Emergent contaminants in the wastewater effluents of two highly populated tropical cities

Sheila M. Soler-Llavina and Jorge R. Ortiz-Zayas

ABSTRACT

Exposure to the anthropogenic chemicals known as endocrine disrupting compounds (EDCs) may result in negative biological effects. Low levels of EDCs in the environment aggravate the problem as exposure is constant. Urban areas concentrate pollution as greater volumes are released from human activities. Water for public supply is particularly vulnerable as the sewage treatment facilities may not eliminate EDCs. The goal was to assess estrogenicity and effectiveness of removal of phthalates in primary and tertiary wastewater treatment facilities in urban cities in the tropical island of Puerto Rico. A yeast bioassay used to measure estrogenicity showed higher removal with tertiary treatment. However, results in the picomolar range suggest low doses of estrogenic compounds were being released to receiving waters. For the phthalates, solid phase extraction and gas chromatography-mass spectrometry analyses revealed removals ranging from 42.9% to 92.4% with tertiary treatment. More than 90% removal was achieved for benzylbutyl phthalate, dibutyl phthalate and bis-2-ethylhexyl phthalate. However, concentrations ranging from 0.86 to 1.29 ppm for the phthalates in the outflow were detected even at the tertiary waste water treatment plant effluent implying failure of EDC removal. These results can assist managers in evaluating pollution control technologies to ameliorate the impacts of EDCs in the tropics.

Key words | emerging pollutants, estrogenic activity, phthalates, receiving waters, wastewater treatment

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NOMENCLATURE

°C	Celsius degrees
ft	feet
GC/MS	gas chromatography mass spectrometry
hr	hour
km^2	square kilometers
m	meters
MGD	million gallons per day
mL	milliliter
mol/L	moles per liter
µg/L	micrograms per liter
µM	micromolar
ng/L	nanograms per liter
pM	picomolar
ppm	parts per million
R^2	goodness of fit
SPE	solid phase extraction
WWTP	wastewater treatment plant

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INTRODUCTION

Growing evidence associates synthetic chemicals with changes in water quality and detrimental biological effects in aquatic ecosystems. Environmental and industrial chemicals may interfere with the endocrine systems of both humans and wildlife hence they are termed endocrine disrupting compounds (EDCs) (Colborn & Thayer 2000).

Effects include alteration of the normal biological signaling that controls development and reproduction among other internal functions controlled by the endocrine system (Cooper & Kavlock 1997; Anway *et al.* 2005; Barber *et al.* 2007) such as reduced fertility, feminization, reproductive organ anomalies and changes in the sexual behavior of various aquatic organisms (Pal *et al.* 2010). Detrimental effects have also been observed in the human population thus posing a risk to public health (Fein *et al.* 1984; Hatch *et al.* 1998, 2001; Palmer *et al.* 2001; Lathers 2002; Focazio *et al.* 2008; Rudel *et al.* 2011). A wide variety of EDCs, including plasticizers such as phthalates, are common in surface waters as mixtures of high concentrations of low-potency disruptors and small amounts of very powerful ones (Kolpin *et al.* 2002; Focazio *et al.* 2008).

The annual global production of plastics has been estimated as about 150 million tons (Li *et al.* 2016). The phthalates are widely used in many everyday materials as plasticizers and also in non-plastic products (Zota *et al.* 2014). Plasticizers are merged with glasslike materials to increase their flexibility (Graham 1973); however, as this merge is not a strong chemical bond, phthalates can leach out of products and be released into the environment (Zota *et al.* 2014). These compounds have been found in all types of environmental and biological samples (Fromme *et al.* 2002; Vethaak *et al.* 2005) and in the effluents of wastewater treatment plants (WWTPs) (Fromme *et al.* 2002; Clara *et al.* 2010). As EDCs, including phthalate compounds, are not removed by WWTPs, they are left free to interact with humans and other organisms that may ingest them downstream. In humans, phthalate esters have been found in blood, seminal fluid, amniotic fluid, breast milk, saliva and urine (Bouma & Schakel 2002; Calafat *et al.* 2004; Silva *et al.* 2004; Hogberg *et al.* 2008; Pant *et al.* 2008; Zota *et al.* 2014). The enormous volume of plastics in consumer and industrial products, their persistence and their routine disposal to the environment helps to explain why human exposure to phthalate esters is nearly ubiquitous.

