Supplementary Information:

Antibiotic resistance gene abundances associated with waste discharges into the Almendares River near Havana, Cuba

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Includes: Eight pages, 2 tables and 1 figure

CONCENTRATIONS OF ORGANOCHLORIDE PESTICIDES IN SEDIMENT PORE WATERS

MATERIALS AND METHODS. Organochlorine pesticides (OCP) and metabolites analyzed in this work were: α, β, γ and δ hexachlorocyclohexane (α, β, γ and δ HCH), aldrin, dieldrin, α endosulfan, endosulfan sulphate, endrin, endrin aldehyde, endrin ketone, heptachlor, heptachlor epoxide and methoxychlor, p,p' dichlorodiphenyltrichloroethane (DDT), p,p' dichlorodiphenyldichloroethane (DDD), and p,p' dichlorodiphenyldichloroethylene (DDE), which were obtained in an EPA TCL mix (Sigma Aldrich, Dorset, UK) at individual concentrations of 2000 μg mL^{-1} in 50% hexane in toluene. Sediment porewater concentration of these OCP was derived with the help of passive samplers**.** 26 μm thick PE sheets cut from plastic bags (VWR International, Leicestershire, UK) were soaked in hexane, methanol and deionised water, in series, each for 24 hr, then dried and cut into 0.15 ± 0.01 g pieces. These PE passive samplers were equilibrated with 100 g of sediment in continuously agitated capped amber glass vials for 14 days.

After this exposure/equilibration PE passive samplers were removed from batches, rinsed until visibly clean, dried with kimwipes and extracted in 20 mL of 20% acetone in hexane for two 24 hr periods. Solvents from each sample extraction were combined and concentrated to 1 mL under a gentle stream of nitrogen. Samples were cleaned up using a modification of USEPA method 3620C. A glass column was packed with 3 g of activated Florisil, topped with 1 cm of anhydrous sodium sulphate and precleaned with 30 mL of 20% acetone in hexane. The 1 mL extract was then transferred to the top of the column and eluted with a further 30 mL of 20% acetone in hexane, which was collected and solvent switched to pure hexane before gas chromatography analysis. An internal standard of 2,2'3,3'4,4'5,5'6,6'decachlorobiphenyl (PCB 209) was added to all samples prior to analysis. OCP quantification was carried out on an Agilent 7890A gas chromatograph with an electron capture detector. Separation was performed on a fused silica capillary column (30 m x 0.25 mm i.d) coated with a 0.25 μm dimethyl polysiloxane (HP-5) phase (Agilent Ltd, Wokingham, Berkshire, UK). Instrumental quantification was calibrated using dilutions of the EPA TCL mix for a five point calibration. Retention times of the OCP were verified by gas chromatography mass spectrometry on a Hewlett-Packard 6890 gas chromatograph and also with a 0.017 μg mL^{-1} lindane/aldrin standard (Agilent Technologies, Palo Alto, USA). OCP concentrations in PE passive samplers were

translated into corresponding sediment porewater concentrations using PE-water partitioning coefficients K_{PE} published by Hale et al. (1).

RESULTS AND DISCUSSION:

All OCP compound were at most present at very low concentrations in the range of up to a few ng/L. Only DDTs could be reliably identified based on the presence of three major peaks in the ECD chromatographic trace corresponding to the retention times of p,p' DDT and its major metabolites p,p' DDD and p,p' DDE. Results are summarized in Table S1.

Table S1. DDTs concentration for the different sampling stations (n.d. = not detectable). All other tested pesticides were below detection limits.

Sample	p,p' DDT (ng/L)	p,p' DDD (ng/L)	p,p' DDE (ng/L)
Blank	n.d	n.d	n.d
Sta. 3A	0.12	0.36	0.08
Sta. 4	0.02	2.21	0.44
Sta. 5		Multiple peaks (PCBs?)	
Sta. 5A	n.d	0.26	0.06
Sta. 6	n.d	n.d	0.04
Sta. 7	n.d	n.d	0.04
Sta. 8	n.d	0.11	0.06
Sta. 9	n.d	n.d	n.d

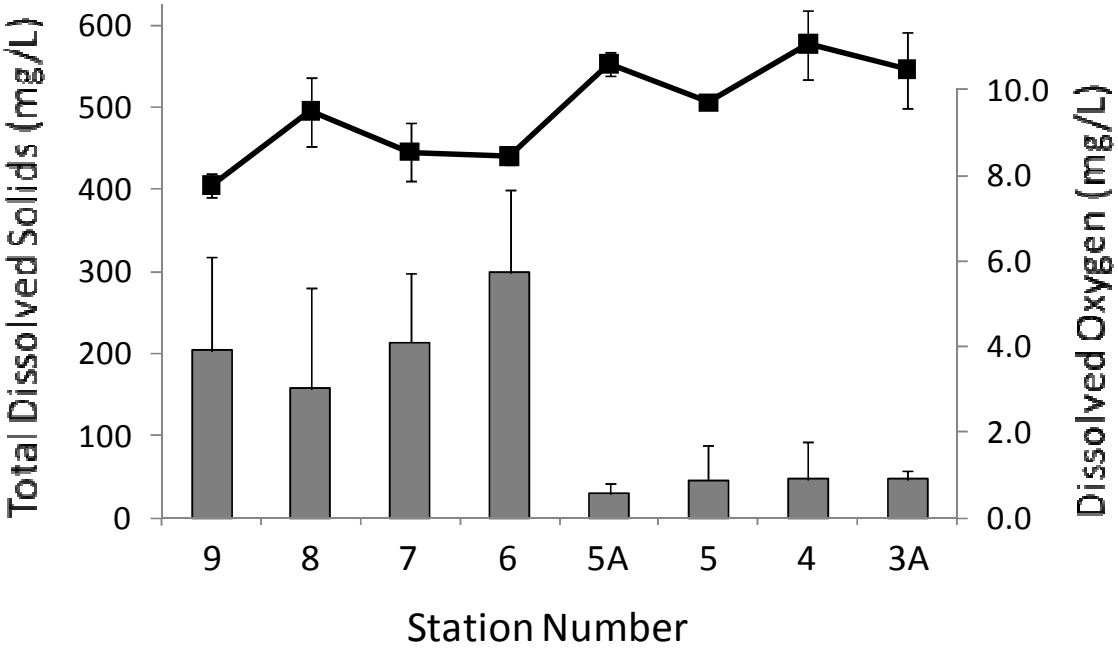
Table S2. Primer and probe sequences used in the study, and PCR reaction conditions.

