Heliyon 8 (2022) e09534

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Bioanalytical and chemical-specific screening of contaminants of concern in three California (USA) watersheds

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ARTICLE INFO

Keywords:
Bioanalytical screening
Water quality
Estrogen receptor
Glucocorticoid receptor
Contaminants of emerging concern (CECs)
Aquatic ecosystems

ABSTRACT

To broaden the scope of contaminants monitored in human-impacted riverine systems, water, sediment, and treated wastewater effluent were analyzed using receptor-based cell assays that provide an integrated response to chemicals based on their mode of biological activity. Samples were collected from three California (USA) watersheds with varying degrees of urbanization and discharge from municipal wastewater treatment plants (WWTPs). To complement cell assay results, samples were also analyzed for a suite of contaminants of emerging concern (CECs) using gas and liquid chromatography-mass spectrometry (GC- and LC-MS/MS). For most water and sediment samples, bioassay equivalent concentrations for estrogen and glucocorticoid receptor assays (ER- and GR-BEQs, respectively) were near or below reporting limits. Measured CEC concentrations compared to monitoring trigger values established by a science advisory panel indicated minimal to moderate concern in water but suggested that select pesticides (pyrethroids and fipronil) had accumulated to levels of greater concern in river sediments. Integrating robust, standardized bioanalytical tools such as the ER and GR assays utilized in this study into existing chemical-specific monitoring and assessment efforts will enhance future CEC monitoring efforts in impacted riverine systems and coastal watersheds.

1. Introduction

Over the past two decades, a profound shift has occurred in the classes of chemicals targeted for monitoring of aquatic systems. In the last half of the 20th century, persistent organic pollutants, hydrocarbons of combustion and petrogenic origin, and trace metals were prioritized, but their dissipation and/or mitigation have reduced the need to measure these legacies of past insult. In their place has emerged an ever-growing list of contaminants of emerging concern (CECs), particularly those that occur in receiving waters impacted by municipal wastewater discharge, stormwater runoff and agricultural land use (Kolpin et al., 2002; Lao et al., 2010; Bai, 2018). Among those, steroidal hormones and pesticides in current use are often at the top of the list of CECs prioritized for monitoring due to their ability to impact aquatic life at relatively low concentrations (Maruya et al., 2014; Brack et al., 2015). Other broad-use pharmaceuticals (e.g., anti-inflammatory drugs) and industrial chemicals (e.g., perfluoroalkyl substances (PFAS) are also increasingly investigated in waterways (Bradley, 2017; Fang et al., 2019).

Establishing robust analytical methods to address this shift in chemicals of interest is a challenging endeavor, particularly as the list of parent CECs, possible metabolites and transformation products continues to evolve. To enhance chemical monitoring practices and better evaluate mixture toxicity, the development and application of rapid high-throughput bioanalytical tools has gained momentum for assessing water quality (Leusch and Snyder, 2015). Bioanalytical tools are receptor-based cell assays that respond to chemicals eliciting a common mode of biological activity (Leusch et al., 2018; Mehinto et al., 2017; Van der Linden, 2008). Studies have shown that standardized cell assays have the potential to serve as robust methods for water quality assessment (Escher, 2014; Mehinto et al., 2015). Two examples of cell assays frequently used for water quality assessment target estrogenic chemicals and glucocorticoids that are commonly found in treated wastewater effluent (Mehinto et al., 2016; Leusch et al., 2018).

In California (USA), home to 30 million people, surface (fresh) waters are impacted by a wide range of human activities and subject to extensive water quality monitoring (https://www.waterboards.ca.gov/water_iss

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https://doi.org/10.1016/j.heliyon.2022.e09534
Received 17 January 2022; Received in revised form 4 March 2022; Accepted 19 May 2022

ues/programs/swamp/). In Southern California, treated municipal wastewater effluent and stormwater runoff from highly urbanized landscapes are collected in channelized waterways that flow into the ocean (Fono et al., 2006; Sengupta et al., 2014). In contrast, watersheds in the northwestern part of the state are typically heavily forested (i.e., much less populated) with minimal hydromodification and many support agricultural land uses. Common to both watershed types is the potential impact of CECs in effluent discharge and runoff from urban and/or rural landscapes (Schlenk et al., 2012).

In previous studies on California watersheds, individual CEC concentrations were evaluated in water, sediment and fish tissue, and when available, evaluated against toxicity thresholds of interest to assess the need for follow-up monitoring (Sengupta et al., 2014; Maruya et al., 2016a, Mehinto, 2021). The goals of the present study were to 1) apply and evaluate the utility of standardized receptor-based cell assays for screening of water quality; and 2) measure and compare the concentrations of individual, high priority CECs to established monitoring thresholds. To accomplish these goals, river water, sediment, and effluent from wastewater treatment plants in three watersheds with varying degrees of urbanization were collected and analyzed by bioanalytical and targeted mass spectrometric methods.

