



ENVIRONMENTAL CHEMISTRY, POLLUTION & WASTE MANAGEMENT | RESEARCH ARTICLE

Wastewater from Mexico City contains organotin compounds and organotin-resistant bacteria

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Abstract: Organotin compounds are man-made chemicals used worldwide for diverse applications. These organometallic compounds may be released from anti-fouling paints, polyvinyl chloride and other materials into terrestrial and aquatic environments. Mexico City is a highly populated and industrialized city with many potential sources of environmental pollution. We evaluated the content of butyltins, triphenyltin and bacteria in water from various sites with different water quality in Mexico City, Pachuca City and two towns located between these two cities. Butyltins and/or triphenyltin were detected by gas chromatography and mass spectrometry analysis in samples from 4/4 sites containing wastewater, 1/2 sites containing reclaimed water and 2/5 sites holding accumulated water in open-air deposits. Neither organotin compounds nor bacteria were found in household water samples. However, 80 bacterial strains were isolated from partially treated or untreated wastewater and 72 of them were identified at the genus and species levels by the automated Mass Spectrometry Identification System VITEK MS. *In vitro* growth of 16 of 28 tested strains was not inhibited by 1 mM tributyltin or triphenyltin, indicating that many environmental bacteria are highly tolerant to organotin compounds.

ABOUT THE AUTHORS

The utilization of organotin compounds (OTCs) represents enormous cost savings to the industry, agriculture and merchant marine. Great amounts of these chemicals are annually produced but their release into the environment poses a potential risk for human health. Professor Miguel Aguilar Santelises and his group are currently dedicated to investigate the presence of OTCs in the environment from Mexico City and their effects on bacteria. Previously, we have analysed the effect of OTCs on human normal cells and cell lines. We have isolated several bacteria that are *in vitro* tolerant to high concentrations of OTCs and demonstrated that human normal lymphoid cells are far more sensitive than many bacteria. It is our hope that our results will contribute to increase awareness of the environmental pollution with OTCs and to promote further investigations and administrative actions leading to a better protection of the environment.

PUBLIC INTEREST STATEMENT

Mexico City is highly polluted with a diversity of contaminants, which are not as easily recognizable as the smoke and particulate residues that are produced by fuel combustion. Organotin compounds (OTCs) are synthetic compounds, formed by tin linked to hydrocarbons. OTCs have been widely used as biocides for agricultural purposes, as plastic stabilizers, and to prevent incrustation of algae and molluscs on the hulls of the ships. However, tiny amounts of OTCs released into water induce sex alterations and other abnormalities in marine organisms. OTCs may also be released into inland aquatic environments from PVC, plastic and other industrial and domestic sources. OTCs may also affect humans. Here, we demonstrate that wastewater from Mexico City contains both OTCs and OTCs-resistant bacteria. Our results suggest the need for better control of OTCs utilization and discharge and the importance of developing processes to degrade harmful OTCs to less toxic compounds.

Subjects: Environment & Agriculture; Bioscience; Environmental Studies & Management; Health and Social Care

Keywords: organotin; bacteria; wastewater

1. Introduction

Organotin compounds (OTCs) are organometallic compounds with one or several tin-carbon covalent bonds. OTCs are used worldwide as polyvinyl chloride (PVC) stabilizers, antifouling paints, catalytic agents and biocides for crop protection and other purposes. Their eventual release into the environment has generated global contamination with many opportunities for human exposure (Kotake, 2012; Rose, Fernandes, Mortimer, & Baskaran, 2015; Sousa et al., 2017). The *in vitro* toxicity of OTCs has been demonstrated in human primary cells and human cell lines (Asanagi et al., 2016; Hiromori, Yui, Nishikawa, Nagase, & Nakanishi, 2016; Rana & Whalen, 2015). Their *in vivo* toxicity have been observed in accidentally or occupationally exposed individuals, and their effects vary, from skin irritation, headache, nephropathy, hepatitis and pancreatitis to severe neurologic or immunologic alterations and death (Guo, Lu, & Xu, 2010; Tang et al., 2013). OTCs can be found in human biological liquids and organs (Rantakokko et al., 2013; Valenzuela, Lespes, Quiroz, Aguilar, & Bravo, 2014), but the human health risks derived from human exposure to small amounts of OTCs are difficult to assess (Gueguen et al., 2011). OTCs remain on the market (Turner & Glegg, 2014), and new cases of human intoxication due to occupational exposure continue to occur (Lee et al., 2016).