Urban-impacted streams tend to receive larger loading rates of synthetic chemicals compared to pristine rivers, even at low levels of catchment urbanization (Kolpin *et al.* 2002; Hatt *et al.* 2004). Urbanized areas are more prone to discharge their wastes into wastewater collection and treatment systems which, depending on their treatment infrastructure, can remove some pollutants from water. Puerto Rico is a tropical US territory with one of the highest population densities in the world (447.669 inhabitants/km^2 (http://factfinder.census.gov) and has experienced rapid rates of urbanization. Pollution of aquatic resources became a critical issue in the island, especially after the 1950s when industry began to surpass agriculture as the base of the economy, mainly those related to pharmaceuticals, electronics, textiles and clothing, petrochemicals, processed foods and tourism (Hunter & Arbona 1995; Grau *et al.* 2003). Industrialization led to rapid population growth and expansion of metropolitan areas, especially San Juan (Pares-Ramos *et al.* 2008). This represents a hotspot of energy and material consumption contributing to the load of pollutant discharges to the surface waters, most likely including estrogenic compounds. Puerto Rico treats only about 57% of its sewage in WWTPs and, of that, 71% receives primary treatment in regional facilities located near the coasts that discharge treated effluents through ocean outfalls (F. Quiñones, personal communication). The effluents from these facilities are rich in nitrogen, organic matter and suspended solids (Ortiz-Zayas *et al.* 2006) but EDC loads have not been determined. Nationally, although the Environmental Protection Agency has established criteria for compounds that end up as EDCs, there are no formal regulations against their effects in living organisms thus, it is critical to regulate environmental concentrations since exposure to these compounds is constant. Moreover, WWTPs are not designed to remove EDCs that could be persistent and, thus, not metabolized or bioremediated, releasing them back into the environment (Basile *et al.* 2011). Given their high operational costs, tertiary treatment plants are not common in Puerto Rico, although efforts have been made recently to improve the operation of the WWTPs, efforts mainly being directed to nutrient loads and not EDCs. In Puerto Rico, there are only three major WWTPs that provide tertiary treatment and with regulated discharge to rivers and creeks that do not include potential EDCs. Although some reduction in EDC loads from WWTPs occurs with secondary and tertiary treatment (Basile *et al.* 2011), the extent of this reduction and the contribution that these effluents make to EDC loads in streams are not well known, especially in tropical countries.

Despite the efforts made to improve the quality of effluents in Puerto Rico, WWTP effluents still affect water quality of receiving streams (Figueroa-Nieves *et al.* 2014). Inputs from WWTPs to streams contribute substantially to changes in water quality, thus potentially affecting downstream ecosystems. Puerto Rico has set as a critical research priority the evaluation of the impacts of high nutrient loads from WWTPs, where coastal ecosystems are highly vulnerable to nutrient inputs due to high population densities and rapid nutrient transport from land to the ocean (Ortiz-Zayas *et al.* 2006). EDC loads in the effluents and its possible effects are unknown.

MATERIALS AND METHODS

Study sites

Two regional WWTPs (RWWTPs) located in two large cities in Puerto Rico were sampled. These facilities provided either primary or tertiary treatment for large urban areas.

Primary treatment

The Puerto Nuevo RWWTP (Figure 1) provides primary treatment of wastewater generated in the San Juan Metropolitan Area and discharges to the Atlantic Ocean.

Tertiary treatment

The Caguas RWWTP (Figure 2) is intended to meet rigorous effluent quality standards including significant nutrient removal. The effluent is discharged into Río Bairoa, a tributary of Río Grande de Loíza and Lago Loíza, one of the most important sources of raw water for the San Juan Metropolitan Area aqueduct system.

Sample collection

Influent and effluent samples from each plant were assayed for estrogenic activity and the efficiency of removal of estrogenic activity by the WWTPs was determined based on the differences between influent and effluent estrogenic activity.

