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Reconnaissance of selected PPCP compounds in Costa Rican surface waters

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ABSTRACT

Eighty-six water samples were collected in early 2009 from Costa Rican surface water and coastal locations for the analysis of 34 pharmaceutical and personal care product compounds (PPCPs). Sampling sites included areas receiving treated and untreated wastewaters, and urban and rural runoff. PPCPs were analyzed using a combination of solid phase extraction and liquid chromatography tandem mass spectrometry. The five most frequently detected compounds were doxycycline (77%), sulfadimethoxine (43%), salicylic acid (41%), triclosan (34%) and caffeine (29%). Caffeine had the maximum concentration of 1.1 mg L^{-1}, possibly due to coffee bean production facilities upstream. Other compounds found in high concentrations include: doxycycline (74 μg L^{-1}), ibuprofen (37 μg L^{-1}), gemfibrozil (17 μg L^{-1}), acetominophen (13 μg L^{-1}) and ketoprofen (10 μg L^{-1}). The wastewater effluent collected from an oxidation pond had similar detection and concentrations of compounds compared to other studies reported in the literature. Waters receiving runoff from a nearby hospital showed higher concentrations than other areas for many PPCPs. Both caffeine and carbamazepine were found in low frequency compared to other studies, likely due to enhanced degradation and low usage, respectively. Overall concentrations of PPCPs in surface waters of Costa Rica are inline with currently reported occurrence data from around the world, with the exception of doxycycline.

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1. Introduction

The active ingredients in pharmaceutical and personal care products (PPCPs) and veterinary antibiotics have increasingly been detected in a wide variety of environmental matrices. These include surface and groundwaters, wastewater treatment end products (effluents, reclaimed waters and sludges), soils and biota in the United States and Europe (Kolpin et al., 2002; Loos et al., 2009; Martìnez-Carballo et al., 2007; Ramirez et al., 2009). The widespread therapeutic and preventative use in both human and animal populations of products containing these active ingredients and their incomplete elimination in both the body and conventional wastewater treatment has resulted in their environmental introduction. Major pathways into the aquatic environment for these compounds include runoff from areas where both animal and human waste is not confined and treated (i.e landfills, manure piles, land application of sewage sludges),

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and the direct discharge of untreated wastewater and treated wastewaters (effluent) into surface waters. Once released into the environment many of these compounds persist and can possibly be transported to locations far from the source (e.g. Walters et al., 2010; Wu et al., 2009).

Research has mostly focused on the occurrence of PPCPs in temperate environments, with only limited studies being conducted in the warm and humid sub-tropical and tropical climates. Managaki et al. (2007) analyzed the occurrence of twelve veterinary antibiotics in waters from the Mekong Delta, Viet Nam and the urban Tamagawa River in Japan. Increased detections and higher concentrations were found for the Japanese sampling compared to the Mekong Delta for most analyzed compounds, although some PPCPs were common in both locations. Locatelli et al. (2010) surveyed Brazilian surface waters for eight antibiotic compounds and found that usage and sample collection during dry versus wet season determined the distribution and occurrence patterns. Martins et al. (2008) analyzed Brazilian hospital effluent for the presence of ciprofloxacin, a fluoroquinolone antimicrobial agent. The high concentrations detected caused the authors to conclude that the risk associated with the use and emission of pharmaceuticals into the environments of developing countries might be higher than in developed countries. Siemens et al. (2008) reported detection of several acidic and basic pharmaceutical compounds surviving the treatment process in reclaimed wastewaters used as an irrigation source in Mexico City. The inefficient or lack of treatment of wastewaters in developing countries can lead to increased introduction into the environment of these "emerging" contaminants.

Costa Rica is a moderately developed country with major urban and vast rural areas. Undeveloped land, rural agricultural areas and highly contaminated sites where identified during previous studies on PCB and pesticide residues (Spongberg and Davis, 1999; Spongberg, 2004). Wastewater treatment in Costa Rica can range from modern regional or city level treatment plants, to areas with primary treatment only, to discharge of untreated waste into local waterways by runoff or pipe. No previous study has been conducted in Costa Rica to assess the occurrence of PPCPs in surface waters. The goal of this study was to detail the occurrence of PPCPs in Costa Rican surface waters, a tropical country, with relation to previous sampling sites used for PCB and pesticide determination, wastewater sources and potentially contaminated runoff. Thirty-four PPCPs ranging in therapeutic class and usage were chosen for analysis. Compounds were selected based on occurrence data reported in other similar large-scale studies, established analytical methodology in our laboratory and suspected usage in Costa Rica.

2. Materials and methods

2.1. Nationwide sampling

Eighty-six sampling locations, along with one wastewater effluent sample, were selected from a wide range of localities within Costa Rica and are presented in Fig. 1 with detailed descriptions of the sites listed in Table 1 and S-1 (supplementary material). Site locations were based on access, previous survey for other compounds, correlation with other studies, or location near a possible source of contamination (wastewater treatment plant, city waste discharge, hospital discharge, urban runoff etc.). Sampling points were recorded using a GPSMAP 76 global positioning satellite receiver (Garmin, Olathe, KS, USA).