The immunotoxic and neurotoxic effects of OTCs have also been thoroughly demonstrated in experimental animals (Pagliarani, Nesci, & Ventrella, 2013). Furthermore, these chemicals may severely decrease reproduction capacity, from aquatic organisms to rodents (Revathi, Iyapparaj, Vasanthi, Munuswamy, & Krishnan, 2014; Si et al., 2013). Very low (nM) concentrations of tributyltin induce imposex in gastropods after release from TBT-based antifouling paints (Abidli et al., 2013). Therefore, the International Maritime Organization (2016) has banned the use of harmful OTCs for antifouling purposes on marine vessels shorter than 25 m to diminish their release into marine environments. However, OTCs can also be released from PVC water pipes and industrial effluents into inland waters, where they may contaminate drinking water and wastewater (Fristachi et al., 2009; Landmeyer, Tanner, & Watt, 2004; Rose et al., 2015; Sadiki & Williams, 1999; Teuten et al., 2009).

OTCs in wastewater may interact with micro-organisms in various ways. The scenarios created by diverse environmental circumstances in polluted systems contribute to determine if micro-organism-organotin interactions end in bacterial death or survival (Martin-Rodriguez et al., 2014). Moreover, the amount of the highly toxic tri-substituted compounds may be altered by surviving micro-organisms with the capability to degrade them (by removal of organic moieties) or transform them (by debutylation or methylation) to less toxic compounds (Gadd, 2000).

Pollution due to the release and persistence of OTCs in aquatic and terrestrial environments is a major concern on a global scale (Ribeiro, Nunes, Pereira, & Silva, 2015). A high number of inhabitants and a strong industry provide Mexico City with numerous potential sources of contamination. Mexico City has significant air pollution and growing difficulties in water supply and wastewater disposal (Calderon-Garcidueñas, Kulesza, Doty, D'Angiulli, & Torres-Jardon, 2015; Fonseca-Salazar, Diaz-Avalos, Castañon-Martinez, Tapia-Palacios, & Mazari-Hiriart, 2016). However, there has been no study of water pollution by OTCs in Mexico. Therefore, we investigated if some of the most common and toxic OTCs (butyltins and triphenyltin) are present in aquatic environments in Mexico City. Additionally, we searched for bacteria present in the same environments, obtained axenic cultures and tested several strains to learn about their *in vitro* sensitivity or resistance to TBT and triphenyltin at concentrations to which other bacterial strains are tolerant (Abubakar et al., 2015; Cruz, Moreira, & Mendo, 2014).

2. Materials and methods

2.1. Reagents

Monobutyltin chloride (MBT), dibutyltin chloride (DBT), tributyltin chloride (TBT) and triphenyltin chloride (TPT) were acquired from Merck (Darmstadt Germany). Sodium tetraethyl borate (NaBEt_4) was purchased from Strem Chemicals (Newburyport, MA, USA). Stock solutions of organotin were prepared by dissolving the corresponding salts in ethanol. Tin metal, enriched in ^{119}Sn was acquired from the Cambridge Isotope Laboratories (Andover, MA, USA). Tin metal, enriched in ^{124}Sn was obtained from the Oak Ridge National Laboratory (Oak Ridge, TN, USA). All organometallic standard solutions were stored in the dark at 4°C , and diluted solutions were prepared daily before analysis. Blood agar, MacConkey agar, salt mannitol agar and chocolate agar were purchased from bioMérieux (Hazelwood, MO, USA). Antibiotic medium No. 3 was from BD Diagnostic Systems (Circle Sparks, MD, USA). Dimethyl sulfoxide (DMSO) and ethanol of analytical grade were acquired from Sigma-Aldrich Corp. (St. Louis MO, USA).

