds that have been previously studied, toxicity thresholds are much higher than the ERL concentrations (Weston 1996, McPherson and Chapman 2000, Anderson *et al.* 2008). A previous preliminary study of BCE sediments indicated that organic compounds were the likely cause of the observed toxicity to the amphipod *E. estuarius* (Bay *et al.* 2005). Further, pyrethroid pesticides were identified as possible causative toxicants in these sediments (Lao *et al.* 2010). Subsequently, alternate TIE methods for identifying toxicity caused by pyrethroids were developed (Weston and Amweg 2007, Weston *et al.* 2009). Pyrethroids are not on the BCE target list, but are a contaminant of increasing concern in southern California urban areas (Holmes *et al.* 2008).

A site specific approach is likely to yield a different set of target chemicals and cleanup thresholds. The use of investigative tools such as TIEs (Ho *et al.* 2009) and site specific bioavailability (Doig and Liber 2000, Maruya *et al.* 2009) analysis would be such an approach and allow for comparison to the SQG based methodology.

The aim of the current study was to first determine the current level of toxicity and concentrations of chemicals in the Estuary. Whole sediment and pore water TIEs were then used to establish the likely causes of toxicity. This also provided an opportunity to examine the efficacy of the various TIE treatment methods. The results of the TIEs were confirmed with other lines of evidence, such as spiked sediment exposures and passive sampling devices. The results of the TIEs and other lines of evidence were then compared to the SQG thresholds to determine which approach was better suited to provide targets likely to reduce sediment toxicity in the BCE.

METHODS

Study Design

The study was organized in three phases of integrated field and laboratory research conducted from 2007 to 2009. For the first phase, sediment samples were collected and analyzed for chemistry and toxicity to determine spatial patterns of toxicity and contaminants. Results from the first phase were used to select stations for TIEs which constituted the second phase. The second phase also included deployment of passive samplers to determine the bioavailable fractions of organics and metals. The third phase of the study was to confirm probable causes of toxicity using spiked sediment exposures.

Sediment Sampling

Field sampling in BCE occurred annually from 2007 through 2009. Samples were collected at six locations within the Estuary (Figure 1; Supplemental Information (SI) Table SI-1; ftp://ftp.sccwrp.org/pub/download/DOCUMENTS/AnnualReports/2013AnnualReport/ar13_169_181SI.pdf). Sediment samples were collected by a variety of methods dictated by station location and study phase. Station BCE1 was sampled from a large research vessel with a Van Veen grab. Stations 2 through 4 were sampled from an inflatable boat using a petite Ponar grab. Stations 5 and 6 were sampled by wading into the creek and collecting sediment with a stainless steel shovel. At the time of passive sampler deployment and retrieval, Stations 1 through 3 were sampled by a diver using a stainless steel trowel. Regardless of sampling method only the top 5 cm of sediment were collected for analysis. For all collection methods, sediment from multiple samples were composited to provide adequate volume. Samples for toxicity were stored in polyethylene jars at 5°C until analyzed. Chemistry samples were stored in glass jars at -20°C until analyzed.

Passive Sampling and Analysis

Sediment pore water metals were sampled using equilibration cells or “peepers” consisting of 50 ml low density-polyethylene snap cap vials

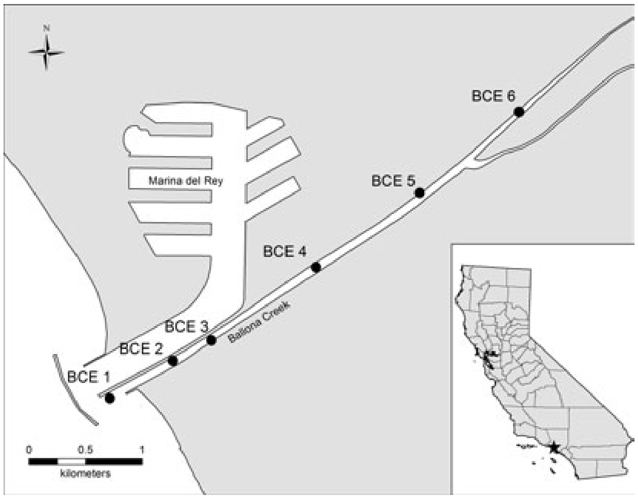

Figure 1. Sampling stations in Ballona Creek, California.

filled with deoxygenated, deionized water. A 0.45 µm polyether-sulfone (PES) filter membrane was placed over the perforated cap (Brumbaugh *et al.* 2007). The peepers were stored in deoxygenated, deionized water until deployment. New 100 µm poly(dimethylsiloxane) coated solid phase microextraction (SPME) fibers were used to sample hydrophobic organic chemicals from sediment pore water (Zeng *et al.* 2004, Maruya *et al.* 2009). The fibers were housed in copper tubes to prevent fouling and were attached to a deployment fixture.