2.2. Sample collection

One liter of surface water from both salt and freshwater environments was collected in high-density polyethylene (HDPE) sampling bottles (Fisher Scientific, Pittsburg, PA, USA). Prior to sampling bottles were washed with dilute hydrochloric acid and methanol. At each sampling site bottles used were rinsed and shaken twice with a full volume of surface water. When necessary bulk samples were collected using a 5-liter bucket, then transferred to the 1-liter HDPE containers. All samples were stored on ice until being processed at the Centro de Investigacion en Contaminacion Ambiental (CICA), located at the University of Costa Rica (typically less than 48 h). Once in the laboratory samples were filtered through 47 mm, 0.7 micron glass fiber filters (Fisher Scientific, Pittsburg, PA, USA) using a vacuum apparatus, containers were then rinsed with a 50% (v:v) methanol in water solution, combined and subsequently extracted using solid phase extraction.

2.3. Chemicals and reagents

All pharmaceutical standards (purity, 90%~99%) were purchased from Sigma–Aldrich (St. Louis, MO), except clarithromycin (purity, 98%), obtained from Abbott (Chicago, IL). Instrumental internal standards $^{13}C_3$-Caffeine (purity, 99%), josamycin (purity, 98%), and 2-(3-chlorophenoxy) propionic acid (purity, 99%) were also obtained from Sigma–Aldrich (St. Louis, MO) and simatone was obtained from AccuStandard (New Haven, CT). All other chemicals and solvents were American Chemical Society certified or HPLC grade and supplied by Fisher Chemicals (Fair Lawn, NJ). Deionized water (18.3 MΩ) was provided by a Barnstead NANOpure® Infinity Water System (Dubuque, IA).

2.4. Solid phase extraction (SPE)

SPE was conducted according to the method reported in Wu et al. (2008). For all samples an aliquot of 350 mL was transferred to a glass container. Twenty-eight milligrams of Na_2-EDTA was added and allowed to dissolve while mixing. Sample pH was then adjusted to 5 using H_2SO_4 and/or 5% (v:v) NH_4OH in water. For SPE, Phenomenex Strata X polymeric cartridges, 6 mL 200 mg packing (Torrance, CA, USA), were conditioned three times with 2 mL methanol then three times with 2 mL deionized water containing 1% (w/v) Na_2-EDTA in water. Each 350 mL sample aliquot was loaded into the SPE cartridge at a rate of 10 mL min^{-1} using large volume sampling tubes connected to a 24 port SPE vacuum manifold (Phenomenex, Torrance, CA, USA). After loading, cartridges were washed with 2 mL of 5% (v/v) methanol in water and dried under vacuum for 2 min. The analytes were then eluted twice with 3 mL methanol without the use of