2.2. Samples recollection

Water samples were collected between April and June 2015 at 11 sites in four districts, namely Mexico City, Pachuca City and two towns located between these cities (Tizayuca and Zumpango). Popotla, Ticoman, Remedios and Xochiaca are located in Mexico City and therefore were included as a single district. Jilotzingo, San Bartolo, Tizayuca and El Carmen formed the second district. Zumpango's lagoon and the Zumpango-derived irrigation channel formed the third district. Pachuca City was the four district. Samples were collected at least twice at every site and analysed in triplicate to detect the presence of butyltins (MBT, DBT and TBT) and/or TPT and measure their quantity in positive samples. Water discarded from residential dwellings, commercial buildings and industrial facilities, not submitted to wastewater management to reduce the level of pollutants before reuse or disposal into the environment is registered as wastewater. Reclaimed water is wastewater that has been treated to standards that allow safe reuse for most uses except human consumption. Accumulated water, as defined in the present report, is rainwater collected in small or large open-air deposits without known industrial or domestic discharges. Household water samples were also collected from the four districts and similarly tested for their content of OTCs and bacteria (Figure 1).

Figure 1. Sampled sites in or between México city and Pachuca City * Km to downtown México city.



Bacterial contamination of the samples and butyltin adsorption on the sample container were prevented by recollection of water samples in sterile glass flasks. (Centineo et al., 2006). One aliquot of every sample was immediately seeded in microbiological media, with aseptic techniques, in order to grow the bacteria present in the sample. Another aliquot of every sample was acidified by adding 1 mL glacial acetic acid to 100 mL of water and stored at 4°C in the dark for no more than 5 days before GC-MS analysis to determine their content of butyltins and TPT (Centineo et al., 2006).

2.3. Detection of organotin compounds

A Thermo Scientific Finnigan Trace Ultra Gas Chromatograph (GC) coupled to a Polaris Q mass spectrometer (MS; Waltham, MA, USA) was used to detect MBT, DBT, TBT and TPT in the water samples. The GC was equipped with a 30 m × 0.25 mm internal diameter × 0.25 μm RTX-5MX diphenyl-dimethylpolysiloxane (5:95) capillary column. The volume of the injected sample was 1 μL. The column temperature was held at 70°C for the first minute and increased 20°/min until reaching 250°C. The splitless injector port was maintained at a temperature of 250°C, and the transfer line was maintained at 300°C. Helium (He) was used as the carrier gas (1 mL/min). The MS was operated with an electronic impact (70 eV) ion source at a temperature of 180°C in total ionization mode, and selected ion monitoring signals were recorded.

¹¹⁹Sn-enriched butyltins and ¹²⁴Sn-enriched TPT stock solutions were prepared by solving appropriate amounts of each OTC in ultrapure ethanol, adding 5 μL of ¹¹⁹Sn or ¹²⁴Sn and further diluting with water until reaching a 0–10 ng mL⁻¹ concentration. Then, 10, 50 100 and 200 μL from each of these solutions were mixed with 2 mL isooctane and shaken during 10 min. The organic layer was taken after separation and placed in a glass flask for GC-MS analysis. A standard curve was obtained with the results.

To assess the quality analysis (QA) and quality control (QC) of the methods, the detection limits and percentages of recovery were determined in 10 different experiments with mixed spiked solutions containing ¹¹⁹butyltins and ¹²⁴TPT in distilled water for isotope dilution analysis. The percentages of recovery (±1 SD) from the spiked samples were 97 ± 8 for MBT, 98 ± 10 for DBT, 96 ± 10 for TBT and 96 ± 7 for TPT. The detection limits, obtained from the quantitative analysis of the calibration curves were found similar to those reported by Ruiz-Encinar, Rodriguez-Gonzalez, Garcia-Alonso, and Sanz-Medel (2002). Specific detection limits ±3 SD were: MBT (0.23 ± 0.129 ng/L), DBT (0.30 ± 0.148 ng/L), TBT (0.55 ± 0.117 ng/L) and TPT (0.74 ± 0.204 ng/L). The accuracy, repeatability, specificity, linearity, ranges of detection and quantitation limits validated the method that was applied to analyse the samples.

One hundred millilitres of water were mixed with 50.0 ng of ¹¹⁹Sn-enriched butyltins or ¹²⁴Sn-enriched TPT and left undisturbed for 1 h. Then, the pH was adjusted at 5.4 with 1 mL of 1 M acetic acid/sodium acetate buffer, and 200 μL of 2% NaBEt₄ in 0.1 M NaOH was added to the mixture to achieve MBT, DBT, TBT and TPT ethylation. The mixture was shaken for 15 min at 10°C, followed by the addition of 1 mL of hexane and continued shaking for 10 min. The organic phase was removed with a Pasteur pipette, transferred to a glass flask and concentrated under a dry nitrogen stream to a volume of 50 μL. A 1 μL aliquot was withdrawn and injected into the GC as described (Nguyen, Muppala, Frech, & Tesfalidet, 2006; Ruiz-Encinar et al., 2002).