Peepers and SPME samplers were deployed in triplicate by diver at an approximate depth of 5 cm below the sediment-water interface at three sites (BCE1, 2, and 3) for 29 days in November-December 2009. Peepers and SPME were also deployed by wading into the estuary at BCE5 during the same timeframe. At the end of the deployment period, these devices were removed from the sediment and gently rinsed with deionized water; SPME fibers were immediately placed in pre-cleaned glass vials and stored on ice. The contents of each peeper were filtered through a 0.45 µm PES syringe filter and analyzed for dissolved metals by Inductively Coupled Plasma Mass Spectronmetry using EPA method 1640 at CRG Marine Laboratories (Torrance, CA). Blanks for the peeper samples were found to be contaminated with zinc.

A second method of pore water metals characterization was conducted in the laboratory. Samples of whole sediment were centrifuged at 3000x g for 30 minutes to extract pore water. The overlying water was removed with a glass pipette and re-centrifuged for an additional 15 minutes at 5000x g. The pore water was then analyzed for metals using the same methods as the peeper water. The SPME fibers were directly analyzed by thermal desorption Gas Chromatograph-Mass Spectronmeter (GC-MS) using an external calibration curve for quantification of target analytes (Maruya *et al.* 2009).

Toxicity Testing

Sediments for spatial characterization were tested for toxicity with a 10-day amphipod (*E. estuarius*) survival test (USEPA 1994). Sediment samples were passed through a 2 mm sieve prior to testing to remove large sediment particles and infauna. These tests were conducted in 1 L glass jars with 2 cm (150 ml) of sediment and approximately 800 ml of overlying water at a salinity of 20%. Exposures were conducted at 15°C, with constant light and gentle aeration. Each sample had 5 replicates with 20 animals per chamber. At the end of the experiment, sediment was passed through a 0.5 mm sieve and surviving animals were enumerated.

Toxicity Identification

Table SI-2 lists the sample manipulations that were employed for either sediment or pore water. Treatments applied to each sample were dependent on sample volume available and specific goals associated with each sampling period. Toxicity testing for whole sediment TIEs was accomplished with *E. estuarius* as above, but with reduced volumes. Exposures were conducted in 250 ml beakers with 2 cm (50 ml) of sediment and 150 ml of overlying 20% water. Each treatment had 3 to 5 replicates with 10 amphipods in each.

Samples of pore water were extracted using the centrifugation method described above. Untreated (baseline) and TIE-treated pore water samples were evaluated for toxicity using *E. estuarius* 10-day survival tests. Exposures were conducted in 22 ml glass shell vials with 10 ml of pore water under static conditions with no aeration. Each treatment had three to five replicates, each with five amphipods. The exposures were performed at 15°C and in the dark to reduce stress on the animals in the absence of sediment. Amphipod survival was recorded daily.

Sediment Spiking

A series of spiked sediment experiments were conducted in order to establish toxicity thresholds for selected organic chemicals of concern in BCE. Sediment used for spiking was collected from an offshore station located about 6 km north-west of the mouth of Ballona Creek. This sediment had low contaminant concentrations and low toxicity. The TOC and grain size were 0.63 and 30% fines, respectively, similar to sediments from some stations in BCE. The results of the spiking experiments were expressed both on a dry weight and organic carbon normalized basis to facilitate comparisons of sediments with variable TOC concentrations.

In separate experiments, batches of sediment were spiked with 4,4' DDT, 4,4' DDE, alpha chlordane, and cyfluthrin at multiple concentrations. A separate stock solution was made for each exposure concentration so the acetone carrier to sediment ratio was the same for each treatment. For each treatment, silica sand was added to a glass jar at a ratio of 10 g of sand per 1.5 L of sediment. A glass syringe was used to add 1 ml of stock solution for every 10 g of silica sand. The acetone was allowed to evaporate for 1 hour in a fume hood. Sediment was then added to the jars containing the spiked sand. The jars were then placed on a roller table at 15ºC in the dark for 24 hours. After the rolling period, the jars were stored at 5ºC in the dark. The jars were rolled for 2 hours once per week during the 28-day equilibration period. At the end of the equilibration period, a sample of each concentration was taken for chemical verification and sediment toxicity was evaluated using the *E. estuarius* 10-day survival test, in 1 L chambers as described previously. Included in each exposure series was an acetone blank that consisted of 1 ml of acetone added to sand and otherwise handled in the same manner as the spiked sediments.

Chemical Analysis of Sediment

Trace metals in sediments (Cd, Cu, Pb, Ag, Zn) were analyzed using EPA Method 200.7 on either a ICP Varian Vista Pro or a ICP Thermo ICAP 6500 Duo. Samples for target organic analytes were prepared according to EPA method 3545 (pressurized fluid extraction) using a Dionex Accelerated Solvent Extraction (ASE) system. Analysis of PAHs was performed in accordance with EPA method 8270C using an Agilent 6850 GC coupled to a 5975 Mass Selective Detector. Organochlorines (pesticides and PCBs) were analyzed using EPA Methods 8081 and 8082 with a Varian 3800 GC equipped with dual Electron Capture Devices (ECD). Sediment total organic carbon (TOC) was determined following Standard Method 5310B, and grain size was measured using a Beckman Coulter LS 13-320.

