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Accepted Article

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Treated wastewater effluents as a source of pyrethroids

TREATED WASTEWATER EFFLUENTS AS A SOURCE OF PYRETHROIDS AND FIPRONIL AT TODOS SANTOS BAY, MEXICO: ITS IMPACT ON SEDIMENTS AND ORGANISMS

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Abstract: Pyrethroids are insecticides widely used to control pests and disease vectors in residential areas and agricultural lands. As pyrethroids are emerging pollutants, their use is a growing concern due to their toxicity potential on aquatic organisms. Todos Santos Bay and Punta Banda estuary, two coastal bodies located to the south of Southern California Bight, were studied to establish a baseline of the current conditions of pollution by pyrethroids and fipronil. Eight pyrethroids along with fipronil and its two metabolites were determined in effluents from wastewater treatment plants (n = 3), surface sediments (n = 32), and three locations with mussels [Mytilus californianus] (n = 9). Bifenthrin, permethrin, and cypermethrin were the most common pyrethroids found in the study areas and were widespread in sediments, mussels, and wastewater treated effluents. Fipronil and its metabolites were detected in mussels and wastewater treated effluents only. The total pyrethroid concentrations in sediments ranged from 0.04 to 1.95 ng/g d.w. in Punta Banda estuary (n = 13) and from 0.07 to 6.62 ng/g d.w. in Todos Santos Bay (n = 19). Moreover, the total pyrethroids in mussels ranged from 1.19 to 6.15 ng/g w.w. Based on the toxic unit data calculated for pyrethroids and fipronil for Eohaustorius estuarius and Hyallela azteca, little to no impact is expected to the benthic population structure. This article is protected by copyright. All rights reserved

Keywords: Pyrethroids; Municipal effluents; Insecticide; Fipronil; Mussels; Wastewater effluents
INTRODUCTION

Pyrethroids are synthetic insecticides used in agriculture and in household products, especially for vector control of Anopheles mosquitoes. Products containing pyrethroids are widely used in residential areas because of their low toxicity for humans, mammals, and birds. Although humans can convert the pyrethroids into nontoxic metabolites such as 3-phenoxybenzoic acid (3-PBA), aquatic organisms such as fish and invertebrates tend to manifest adverse effects due to the exposure to pyrethroids [1,2]. Toxic effects for exposure to sublethal concentrations of pyrethroids such as esfenvalerate can; affect the optomotor responses, induce swimming abnormalities and increase the predation risk in early life stages of fishes such as fathead minnow [3]. Moreover, phenylpirazole insecticide fipronil is frequently used for pest control against insects tolerant to pyrethroids and other insecticides. Besides that, fipronil is used for veterinary purposes, mainly in the tick and flea control in domestic pets. The use of pyrethroids and fipronil is a subject of concern because exposure to even low concentrations causes high toxicity effects to nontarget organisms, particularly aquatic invertebrates [4,5].

The main routes by which pyrethroids and fipronil are incorporated into the marine environment include agricultural and urban runoff spills and municipal and industrial wastewater discharges. Due to their high hydrophobicity, pyrethroids as well as fipronil are most likely to be associated through sorption processes to the sediment, soil or in suspended matter [2,6]. The Official Mexican Standards (NOM) were created to establish the maximum pollution limits for the discharge of wastewater from municipal and industrial processes into water bodies, where physicochemical and bacteriological parameters as well as some inorganic pollutants are monitored from their effluents [7].
Due to the severe drought condition prevailing in the State of Baja California where the average total annual precipitation is approximately 200 mm per year [8], the contributions of fresh water by creeks are intermittent at best and usually occur during the winter season when rainfall occurs. Therefore, we assume that the contribution of pyrethroids to the coast is mainly by wastewater treatment plants (WWTPs) discharges because the surface runoff into Todos Santos Bay (TSB) is scarce during the year. The main purpose of this work was to characterize the input of pyrethroids and fipronil discharged into TSB and Punta Banda (PB) estuary through effluents from WWTPs from the city of Ensenada to assess the extent of the impact of discharges of these pollutants into the marine environment. Surface sediments and mussels were evaluated to establish a precedent of the current conditions due to the lack of information of these pollutants in the region and in the country and to understand the behavior, fate, and distribution of pyrethroids and fipronil.