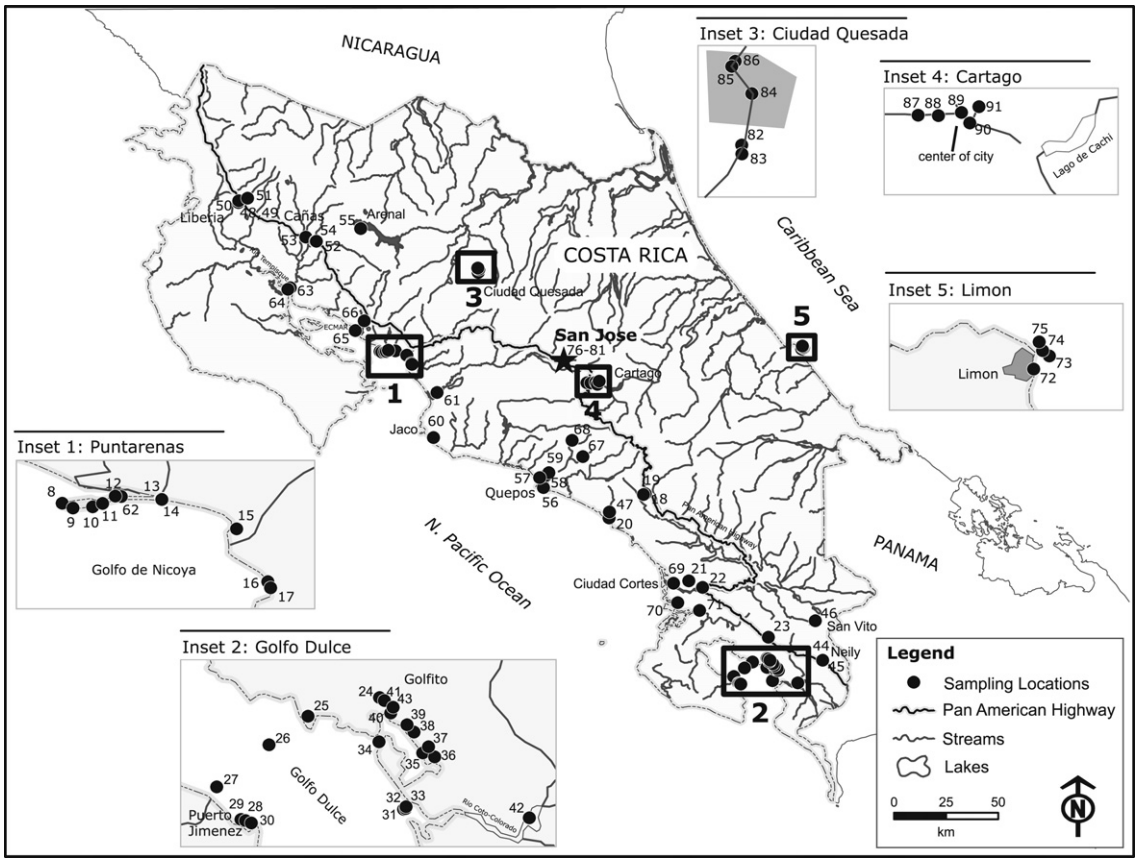

Fig. 1 – Overall map showing sampling locations, surface hydrography and points of interest in Costa Rica, Central America (10N, 85W).

vacuum. The eluate was collected in a glass conical vial and evaporated using nitrogen and a water bath at 40 °C to around 200 μL using a Turbo Vap LV (Caliper Life Sciences, Hopkinton, MA, USA). Samples were spiked with 100 ng instrumental internal standards, reconstituted to 0.45 ml using 50% (v/v) methanol in water, vortexed and transferred to 2 ml amber glass vials. SPE extracts were stored at −20 °C until instrumental analysis.

2.5. *Liquid chromatography mass spectrometry analysis (LC-MS/MS)*

The LC-MS/MS system consists of a ProStar® 210 solvent delivery module with a ProStar 430 autosampler and a 1200L triple-stage quadrupole mass spectrometer with a dual off-axis electrospray ionization interface (Varian Inc., Walnut Creek, CA). Analytes were separated using a Supelco Discovery® HS C18 column (150 × 4.6 mm, 3 μm). The column was maintained at 25 °C using a ThermaSphere TS-430 Column Chiller/Heater (Phenomenex, Torrance, CA, USA). Mobile phase A was 0.1% (v/v) formic acid in water, mobile phase B was 100% acetonitrile and the total flow rate was 0.3 mL min^{-1}. The gradient started with 5% B, held for 2 min, ramped to 100% in 18 min, held 10 min, dropped to 5% B in 2 min, and equilibrated for 8 min. The precursor ions and two most abundant transition ions are provided in Table 2. Detailed MS/MS parameters and method development procedure is presented elsewhere (Wu et al., 2008).

2.6. *Quantification and method validation*

Instrument control, peak detection and integration were accomplished using Varian MS Workstation (Version 6.8). Data acquisition was performed under multiple reaction monitoring (MRM) mode. Identification of the target analytes was based on the presence of two MRM transitions and match of retention time with the reference standard. The ratio of two MRM transitions was used for confirmation. The most abundant transition was selected for quantification. Instrumental internal standards (Wu et al., 2008) were added prior to LC-MS/MS analysis, but after SPE, to compensate for instrumental and ionization variation (matrix effect). Ratios of the analyte peak areas to appropriate internal standard peak area were used to construct calibration curves and for sample extract quantification. Powdered standards were dissolved in 450 μL of methanol at five concentration levels (10–500 μg L^{-1}), including 100 ng internal standards, to create calibration curves for external quantification. All calibration curves were linear ($r^2 > 0.98$) between 10 and 500 μg L^{-1}. Method blanks and reagent water used in the extractions were also run for quality assurance, and data were adjusted for any carryover and background accordingly. A quality control sample (50 μg L^{-1} calibration standard) was run every six injections, and the

Table 1 – Detailed sampling location information for Costa Rica study.