2. Experimental section

2.1. Materials

High-purity dichloromethane (DCM), dimethylsulfoxide (DMSO) and methanol were purchased from Fisher Scientific (Pittsburgh, PA, USA). Ascorbic acid, sodium azide, 17β-estradiol (E2) and dexamethasone (DEX) were purchased from Sigma Aldrich (St. Louis, MO, USA), and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, dioxin) was obtained from AccuStandard (New Haven, CT, USA). Cells, media, and reagents for *in vitro* cell bioassays were obtained from Life Technologies/Thermo Scientific (Carlsbad, CA, USA). Authentic standards of PAHs as well as perdeuterated PAHs used to track analyte recovery were purchased from Ultra Scientific (North Kingstown, RI, USA).

2.2. Study sites

The Russian River (RR) is a predominantly rural watershed that drains 3850 km^2 in northern California (Fig. S1). The watershed is composed of ~80% forested areas, ~10% agricultural lands (mostly viticulture), and ~10% developed landscape. Several municipal wastewater treatment plants (WWTP), serving a total population of ~500,000 residents, discharge treated effluents into the RR watershed. In contrast, the Los Angeles River (LAR) and San Gabriel River (SGR) are two urban waterbodies in southern California with watershed areas of 2170 and 1800 km^2, respectively (Figs. S2 and S3). The LAR and SGR are composed of ~48–56% developed landscapes, highly modified concrete river channels, and extensive flow diversions in the upper and middle reaches of the SGR. Both rivers receive treated effluents from multiple WWTPs serving populations of over 5 million collectively. During dry weather conditions, WWTP effluents are the primary source of water in the LAR and SGR (Sengupta et al., 2014).

2.3. Sample collection

A total of 16 river water, 16 sediment and 5 treated wastewater effluent samples were collected for this study (Table 1). Grab samples of surface water were collected from 8 RR stations in March 2016, and from 3 LAR stations and 5 SGR stations in July through October 2016 (Figs. S1, S2, S3). Timing of sample collection was selected to evaluate conditions when both these watersheds are most impacted by contaminated runoff and/or discharges. Water samples were collected in a pre-cleaned stainless-steel bucket and transferred into sterile 1-L and 4-L amber bottles containing ascorbic acid and sodium azide as preservatives. Bed sediment (to 5 cm depth) was collected at each station, where present and accessible, using a pre-cleaned stainless-steel scoop and placed in 250 mL glass jars with Teflon-lined lids. Additionally, final WWTP effluent samples (24-h composites) were collected in pre-cleaned 1-L amber bottles with preservatives from two WWTPs discharging to the RR in April 2016; and 3 WWTPs discharging to the SGR in August 2016. All samples were kept in the dark on ice and delivered to the analytical lab within 48 h of collection. For each sampling event, a field blank for water collection was prepared by pouring 1-L of Milli-Q grade water into the stainless-steel collection bucket and then into a sterile 1-L amber glass bottle with preservative.

Table 1. Sample identification matrix for targeted CEC analysis and bioanalytical screening of water, sediment and WWTP effluent samples from the Russian River watershed.

Station name	Sample Matrix
Russian River Watershed	
City of Ukiah WWTP	effluent (tertiary)
El Roble	River water and sediment
City of Cloverdale WWTP	effluent (secondary)
Airport	River water and sediment
Lytton Springs Creek	River water and sediment
Pull Out	River water and sediment
Riverfront	River water and sediment
Mirabel	River water and sediment
Monte Rio	River water and sediment
Piner Creek	River water and sediment
Santa Rosa Creek	River water and sediment
Los Angeles River	
LAR REF	River water and sediment
LA1	River water and sediment
LA3	River water and sediment
San Gabriel River	
SGR REF	River water and sediment
San Jose Crk, Los Coyotes & Long Beach WWTPs	effluent (tertiary)
SJC1	water and sediment
SG4	River water
SG5a	River water and sediment
SG6	River water and sediment

2.4. Bioanalytical screening

Aqueous samples were processed within 72 h of collection following procedures described in Mehinto et al. (2016). Briefly, aqueous samples were passed through 1.6 μm glass fiber filters (Whatman GF/A) prior to extraction using 200 mg Oasis HLB solid phase extraction (SPE) cartridges. After loading each cartridge with sample, CECs were eluted with 10 mL methanol and 10 mL acetone:hexane (1:1, v/v), concentrated under a stream of nitrogen gas and exchanged to DMSO. Sediment samples were processed using accelerated solvent extraction (ASE) with DCM under elevated temperature and pressure as described in Lao et al. (2010), and subsequently exchanged to DMSO. All sample extracts were stored at -20 °C until further analyses.