MATERIALS AND METHODS

Description of the study area

Southern California Bight (SCB) covers a binational wide coastal strip that extends from Point Conception, California, United States, to Cabo Colonet, Baja California, Mexico. The study area in this work is limited to TSB (31.8206 °N, 116.7061 °W) and PB estuary (31.739 °N, 116.632 °W), two coastal bodies located to the south of the Southern California Bight (Figure 1). TSB is located 100 km to the south of the United States–Mexico International border, has an approximate extent of 180 km², and has a depth less than 50 m except in the submarine canyon located between PB and Todos Santos Island [9]. The city and port of Ensenada is located adjacent to the bay and the estuary and has more than 300,000 inhabitants according to the
Population and Housing Census 2010 [10]. In addition, this region is recognized nationally for its wine production and for the developing aquaculture of bivalves on its shores, mainly mussels and oysters. Due to the prevailing drought in the region, surface runoff from creeks is scarce and is limited only to the winter season when there is more rainfall. Moreover, the average annual precipitation for this region is approximately 200 mm per year [8]. Consequently, we assume that the main surface discharges into the bay and estuary waters are mainly the treated wastewater effluents discharged in the creeks and are to a lesser extent from nonpoint sources coming from the agricultural and urban runoffs.

**Sampling protocols**

*Surface sediments.* Thirty-two surface sediment samples were collected in October 2013 as part of the “Bight 13” sampling campaign conducted in the southern part of the Southern California Bight, which extends from the US–Mexico border and includes TSB and PB estuary (Figure 1). Sites were selected using a stratified random sampling design [11]; the same sampling protocol has been described for the Southern California Bight [12] and previously for the study area by Macías-Zamora et al. [13]. Briefly, the top two centimeters’ layer of unperturbed sediment samples were collected at each site in TSB aboard Oceanographic Research Vessel *Alguita*, using a Van Veen grab of 0.1 m². Sediment samples collected in PB were sampled aboard a Zodiac Mark II boat, using a Petite Ponar grab (Wildco, Inc.) with a sample area of 0.025 m². All samples for pyrethroids determination were collected in pre-cleaned amber glass containers. Furthermore, subsamples for the determination of total organic carbon (OC) and grain size were collected in 50-mL Falcon® polypropylene tubes. Finally, all samples were kept frozen at -20 °C until analyzed, for a period no longer than nine months.
Mussel tissues (*Mytilus californianus*). Mussels (*Mytilus californianus* Conrad, 1837) were collected from three rocky intertidal locations along the coast of TSB during November 2014. At each site, a total of at least 180 mussels of commercial size (shell length: 6 to 10 cm) were collected from three intertidal mussel patches with a minimum separation distance of 20 m and not more than 200 m [14]. All samples were properly identified and then kept cool in an ice chest until their arrival to the laboratory. Then, the mussels were cleaned to rid from any debris or seaweed on their outer shells, while that the mussel’s byssus threads were carefully removed from the tissue. It should be noted that the mussels were not depurated prior analysis. Finally, the tissues were homogenized using a Tekmar Tissumizer Homogenizer Model SDT-1810S1 (Tekmar Co.); therefore, three composite mussel samples of 60 mussels were prepared for each location. Composite samples were kept frozen at -20 °C until analyzed, within a period no longer than six months.

Municipal wastewater treated effluents. We collected 24-hour composite samples for treated effluents of three WWTP located in Ensenada, Mexico, to characterize the input of pyrethroids to the bay. These WWTP (El Gallo “GAL”, El Naranjo “NAR”, and El Sauzal “SAU”) were built as secondary treatment plants with an activated sludge process to remove most of the suspended solids and reduce biochemical oxygen demand to values under the permissible limits established on the NOM. Four individual grab samples were collected from the treated effluent according to the criteria established in NOM-002-SEMARNAT-1996 to prepare 18 L of one composite sample from each WWTP [7], in which the volume of each individual grab sample must be proportional to the discharge rate at the time of the sampling. All samples were collected in 19 L pre-cleaned glass containers. The samples were preserved using a
saturated solution of mercuric chloride to inhibit bacterial growth and were refrigerated at 4 °C until filtration. To recover suspended solids, each composite sample was filtrated through a pre-ashed 47-mm Whatman® GF/D and successively through a 47-mm Whatman® GF/F filter. Then, the filtered samples were discarded appropriately, and the suspended solids collected on glass filters were oven dried at 50 °C for 48 h for further analysis. We realize that a small portion of these compounds is present in water but because their log $K_{ow}$ $\geq$ 3.5 [5], the detection of these compounds in water is more difficult as their concentration is smaller.