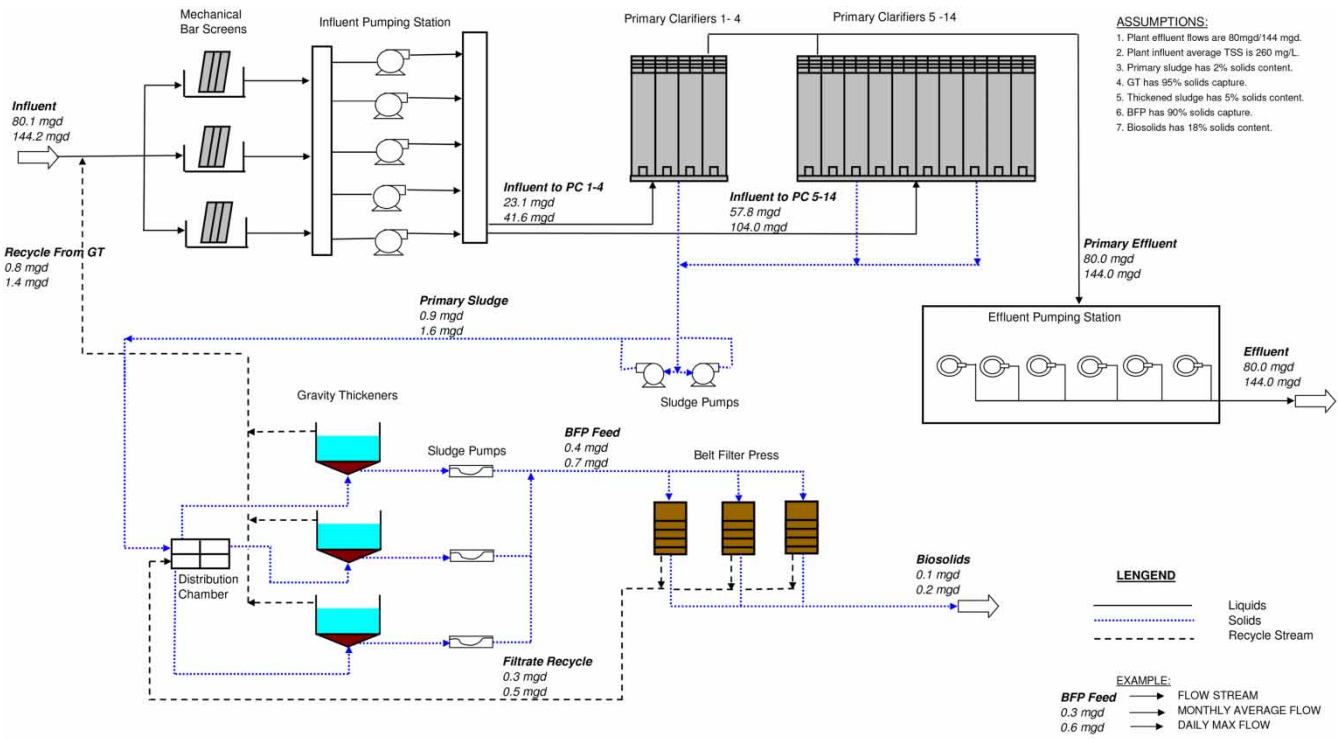

Figure 1 | Flow chart of the Puerto Nuevo WWTP (www.epa.gov/region02/water/permits.html).

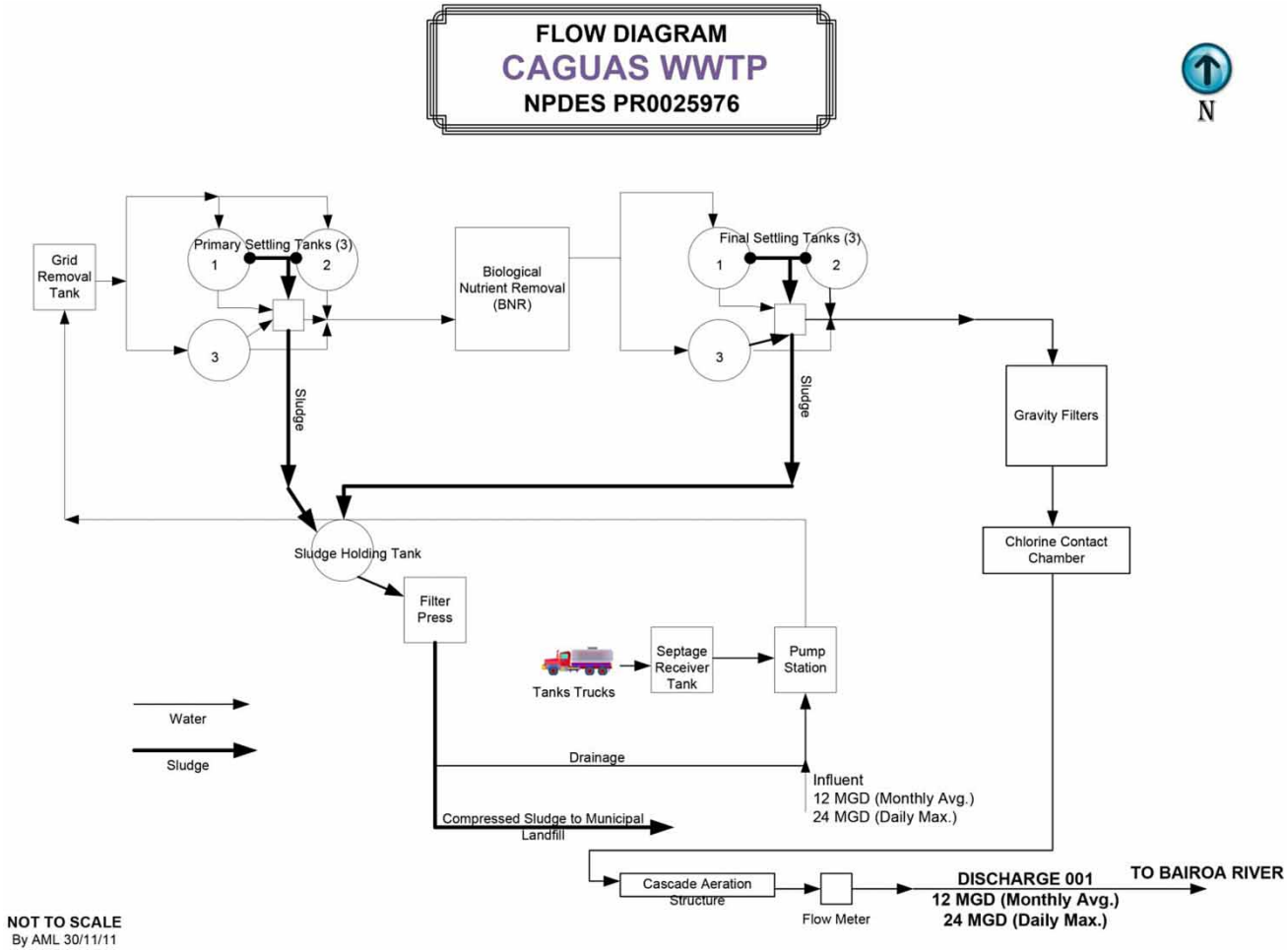

Figure 2 | Flow chart of the Caguas WWTP (www.epa.gov/region02/water/permits.html).