Water and sediment extracts were analyzed using the GeneBLAzer estrogen receptor-alpha (ER) and glucocorticoid receptor (GR) cell transactivation assays following the procedures described in Mehinto et al. (2016) and Mehinto et al. (2017). Briefly, division-arrested HEK 293T cells were diluted in assay media, seeded into 96-well plates, and exposed to serial dilutions of sample extracts (final DMSO concentration <0.5%). After overnight incubation at 37°C and 5% CO_2, a loading substrate (for bioactivity) and PrestoBlue (for cytotoxicity) were added to each well and the plates were incubated for 2 h in the dark at room temperature. Using a Synergy H1 Hybrid microplate reader (BioTek)

configured to measure fluorescence, bioactivity was measured in the blue (409/460 nm; excitation/emission wavelengths) and green (409/530 nm; excitation/emission wavelength) channels, while cytotoxicity was measured at 560 nm Ex/590 nm Em. All sample extracts were analyzed in triplicate wells at 1.25 to 10 times their original sample concentration. Assay-specific dose-response curves based on serial dilutions of E2 for ER and DEX for GR, were utilized to express the results as bioanalytical equivalent concentrations (BEQs) in ng/L (water) or ng/g dry weight (sediment). The average fluorescence ratios measured for cell-free and cells-only controls, also analyzed in triplicate, were subtracted from fluorescence ratios measured for sample extracts prior to estimation of BEQs.

2.5. Targeted chemical analyses

Individual CECs prioritized for monitoring in California waterways (Maruya et al., 2014) were analyzed in aqueous and sediment samples by the Los Angeles County Sanitation Districts Water Quality Research Lab (LACSD); Eurofins Eaton Analytical (EEA); and the Southern California Coastal Water Research Project (SCCWRP). Target analytes and methods used by each laboratory are summarized in Table S1. PPCPs were extracted from two 200 mL aliquots of aqueous sample by SPE using Oasis HLB cartridges, and extracts in methanol were analyzed by HPLC MS/MS. Steroids and alkylphenols were extracted from a 500-mL aliquot of aqueous sample by SPE using Strata-X cartridges, followed by HPLC-MS/MS analysis. Galaxolide and fipronil were extracted from a 500-mL aliquot of aqueous sample by SPE using C18 columns, followed by GC/MS in SIM mode. Pyrethroids were extracted from a 1-L aliquot of aqueous sample by SPE (C18 columns), followed by HPLC-MS/MS. PFOS was analyzed by direct injection HPLC/MS-MS. Sediment samples were extracted for the above analytes using QuEChERS, followed by the corresponding instrumental technique described above for aqueous sample extracts. EEA analyzed bifenthrin, permethrin, fipronil and galaxolide for LAR and SGR water samples only, using liquid-liquid extraction followed by GC-triple quadrupole MS (QQQ). SCCWRP analyzed additional CECs in sediment (pyrethroids and fipronil-related analytes) using a GC-MS-based method, after extraction of a 5 g freeze dried aliquot using ASE.

2.6. Data analysis and validation

Measured concentrations of CECs were reported individually and as their respective sums in units of ng/L (aqueous) and ng/g dry weight (sediment). Maximum concentrations of CECs were compared to their corresponding monitoring trigger levels (MTLs), derived from toxicity thresholds for ecological receptors, to compute monitoring trigger quotients (MTQs) using Eq. (1):

$$MTQ = MEC_{max}/MTL \quad (1)$$

where MEC_{max} is the maximum measured environmental concentration. Chemical-specific MTLs were established by the State of California's science advisory panel as the basis of a screening level interpretation framework for occurrence data in water and sediment (Maruya et al., 2014). The resulting values of MTQs were assessed to determine the need and extent for future monitoring and assessment.

A performance-based QA/QC approach, adopted from Dodder et al. (2015), was followed to ensure that data generated were of high quality. Data quality for cell assays were validated against pre-set criteria for calibration, blank, DMSO control, cytotoxicity (cell viability) and sample dose-response. Instrumental methods for individual CECs were selected and/or optimized to meet minimum reporting limits (RLs) recommended by the science advisory panel. These data were validated against criteria for instrument calibration, analysis of blanks, matrix spikes and duplicate samples.

3. Results

3.1. Bioanalytical screening

Lab and field blanks for all matrices of interest and study watersheds were at or below RL for estrogenic and glucocorticoid receptor activities (ER- and GR-BEQ, respectively) (Tables 2, S2 and S3). ER- and GR-BEQ for aqueous matrix spike samples (i.e., those fortified with either E2 or DEX) were all above RLs, with over 60% recovery of spiked mass for 4 of 6 measurements Bioanalytical results for the Lytton Springs Creek water sample and its duplicate were both below RLs (Table 2). It should be noted that one set of samples (LAR and SGR, event 2) had low recovery (<60%) of the spiked chemicals, indicative of poor extraction efficiency which could lead to underestimation of BEQ values in river samples extracted with this batch.