**Sample extraction and clean-up**

Marine sediments, suspended solids obtained from the filtration of 18 L composite samples from WWTPs, and the homogenized mussel tissues were analyzed following a modified method for pyrethroids and fipronil described for Lao et al. [6]. Briefly, 10 grams of freeze-dried sediment, three grams of homogenized mussels, or the WWTP suspended solids collected by filtration were spiked with a recovery surrogate mixture with BDE-33 and PCB-209 (UltraScientific, Inc.). Then, the samples were Soxhlet extracted for 16 hours using HPLC grade dichloromethane (BHD. VWR, Inc.). Acid-activated copper was used to remove elemental sulfur interferences in the pyrethroid analysis. Then, the extracts were concentrated and subjected to column clean-up using 10 grams of florisil (deactivated at 6%) before the GC-MS/MS analysis was conducted. The elution was conducted with 15 mL of hexane and 80 mL of hexane:ethyl ether (7:3, v/v). The second eluate was further evaporated and concentrated to a final volume of 0.1 mL. Procedural and fortified blanks were prepared for quality control in each analyzed set of samples. Mean surrogate recoveries in the sediment, WWTP, and mussel samples were 83.1 ± 11.4%. The method detection limits for individual target compounds were from 0.002 to 0.033.
ng/g dry weight (d.w.) in sediment samples, 0.07 to 0.11 ng/g wet weight (w.w.) in mussels, and 0.001 to 0.018 ng/L in WWTP composite samples. Finally, fat content on mussels was gravimetrically determined according the AOAC protocol using Soxhlet extraction and hexane as the extraction solvent [15].

Gas chromatography–tandem mass spectrometry (GC-MS/MS)

Samples were analyzed using an Agilent 7890A gas chromatograph coupled to an Agilent GC/MS Triple Quad 7000 in the negative chemical ionization mode using methane as the reagent gas. A DB-XLB column (30 m × 0.25 mm × 0.1 µm) was used in the pyrethroid analysis. The initial oven temperature was set to 150 °C for 0 min, then raised to 220 °C at 30 °C/min for 1 min, and then rose to 290 °C at 5 °C/min and hold for 0 min for a run time of 17.33 min. A backflush program of 300 °C for 5 min was established after each run to prevent further ion source contamination between samples. Helium was used as the carrier gas at a constant flow of 1.2 mL/min.

For the MS/MS, several parameters were optimized to reduce detection limit including collision energies of ions, MRM transitions, dwell time and specific gains for ions. In addition, an injection volume of 2 µL was used in the pulsed splitless mode, whereas a temperature of 240 °C was programmed for the multimode inlet. In both cases, a temperature of 150 °C was used for the ion source and the quadrupoles. The flow of 2.25 and 1.5 mL/min of helium and nitrogen were used as quench and collision gases, respectively.

Pyrethroids were identified and quantified using a multiple reaction monitoring method, while 2,2′,5,5′-BDE and PCB-205 were used as internal standards [see Supplemental Data]. Target compounds included eight pyrethroids (bifenthrin, fenpropathrin, lambda-cyhalothrin, ...
permethrin, cypermethrin, cyfluthrin, esfenvalerate, and deltamethrin), as well as fipronil and its two common metabolites (fipronil sulfide and fipronil sulfone).

**Grain size and total organic carbon**

OC was measured in sediment samples using a LECO CHNS-932 elemental analyzer after the removal of inorganic carbon using an acid treatment with HCl 0.1M [16]. Moreover, grain size analysis was performed using a particle size distribution analyzer HORIBA Model LA-910 to determine the fine grain size fraction (<63-µm) on the sediment samples.

**RESULTS AND DISCUSSION**

All sediment samples, mussels, and the wastewater collected from effluents of WWTPs that were analyzed in this work showed at least three or more compounds at detectable concentrations.