Four sampling events occurred from September to December 2012 at each plant (Table 1). Composite water samples were collected at regular time intervals over a 24 h period at each inflow and outflow station. The samples were collected by personnel of the Puerto Rico Aqueduct and Sewer Authority and handed to us immediately after collection. The composite sample was later analyzed in triplicate for estrogenic activity and concentration of phthalates. For quality control purposes, all samples were taken in amber glass bottles and stored below 4 °C during transportation. Glassware was cleaned using a rigorous cleaning process to eliminate interference and minimize microbial degradation of the analytes. Glassware was cleaned with Alconox® soap and rinsed with tap water, soaked in 10% HCL for 5 minutes and rinsed three times sequentially with tap, distilled and ultra-pure water and left to air dry, upside down. In the field, water bottles were rinsed with sample water three times before collecting the sample. At the lab, samples were immediately filtered using a 0.45 μm glass fiber filter. Samples were stored at 4 °C for no more than two days before estrogenic activity analysis.

Table 1 | Description of sampling events at each WWTP

Location	Type of treatment	Sampling event	Date
Puerto Nuevo	Primary	1st	11/6/2012
		2nd	11/8/2012
		3rd	11/13/2012
		4th	11/15/2012
Caguas	Tertiary	1st	9/21/2012
		2nd	11/27/2012
		3rd	11/29/2012
		4th	12/18/2012

Recombinant yeast assay

A receptor-mediated β-galactosidase reporter yeast assay was used as previously described (Balsiger *et al.* 2010) to detect estrogenic activity in wastewater samples. A standard calibration curve of 17β-estradiol (E2 mol/L) was prepared in ethanol and assayed along with the samples following the same procedure. The total estrogenic activity of the unknown samples was determined according to the response of the assay and interpolated to a dose–response curve of the standard compound E2 in mol/L and appropriately converted to ng/L of 17β-estradiol estrogen equivalents (EEq). The plates were read in a Tecan Infinite 200Pro luminometer.

Chemical analyses

Extraction of phthalates compounds

We focused on five phthalates compounds: dimethylphthalate (DMP); diethyl phthalate (DEP); dibuthyl phthalate (DBP), benzylbutyl phthalate (BBP) and bis-2-ethylhexyl phthalate (DEHP). The concentrations of these phthalates were determined in samples taken at two RWWTPs from September to December 2012. Water samples were pre-concentrated using solid phase extraction (SPE). Envi-Chrom P 500 mg glass cartridges were conditioned with 6 mL ethyl acetate, 6 mL methanol and 6 mL nanopure water in sequence. Then, 100 mL of sample was loaded to the cartridge. After passing the sample, the cartridge was washed with 3 mL nanopure water to eliminate possible polar interferences from the matrix. Cartridges were dried under vacuum for 15 minutes, centrifuged for 30 minutes and exposed to a N_2 flow for 30 minutes. Analytes were eluted with 6 mL ethyl acetate and evaporated to 0.5 mL by a gentle stream of nitrogen gas and reconstituted to a final volume of 1 mL in ethyl acetate.

Detection

The concentrated extract (1 μL) was injected into the gas chromatography mass spectrometry (GC/MS) system. Samples were heated to an initial temperature of 50 °C with an 8 °C/min. ramp to 260 °C and held for 40 minutes with ultrapure helium as carrier gas. Target compounds were measured based on the following quantification ions: DMP: m/z = 163; DBP, DEP, BBP and DEHP: m/z = 149 (Figure 5). Data acquisition was performed in the full scan mode measuring from m/z 50 to 550. Six-point calibration curves were conducted in the range 1–100 ppm. The linear response of the curves produced goodness of fit (R^2) higher than 0.99 for all compounds.

Statistical analyses

The reported data are the result of four independent experiments with all samples measured in triplicate within each experiment. Graphpad Prism trial version 6 and PAST version 3.01 were used for the paired *t*-tests for differences between inflow and outflows and repeated measures one way analysis of variance (ANOVA) followed by a post-hoc Tukey test for differences between treatment technologies. A p value <0.05 was used to represent a statistically significant difference.