CRP	Type	Influence	Description	CRP	Type	Influence	Description
1	Fresh	Rural	Río Pará	48	Fresh	Urban	Liberia, near oxidation ponds
2	Fresh	Rural	Tributary to Río Virilla	49	Waste	Effluent	Liberia, WWT oxidation pond
3	Fresh	Rural	Río San Miguel	50	Fresh	Urban	Liberia, Río Liberia, upstream of WWT
7	Tap	Urban	UCR Campus	51	Fresh	Urban	Río Liberia, near hospital
8	Salt	Urban	Puntarenas Estuary, exit, near the Ferry	52	Fresh	Rural	Cañas, Tilapias El Sol, irrigation canal
9	Salt	Urban	Puntarenas beach, near cruise ship	53	Fresh	Rural	Cañas, Río Corobicí
10	Salt	Urban	Puntarenas dock	54	Fresh	Rural	Cañas, irrigation channel
11	Salt	Urban	Puntarenas, 1 km east of dock	55	Fresh	Rural	Arenal, Bahía San Luis
12	Salt	Urban	Puntarenas, Fertica channel, dry dock	56	Salt	Rural	Parque Nacional Manuel Antonio, beach
13	Salt	Urban	Puntarenas, Fertica channel	57	Fresh	Urban	Quepos, downstream of hospital
14	Salt	Urban	Puntarenas, north side of peninsula	58	Fresh	Rural	Quepos, upstream of hospital
15	Fresh	Rural	Río Barranca, end of low tide	59	Fresh	Urban	Quepos, downstream of city, low tide
16	Salt	Rural	Puntarenas, Port Caldera	60	Fresh	Urban	Jaco
17	Fresh	Rural	Puntarenas, Port Caldera, Mata de Limón	61	Fresh	Rural	Río Tárcoles
18	Fresh	Urban	San Isidro, upstream of wastewater	62	Fresh	Urban	Puntarenas, Talmana Estuary, residential
19	Fresh	Effluent	San Isidro, downtream of wastewater	63	Fresh	Rural	Río Bebedero, falling tide
20	Fresh	Rural	Dominical Beach	64	Fresh	Rural	Río Tempisque
21	Fresh	Urban	Cortés, Río Balsar, upstr Osa hospital	65	Salt	Rural	Golfo de Nicoya, ECMAR dock, high tide
22	Fresh	Rural	Río Térraba, Palmar Norte	66	Fresh	Rural	Río Lagarto
23	Fresh	Rural	Río Esquinas, from Road	67	Fresh	Rural	Santa Marta, Térraba-Sierpe wetland
24	Fresh	Urban	Golfito, Río Cañazas	68	Fresh	Rural	El Caite, Térraba-Sierpe wetland
25	Salt	Urban	Golfo Dulce, Punta Gallardo	69	Fresh	Rural	Río Térraba, Samu, Térraba-Sierpe wetland
26	Salt	Open water	Golfo Dulce, Punta Gallardo	70	Fresh	Rural	Isla Loros, Térraba-Sierpe wetland
27	Salt	Urban	Golfo Dulce, Río Tigre, coral reef	71	Fresh	Rural	Río Sierpe, Térraba-Sierpe wetland
28	Salt	Urban	Golfo Dulce, Puerto Jiménez	72	Salt	Urban	Limón, Cieneguita
29	Salt	Urban	Golfo Dulce, Puerto Jiménez	73	Salt	Urban	Limón, Vargas Park
30	Salt	Urban	Golfo Dulce, near Hotel Cocodrilo	74	Salt	Urban	Limón, near hospital, accumulation of waste
31	Salt	Rural	Golfo Dulce, Río Coto-Colorado	75	Salt	Urban	Limón, Moím pier
32	Salt	Rural	Golfo Dulce, Río Coto-Colorado, inland	76	Fresh	Urban	Stream running through UCR campus
33	Salt	Rural	Golfo Dulce, Coto-Colorado mouth	77	Fresh	Urban	Río Torres, Barrio Tournon
34	Salt	Urban	Golfo Dulce, Golfito, end of low tide	78	Fresh	Urban	River at end of airport runway, Juan Santamaría
35	Salt	Urban	Golfo Dulce, Golfito, Isla Pelícano	79	Fresh	Urban	Río San Joaquín de Flores, near medical clinic
36	Salt	Urban	Golfo Dulce, Golfito, Purruja Estuary	80	Fresh	Urban	Río Pirro
37	Salt	Urban	Golfo Dulce, Golfito	81	Fresh	Urban	Río Bermúdez, btwn San Pablo-Santo Domingo
38	Salt	Urban	Golfo Dulce, Golfito, near cemetary	82	Fresh	Rural	Ciudad Quesada, upstream of city
39	Salt	Urban	Golfo Dulce, Golfito, municipal pier	83	Fresh	Urban	Ciudad Quesada, upstream of city, residential
40	Salt	Urban	Golfo Dulce, Golfito, hospital	84	Fresh	Urban	Ciudad Quesada, upstream, before confluence
41	Fresh	Urban	Golfo Dulce, Golfito, urban drainage	85	Fresh	Urban	Ciudad Quesada, Río Platanar, in city
42	Fresh	Urban	Golfo Dulce, Río Coto-Colorado, near ferry	86	Fresh	Urban	Ciudad Quesada, Río Platanar, hospital and residential drainage
43	Fresh	Urban	Golfo Dulce, Golfito, ditch drainage for hospital	87	Fresh	Urban	Cartago, Río Purires
44	Fresh	Urban	Río Corredores, Neily, downstream of hospital	88	Fresh	Urban	Cartago, Río Reventado, downstream of slum homes
45	Fresh	Urban	Río Corredores, Neily	89	Fresh	Urban	Cartago, Quebrada Creek,center of city
46	Fresh	Urban	Río Java, San Vito, near animal feed factory	90	Fresh	Urban	Cartago, Río Agua Caliente, la Ciudad de los Niños (Hervidero)
47	Fresh	Rural	Río Térraba	91	Fresh	Urban	Cartago, Río Toyogres, drains san Rafeal de Oreamuno

response factor was found to vary less than <5% RSD (relative standard deviation) for all compounds. Sample extracts with compound concentrations outside of the linear range (previously given) were diluted with methanol and rerun. Final concentrations presented here have been adjusted for any dilution and concentration factors utilized during analysis.