**Surface sediments**

The spatial distribution on surface sediments for the total pyrethroid concentration (sum of eight pyrethroids and the sum of fipronil and its two metabolites) in TSB and PB estuary are shown in the Figure 2.

Mean total pyrethroid concentrations found in sediments were 0.45 ng/g dry weight (d.w.) in PB estuary (n = 13; min–max: 0.08–1.97 ng/g d.w., SD = 0.49) and were 0.62 ng/g d.w. (n = 19, min–max: 0.10–6.66 ng/g d.w., SD = 1.49) in TSB. The lowest total pyrethroid concentrations were observed in sites closer to the mouth of PB estuary, a tide-dominated area characterized by sandy sediments with <63-µm grain size fraction near to 1%, in addition to low organic carbon content.
Pyrethroids such as esfenvalerate, deltamethrin, and fenpropathrin and fipronil and its two metabolites were found in concentrations below the detection limits in sediments of TSB and PB estuary. However, only the station E6 located on the head of PB estuary (Figure 1) showed detectable concentrations of deltamethrin and fipronil sulfide, while esfenvalerate was detected in seven sites along the estuary (Figure 3). This could be suggesting that local non-point sources as runoff or spray drift and misdirected applications of pyrethroids from the agricultural fields located in the vicinity could be the most expected source of pyrethroids in PB estuary, as suggested by Hall et al. as highly probable sources to surface water bodies in California [17]. In TSB, the highest concentrations found on sediments were located near the mouth of the submarine canyon between PB and Todos Santos Island. A similar trend was observed previously in the spatial distribution for polycyclic aromatic hydrocarbons in sediments [18]. OC content for sediments of the sampled sites ranged from 0.16% to 1.93% in TSB (n = 19, 0.71 ± 0.58%; mean ± SD) and from 0.04 to 1.31% (n = 13, 0.62% ± 0.44; mean ± SD) for PB estuary. Sites near to the mouth of the estuary showed the lowest OC contents on sediments, which differ with inner estuary sites where we found values of OC to be approximately 1% meanwhile values near to 1 for OC were found at sites near to the submarine canyon between Todos Santos Island and Punta Banda. This is expected because deeper sites have less impact of surface currents and waves.

Moreover, fine grain size composition (<63-µm fraction) for sediments ranging from 0.46% to 77.4% in TSB (n = 19, 26.2% ± 23.9; mean ± SD) and from 0.05 to 53.05% in PB estuary (n = 13, 27.7% ± 19.4; mean ± SD). Moderately strong correlation was observed between fine grain size fraction (<63-µm) and OC for PB estuary (r² = 0.73) and TSB (r² = 0.75), but no
relationship was found between the total pyrethroid concentration and <63-µm grain size fraction (or OC contents) on sediments of PB estuary and TSB (r² < 0.1). This trend is unexpected due to the strong affinity of pyrethroids with organic matter and fine grain sized fraction (<63-µm) and has been observed on other organic pollutants that are highly hydrophobic like organochloride pesticides, polychlorinated biphenyls (PCBs), or polycyclic aromatic hydrocarbons (PAHs) [18]. Therefore, this pattern could be explained by the proximity to runoff sources, mainly to WWTP effluents, where urban and agricultural activities could have a bigger impact in the study area and also can be reported in the northern area of Southern California Bight [19].

Typical composition on WWTPs, mussels and sediments

The typical compositions of pyrethroids found on the samples of WWTP effluents, mussels, and in the sediments of TSB and PB estuary are shown in Figure 3. Cypermethrin > permethrin > bifenthrin were the three main compounds found in all the samples of sediments and mussels in slightly different proportions on the study area and contribute with more than 90% of the total pyrethroid concentrations found on each site. No important difference in composition between sediment and mussels was observed.