Synthetic pyrethroids and fipronil were extracted from sediment samples using ASE and the resulting extracts analyzed by GC-ECD and negative chemical ionization mass spectrometry (NCI-MS) as described in Lao *et al.* (2010). Acid volatile sulfides and simultaneously extracted metals (AVS-SEM) were analyzed in accordance with standard EPA methods (Allen *et al.* 1991).

Data Analysis

Analysis of the spiked sediment toxicity data included calculation of the 20th and 50th percentile lethal concentration (LC20 and LC50) values. The LC20 value was chosen as indicator of low effect and was based on the methodology of deBruyn and Elphick (2013). Previous work has established a PMSD of 18% for *E. estuarius* survival (Greenstein and Bay 2011), which was rounded to 20 for ease of calculation; this calculation was performed using Toxstat Version 3.5 (West, Inc., Cheyenne, WY). The LCx calculations were conducted by probit analysis using unadjusted controls. The LCx values were calculated on both a dry weight and an organic carbon normalized basis. Toxicity units for *E. estuarius* (sediment concentration (µg organic carbon)/LC50 (µg organic carbon)) were calculated for bifenthrin, permethrin and cypermethirin using LC50 data from Anderson (2008) and cyfluthrin with the LC50 from this study.

Porewater and peeper metals concentrations were compared to California Toxics Rule (CTR) values. These water quality standards were set by the State and the USEPA to protect aquatic life in inland waters and enclosed bays and estuaries (Federal Register, May 18, 2000).

RESULTS

Toxicity Testing and Sediment Chemistry

Toxicity to amphipods was widespread at the six stations in BCE, but had high temporal variation (Figure 2). Twenty-nine of 31 samples were toxic, causing 11 to 100% amphipod mortality. Stations BCE1 and BCE4 had the widest range of response, being not toxic at times, but having less than 20% survival at others. The other four stations were always found to have some degree of toxicity to the amphipods, with variable ranges of survival but similar means.

The chemistry data for the compounds of interest indicated their concentrations often exceeded

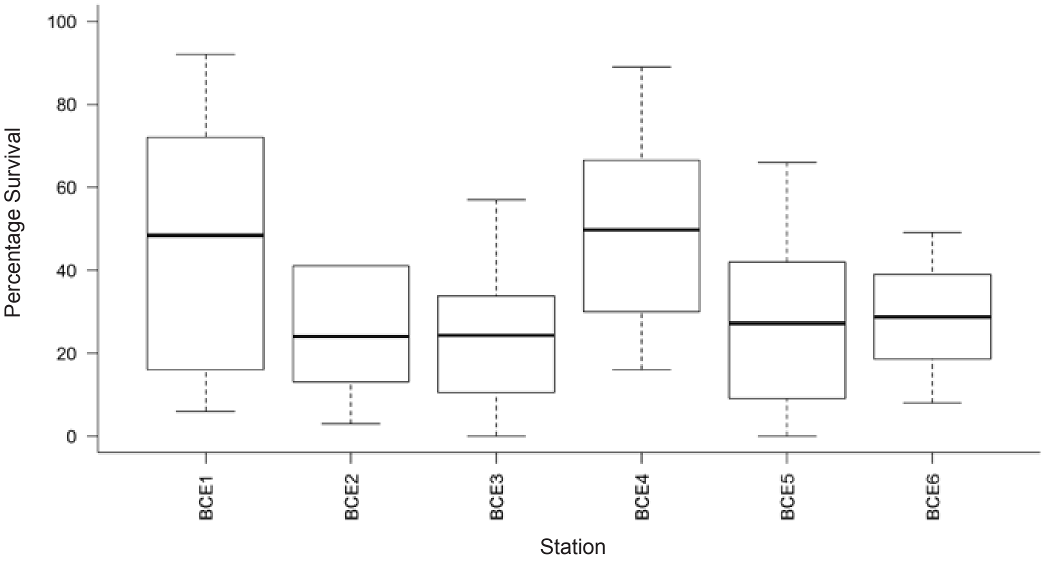

Figure 2. Mean, 25th and 75th percentile and range of *Eohaustorius estuarius* survival results for all Ballona Creek Estuary samples collected between 2007 and 2009. Results are expressed as a percentage of the control value for each exposure.

the ERL-based cleanup thresholds (Figure 3). Concentrations were temporally variable. All five metals were above their respective target concentration in at least one of the samples. The mean concentrations of copper and zinc were above the ERL with 33 and 28% of the samples in exceedance. While the mean concentrations for cadmium, lead and silver were below the ERL, it was exceeded for 17, 22 and 17% of the samples, respectively. For the organic compounds, chlordanes were above the cleanup thresholds for every sample, while no samples were above the target for PAHs or PCBs. The mean value for total DDTs was well above the target and 60% of the samples were in exceedance.