SPE extraction recoveries using fresh and saltwater were generated by fortifying 350 mL of each matrix in triplicate with

175 μL of a 200 μg L^{-1} standard mix solution, yielding a final spiked concentration of 100 ng L^{-1}, within reported environmental ranges for surface waters for these analytes. Conductivity, total dissolved solids and salinity for the fresh and saltwater used for extraction recovery calculations were 0.78 μS m^{-1}, 0.26 g L^{-1} and 0.35 g L^{-1}, and 32 mS m^{-1}, 28.58 g L^{-1} and 19.57 g L^{-1}, respectively. Recoveries were typically above 70%, and variation was within 10% between fresh and saline matrices, with some exceptions. Limit of quantitation (LOQ) was calculated using the lowest detectable concentration on the instrument (IDL) with a signal to noise ration of 3 multiplied by the concentration factor divided by the sample volume. LOQs varied considerably between the first and second block of samples collected and analyzed (block 1; 1/9/2009 to 1/29/2009; block 2; 2/13/2009 to 2/16/2009). This difference was due to instrumental sensitivity problems during analysis of block 2 samples; therefore, two LOQ's are given in Table 2.

3. Results and discussion

3.1. General trends

Surface waters collected from coastal and interior areas were analyzed for 16 classes of PPCPs, and Fig. 2 presents the percent of total measured concentration for each class. The predominant PPCP class detected in surface water samples was dominated by the central nervous system (CNS) stimulant (caffeine). This is not surprising since caffeine has been shown to be ubiquitous in waters affected by domestic wastewater pollution (Buerge et al., 2003), and is commonly found as an ingredient in food products. Other classes of note, in order of contribution, included tetracycline antibiotics, non-steroidal anti-inflammatory drugs (NSAIDs), the lipid regulator gemfibrozil (tradename Lopid) and the analgesic acetominophen. These trends are not surprising since all of

Table 2 – Classification, MS/MS ions, method extraction recovery (mean ± sd) and limits of quantitation (LOQ) for the analytes of interest in this study.

Classification[a]	Compound	MS Precursor	Transition ions		Recovery [%]		LOQ[c] [ng L^{-1}]	
			Quant	Confirm	Fresh water	Salt water	Fresh water	Salt water
Analgesic, antipyretic	Acetominophen	152.2	110.0	93.0	97 ± 17	43 ± 6	7, 37	15, 17
Anticonvulsant, antidepressant	Carbamazepine	237.1	194.0	192.0	115 ± 4	95 ± 6	1, 3	1, 3
Antimicrobial	Triclosan	286.8/289.0	35.1		79 ± 6	47 ± 25	10, 91	6152
Antohypertensive	Diltiazem	415.1	178.0	149.9	82 ± 3	83 ± 13	1, 1	1, 1
Bacteristatic	Trimethoprim	291.1	230.0	230.0	85 ± 8	64 ± 4	7, 10	5,13
CNS Stimulant	Caffeine	192.2	138.0	110.0	85 ± 9	110 ± 34	18, 498	24, 386
Caffeine metabolite	Paraxanthine	181.2	124.0	96.0	78 ± 8	88 ± 10	8, 12	7, 14
Fluoroquinolone	Ciprofloxacin	332.1	314.0	230.9	55 ± 8	103 ± 5	21, 251	31, 133
	Norfloxacin	320.1	302.0	230.9	51 ± 8	100 ± 10	20, 498	38, 255
	Ofloxacin	362.1	318.0	344.0	64 ± 2	70 ± 3	22, 90	14, 82
Histamine H_2-Receptor antagonist	Cimetidine	253.1	159.0	117.0	78 ± 13	112 ± 14	6, 9	6, 8
	Ranitidine	315.1	176.0	130.0	54 ± 3	79 ± 9	5, 9	6, 7
Lincosomide	Clindamycin	425.2	126.0		87 ± 2	97 ± 21	3, 12	3, 11
	Lincomycin	407.1	126.0	359.1	102 ± 11	67 ± 8	1, 2	1, 2
Lipid regulator, metabolite	Gemfibrozil	249.2	121.1		91 ± 16	77 ± 5	41, 275	35, 325
	Clofibric acid	213.1	126.8	85.0	89 ± 2	110 ± 4	11, 18	14, 15
Macrolide	Clarithromycin	748.5	158.0	590.3	79 ± 10	90 ± 10	5, 10	5 10
	Erythromycin[b]	716.4	158.0	558.0	99 ± 8	77 ± 14	8, 55	11, 42
	Roxithromycin	837.6	158.0	679.3	90 ± 13	77 ± 10	116, 157	100, 183
	Tylosin	916.4	174.0	156.0	70 ± 5	81 ± 42	974, 4909	844, 5666
NSAIDs	Diclofenac	293.8	249.8		113 ± 3	103 ± 7	12, 15	14, 14
	Ibuprofen	204.9	161.0	159.0	98 ± 3	100 ± 5	5, 984	5, 969
	Indomethacin	358.0	139.0	174.0	95 ± 2	82 ± 7	7, 9	6, 10
	Ketoprofen	255.1	209.0	105.0	75 ± 8	90 ± 5	6, 20	7, 17
Sulfanomide	Sulfadimethoxine	311.1	156.0	108.0	82 ± 5	76 ± 5	1, 6	1, 6
	Sulfamethazine	279.1	186.0	124.0	74 ± 3	84 ± 4	3, 7	4, 6
	Sulfamethoxazole	254.1	156.0	92.0	88 ± 4	63 ± 4	11, 14	8, 19
	Sulfathiazole	256.1	156.0	92.0	103 ± 6	71 ± 9	4, 7	5, 6
Tetracyclines	Doxycycline	445.1	428.0	320.8	79 ± 14	75 ± 10	18, 1607	19, 1693
	Oxytetracycline	461.2	426.0	443.1	109 ± 11	101 ± 9	<1, 156	<1, 144
	Tetracycline	445.1	410.0	153.9	79 ± 8	75 ± 6	44,4133	2, 4353
β-lactam	Oxacillin	402.1	143.9	186.0	77 ± 8	70 ± 3	63, 6750	70, 7425
	Penicillin G	335.1	127.9	160.0	65 ± 6	75 ± 13	84, 100	86, 97
Skin care product ingredient	Salicylic acid	136.8	92.8		60 ± 12	110 ± 20	11, 19	6, 47