All 8 water samples from the RR rural watershed showed no measurable ER or GR activity (Table 2). The ER- and GR-BEQ for the Cloverdale WWTP effluent sample were below RLs (<0.4 ng E2/L and <22 ng DEX/L, respectively), in contrast to the detectable levels for the Ukiah WWTP effluent sample (1.9 ng E2/L and 61 ng DEX/L). Sediment samples from the RR also showed no or minimal detectable ER and GR response (Table S2), with only a single sample eliciting a detectable ER

Table 2. Bioassay equivalent concentrations (BEQs) for ER and GR assays for aqueous samples from the Los Angeles and San Gabriel River watersheds. Samples were collected in Jul–Aug (Event 1) and Sep–Oct (Event 2) of 2016.

Station	ER-BEQ (ng E2/L)	GR-BEQ (ng DEX/L)
LA River – Event 1		
Field Blank 1	<0.2	<26
LAR Ref	0.8	<26
LA1	0.9	<26
LA3	0.4	<26
LA3 Matrix Spike	9	162
LA River – Event 2		
Field Blank 2	<0.2	<29
LAR Ref	<0.2	<29
LA1	<0.2	<29
LA3	<0.2	<29
LA3 Matrix Spike	4.2	103
SG River – Event 1		
Field Blank 1	<0.2	<26
SGR Ref	<0.2	<26
SG4	<0.2	81
SG5a	<0.2	<26
SG6	0.4	<26
Field Blank 2	<0.2	<26
SJC1	0.4	99
Effluent Blank[1]	<0.2	<29
San Jose Crk WWTP[1]	<0.2	98
Los Coyotes WWTP[1]	<0.2	98
Long Beach WWTPt[1]	<0.2	94
SG River – Event 2		
Field Blank 3	<0.2	<29
SGR Ref[2]	<0.2	<29
SG4	0.24	86
SG5a	<0.2	<29
SG6	<0.2	<29
SJC1	<0.2	<29
SGR Ref Matrix Spike	4	54

< not detected (value is reporting limit); E2 – 17β-estradiol; DEX - dexamethasone.
[1] collected on 8/3/16.
[2] collected on 10/6/16.

response (0.09 ng E2/g for Piner Creek). All 8 RR sediment samples were below the RL for the GR assay.

In contrast, ER- and GR-BEQ for water and sediment from the LAR and SGR urban watersheds were more frequently above RLs and higher in magnitude than the RR samples (Tables 2 and S3). For water, responses were above RLs for 6 of 16 samples for ER-a, and for 3 of 16 samples for GR. The magnitude of response ranged from non-detect (<0.2 ng E2/L) to 0.9 ng E2/L for ER-BEQ; and from non-detect (<26 ng DEX/L) to 99 ng DEX/L for GR-BEQ (Table 3). Only 2 water samples from the SGR (SJC1/Event 1 and SG4/Event 2) showed measurable response for both ER and GR; none of the LAR water samples showed measurable response for both endpoints. Measurable ER-BEQs were observed for 6 of 14 sediment samples, narrowly ranging from 0.12 to 0.52 ng E2/g (Table S3). However, no sediment sample showed measurable GR response (<10 ng DEX/g). No measurable ER-a response was observed for effluent samples from the 3 WWTPs discharging to the SGR. In contrast, GR-BEQs for these same effluent samples were several-fold higher than the RL (94–98 ng DEX/L; Table 2) and were similar in magnitude to levels of GR activity detected at the SG4 and SJC1 stations.

3.2. Analysis of individual CECs in river and effluent samples

Aqueous concentrations (dissolved phase) of target CECs in the RR water samples are summarized in Figure 1 and Table S4. Galaxolide and 4-nonylphenol were detected in all samples, including laboratory and field blanks (Table S5). River water concentrations of galaxolide and 4-nonyphenol were 1–4 times the measured blank concentrations, indicating that blank contributions were not trivial. No other target CEC was detected in aqueous dissolved phase blanks. The analysis of duplicate water samples collected at the Lytton Springs Creek indicated consistent and reproducible results (Tables S4 and S5).

The greatest number of detectable CECs were found in water samples from Piner Creek, Santa Rosa Creek and Mirabel (Figure 1), all located in the southeastern RR watershed near the city of Santa Rosa. Among the CECs detected, PFOS was found in all but one (Riverfront) sample, whereas fipronil, permethrin and bifenthrin, bisphenol A and estrone were detected at lower frequency (Table S4). In the wastewater effluent samples discharging in the RR watershed, all 12 CECs were detected in the Ukiah sample, whereas 5 of 12 analytes were found in the Cloverdale effluent sample (Figure 1, Table S5). It should be noted that galaxolide concentrations in these samples were 10–130 times higher than blank levels.