Pyrethroids were detected at all six stations and at every time point (Figure 4). Station BCE3 had the greatest concentration. For all stations combined, permethrin was the dominant by mass of the eight individual pyrethroids analyzed, accounting for about half of the total concentration. Bifenthrin, cyfluthrin, and cypermethrin were the next highest contributors at 16, 12 and 12% of the total, respectively. The

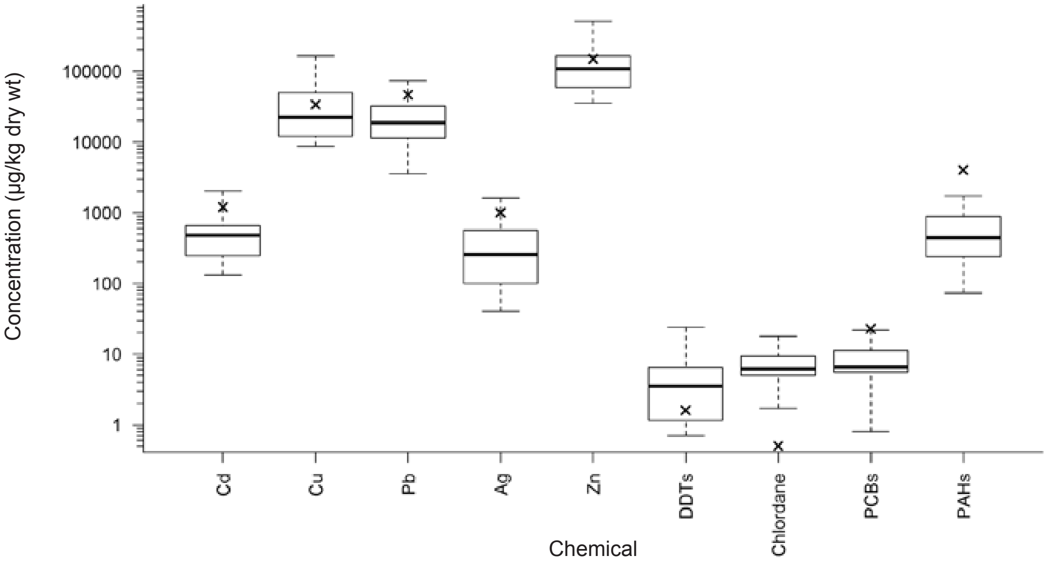

Figure 3. Mean, 25th and 75th percentile and range of concentrations for trace metals and organics of concern in the Ballona Creek Estuary. "X"s indicate cleanup targets based on Effects Range-Low values.

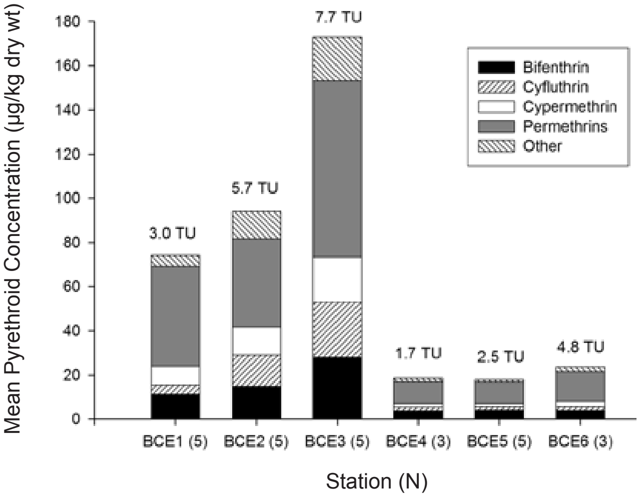

Figure 4. Mean concentration (µg/dry kg) of pyrethroids in whole sediments from Ballona Creek Estuary and mean toxic units (TU) for sum of bifenthrin, cyfluthrin, cypermethrin, and permethrins.

remaining compounds included in the total were fenpropathrin, lamda-cyhalothrin, esfenvalerate, and deltamethrin. Each of these pyrethroids accounted for less than 5% of the total. Every sediment sample contained at least 0.3 toxic units of pyrethroids (sum of permethrin, bifenthrin, cyfluthrin, and cypermethrin), with 27 samples containing more than one toxic unit (range of 1 - 30 TUs). Mean summed toxic units per station ranged from 1.7 at BCE5 to 7.7 at BCE3 (Figure 4).

Total fipronil (sum of the parent compound and its desulfinyl, sulfide, and sulfone derivatives) averaged 3.0 µg/kg (standard deviation = 3.9). Concentrations ranged from 0.2 to 12.2 µg/kg. At least one fipronil compound was detected in all 26 samples analyzed across all stations and time points.