In addition, fipronil sulfide was the predominant compound found in the particulate matter obtained from two of the three main effluents discharged in TSB (El Gallo and El Naranjo WWTPs). Usually, fipronil sulfide has been reported as the dominant degradation product of fipronil found in biosolids of WWTPs, in the interstitial water of anaerobic sediments, and as one of the two major products by hydrolysis under aquatic conditions [5,20]. Under experimental conditions, fipronil is reported to be completely converted to fipronil sulfide in sediment samples under strongly reduced anaerobic conditions [21]. Moreover, fipronil and its degradates usually
remain without changes after the conventional treatment of sewage in municipal WWTPs, showing a limited settling in the clarifiers despite their high octanol-water partitioning coefficient (log $K_{ow}$ 3.75) and low solubility in water (3.78 mg/L) [20,22,23]. Under conventional wastewater treatment process with activated sludges, the mean aqueous-phase removal efficiencies for fipronil is 18% ($\pm$ 22%; $\pm$ 95% CI), according to the reported for 25 WWTP facilities located in 18 US states [24]. Furthermore, the transformation rate of fipronil during a conventional wastewater treatment process is estimated around 25%, with at least 1% being removed from water by the solids presents in waste activated and primary sludge [20].

Regarding fipronil sulfide levels found in WWTP effluents, the sediment concentrations for fipronil and its metabolites in TSB and PB estuary were below detectable levels (below 0.01 ng/g d.w.) in all samples. The absence of fipronil and its metabolites in surface sediments could suggest fast environmental degradation in aqueous media through photolysis, hydrolysis, or by another degradation pathways [5,6]. In sediments, the indigenous microbial populations and the physicochemical properties of the sediments is reported to contribute in the regulation of the degradation rate of fipronil, showing slower degradations rates in sediments with lower pH and higher organic matter content [21]. In addition, the sorption coefficients for fipronil sulfide and fipronil sulfone on sediments and soils are generally 3 to 10 times higher than that for fipronil. Also, the sorption of fipronil and its degradates are highly correlated with organic carbon content, so it’s expected that a fraction of fipronil sulfide and fipronil sulfone is partitioned into the sediments [25]. Although the typical composition of pyrethroids found in urban runoffs and sediments in residential areas at northern California (such as San Francisco or Sacramento) includes bifenthrin, cyfluthrin, and cypermethrin as the main pyrethroids used [26,27], in our
work we found that the southern part of Southern California Bight presents a composition where cypermethrin, permethrin, and bifenthrin were among the pyrethroids most commonly detected in the sediments. We suspect that the differences may be related to differences in usage of commercial products between the two populations at both sides of the international frontier.

*Area-weighted mean concentrations on sediments*

Area-weighted mean (AWM) concentrations were calculated to evaluate the total mass inventory for pyrethroids and fipronil and its metabolites distributed in the study area according to the previously published for pyrethroids and other organic pollutants [12,19] (See supplemental data). For this purpose, a total area of 423.5 km$^2$ was used in the estimation of the total inventory of pyrethroids for TSB, while an area of 21.4 km$^2$ was considered for the estimations of area-weighted concentrations of pyrethroids in PB estuary. The overall AWM concentration of pyrethroids for TSB and PB were 484.6 and 365.7 ng/kg, respectively. This AWM concentrations were one order of magnitude lower than the overall AWM concentrations reported for Southern California bight embayment sediments in USA [19]. The total mass inventory of pyrethroids on surface sediments of TSB and PB estuary were estimated to be 6.16 and 0.23 kg, respectively. This mass is at least six-fold lower that the total mass inventory reported in Southern California bight embayment sediments in USA, which was estimated to be 36 kg for a total area of 94.1 km$^2$ [19]. These inventories are provided to facilitate direct comparison of size or magnitude to other inventories at different sites.

The main composition of pyrethroids were dominated by three compounds: cypermethrin, bifenthrin, and permethrin, which contribute 0.13, 0.05, and 0.04 kg to the total mass of pyrethroids that were found in the estuary. Moreover, the contribution of cypermethrin,
bifenthrin, and permethrin to the total mass inventory of pyrethroids on sediments in the area of TSB were estimated to be 4.52, 0.39, and 1.1 kg, respectively. For cypermethrin and permethrin, their respective half-life are oscillating from 6-20 d and 39.5 d, respectively, depending on the particular conditions of preservation. This implies that in our particular case, cypermethrin and permethrin could be attributable to recent events of use or application, which may be associated to the harvest season and pest control for ticks’ plagues in the summer.

Typically, from the commercial formulations of insecticides containing pyrethroids as cypermethrin, the most abundant compound found in our work are manufactured with 0.1% to 2% of active ingredient for home pest control and in emulsifiable concentrates formulations with 10 to 25% of active ingredient for agricultural or commercial uses. Therefore, the total mass of cypermethrin distributed in the top layer of surface sediments in TSB is equivalent to 110 aerosol cans of 12 oz. with 0.1% of active ingredient. However, the total mass of cypermethrin for PB estuary, represent one aerosol can.