Table 3. Monitoring trigger quotients (MTQs) for CECs in Russian River sediments.

Analyte	MTL River	MTL Bay	MEC_{max} RR	MTQ RR	MTQ RR Estuary
17β-Estradiol (E2)	n/a	n/a	0.23	n/a	n/a
Estrone (E1)	n/a	n/a	1.3	n/a	n/a
Diclofenac	n/a	n/a	<1.0	n/a	n/a
Ibuprofen	n/a	n/a	<1.0	n/a	n/a
Triclosan	n/a	n/a	6.8	n/a	n/a
4-Nonylphenol[1]	n/a	n/a	34	n/a	n/a
Bisphenol A	n/a	n/a	15	n/a	n/a
PFOS[2]	n/a	n/a	4.1	n/a	n/a
Bifenthrin	n/a	0.052	130	n/a	**2500**
Permethrin	n/a	0.073	4.9	n/a	*67*
Fipronil[3]	0.09	6.5	3.4	*38*	0.52

MTL - monitoring trigger level (ng/g).
MTQ - monitoring trigger quotient.
MEC_{max} - maximum measured environmental concentration (ng/g).
Italic highlighting denotes 1 < MTQ<100; Bold highlighting denotes MTQ>100.
[1] technical mixture.
[2] perfluorooctane sulfonate.
[3] parent, desulfinyl, sulfide or sulfone.

Aqueous CEC concentrations for the LA and SG river samples are shown in Tables S6, S7, S8. Analytes typically found in municipal discharges were detected in more than 50% of the LAR water samples, including 4-nonylphenol, bifenthrin, diclofenac, estrone, PFOS, permethrin, fipronil and galaxolide (Table S6). Similar detection frequencies for the above CECs were noted for the SGR, except for fipronil (4 of 10 stations) (Tables S7 and S8). As was observed for the RR samples, 4-nonylphenol and galaxolide were detectable in blanks, and along with bisphenol A, remain a challenge for low level determination. It should be noted that concentrations of bifenthrin, permethrin and PFOS were several-fold higher at LAR reference site compared to the downstream stations LA1 and LA3 receiving WWTP effluent discharges. Similary, higher concentrations of bifenthrin, permethrin and PFOS were measured at SG5a (Coyote Creek), a tributary not influenced by WWTP discharge suggested that these CECs are associated with surface runoff and/or infiltration. Moreover, most CEC concentrations in water from SGR reference site, located at elevation well upstream of any WWTP, were < RLs, confirming that CEC input in the SGR occurs largely in the lower reaches of this watershed.

3.3. Analysis of individual CECs in sediment samples

Sediment concentrations for the 20 target CECs are summarized in Figure 2 and Tables S9, S10, S11. Analyte-specific levels in blanks were uniformly at or below RLs. Similar to water chemistry data, detection frequencies and concentrations were greatest in RR sediments from Piner and Santa Rosa Creeks and Mirabel (Table S9). The maximum concentration for 9 of 20 analytes (mostly pyrethroids up to 130 ng/g) were measured in Piner Creek. Bisphenol A, 4-nonylphenol and PFOS were detectable in most samples at maximum concentrations of 34, 15 and 4.1 ng/g, respectively. CEC concentrations for the LAR REF sediment sample were higher for many analytes (e.g. pyrethroids) than corresponding levels in sediments from the stations downstream from the WWTPs (Table S10, Fig. S2). Exceptions were noted for E2, estrone (E1) and triclosan, which appeared to be higher in the downstream sediments (i.e. LA1 and LA3). Nearly every CEC was detected in sediments from SJC1/SGR, located adjacent to the San Jose Creek WWTP (Table S11). Although CECs were also detected in sediments from other SGR stations, their concentrations were one or two order of magnitude lower compared to those for SJC1 sediment.

3.4. Chemical prioritization and estimated risk quotients

Monitoring trigger quotients (MTQs) computed for CECs measured in water and sediment are summarized in Tables 3 and S12 for the RR samples, and Tables 4 and S11 for the LAR and SGR samples. Because of conservative assumptions used in establishing MTLs, the guidelines used for interpreting MTQs were: MTQ <1.0 “minimal concern”; 1 < MTQ <100 “moderate concern; MTQ >100 “elevated concern”. Due to the dearth of effects information for sediment-associated CECs, MTQs were only considered for three CECs: fipronil in freshwater habitats, and the pyrethroids bifenthrin and permethrin in estuarine habitats. Although the majority of sampling locations in the present study are freshwater, it is worth noting that station SGR6 was within the tidally influenced reach of the SGR watershed and is thus classifiable as estuarine habitat. For fipronil in freshwater, MTQs were 38 (RR), 57 (SGR) and 180 (LAR). For bifenthrin and permethrin, respectively, MTQs were 519 and 274 (LAR); 942 and 790 (SGR); and 2500 and 67 (RR) (Tables 3 and 4).