Toxicity Identification

Addition of piperonyl butoxide (PBO) to the overlying water of the sediment always reduced the survival of the amphipods (Figure 5a), even for samples with high baseline survival (e.g., BCE5 in 2008). Addition of coconut carbon to the sediment increased survival in samples having low baseline survival (Figure 5b; BCE2 in 2007 and BCE3 in 2009). Addition of cation exchange resin elicited a slight increase in survival for three samples (Figure 5c). However, the increase was similar to that observed by dilution with clean sediment at similar ratio as used for resin addition (Figure 5d). The addition of carboxylesterase (CE) caused small increases in survival for samples with relatively low levels of toxicity (Figure 5e; BCE3 in 2008 and BCE5 in 2009), but had no effect on highly toxic samples. The remaining treatments had small or inconsistent effects (Figures SI-1 through SI-5; Table SI-3).

Baseline pore water toxicity did not always match well with the whole sediment results (Figure 5; Table SI-4). The most extreme example was BCE5 in 2008 where there was no survival in untreated pore water, but 88% survival in the whole sediment. As with the whole sediment results, the only treatment having an effect on all samples tested was the addition of PBO. The PBO treatment always reduced amphipod survival. Solid phase extraction with C-18 usually increased survival. While all of these treatments might indicate pyrethroid pesticides as a cause of toxicity, addition of CE was only slightly effective in one moderately toxic sample (BCE2 in 2008). Zeolite reduced toxicity in the 2008 sample from BCE5, indicating ammonia could be contributing to toxicity (Figure SI-1). The untreated pore water sample contained 0.645 mg/L un-ionized ammonia, which is just below the recommended maximum concentration in overlying water for *E. estuarius* (USEPA 1994).

Confirmation of Toxic Constituents

All of the peeper metal concentrations were less than 1 µg/L (Figure SI-6). Porewater metals concentrations from centrifuged sediment samples were generally higher than those from the peepers (Table SI-5). Concentrations of the target metals in both peeper and pore water were mostly below California Toxics Rule (CTR) thresholds. Porewater copper from BCE5 was the only sample and constituent above the CTR. Concentrations of cadmium and lead were one to two orders of magnitude below the CTR. Peeper blank samples were contaminated with zinc and therefore peeper zinc data are not presented.

Most sediment samples had AVS concentrations that exceeded SEM (Table SI-6). In the few instances where SEM was greater, the exceedance was small. In no case did the SEM exceed AVS by more than 0.3 µmoles/g. The samples having two of the three highest zinc and the highest two copper concentrations in sediment accounted for all three samples where SEM exceeded AVS.