RESULTS

Estrogenic activity

Table 2 summarizes the removal capacity of estrogenic activity for both the primary and tertiary RWWTPs. Estrogenic activity removal was significantly different between inflow and outflow in the tertiary RWWTP (paired sample $t(22) = 5.062$, $p = <0.001$), as opposed to the primary RWWTP where the inflow and outflow showed no significant differences between them (paired sample $t(22) = -0.617$, $p = 0.543$) (Figure 3).

Comparing the outflows of both levels of treatment, the *t*-test shows statistically significant differences between the

Table 2 | Estrogenic activity (ng/L EEq) in the inflow and outflow of a primary and tertiary RWWTPs ($n = 12$)

Treatment	Mean	SE
	Inflow	
Primary	0.7823	0.0558
Tertiary	0.9133	0.0580
	Outflow	
Primary	0.8256	0.0424
Tertiary	0.4998	0.0687

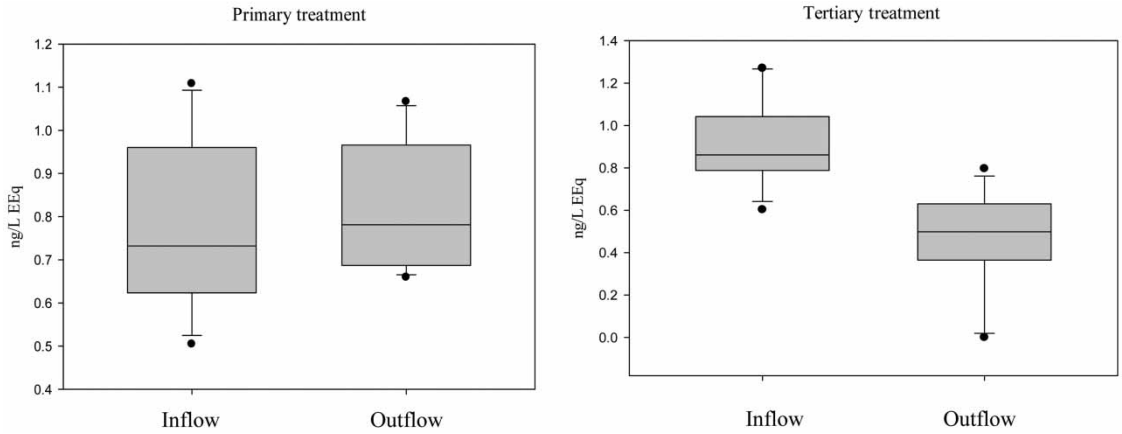

Figure 3 | Mean comparison for estrogenic activity ($n = 12$) in the inflow and outflow at each level of treatment technology. The error bars represent the standard error.

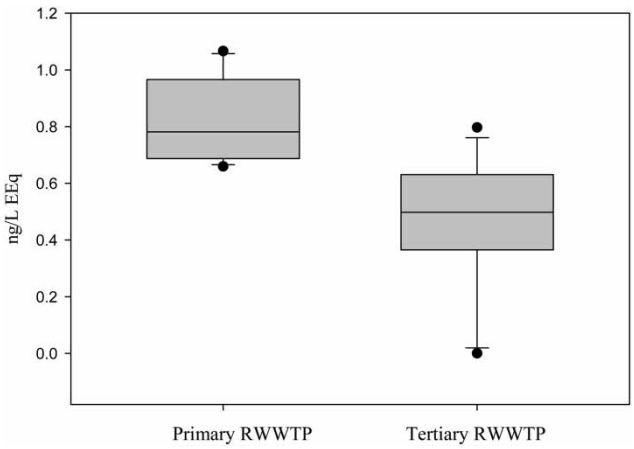

Figure 4 | Comparison between outflow estrogenic activity by level of treatment (mean and standard error are shown, $n = 12$).

outflows of the two WWTPs (paired sample $t(21) = -4.498$, $p = <0.001$) (Figure 4). Therefore, the tertiary RWWTP was more effective in reducing estrogenic activity from the wastewater than the primary wastewater treatment technology.

Four sampling events in each RWWTP were performed independently. To test for temporal differences between events, a repeated measures one way ANOVA was performed. For the primary RWWTP, the test for the inflow showed no statistically significant differences, ($F\ (3,6) = 1.757$, $p = 0.255$), as well as the outflow, ($F\ (3,6) = 2.460$, $p = 0.160$) where there were no significant differences between sampling events. The tertiary RWWTP repeated measures one way ANOVA showed statistically significant differences between sampling events for the inflow, ($F\ (3,6) = 7.061$, $p = 0.021$). Post hoc comparison using the Tukey test revealed that the 2nd vs. 4th events and 2nd vs. 1st events differed significantly from the other sampling events. The outflow of the tertiary WWTP showed no statistically significant differences ($F\ (3,6) = 2.201$, $p = 0.189$).