2.4. Isolation and identification of bacteria

Enriched, selective and differential culture media were used to isolate bacteria from wastewater (Remedios, Xochiaca, Zumpango's lagoon and a Zumpango-derived irrigation channel), reclaimed water (Ticomán and Pachuca City) and accumulated water (Popotla, Jilotzingo, San Bartolo, Tizayuca and El Carmen). Bacterial strains were isolated after incubation for 48 h at 37°C in bacteriological culture media and identified by the VITEK MS (bioMérieux, Hazelwood, MO), a fully automated microbiology identification system that uses matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) and mass spectrometry to provide specific, reliable and accurate results, which are

confirmed by bacterial identification with manual methods in multiple studies (Wang et al., 2014), including our own.

2.5. Bacterial susceptibility to OTCs

Axenic bacterial cultures were inoculated in Erlenmeyer culture flasks containing 50 mL of antibiotic medium No. 3 and incubated overnight at 37°C. The bacterial mass was harvested, washed and re-suspended in broth medium No. 3. One hundred and eighty µL of a 22.2×10^6 UFC/mL suspension of each strain was placed in each well of a 96-microwell plate to analyse the sensitivity to OTCs. TPT or TBT was added to each well at a final concentration of 10 µM, 100 µM or 1 mM. The final dilution of TBT and TPT in ethanol or DMSO was $\geq 1:1000$, and the control wells received medium or solvents at similar dilutions without OTCs. Experiments were performed twice in triplicate to ensure accuracy and reproducibility. Plates were incubated with agitation in a humid atmosphere overnight at 37°C. The optical density was read at 600 nm at 0, 24 and 48 h, and the OD averages were used to calculate the percentages of growth inhibition.

3. Results

3.1. OTC detection

We examined samples with different water quality from several sites in four districts (México City, Tizayuca, Zumpango and Pachuca City, Figure 1). Neither butyltins nor triphenyltin were detected in household water samples. By contrast, DBT and MBT were detected in samples of reclaimed water from Pachuca City, whereas TBT was found in samples from 1/4 sites containing wastewater (from Xochiaca, in Mexico City), 1/2 sites with reclaimed water (Pachuca City) and 1/5 sites holding accumulated water (from Jilotzingo, in the district of Tizayuca) (Figure 1 and Table 1). TPT was detected in 4/4 sites containing wastewater (from Mexico City and Zumpango), 1/2 sites with reclaimed water (from Pachuca City) and 1/5 sites holding accumulated water (Popotla, in Mexico City). The highest concentrations of TBT were detected at one site carrying wastewater (Xochiaca) and one site with accumulated water (Jilotzingo), whereas TPT was highest at one site containing wastewater (Zumpango's lagoon) (Table 1).

Table 1. Organotin compounds detection

Water quality	Site	MBT	DBT	TBT	TPT
Household water	Mexico City	0	0	0	0
	Zumpango's town	0	0	0	0
	Tizayuca's town	0	0	0	0
	Pachuca City	0	0	0	0
Wastewater	Remedios	0	0	0	12 ± 4
	Xochiaca	0	0	7 ± 2	11 ± 3
	Irrigation channel	0	0	0	12 ± 5
	Zumpango's lagoon	0	0	0	27 ± 3
Reclaimed water	Ticomán	0	0	0	0
	Pachuca City	0.21 ± 0.02	0.30 ± 0.06	0.57 ± 0.1	17 ± 4
Accumulated water	Popotla	0	0	0	13 ± 3
	Jilotzingo	0	0	8 ± 3	0
	San Bartolo	0	0	0	0
	Tizayuca's town	0	0	0	0
	El Carmen	0	0	0	0

Notes: ng/L monobutyltin (MBT), dibutyltin (DBT), tributyltin (TBT) and triphenyltin (TPT) detected by GC-MS in samples of water.