a CNS: central nervous system; NSAIDs: non-steroidal anti-inflammatory drugs.
b Determined as Erythromycin-H_2O.
c Two LOQ's given based on change in instrument sensitivity for samples processed after 2/10/2009.

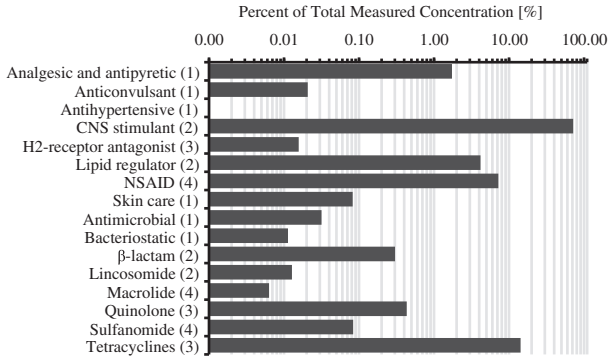

Fig. 2 – The percent of total measured concentration by drug class. Value in parenthesis indicates number of compounds in class.

these are widely available and relatively cheap, with possible heavy use in Central American countries.

Table 3 provides the summary statistics for PPCP compounds analyzed in surface waters in this investigation. Complete data for each location can be found in Table S-2 in the supplementary material. Of the 34 compounds chosen for analysis, seven were not detected in any of the environmental water samples above the LOQ; clofibric acid, diltiazem, erythromycin, penicillin G, ranitidine, roxithromycin, and tylosin. The five compounds detected with greatest frequency were doxycycline (77%), sulfadimethoxine (43%), salicylic acid (41%), triclosan (34%) and caffeine (29%). The six compounds found in highest concentrations include: caffeine (1.12 mg L^{-1}), doxycycline (74 μg L^{-1}), ibuprofen (37 μg L^{-1}), gemfibrozil (17 μg L^{-1}), acetominophen (13 μg L^{-1}) and ketoprofen (10 μg L^{-1}). Detection frequency in general for all other compounds was low (<30%), and median values were below the LOQ for all but one compound (doxycycline). This presents the heterogeneous nature of PPCP contamination in Costa Rica, linked to untreated wastewaters from both urban and rural sources. This trend is similar to what has been found in other studies around the globe.

3.2. *Caffeine*

Caffeine is often the compound reported with the highest frequency in similar studies and has previously been used as an indicator of anthropogenic contamination (Seiler et al., 1999; Buerge et al., 2003). In South Korea caffeine was detected in eight out of eight surface water samples and six out of seven effluent samples with a mean concentration of 105 ng L^{-1} (Kim et al., 2007). In Costa Rican surface water samples caffeine was detected in only 29% of samples, likely due to rapid biodegradation. The presence of paraxanthine (20%), a metabolite of caffeine supports this idea. The highest concentration detected in any sample was for caffeine, with 1.1 mg L^{-1}, obtained from the sediment-laden waters of Rio Java downstream from San Vito (CRP-46). Upstream from this sampling location, coffee beans are washed frequently during processing, likely the source of the high concentration. Caffeine was found in locations with both rural and urban influences, albeit with low frequency and typically low concentrations. The 75th percentile concentration for caffeine was 21 ng L^{-1}, well below the mean caffeine concentrations reported previously in Brazil (Ferreira, 2005), but similar in magnitude to those reported in surface water in Europe (Loos et al., 2009) and the United States (Kolpin et al., 2002). The concentrations reported here are higher than those typically found in wastewater effluent samples collected in the United States (e.g. Spongberg and Witter, 2008) or Europe (Buerge et al., 2003). Regular influxes of caffeine from point sources present in the drainage areas of rivers sampled, including coffee manufacturing facilities and rural wastewater, are likely, though the degradation properties are largely unknown in this tropical environment.