Phthalate compounds

Measured, % change and p values in phthalate concentrations in raw and treated wastewater of the primary and tertiary WWTPs are listed in Table 3. We used a GC/MS instrument operating in the scan mode to analyze the samples for DMP, DEP, DBP, BBP and DEHP. As confirmed by mass spectral data, four of the peaks in the extracted ion chromatogram corresponded to compounds of the phthalate ester family (Figure 5). Phthalate esters were consistently detected at concentration levels ranging from 0.33 to 9.20 ppm in the inflow of the primary WWTP and from 0.29 to 6.89 ppm in the outflow (BBP > DEHP > DBP > DEP > DMP). A paired t-test shows significant differences in removal for DMP, DBP and DEHP (p values: <0.001, 0.010 and 0.023, respectively).

In the tertiary WWTP, the phthalates were detected in concentrations ranging from 0.52 to 16.92 ppm in the inflow (BBP > DBP > DEHP > DEP > DMP) and from 0.09 to 1.29 ppm in the outflow (BBP > DBP > DEHP > DMP > DEP). Between the inflow and the outflow, concentrations were consistently reduced. The percentage removal ranged from 42.9 to 92.4% (BBP = DBP > DEHP > DEP >

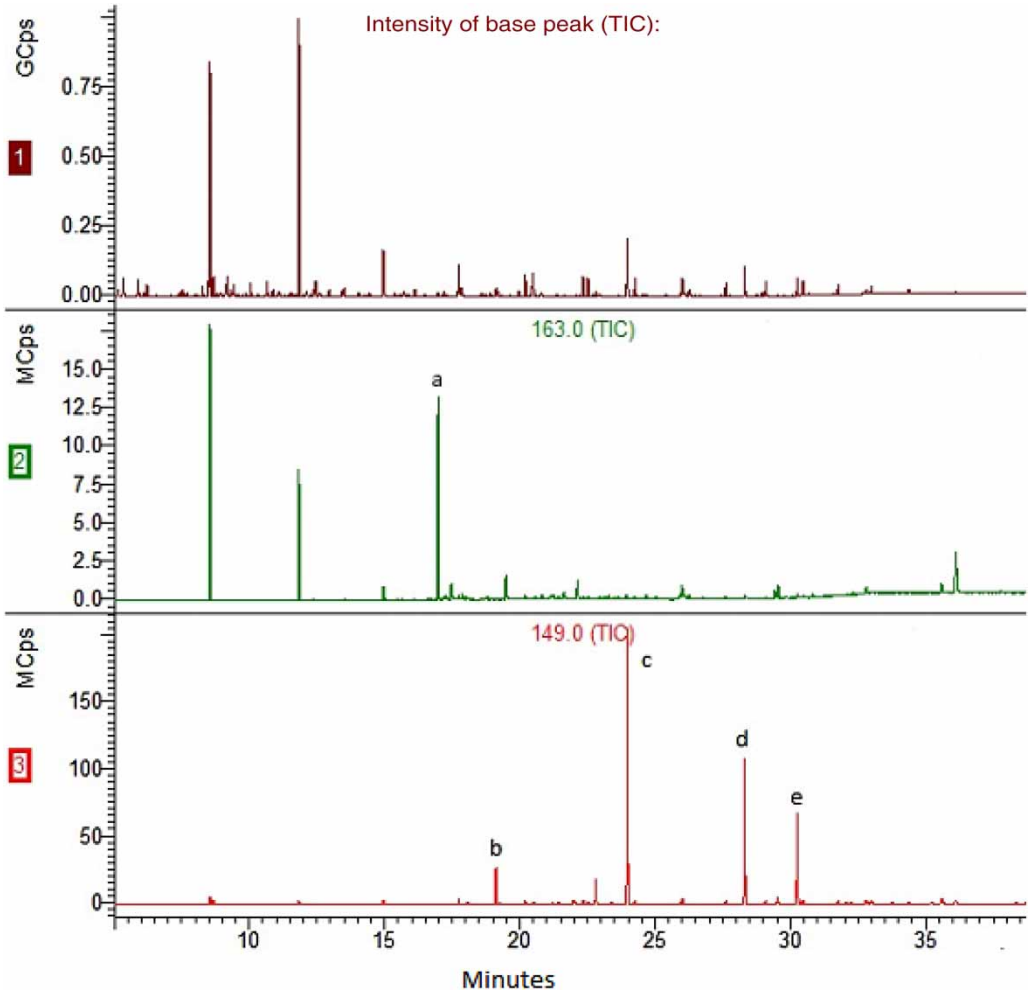

Figure 5 | (1) Total ion chromatogram (TIC) representative of a water sample from the WWTP; (2) and (3) extracted ion chromatograms for m/z = 163 and 149, respectively. The peaks identified as a, b, c, d and e correspond to compounds of the phthalate ester family (DMP, DEP, DBP, BBP, DEHP, respectively).

DMP). A paired-samples *t*-test was conducted to compare concentrations between the inflow and outflow. There was a significant difference between the concentrations of all compounds.