3.2. Isolation and identification of bacteria

All attempts to isolate bacteria from household water failed. Instead, 80 bacterial strains were isolated from water sampled at 11 different sites containing wastewater, reclaimed water or accumulated water. Seventy-two of these strains were identified at the genus and species levels by the VITEK MS system, which uses a state-of-the-art technology for specific and reliable identification of a high number of bacteria, including those isolated from aquatic environments (Popovic, Kazacic, Struniak-Perovic, & Coz-Rakovac, 2017; van Veen, Claas, & Kuijper, 2010). Eight strains were not recognized by the instrument (Table 2).

Table 2. Bacterial strains isolated

Strain	Water quality									
	Wastewater			Reclaimed water		Accumulated water				
	Re	Xo	Ic	Tc*	Pa	Po	Cr*	Ba*	Tz*	Ji
<i>Acinetobacter iwoffii</i>						X				
<i>Acinetobacter johnsonii</i>										X
<i>Acinetobacter junii</i>							X**			
<i>Aerococcus viridans</i>					X					
<i>Aeromonas caviae</i>									X	
<i>Aeromonas hydrophila</i>		X	X			X		X**	X**	
<i>Aeromonas salmonicida</i>		X**								
<i>Aeromonas sobria</i>			X		X		X**			
<i>Aeromonas veronii</i>										X
<i>Bacillus firmus</i>						X				
<i>Bacillus mycoides</i>		X				X				
<i>Bacillus pumilus</i>					X					
<i>Bacillus sp.</i>				X						
<i>Bacillus subtilis</i>						X**				
<i>Brevundimonas vesicularis</i>			X							
<i>Chromobacter violaceum</i>									X	
<i>Citrobacter braakii</i>			X							
<i>Citrobacter freundii</i>		X								
<i>Clostridium sporogenes</i>										X
<i>Comamonas testosteroni</i>	X									
<i>Enterobacter asburiae</i>									X	
<i>Enterobacter cloacae</i>					X					

(Continued)

Table 2. (Continued)

Strain	Water quality									
	Wastewater			Reclaimed water		Accumulated water				
	Re	Xo	IC	Tc*	Pa	Po	Cr*	Ba*	Tz*	Ji
<i>Grimontia hollisae</i>									X	
<i>Klebsiella pneumoniae</i>			X							
<i>Kocuria rosea</i>					X					
<i>Lactococcus lactis ssp lactis</i>	X									
<i>Morganella morgani</i>		X**								
<i>Pantoea agglomerans</i>									X	
<i>Proteus penneri</i>								X		
<i>Pseudomonas alcaligenes</i>					X				X	X
<i>Pseudomonas fluorescens</i>					X		X		X	X
<i>Pseudomonas mendocina</i>			X**							
<i>Pseudomonas oleovorans</i>			X	X			X		X	
<i>Pseudomonas oryzae</i>									X	X
<i>Pseudomonas putida</i>				X						
<i>Pseudomonas stutzeri</i>	X		X**				X		X**	
<i>Pseudomonas veronii</i>		X**								
<i>Pseudomonas viridiflava</i>									X	
<i>Staphylococcus hominis</i>					X					
<i>Shewanella putrefaciens</i>								X**		
<i>Yersinia frederiksenii</i>		X								

Notes: Seventy-two strains isolated from water samples taken at sites with different water quality were identified by the VITEK MS system. Eight additional unidentified strains were isolated from water samples taken at Zumpango's lagoon. Po: Popotla, Tc: Ticoman, Re: Remedios, Xo: Xochiaca, Ji: Jilotzingo, Ic: Irrigation channel, Ba: San Bartolo, Tz: Tizayuca, Cr: El Carmen, Pa: Pachuca.

*Sites where OTCs were not detected.

**Species repeatedly isolated in different samples from the same site.

Forty-three strains were isolated from water samples that contained OTCs, whereas 29 other strains were isolated from water samples that did not contain OTCs. The isolated strains belonged to 21 genera, including 15 Gram negative and 6 Gram positive. Eleven of these genera are aerobic or aerobic facultative and 10 are anaerobic or anaerobic facultative. *Acinetobacter*, *Aeromonas*, *Bacillus* and *Pseudomonas* were the most frequently isolated genera, whereas *Aeromonas hydrophila*, *Aeromonas sobria*, *Pseudomonas alcaligenes*, *Pseudomonas fluorescens*, *Pseudomonas oleovorans* and *Pseudomonas stutzeri* were the most frequently isolated species (Table 2). Some bacterial

genera (*Aerococcus*, *Brevundimonas*, *Citrobacter*, *Clostridium*, *Comamonas*, *Klebsiella*, *Kocuria*, *Lactococcus*, *Staphylococcus* and *Yersinia*) were exclusively isolated from OTC-contaminated water, whereas others (*Chromobacter*, *Grimontia*, *Pantoea*, *Proteus* and *Shewanella*) were only isolated from non-OTC-contaminated water.