**Potential toxicity on sediments**

An analysis to assess the potential toxicity and environmental risk by pyrethroids in sediments was performed using the estimation of the toxic units (TU) for each compound. For this, we used the organic carbon-normalized sediment concentrations for each pyrethroid and the lethal concentration exposure to pyrethroids in sediments (LC50) [19,28,29]. Mean OC-normalized values for 10-day LC50 reported for the amphipods *Hyallela azteca* and *Eohaustorius estuarius* were used as an estimation of the toxic units. Sediment LC50 values of 1.03, 1.41, and 17.9 µg/g OC for *E. estuarius* [30] and values of 0.25, 0.38 and 9.8 µg/g OC [19], respectively, for *H. azteca* were used in the estimation of TU for the three most abundant

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pyrethroids measured in TSB and PB estuary: bifenthrin, cypermethrin, and permethrin (Figure 4). These values presented in the Figure 4 are first indirect expected toxicities for local populations bases solely on measured concentrations of these substances. As such they are used only as reference.

After performing an analysis of pyrethroid concentrations expressed as TU and assuming that toxicity from pyrethroids is additive [28], two of the 32 sites (6%) had TU values greater than 1, while three sites (9%) showed TU values of approximately 0.5. For these sites, we would expect little to no impact on population structure in susceptible benthic organisms as *H. azteca* and even into lesser extent in more tolerable organisms as *E. estuarius*.

TU values for *H. azteca* were estimated in the range of 0.02 to 1.16 for TSB and 0.06 to 1.36 for PB estuary. For *E. estuarius*, the TU values estimated was from 0.05 to 0.32 for TSB and from 0.016 to 0.35 for PB estuary. Data suggest that cypermethrin was the main contributor in sites where the expected toxicity was found. In general, the distribution of possibly perturbed sites in TSB was near the coastline except for two sites that were located near the submarine canyon. Moreover, higher TU values were found at the mouth of PB estuary, a high-energy environment with low OC content (0.04 to 0.07). In these particular sites, this only represent the effect of the normalization procedure at low OC content. Although l-cyhalothrin and cyfluthrin were found at detectable concentrations on sediments in TSB and PB estuary, both were minor contributors to the expected toxicity of pyrethroids.

*Mytilus californianus*

Punta Morro (PM), Salsipuedes (SL), and Todos Santos Island (TS), the three locations where *Mytilus californianus* were collected, presents a sum of total pyrethroid concentrations of...
4.7 \( (n = 3, \ SD = 0.96) \), 4.9 \( (n = 3, \ SD = 1.14) \), and 1.7 ng/g w.w. \( (n = 3, \ SD = 0.43) \), respectively. Moisture content in mussels were estimated to be 87.3 \( (n = 3, \ SD = 2.4) \), 87.0 \( (n = 3, \ SD = 1.2) \), and 89.6\% \( (n = 3, \ SD = 1.6) \). The lipid content (based in wet weight) in the mussels’ tissues was determined for the same three locations and has mean values of 0.38 \( (n = 3, \ SD = 0.08) \), 0.43 \( (n = 3, \ SD = 0.06) \), and 0.32\% \( (n = 3, \ SD = 0.09) \), respectively. Note that low lipid contents in the mussels could be attributed to premature spawning by the unusual warmer surface waters in the Pacific coastal region that has persisted since 2013 [31]. Permethrin was the main contributor of pyrethroids in mussels with average concentrations of 2.19 ng/g w.w. \( (n = 3, \ SD = 0.54) \) in Punta Morro, 2.6 ng/g w.w. in Salsipuedes \( (n = 3, \ SD = 1.1) \), and 0.22 ng/g w.w. in Todos Santos Island \( (n = 3, \ SD = 0.22) \).

Fipronil and fipronil sulfide were measured at detectable concentrations on mussels (0.04 ng/g w.w.) indicating a continuous discharge of these compounds in the area, mostly attributable to the effluents from the WWTPs. Furthermore, their presence could be associated mostly with wastewater effluents and to other non-point sources located nearby. However, their absence in sediments could be attributed to fast degradation processes in the water column as was previously described.