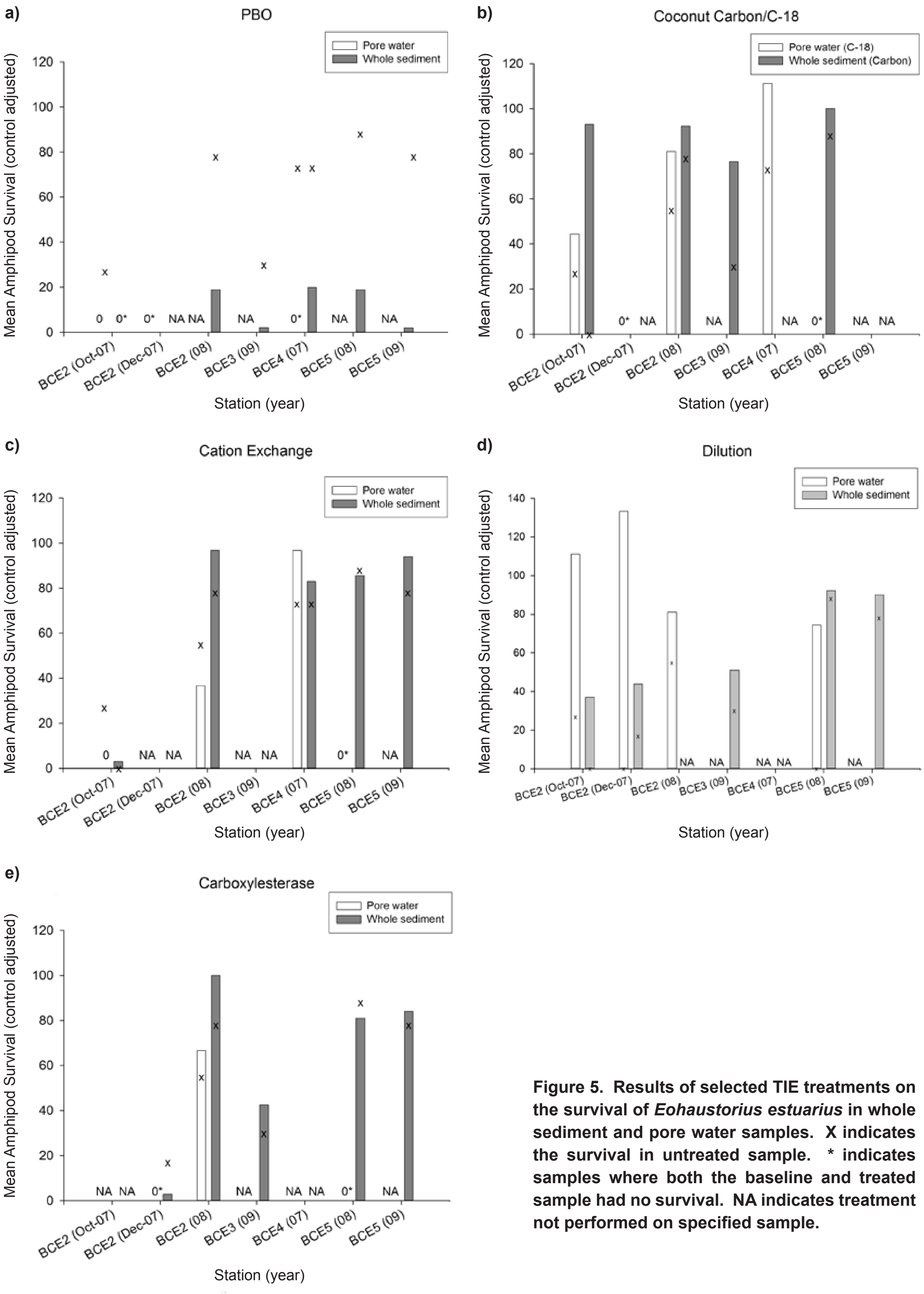

Figure 5. Results of selected TIE treatments on the survival of *Eohaustorius estuarius* in whole sediment and pore water samples. X indicates the survival in untreated sample. * indicates samples where both the baseline and treated sample had no survival. NA indicates treatment not performed on specified sample.

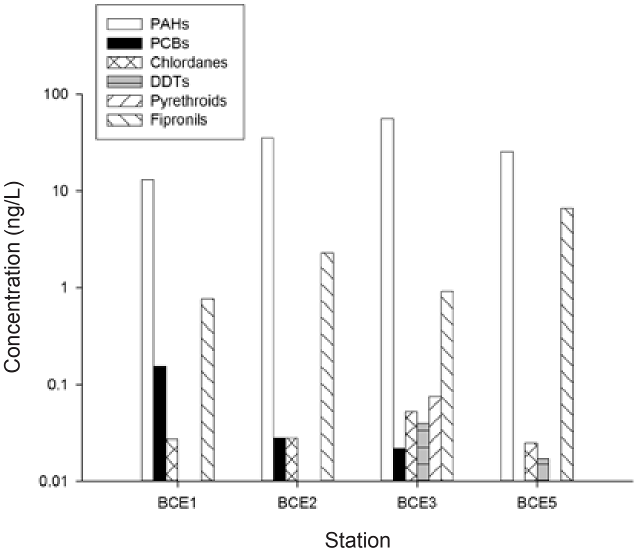

Figure 6. Concentrations of organic chemicals in pore water based on solid phase microextraction (SPME) fiber deployment in Ballona Creek Estuary sediments.

Chlordanes, PAH and fipronil and its derivatives were detected in sediment pore water at each of the four stations sampled using SPME (Figure 6). PAH compounds were found at the highest concentrations (>10 ng/L), followed by fipronil and its derivatives (0.77 to 6.6 ng/L). Pyrethroids were only detectable at BCE3 which was also the only station having detectable concentrations of all six classes of compounds targeted for analysis.

Spiked sediment exposures resulted in estimates of LC20s and LC50s for two of the four evaluated chemicals. An LC50 of 1680 µg/kg was established for 4,4’-DDT and 2.07 µg/kg for cyfluthrin (Table 2; Figure SI-7). The organic carbon normalized LC20s for these 2 compounds were 121 and 0.15 µg/g organic carbon (OC). Comparison to maximum concentrations in BCE whole sediments of 1.17 and 4.27 µg/g OC, respectively, indicated 4,4’ DDT was not a likely cause of toxicity, but cyfluthrin potentially could cause toxicity. The highest concentrations tested for 4,4’-DDE and alpha-chlordane were 3050 and 2120 µg/g OC, respectively. These concentrations were several orders of magnitude greater than those encountered in BCE sediment, but no toxicity was observed in the spiked sediments for either compound. Preliminary 10-day range finding experiments in the laboratory using *E. estuarius* have found the LC50 for fipronil to be approximately 30 µg/kg.

Table 2. Expected toxicity concentrations ±95% confidence interval for *Eohaustorius estuarius* obtained from spiked sediment tests. Results are expressed both on a dry weight basis and normalized to sediment organic carbon (OC).

Chemical	LC20[1]		LC50[2]	
	µg/kg	µg/g OC	µg/kg	µg/g OC
4,4’-DDE	≥19300	≥3050	>19300	>3050
4,4’-DDT	766 ±64	121 ±10	1680 ±72	266 ±11
Alpha-chlordane	≥13400	≥2120	>13400	>2120
Cyfluthrin	0.92 ±0.09	0.15 ±0.01	2.07 ±0.09	0.33 ±0.01

[1] LC20 = The concentration that would be expected to cause 20% mortality.