3.3. *Carbamazepine*

Carbamazepine is a prescription medication used to control certain types of seizures, mental illnesses and depression and has been shown to persist in the aquatic and terrestrial environment (i.e. Tixier et al., 2003). Along with caffeine it has been proposed as an anthropogenic waste marker (Clara et al., 2004) and is typically detected in a majority of aquatic samples. Carbamazapine was detected in seven out of eight surface waters in South Korea with a mean concentration of 25 ng L^{-1} (Kim et al., 2007). In Costa Rican surface waters carbamazepine was detected in only 10% of samples, with a maximum concentration of 82 ng L^{-1}(site 59, downstream of a large tourist area). Concentrations at other sampling sites were typically close to the LOQ (1–3 ng L^{-1}), indicating low usage for this compound in Costa Rica. This could be due to the availability and cost of the drug in this country. Prescription records and pricing were not readily available.

3.4. *Antibiotics*

Antibiotic residues from varying classes have been reported in surface waters around the globe. In this study several antibiotic compounds were detected with frequencies greater than 10%: doxycycline (77%), sulfadimethoxine (43%), norfloxacin (28%), tetracycline (22%), ciprofloxacin (15%) and sulfamethazine (12%). Doxycycline had a maximum concentration of 74 μg L^{-1}. Neither Hirsch et al. (1999) in Europe or Focazio et al. (2008) in the United States detected any doxycycline in aquatic samples in sewage treatment plant discharge or surface waters. Tetracycline antibiotics have been shown to dissipate rapidly in the aqueous environment, and the relatively high occurrence and concentration found here likely indicates high usage in Costa Rica compared to previously reported study areas. The broad-spectrum antibiotic ciprofloxacin was detected with a maximum concentration of 740 ng L^{-1} Martins et al. (2008) provides a detailed table of comparison values for ciprofloxacin, with concentrations in surface waters as high as 6.3 μg L^{-1}, much higher then observed during our sampling campaign. Sulfamethoxazole was only detected above LOQ in 4% of samples, unlike similar studies in Taiwan (96%, maximum concentration of 5.8 μg L^{-1}, Lin et al., 2008) and in Vietnamese and Japanese waters (Managaki et al., 2007).

The input and persistence of antibiotics and antimicrobials, such as doxycycline, into the environment is of major concern due to the possible development of bacterial resistance to these compounds (e.g. Khachatourians, 1998). Further

Table 3 – Summary statistics for PPCP compounds detected in Costa Rican surface water samples ($n = 86$).

Compound	% Frequency detected	Maximum [ng L^{-1}]	Median [ng L^{-1}]
Acetominophen	27	13,216	<15
Caffeine	29	1,121,446	<24
Carbamazepine	10	82	<1
Cimetidine	8	63	<6
Ciprofloxacin	15	740	<31
Clarithromycin	3	63	<5
Clindamycin	6	8	<3
Diclofenac	8	266	<14
Doxycycline	77	73,722	74
Gemfibrozil	26	17,036	<41
Ibuprofen	19	36,788	<5
Indomethacin	20	2323	<7
Ketoprofen	27	9808	<7
Lincomycin	5	11	<1
Norfloxacin	28	1744	<38
Ofloxacin	3	335	<22
Oxacillin	2	7571	<70
Oxytetracycline	3	428	<1
Paraxanthine	20	592	<8
Salicylic acid	41	274	<11
Sulfadimethoxine	43	20	<1
Sulfamethazine	12	1626	<4
Sulfamethoxazole	3	56	<11
Sulfathiazole	5	39	<5
Tetracycline	22	93	<44
Triclosan	34	263	<11
Trimethoprim	9	122	<7

Compounds not detected above LOQ: Clofibric acid, Tylosin, Ranitidine, Diltiazem, Roxithromycin and Penicillin G.

study is needed to identify if input is larger or if any conditions prevalent in Costa Rica and tropical systems slow the environmental persistence of antibiotics.