DISCUSSION

Anthropogenic chemicals are clearly present in the effluents of urban RWWTPs in Puerto Rico reflecting a strong level of urban influence. A large contribution of EDCs to aquatic ecosystems is attributed to the discharges of wastewater effluent from sewage treatment facilities (Harries *et al.* 1996; Ternes *et al.* 1999; Sando *et al.* 2005; Auriol *et al.* 2006; Kasprzyk-Hordern *et al.* 2009; Basile *et al.* 2011; Quinn-Hosey *et al.* 2012). In this study, low concentrations of estrogenic activity and phthalate compounds were detected in the effluents of both primary and tertiary treatment facilities making them contributors of EDCs to the environment. The type of technology at the RWWTPs appeared to have a notable effect on the estrogenicity of the effluents.

Efficiency of wastewater treatment technologies

Primary treatment facility

Our results show that the primary RWWTP discharges an effluent with estrogenic activity significantly higher than the tertiary RWWTP. For the phthalate compounds, DMP,

Table 3 | Measured phthalate concentrations (shown are mean and standard error) in raw and treated wastewater of the primary and tertiary RWWTPs ($n = 12$)

	Primary						Tertiary					
	Inflow (ppm)		Outflow (ppm)				Inflow (ppm)		Outflow (ppm)			
	Mean	SE	Mean	SE	% change	*p*	Mean	SE	Mean	SE	% change	*p*
DMP	0.33	0.08	0.29	0.04	−12.1	<0.01	0.52	0.21	0.29	0.13	42.9	0.02
DEP	0.466	0.03	0.45	0.04	−0.9	0.79	0.62	0.05	0.09	0.01	86.1	<0.01
DBP	8.07	6.16	5.15	2.88	−36.2	0.01	13.02	5.30	1.17	0.43	92.3	<0.01
BBP	9.20	7.69	6.89	4.50	−25.1	0.21	16.92	7.98	1.29	0.89	92.3	0.01
DEHP	6.25	5.27	5.62	3.01	−10.2	0.02	7.49	3.28	0.65	0.33	91.3	0.01

DBP and DEHP were significantly removed by the primary treatment but not DEP and BBP. It should be noted that, although the outflow showed a reduction in concentration in some of the compounds, primary WWTPs are not designed to eliminate chemical/toxic substances in the process (Davis & Cornwell 2008). However, adsorption to particulate matter and/or complex or micelle formation and posterior sedimentation of suspended particulate matter could aid in the removal of these compounds during the process. As previously reported, DEHP showed reduction through the sorption process (Dargnat *et al.* 2009). Nevertheless, these compounds will eventually be deposited in the environment through the sludge collected.

Tertiary treatment

The Caguas tertiary RWWTP showed a significantly higher removal of estrogenic activity and phthalate compounds. More than 90% removal was achieved for BBP, DBP and DEHP. The activated sludge process makes it more efficient in removing pollutants although soluble organic compounds resistant to biological degradation may persist in the effluent. Additionally, microbial species capable of degrading these compounds may not be present in the bioreactor, making them available in the effluent (Basile *et al.* 2011). Dual media effluent filters are efficient in removing particulates and thus, pollutants adsorbed to suspended solids but not soluble compounds. Therefore, soluble compounds and those that are not biologically degraded are prone to persist in the effluent and are later discharged into the receiving stream. The disinfection step through chlorination at the tertiary facility could also aid in the removal of EDCs via the oxidation process (Schilirò *et al.* 2009). Chlorine has the potential to react with some EDCs, namely antibiotics and estrogens, although chlorine oxidation is better achieved in acidic media as the reaction is pH dependent (Basile *et al.* 2011). However, this oxidation reaction is toxic and causes the formation of carcinogenic byproducts (Davis & Cornwell 2008; Schilirò *et al.* 2009). The effluent of the tertiary RWWTP is discharged into Río Bairoa, a tributary of Río Grande de Loíza which feeds Lago Loíza, a major drinking water source for the San Juan Metropolitan Area. Hence, the importance of producing high-quality water effluent.

Managing emerging contaminants in tropical settings

Inland effluents

Our results show that WWTPs can be a significant source of EDCs in receiving waters of tropical streams, as has already been demonstrated in temperate streams (Dargnat *et al.* 2009; Clara *et al.* 2010; Zolfaghari *et al.* 2014). Inputs from WWTPs have been shown to contribute to the estrogenic loads in receiving streams although there is high variation between sites and sampling seasons (Martinovic-Weigelt *et al.* 2013; Baldigo *et al.* 2014). Although EDCs in effluents and receiving surface waters are of increasing concern worldwide, it is still poorly understood how these emerging contaminants are persistent in the environment (Deblonde *et al.* 2011).