[2] LC50 = The concentration that would be expected to cause 50% mortality.

DISCUSSION

Toxicity in the BCE was widespread, but with considerable temporal and spatial variability. This variability is most likely related to the wide fluctuation in contaminant inputs to the Estuary from Ballona Creek, which in turn is related to year-to-year variation in rainfall in southern California. The target chemicals often exceeded cleanup threshold concentrations, but were at concentrations not likely to cause toxicity. Pyrethroid pesticides, which were not on the target chemical list, were found to be a more likely cause of observed toxicity than the target chemicals. Pyrethroids were often in sediments at concentrations high enough to cause toxicity (Figure 4). Fipronil was also at concentrations that, while not likely toxic to the amphipod used in our toxicity tests, were potentially toxic to other organisms (Lao *et al.* 2010). This pesticide of emerging use should be monitored closely as its application increases in southern California.

The use of sediment quality guidelines was not an effective method for identifying chemicals likely to be responsible for the observed toxicity in BCE. The contaminants with the greatest exceedances of ERL-based targets, chlordane and DDTs, showed little potential for toxicity in the spiking experiments. Chlordane and DDE were not toxic at concentrations four orders of magnitude above those observed in BCE sediment. The LC20 for DDT was more than 10 times higher than the maximum concentration encountered in BCE sediment. Other researchers have found the estuarine amphipod *Rhepoxynius abronius* to be affected by PCB mixtures at concentrations much higher than those in BCE sediments (Swartz *et al.* 1988, Murdoch *et al.* 1997). The same amphipod species was also unaffected by PAHs at concentrations greater than those in BCE sediments (Swartz *et al.* 1997, Anderson *et al.* 2008).

While use of ERLs resulted in inaccurate stressor identification and cleanup targets for BCE, application of other empirical sediment quality guidelines would have similar limitations. Use of guidelines considered to have greater reliability for predicting the occurrence of toxicity, such as Probable Effects Levels (Smith *et al.* 1996) or Effects Range Medians (Long *et al.* 1995) would have still indicated chlordane and DDTs as stressors of concern and established cleanup targets far below those shown to be nontoxic to *E. estuarius* based on our spiking experiments. In addition, none of these guidelines include values for current use pesticides, such as pyrethroids, which were found to be the most likely cause of BCE sediment toxicity.

Metals were also not present in BCE sediments at concentrations likely to cause toxicity to amphipods. Other studies have found *E. estuarius* to be tolerant to cadmium and copper, with EC50s in excess of 500 mg/kg for each metal (Weston 1996, Anderson *et al.* 2008). Amphipod species may also be tolerant to zinc, with effects only occurring at concentrations greater than 3000 mg/kg in 10-day exposures (King *et al.* 2006). Spiked sediment experiments with the estuarine amphipod *Leptocheirus plumulosus* found a LC50 ≥3400 mg/kg for zinc (Stanley *et al.* 2010). All of these metal effects concentrations are five times or greater above those observed in BCE sediments.

The use of TIE and bioavailability analyses provides a more accurate way of determining chemicals of concern in a watershed. Whereas SQG methods are limited in the number of individual chemicals for which guidelines have been established, TIEs can target a broad spectrum of potential toxicants. Both TIE testing and sediment spiking results support the conclusion that pyrethroid pesticides were a more likely cause of toxicity in BCE than the chemicals on the target list. The spiked exposure to cyfluthrin showed this chemical to be one of the most toxic pyrethroids to *E. estuarius*. The concentration of cyfluthrin in BCE sediments usually exceeded the LC50. Concentrations of bifenthrin and cypermethrin also were often above the LC50s for *E. estuarius* that have been established by other researchers (Anderson *et al.* 2008). A concurrent study on BCE sediments found pyrethroid toxic units (calculated using thresholds for a freshwater amphipod) exceeded 1 for every sample examined and ranged as high as 34, indicating a great potential for pyrethroid toxicity (Lao *et al.* 2010). That study also found a correlation between pyrethroid toxic unit values and toxicity to *E. estuarius*.

Cleanup target concentrations based on toxicity dose-response relationships of chemicals provide more realistic thresholds. Sediment quality guideline-based targets often provide values well below concentrations known to cause any effects to organisms. The ERLs used in the BCE were designed to identify sediments having low probability of toxic effects, not to be used as cleanup targets (Long *et al.* 1998). When toxicity data acquired either through new spiking experiments or from literature sources is used, thresholds can be set that are both protective and relevant to the type of impact (e.g., toxicity). Site specific cleanup thresholds should also consider bioavailability as it relates to sediment characteristics (e.g., TOC and black carbon content) of the location.