3.3. Bacterial susceptibility to OTCs

Bacteria were cultured *in vitro* to test their susceptibility to TBT or TPT at concentrations of 1 mM and lower. *In vitro* growth inhibition caused by the lowest (10 µM) and highest (1 mM) concentrations are shown in Table 3. Three strains of *Bacillus* and 2 of *Pseudomonas* were among the 12 inhibited strains. Most of the tested 28 strains were quite insensitive to 10 µM TBT or TPT during 48 h *in vitro* culture. However, there were exceptions, with some strains totally or nearly totally inhibited by 10 µM TBT (*P. fluorescens* and *S. hominis*) or TPT (*B. pumilus*, *B. firmus*, *P. fluorescens* and *S. hominis*). The number of sensitive strains increased when they were incubated with the highest concentration of TBT or TPT, but only 12 of 28 tested strains were inhibited ≥ 40% when cultured in the presence of

Table 3. Inhibition of bacterial *in vitro* growth by organotin compounds

Water quality	Site	Strain	TBT		TPT	
			10 µM	1 mM	10 µM	1 mM
Wastewater	Remedios	<i>C. testosteroni</i>	10	22	11	31
		<i>P. stutzeri</i>	0	46	0	53
	Xochiaca	<i>A. salmonicida</i>	14	12	12	3
		<i>C. freundii</i>	8	10	0	0
		<i>M. morgani</i>	6	4	4	21
	Irrigation channel	<i>A. hydrophila</i>	6	32	0	1
		<i>C. braakii</i>	13	12	7	0
		<i>K. pneumoniae</i>	14	12	7	8
	Reclaimed water	Ticomán	<i>P. oleovorans</i> *	5	4	0
Pachuca City		<i>A. sobria</i>	7	4	0	86
		<i>A. viridans</i>	60	80	56	49
		<i>B. pumilus</i>	56	96	100	86
		<i>E. cloacae</i>	20	49	11	72
		<i>K. rosea</i>	5	81	0	52
		<i>P. alcaligenes</i>	0	51	0	28
		<i>P. fluorescens</i>	100	100	100	88
		<i>S. hominis</i>	82	91	100	84
Accumulated water	Popotla	<i>A. hydrophila</i>	11	9	7	5
		<i>B. firmus</i>	0	0	87	97
		<i>B. subtilis</i>	23	100	49	91
	Jilotzingo	<i>A. johnsonii</i>	25	100	11	57
		<i>A. veronii</i>	9	12	17	11
		<i>P. alcaligenes</i>	0	0	0	11
		<i>P. fluorescens</i>	29	40	5	7
	San Bartolo	<i>A. hydrophila</i> *	4	0	14	8
	Tizayuca's town	<i>A. caviae</i> *	17	15	29	11
		<i>P. agglomerans</i> *	23	31	5	10
	El Carmen	<i>A. sobria</i> *	9	9	9	9

Notes: Percentages of growth inhibition of the *in vitro* growth of 28 bacterial strains isolated from aquatic environments containing different quality water from Mexico City, Pachuca City and sites located between these cities. Bacterial growth was measured after 48 h of incubation with or without TBT or TPT at a concentration of 10 µM or 1 mM.

*Strains isolated from not OTC-contaminated sites.

1 mM TBT or TPT. The growth inhibition of most sensitive strains was induced by both OTCs. Only *P. fluorescens* was inhibited by TBT but not by TPT, and *A. sobria* was inhibited by TPT but not by TBT (Table 3). The *in vitro* growth of 5 strains isolated from non-OTC-contaminated sites was not significantly inhibited in any case (Table 3).