In rapidly developing tropical countries, managing emerging contaminants is challenging. For instance, the Caguas RWWTP effluent is discharged into Río Bairoa, a tributary of the Río Grande de Loíza, whose waters provide about 100 million gallons per day to the San Juan Metropolitan

Area through the Sergio Cuevas Water Filtration Plant. A wastewater treatment system not efficient in removing persistent chemical compounds could make them readily available in drinking water systems. However, high rates of river metabolism could have a role in minimizing their persistence in tropical rivers. Tropical streams and rivers differ from temperate regions because of their year-round high temperature (Ortiz-Zayas *et al.* 2005). Urbanization increases water temperature and microbial activity in urban tropical streams (Ramirez *et al.* 2009) possibly increasing the degradation of EDCs and thus, the respiration rates in the stream. However, higher respiration can lead to large oxygen fluctuations and oxygen deficits in urban streams (Faulkner *et al.* 2000; Gücker *et al.* 2006). Although respiration is not always directly related with urbanization, it is often elevated in streams receiving wastewater discharges (Gücker *et al.* 2006; Wenger *et al.* 2009). Most of the flow of the Río Bairoa (71–94%) comes from the effluent discharge from the Caguas RWWTP (Figueroa-Nieves *et al.* 2014). Contributions from WWTPs to streams with low flow could have more substantial effects, not only to stream flow but to the estrogenic load into the receiving stream as the estrogenic effluent will dominate the natural river flow. As a result, a larger effect is expected in a stream with low flow and a high amount of sewage input as is the case for Río Bairoa. Our results show estrogenic activity in the effluent of the Caguas RWWTP at the picomolar range. Whether this concentration could have negative impacts on the aquatic life in this site is still unexplored. However, exposure to WWTP effluents with <1 ng/L EEq induced estrogenic effects in the organisms exposed (Jobling *et al.* 2004; Liney *et al.* 2006).

Coastal effluents

Without adequate wastewater treatment, pollution of inland water occurs and coastal waters are also affected. Coastal ecosystems are highly vulnerable to anthropogenic inputs due to high population densities and rapid transport of pollutants from land to the ocean (Ortiz-Zayas *et al.* 2006). In coastal urban centers, such as those in Puerto Rico, wastewaters receive primary treatment only, which is not capable of removing chemical pollutants such as EDCs. The primary treated effluent from the Puerto Nuevo RWWTP is discharged into the Atlantic Ocean. The failure to remove chemical pollutants from the effluent could result in an increased load of EDCs to the ocean. EDCs have been found in seawater and sediments in marine environments worldwide (Atkinson *et al.* 2003; Pinto *et al.* 2005; Gómez-Gutiérrez *et al.* 2007) and in invertebrate and vertebrate marine species (Allen *et al.* 1999; Depledge & Billinghurst 1999; Andrew-Priestley *et al.* 2012). The impacts of EDCs on coastal aquatic ecosystems may differ from temperate ecosystems as has been observed in tropical rivers with high nutrient loadings (Figueroa-Nieves *et al.* 2014). Given these findings, the presence of EDCs in tropical coastal waters such as those near the ocean outfalls in Puerto Rico should be assessed soon.

Environmental EDCs

Associations between environmental pollution and ecosystems and human health are complex and often difficult to characterize (Briggs 2003; Eggen *et al.* 2004). Insufficient detailed monitoring and the variations within population groups make it difficult to establish levels of exposure (Briggs 2003). However, it is well known that low concentrations of continuously present, and an increasing number of, pollutants have chronic effects in the organisms exposed (Eggen *et al.* 2004; Vandenberg *et al.* 2012; Bergman *et al.* 2013).

Although the EPA has established criteria for compounds that end up as EDCs, there are no formal regulations against their effects in living organisms thus, environmental concentrations are also critical to regulate since exposure to these compounds is constant. WWTPs are not designed to remove emerging contaminants that could be persistent and, thus, not metabolized or bioremediated, releasing them back into the environment (Basile *et al.* 2011). Therefore, it is necessary to establish rigorous criteria and enforcement for the adequate management of anthropogenic pollutants in effluents and environmental concentrations of these compounds to protect the health of the ecosystem and human beings. These criteria must recognize latitudinal differences in degradation rates, particularly in tropical waters.

CONCLUSIONS

Although it has been shown worldwide that EDCs are detrimental, the consequences of such impacts on tropical

streams and coastal environments have not been fully evaluated. The extent to which tropical receiving waters may be affected by EDCs and the threat that these compounds pose to aquatic life or human consumption remain largely unknown because comprehensive surveys are lacking. Our data are the first to characterize estrogenic levels in effluents from sewage treatment facilities in Puerto Rico and may be helpful for managers. The comparison of the two treatment technologies indicated that, as expected, tertiary technology is more efficient than the primary in the removal of estrogenic activity and the phthalate esters studied. Insufficiently treated municipal wastewater discharges could be responsible for surface and coastal water contamination with EDCs. Establishing more efficient technologies in WWTPs could improve the quality of the effluent discharge and in turn the quality of the receiving water bodies. Unfortunately, water quality standards for EDCs in the environment do not exist yet. As a first step, the establishment of criteria for EDCs in receiving waters is needed in order to minimize degradation of downstream ecosystems and human health. Management of sewage effluents is critical for the conservation and restoration of tropical inland and coastal waters. Given the economic importance of clean tropical beaches associated with touristic activities, careful water pollution control strategies must be strengthened in tropical islands if a sustainable economic development is to be achieved.

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