MTQs for aqueous samples in all 3 watersheds painted a different picture. For the RR watershed, all MTQs for waterborne CECs were less than 1, indicating minimal concern (Table S12). For the effluent-dominated LAR and SGR watersheds, MTQs for waterborne CECs were also <1.0 except for diclofenac and galaxolide, with values 1.3–1.9 (Table S13).

4. Discussion

Bioanalytical screening tools are now recognized worldwide as valuable tools to improve water quality assessment (Poulsen et al., 2011;

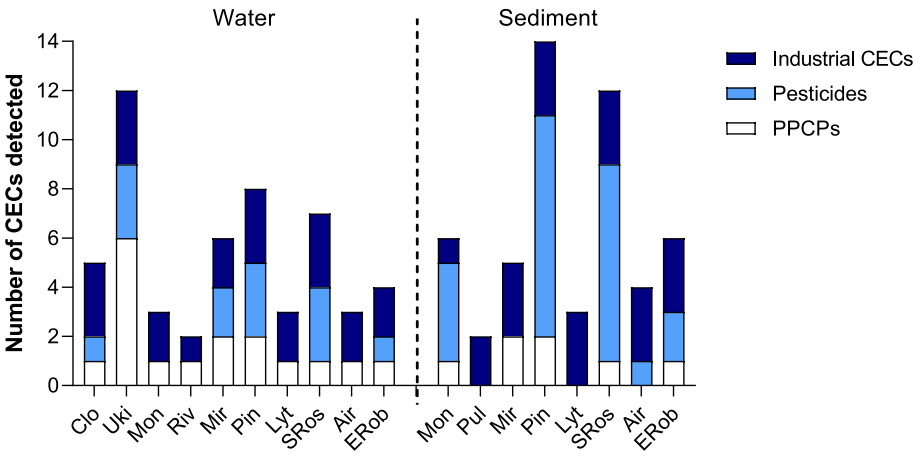

Figure 1. Number of CECs detected in water, sediment and treated wastewater effluent samples from the Russian River watershed. Study stations are abbreviated as: Clo – Cloverdale effluent; Uki – Ukiah effluent; Mon – Monte Rio, Mir – Mirabel, Pin – Piner Creek; Lyt – Lytton Springs Creek; SRos – Santa Rosa Creek; Air – Airport; ERob – El Roble; Pul – Pull Out.

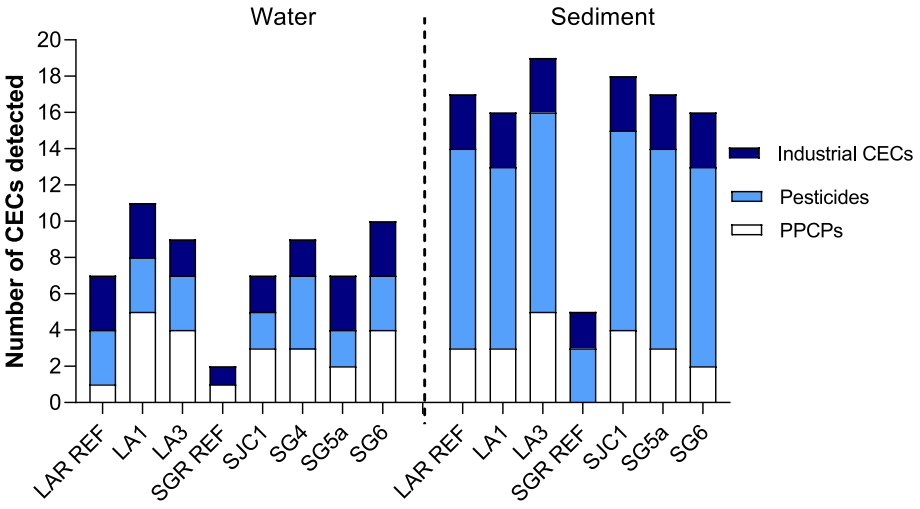

Figure 2. Number of CECs detected in water and sediment samples from the Los Angeles River and San Gabriel River watersheds.

Table 4. Monitoring trigger quotients (MTQs) for sediment phase CECs in the Los Angeles and San Gabriel River watersheds.