**Contribution of pyrethroids and fipronil by WWTPs**

For WWTPs, the discharges of pyrethroids and fipronil to the coastal area were mainly characterized by three compounds: fipronil sulfide, bifenthrin, and cypermethrin. However, four compounds: fipronil, fipronil sulfone, l-cyhalothrin, and esfenvalerate showed lower contribution in the sum of total pyrethroids and fipronil on the three WWTP discharges. Besides, deltamethrin and bifenthrin were only not detected in the effluent of El Gallo WWTP. Among the three
WWTPs, El Gallo has a major contribution of sewage coming from residential and commercial zones located downtown of Ensenada, while El Naranjo WWTP has a notable influence of the residential and some agricultural areas located south of the city.

For the three main WWTPs located in Ensenada, the total pyrethroid concentrations discharged to the bay were 1150, 140.4, and 3321.5 ng/L from El Naranjo, El Sauzal, and El Gallo WWTPs, respectively. The daily mean flows of the treated effluents measured in the sampling campaign were 380, 4121.3, and 18334.1 m$^3$/d for El Naranjo, El Sauzal, and El Gallo WWTPs. An estimated contribution of total pyrethroid concentrations discharged to the bay were calculated to be 60.9, 4.4, and 0.6 g/d for the El Gallo, El Naranjo and El Sauzal WWTPs, respectively. Note that to estimate the contribution of total concentrations of pyrethroids in the study area, the daily mean flows discharged by each WWTP were used. Moreover, it is an important issue to consider that during normal operating conditions, El Naranjo WWTP usually has a daily mean flow that can be as much as one thousand times higher than that reported in the present study. It is noteworthy that during the sampling, Ensenada had a serious water deficit due to the prevailing drought in the region and discharges were intermittent.

CONCLUSIONS

Pyrethroids showed detectable concentrations in sediments, mussels, and wastewater treated effluents samples in sites monitored in this work. In sediments, the main contributors of pyrethroids were bifenthrin, permethrin, and cypermethrin. In addition, mussels also incorporated these compounds in their body burden. Although fipronil sulfide was the main compound discharged to the coast in WWTP effluents, it’s concentration was below the detection limit in sediments and was very low in mussels. Moreover, the highest concentrations
of pyrethroids in sediments were found along the coast, whereas a downward gradient was observed in sites offshore. Additionally, the concentrations of pyrethroids were not correlated with the granulometric parameters or the OC contents on sediments, suggesting that the distribution of these compounds may be regulated by other factors, such as proximity to their sources. In addition, an evaluation of toxic units for *H. azteca* and *E. estuarius* suggests a little to no impact for sediment toxicity for both species at most of the analyzed sites.

*Supplemental Data*—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.xxxx

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*Data Availability*—Supplementary Information available with the submitted manuscript. Other information will be available upon request to the authors (vmacias@uabc.edu.mx).

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Figure 1. Sampling locations for sediments, wastewater treatment plants (WWTP), and mussels in Todos Santos Bay.

Figure 2. Spatial distribution of the sum of total concentrations of pyrethroids and fipronil (ng/g d.w.) in surface sediments of Todos Santos Bay and Punta Banda Estuary. The bar graph represents the mean of the sum of pyrethroids and fipronil found in samples of WWTPs and mussels. SAU = El Sauzal WWTP, NAR = El Naranjo WWTP, GAL = El Gallo WWTP, PM = Punta Morro, SL = Salsipuedes, TS = Todos Santos Island.

Figure 3. Percentage contribution of each pyrethroid and fipronil found in WWTP, mussel, and sediment samples. SAU = El Sauzal WWTP, NAR = El Naranjo WWTP, GAL = El Gallo WWTP. For mussels: SL = Salsipuedes, TS = Todos Santos, PM = Punta Morro.

Figure 4. Sum of toxic units (TU; up), individual TU for cypermethrin (middle) and bifenthrin (down), and the two main contributors to the expected toxicity for *H. azteca* and *E. estuarius* on sediments of Punta Banda Estuary (left) and Todos Santos Bay (right). The dashed line represents a value of TU = 1.

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Figure 1
Figure 3
Figure 4