In the TIE treatments, the use of PBO consistently increased toxicity, but other amendments designed to confirm pyrethroid toxicity were less successful. Other researchers have had success in treating water and sediment samples with CE to reduce pyrethroid toxicity (Wheelock *et al.* 2006, Weston and Jackson 2009, Anderson *et al.* 2010). Addition of CE was effective only on whole sediments having relatively little toxicity and had only a slight effect on toxicity of one pore water sample in our testing (Figure 5). This study's lack of success is likely related to the concentration of CE used. The study had found in earlier testing that using the recommended concentration and frequency of CE addition led to unacceptably high ammonia concentrations and therefore used a lower concentration that apparently was insufficient to remove toxicity. This study also did not find temperature reduction to have much of an effect on survival of *E. estuarius* in the presence of pyrethroids. This may be because a larger temperature differential can be attained for the warm water *Hyalella azteca* (23ºC) which has been used successfully (Weston *et al.* 2009), than for the colder water *E. estuarius* (15ºC).

Passive sampling devices provided insight into the bioavailability of contaminants. Results from the TIE treatments which focus on metals (cation exchange resin addition or extraction or EDTA addition) rarely reduced toxicity. Porewater concentrations of metals that were found using the peepers were low and not in the range expected to cause toxicity. Additionally, the metals that were present in sediments had low bioavailability. Most of the sediment samples had AVS concentrations that exceeded SEM levels. This indicates that there was little in the way of free metals available for uptake by the animals (Di Toro *et al.* 1992). For the samples where SEM exceeded AVS, the difference was small and those excess metals may be bound by other materials, such as organic carbon (Di Toro *et al.* 2005).

Passive samplers that target hydrophobic organic compounds in sediment, including the SPME samplers utilized in the present study, provide an estimate of the freely dissolved concentration that is available for uptake and/or exposure to benthic organisms (Maruya *et al.* 2010). The only station (BCE3) where all classes of organic compounds targeted by SPME were detected also had the lowest mean survival of amphipods in the toxicity tests. However, the PAHs and chlordanes found at the highest concentrations in the SPMEs (Figure 6) were unlikely sources of the observed toxicity. A study of European amphipods found the lowest EC50 among several PAHs tested to be 2.85 µg/L (Sanz-Lazaro *et al.* 2008), more than an order of magnitude greater than the concentrations estimated by SPME. Similarly, the estimated concentration for pyrethroids at BCE3 (0.038 ng/L) is not expected to cause toxicity to *E. estuarius*, being more than three orders of magnitude below the aqueous EC50 for this species (Darrin Greenstein, unpublished data). No aqueous phase toxicity threshold for this test species is available for comparison to the maximum total fipronil concentration estimated in this study (6.6 ng/L), which was more than two orders of magnitude greater than for pyrethroids. This result is not unexpected as the aqueous solubilities of fipronil and its derivatives are two to three orders of magnitude greater than for pyrethroids.

Accurate estimation of sediment porewater concentrations using SPME is contingent on achievement of thermodynamic equilibrium between the sediment and SPME fiber. If equilibrium was not achieved after the 29-day deployment, estimated concentrations would be underestimated, e.g., by a factor of 2 if the extent of equilibrium was 50%. This may account for the low concentrations of pyrethroids observed on our SPMEs, relative to what was expected based on TIE results and whole sediment chemistry. Future passive sampling efforts to characterize the freely dissolved concentrations of hydrophobic organic toxicants should incorporate additional measures to ensure achievement of equilibrium, or alternatively, to correct for incomplete equilibration (e.g., Oen *et al.* 2011).

Performing TIE treatments on porewater samples provided confirmation of the whole sediment results. While porewater is a primary route of exposure for benthic organisms, the challenges with using it as the only matrix for toxicity testing are well documented (Chapman *et al.* 2002). Another study on freshwater organisms found differences in interpretation of TIE results between porewater and whole sediment (Mehler *et al.* 2010). In the present study, the baseline toxicity results for porewater and whole sediment did not always match well (Figure 5). However, porewater treatments with C-18, temperature reduction and especially PBO addition verified results observed with whole sediments. In this study, had only porewater been tested the same general conclusions indicating pyrethroids as a source of toxicity would have been reached as for the whole sediment, but the spatial extent and magnitude of impacts would have differed.

Sediment quality guidelines are a valuable tool for interpreting sediment chemistry data and identifying sites of concern, but they are not sufficient for planning cleanup activities. When toxicity is found in a watershed, target chemicals and cleanup concentrations should be based on dose-response relationships instead of SQGs. Most SQGs are based on a limited number of chemicals which do not include chemicals of emerging concern (CECs). Resources may be wasted by using SQGs to target chemicals that are not likely responsible for toxic impacts, while actual causes of toxicity are ignored. In the BCE, pyrethroids were not targeted because of the reliance on a limited set of SQG values.

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SUPPLEMENTAL INFORMATION

Supplemental Information is available at ftp://ftp.sccwrp.org/pub/download/DOCUMENTS/AnnualReports/2013AnnualReport/ar13_169_181SI.pdf.