ANALYTE	MTL River	MTL Estuary	MEC_{max} LAR	MTQ LAR	MTQ LAR Estuary	MEC_{max} SGR	MTQ SGR	MTQ SGR Estuary
17β-Estradiol	n/a	n/a	0.64	n/a	n/a	1.5	n/a	n/a
Estrone	n/a	n/a	7.4	n/a	n/a	12	n/a	n/a
Diclofenac	n/a	n/a	2.1	n/a	n/a	2.1	n/a	n/a
Ibuprofen	n/a	n/a	190	n/a	n/a	18	n/a	n/a
Triclosan	n/a	n/a	140	n/a	n/a	83	n/a	n/a
4-Nonylphenol[1]	n/a	n/a	800	n/a	n/a	1600	n/a	n/a
Bisphenol A	n/a	n/a	46	n/a	n/a	180	n/a	n/a
PFOS[2]	n/a	n/a	5.5	n/a	n/a	2	n/a	n/a
Bifenthrin	n/a	0.052	27	n/a	**519**	49	n/a	**942**
Permethrin	n/a	0.073	20	n/a	**274**	57.7	n/a	**790**
Fipronil[3]	0.09	6.5	16	**180**	*2.5*	5.1	*57*	0.78

MTL - monitoring trigger level (ng/g); MTQ - monitoring trigger quotient; MEC_{max} - maximum measured environmental concentration (ng/g).
Italic highlighting denotes 1 < MTQ<100; Bold highlighting denotes MTQ>100.

[1] technical mixture.
[2] perfluorooctane sulfonate.
[3] parent, desulfinyl, sulfide or sulfone.

Maruya et al., 2016b, (Brack et al., 2015)). However, routine application as part of monitoring programs remains limited. In the present study, two cell bioassays were applied to evaluate their sensitivity and usefulness in various matrices (water and sediment) from watersheds with varying human impact (i.e., rural and urban). Overall, the ER bioassay responses in water samples were consistent with those previously reported in California freshwater habitats, (Conley, 2017; Mehinto, 2021; Mehinto et al., 2017). GR-BEQs reported for river water samples in this study were ~2-fold higher than those published in Mehinto et al. (2017 and 2021).

As expected, the highest bioassay responses were found in WWTP effluent samples or at downstream sites although ER- and GR-BEQs for the SGR effluent samples were lower by a factor of 2 than those reported previously (Mehinto et al., 2016). It is possible that bioactivity in these samples was underestimated due to incomplete extraction as suggested by the low recoveries in the matrix spike samples. For sediment, comparison of ER and GR screening data is more challenging due to the paucity of such datasets, especially for GR response. That said, our findings were consistent with a previous study of marine sediment that reported up to 0.3 ng E2/g at a reference site and 1.3 ng E2/g at a site contaminated with known/suspected ER agonists (Crago et al., 2016).

Robust interpretive frameworks for bioanalytical screening data are still in development, due in large part to limited analytical methods to measure all bioactive CECs and the lack of consensus in establishing thresholds of concern for environmental matrices. Bioanalytical screening results were partially corroborated by the targeted chemistry results for the ER bioassay. In one instance for the Ukiah WWTP effluent sample, measured concentrations of E2 (11 ng/L, potency factor of 1) and E1 (0.6 ng/L, potency factor ~0.1) explained over 80% of the measured ER bioactivity (ER-BEQ of 1.9 ng/L). Highest ER-BEQs in RR, LAR and SGR sediments also exhibited the highest concentrations of moderate and weak estrogenic chemicals including E1, bisphenol A and 4-nonylphenol. Overall, our ER-BEQ data did not exceed concentrations determined by LC-MS/MS, indicating that the standardized bioanalytical method was not subject to false positive responses. Similar comparisons could not be performed with the GR bioassay as most analytical laboratories do not routinely measure GR bioactive CECs. When comparing ER- and GR-BEQs to published in vivo effects, our data suggest limited potential for ER and GR-related toxicity in fish (Kidd et al., 2007; LaLone et al., 2012; Kugathas et al., 2013, Mehinto, 2018).

Targeted chemistry profiles were consistent with our understanding of the systems studied. The direct discharge of wastewater effluent is only permitted into the mainstem Russian River during the wet (high flow) season, corresponding to the period sampled in the present study. Thus, one would expect the detection of CECs discharged via WWTP effluent in river water downstream. In urbanized channels, pathways for CECs in aquatic environments include wastewater effluents, localized run-off and stormwater discharges (Maruya et al., 2016a; Masoner et al., 2019). Target pesticides were higher at the LA reference station than at the downstream locations suggesting localized (non-WWTP source) input from surface runoff/and or infiltration. Elevated pesticides and PFOS concentrations were also detected in tributary sites of the SGR not influenced by WWTP discharge. The occurrence of pesticides at the LA reference station in particular may be attributed to local usage within the large municipal park and golf course complex adjacent to this location. Additionally, CEC concentrations in water from SGR reference station, located at elevation well upstream of any WWTP, were mostly below detection, confirming that CEC input in the SGR occurs largely in the lower reaches of this watershed.