4. Discussion

The detection of TBT and TPT in wastewater and reclaimed water was not unexpected since Mexico City has a high population density and severe environmental pollution with a variety of contaminants (Calderon-Garcidueñas et al., 2015). Contamination of water with OTCs is usually due to industrial discharges, agrochemical remains and leakage from OTC-treated materials. The detection of OTCs in wastewater and reclaimed water from Mexico City and Pachuca City is in agreement with previous findings in other parts of the world (Gao et al., 2015; Sabah et al., 2016). We detected MBT and DBT in reclaimed water but do not know if their presence was due to TBT biodegradation, photolytic degradation or simultaneous discharge and failure to remove butyltins from the examined water (Cruz, Caetano, Suzuki, & Mendo, 2007; Ye et al., 2013). The presence of these compounds in open-air sites in Mexico City (Popotla) or a small town (Jilotzingo) may be due either to air and soil pollution already existing in the city or to unknown discharges of wastewater or OTC-release from other sources in specific locations. Finally, the absence of TBT and TPT in household water reflects the efficiency of the wastewater treatment plants operating in the examined districts.

The simultaneous presence of chemicals and micro-organisms in water gives rise to a complex net of interactions. Generally, tri-substituted organotins are more toxic than di- and mono-substituted compounds. However, some bacteria are disturbed by low nM concentrations, whereas others remain undamaged by tri-substituted OTCs at high mM concentrations (Abubakar et al., 2015; Cruz et al., 2007; Martins, Jurado, Moreno, & Madeira, 2005; Roy & Nair, 2007). The bacterial capacity to form biofilms and grow over sediment may be determinant for the persistence of OTCs in the environment (Jadhav, Bhosle, Krishnamurthy, & Sawant, 2012; Martin-Rodriguez et al., 2014; Sakultantimetha, Keenan, Beattie, Bangkedphol, & Cavoura, 2011). In addition, bacterial resistance to OTCs might be related or unrelated to the presence of OTCs in their environment (Suehiro et al., 2007). We did not observe an apparent relationship between OTC-induced *in vitro* growth inhibition and the presence or absence of OTCs in the water from which the 28 tested strains were isolated.

Other researchers have analysed the susceptibility to OTCs of 11 strains belonging to some of the bacterial genera that we isolated (Girasolo et al., 2012; Inoue et al., 2003; Wuertz, Miller, Pfister, & Cooney, 1991; Yañez et al., 2015). Strains of *Aeromonas veronii*, *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Shewanella colwelliana* have been shown to resist and degrade TBT or TPT (Cruz et al., 2007; Labare, Coon, Matthias, & Weiner, 1997; Sakultantimetha et al., 2011; Ye et al., 2013). Here, we report on 8 additional genera (*Acinetobacter*, *Aerococcus*, *Brevundimonas*, *Clostridium*, *Comamonas*, *Kocuria*, *Lactococcus* and *Morganella*) that have not been isolated previously from water containing TBT or TPT. We also demonstrated that many of these environmental bacteria grow undisturbed when cultured *in vitro* in the presence of 1 mM TBT or TPT.

A vast difference was observed between the highest concentrations detected in water (8 ng/L TBT = 0.02 nM and 27 ng/L TPT = 0.07 nM) and the concentrations necessary to inhibit (when inhibition occurs) the *in vitro* growth of bacteria (1 mM TBT = 3.25×10^8 ng/L and 1 mM TPT = 3.85×10^8 ng/L). Environmental concentrations of OTCs vary depending upon how, when and where these compounds are released, their residence time, and the types of interactions they have had with micro-organisms present in the same environment (Gao et al., 2015; Martin-Rodriguez et al., 2014). We have only examined the mentioned sites for a limited period of time thus far and lack information about the mentioned factors. It will be necessary to extend our investigations to monitor these and other aquatic environments in the studied areas to determine if the concentrations of OTCs are maintained or differ depending on weather or nearby industrial, agricultural and domestic activities. Since butyltins and phenyltins were detected in untreated or partially treated wastewater and

accumulated water at open-air sites, it would also be of interest to examine the waste management disposal policies as well as air and soil contamination from the inspected districts.

5. Conclusions

Wastewater from Mexico City and neighbouring areas contained butyltins and triphenyltin. Many environmental bacteria grew undisturbed by 1 mM TBT or TPT during a 48 h *in vitro* culture, independently of the content of butyltins or phenyltins in the water from which they were originally isolated. The presence of OTCs in wastewater is in agreement with their sustained use in Mexico City. However, further investigations are required to determine the magnitude of the contamination of water with OTCs and the mechanisms responsible for bacterial *in vitro* survival during exposure to OTCs.

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