3.5. *Geographic trends*

The site with the highest total concentration of all compounds was CRP-46, on the Rio Java. However, this sample was overwhelmed by the high amount of caffeine and has only four other compounds detected above LOQ, norfloxacin paraxanthine (a metabolite of caffeine), salicylic acid and triclosan (an antimicrobial ingredient in many personal care products). Two sites had the highest compound frequencies, both 59%: CRP-43, a ditch located downstream from a regional hospital in Golfito (on Golfo Dulce) collecting runoff from an effluent pipe; and CRP-49, sampled directly at the effluent pipe of a wastewater treatment oxidation pond in the city of Liberia. Compounds with high concentrations in both of these wastewater dominated samples include caffeine, gemfibrozil, doxycycline, paraxanthine, oxacillin, diclofenac, triclosan, and ibuprofen. Four compounds (acetominophen, oxacillin, sulfamethazine and triclosan) were not present in the oxidation pond effluent but were present in the hospital effluent dominated ditch. In general the concentrations in the oxidation pond effluent were 1–2 orders of magnitude higher then in the hospital effluent and the rest of the surface water samples with detections. The range of compounds and concentrations for the oxidation pond effluent are similar with those typically detected in other WWTP effluents (Spongberg and Witter, 2008; Stumpf et al., 1999).

CRP-59, downstream from the tourist destination city of Quepos, sampled at low tide, also had high frequency (56%) and similar concentrations to the Liberia effluent sample. The sample was collected at a large national park (Parqué National Manuel Antonio), indicating the possibility of wastewaters from the surrounding hotels and area contaminating the park environment. CRP 61, taken on the Rio Tarcoles, also had a high frequency of compound detection (44%). The high contamination found at this site poses a risk to the present ecosystem, known as crocodile habitat, because of possible bioaccumulation of some chemicals in the food chain This was also one of the only sites (along with CRP-22 and 23) to have detection for oxytetracycline. Site CRP 89, located in the Moline ravine, was located downstream from the city of Cartago and represented the main drainage for the city. This sample was full of suspended material and had a bad odor at the time of collection. Analysis identified 14 of the 34 compounds above LOQ in this sample. The creek passes by new developments and the local hospital. All samples (CRP 87-91) from the Cartago area had detection frequencies >20% and high concentrations for doxycycline, gemfibrozil and ketoprofen.

The Golfito area was found to be one of the most polluted sites in Costa Rica with respect to PCBs and hydrocarbons (Spongberg, 2004; Spongberg and Davis, 1999). However, most of the sites around Golfito were among the cleanest with respect to PPCPs (~10% frequency), with the exception of the freshwater ditch leaving the hospital mentioned previously (CRP-43). Sites receiving drainage from the city, local cemetery and local hotel were comparable to samples from the middle of Golfo Dulce (CRP-26) used as a clean reference site. Other locations sampled within Golfo Dulce (CRP 25s-33) had fairly low frequency (3–12%), although doxycycline, sulfadimethoxine and triclosan were present in almost all samples. Other sites with clean samples (<6% frequency and low concentrations) include the waters from the irrigation canal at Cañas (CRP-52 and 53), the mouth of the Barranca River in Puntarenas at the onset of rising tide (CRP-15) and the low tide samples taken along the downstream parts of the Rio Coto-Colorado (CRP-32, 33). The Terraba-Sierpe wetland along the Sierpe River (CRP 71) also had low frequency of detection, although the concentration of ketoprofen was very high (10 ug L^{-1}), similar to another site sampled in the wetland.

3.6. *Comparison of fresh and salt water locations*

This study sampled both fresh and saltwater locations. In general, the freshwater samples had higher concentrations and a greater number of detected compounds than the saltwater samples. This reflects both the dilution process into larger saltwater areas, and the selection of many fresh water sample sites based on proximity to potential pollution sources (urban runoff, point sources etc). The average concentration of the 34 PPCPs analyzed in the saltwater samples was 727 ng L^{-1}. However, the average for the Pacific samples was

only 75 ng L^{-1}. Several of the Pacific sites were chosen based on their remote locations and have been used in other studies as reference sites. However, even sites chosen close to shore near potential sources (e.g. Golfito samples) still had low values. The Caribbean sites had an average concentration of 4.5 μg L^{-1}. All of the Caribbean samples were taken near Limón near sites suspected of having a point sources, including near the local hospital (CRP-74). The data seem to reflect the presence of these point sources.

4. Conclusions

Surface water samples and one wastewater effluent sample were collected from saltwater and freshwater environments throughout Costa Rica for the analysis of 34 PPCP compounds in early 2009. Sampling locations were chosen based on previous contaminant studies and proximity to possible sources of PPCP compounds. Although a large number of sites had low PPCP occurrence and concentration, data indicate the potential for abundant contaminant levels in areas were urban and agricultural wastewaters are treated inadequately. Compound concentrations at sites near hospitals and other heavily used waters were higher than those reported in similar studies in other countries, indicating a need to treat these wastewaters. The over the counter painkiller acetominophen, along with tetracycline antibiotics, gemfibrozil and ketoprofen were commonly associated with areas receiving both urban and rural runoff. The high occurrence and concentrations found for doxycycline show the need to further understand both usage and environmental fate of antibiotic compounds in tropical environments. This study has shown that Costa Rica faces the same wastewater treatment and management challenges posed to the rest of the world when dealing with these new emerging contaminants of concern.

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Appendix. Supplementary data

Supplementary data related to this article can be found online at doi:10.1016/j.watres.2011.10.004.

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