In sediment, pesticides most frequently detected included bifenthrin, permethrin and cypermethrin, while most PPCPs were found below detection. Fipronil contamination was most prevalent in the urbanized watersheds (all 6 LAR and 5 of 8 SGR sediment samples). Interestingly, the parent compound was the most abundant (maximum of 16 ng/g) in all LAR samples, but fipronil sulfone was the most abundant in the SGR samples (maximum of 5.1 ng/g). With a relatively short environmental half-life, fipronil is transformed into its more stable metabolites, namely sulfone under aerobic conditions, sulfide under anoxic conditions, and desulfinyl, a photolytic product (Lao et al., 2010). The predominance of parent fipronil in LAR watershed sediments indicates little such transformation, perhaps limited by the natural bottom substrate and relatively dense riparian vegetation that reduces sunlight irradiation in the upper LAR watershed. The elevated pesticides concentrations were comparable to those reported in sediments from an urban southern California estuary that receives no intentional discharge of WWTP effluent (Lao et al., 2010) but were several-fold lower than sediments from some of the most impacted California watersheds (Siegler et al., 2015).

The moisture content of sediment is a well-established proxy for porosity, grain size and total organic carbon (TOC), with a positive association between % moisture and TOC (Dan et al., 2020). Thus, it can be hypothesized that finer grained sediments (i.e. those with high moisture content) have greater capacity to house aqueous phase compounds (i.e. CECs). In the present study, fine-grained sediments from Piner and Santa Rosa Creeks, LAR reference station and LA3, and San Jose and Coyote Creeks (SJC1 and SG5a) that had the greatest moisture content (69–80%) also exhibited the greatest CEC concentrations. In contrast, sediments with low moisture content (10–59%), including Lytton Springs Creek, Monte Rio, Pull Out, LA1 and SG6 are examples of relatively coarse substrates, and would thus be expected to retain lower concentrations of hydrophobic CECs, as they in fact did.

4.1. Implications for environmental quality and future monitoring

In addition to the clear differences in habitat and population density among the 3 study watersheds, the seasonal precipitation patterns across California were a contributing factor to the results reported herein. Sampling of the RR was conducted during the wet (winter) season when contaminant runoff and effluent discharges are most likely to enter the watershed. In LAR and SGR watersheds, sampling was conducted during the dry (summer-fall) season when minimal dilution occurs and contaminants loads in these effluent-dominated systems are likely to be elevated. Under these worst-case scenarios, most CEC levels were sufficiently low as to not warrant extensive CEC monitoring. The findings are supported by the bioanalytical screening results, where the mostly undetectable and/or low levels of ER- and GR activities suggest minimal likelihood of impact. However, the occurrence of 3 specific pesticides, fipronil, bifenthrin and permethrin were deemed of moderate to elevated concern as they exceeded the MTLs adopted by the science advisory panel for toxicity to non-target benthic organisms (Schlenk et al., 2001; Maul et al., 2008). Thus, our results suggest that future targeted monitoring of these 3 pesticides sediments is warranted in these watersheds.

5. Conclusions

Targeted (LC- and GC-MS) analysis of CECs and bioanalytical screening of ER- and GR activities was performed on water, WWTP effluent and sediment samples collected from two different habitats (urban and rural). Various wastewater-derived CECs were detected in effluent and river water, the latter at levels that were considered to be of minimal to moderate concern. In contrast, current use pesticides were detected in sediments from all 3 watersheds at concentrations that warrant further action. ER- and GR-BEQ were at or near reporting limits for both water and sediment, suggesting limited potential for impact due to estrogens and glucocorticoids in these watersheds. ER-BEQ results were largely in agreement with concentrations of targeted estrogens determined by LC-MS/MS, underscoring the potential utility of receptor-based cell assays as robust monitoring tools. Future research is needed to further refine and validate sample extraction protocols, establish water and sediment screening thresholds for existing bioanalytical tools (such as the ER and GR assays described herein) and to expand the toolbox to include the most relevant modes of action and/or chemical stressor groups (e.g. current use pesticides).

Declarations

Author contribution statement

Keith A. Maruya & Alvine C. Mehinto: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Wenjian Lao & Darcy R. Vandervort: Performed the experiments.

Richard Fadness & Michael Lyons: Conceived and designed the experiments.

Funding statement

Keith A Maruya and Alvine C. Mehinto were supported by California State Water Resources Control Board [14-090-270] & [15-048-140].

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2022.e09534.

Acknowledgements

The authors thank E. Nelson, A. Eaton, D. Diehl and members of the LACSD field sampling crew.

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