Target	Primer/probe (concentration)		Sequence (5′–3′) [a]	Annealing conditions	Elongation conditions	Ref.
Total eubacteria	BAC338-F	(500 nM)	ACTCCTACGGGAGGCAG	60 ºC / 30s		(2)
	BAC516-F	(300 nM)	Hex-TGCCAGCAGCCGCGGTAATAC-TAMRA			
	BAC805-R	(500 nM)	GACTACCAGGGTATCTAATCC			
Tet(L)	TetL-F	(900 nM)	GGTTTTGAACGTCTCATTACCTGAT	60 ºC / 30s		(3)
	TetL-Taq	(300 nM)	FAM-CCACCTGCGAGTACAAACTGGGTGAAC-TAMRA			
	TetL-R	(900 nM)	CCAATGGAAAAGGTTAACATAAAGG			
Tet(M)	TetM-F	(900 nM)	GGTTTCTCTTGGATACTTAAATCAATCR	60 ºC / 30s		(3)
	TetM-Taq	(300 nM)	FAM-ATGCAGTTATGGARGGGATACGCTATGGY-TAMRA			
	TetM-R	(900 nM)	CCAACCATAYAATCCTTGTTCRC			
Tet(O)	TetO-F	(900 nM)	AAGAAAACAGGAGATTCCAAAACG	60 ºC / 30s		(4)
	TetO-Taq	(300 nM)	FAM-ACGTTATTTCCCGTTTATCACGG-TAMRA			
	TetO-R	(900 nM)	CGAGTCCCCAGATTGTTTTTAGC			
Tet(Q)	TetQ-F	(900 nM)	AGGTGCTGAACCTTGTTTGATTC	60 ºC / 30s		(4)
	TetQ-Taq	(300 nM)	FAM-TCGCATCAGCATCCCGCTC-TAMRA			
	TetQ-R	(900 nM)	GGCCGGACGGAGGATTT			
Tet(W)	TetW-F	(900 nM)	GCAGAGCGTGGTTCAGTCT	60 ºC / 30s		(4)
	TetW-Taq	(300 nM)	TTCGGGATAAGCTCTCCGCCGA			
	TetW-R	(900 nM)	GACACCGTCTGCTTGATGATAAT			
Erm(B)	ErmB-F	(500 nM)	AAAACTTACCCGCCATACCA	60 ºC / 30s		(5)
	ErmB-R	(500 nM)	TTTGGCGTGTTTCATTGCTT			
Erm(C)	ErmC-F	(500 nM)	GAAATCGGCTCAGGAAAAGG	60 ºC / 30s		(5)
	ErmC-R	(500 nM)	TAGCAAACCCGTATTCCACG			
Erm(E)	ErmE-F	(500 nM)	TGTTCGAGTGGGAGTTCGT	60 ºC / 30s		(5)
	ErmE-R	(500 nM)	GGTACTTGCGCAGAAGCGA			(6)
Erm(F)	ErmF-F	(500 nM)	TCGTTTTACGGGTCAGCACTT	60 ºC / 30s		(6)

Target	Primer/probe (concentration)		Sequence (5′–3′) [a]	Annealing conditions	Elongation conditions	Ref.
	ErmF-R	(500 nM)	CAACCAAAGCTGTGTCGTTT			(5)
bla$_{TEM}$	BlaTEM-F	(400 nM)	TCGGGGAAATGTGCG	50 ºC / 60s	72 ºC / 60s	(7)
	BlaTEM-R	(400 nM)	GGAATAAGGGCGACA			
bla$_{SHV-1}$	blaSHV-F	(400 nM)	TGATTTATCTGCGGGATACG	55 ºC / 60s	76 ºC / 30s	(8)
	BlaSHV-R	(400 nM)	TTAGCGTTGCCAGTGCTCG			(9)
bla$_{CTX-M}$	CTX-M-F	(200 nM)	ATGTGCAGYACCAGTAARGTKATGGC	58 ºC / 60s	72 ºC / 30s	(10)
	CTX-M-1-group probe	(100 nM)	HEX-CCCGACAGCTGGGAGACGAAACGT-TAMRA			
	CTX-M-probe	(100 nM)	FAM-CGACAATACNGCCATGAAMGB-TAMRA			
	CTX-M-R	(200 nM)	ATCACKCGGRTCGCCNGGRAT			
bla$_{OXA-1}$	OXA1B14 (as forward primer)	(400 nM)	CACTTACAGGAAACTTGGGGTCG	55 ºC / 60s	72 ºC / 30s	(11)
	OXA-probe	(200 nM)	HEX-ATCAAGCATAAAAGCCAAGAAAATGC-TAMRA			(5)
	blaOXA1-R	(400 nM)	AGTGTGTTTAGAATGGTGATC			(12)

[a] Sequence modification added: 5′-FAM (6-carboxyfluorescein; fluorophore); 5′-HEX (hexachlorofluorescein; fluorophore); 3′-TAMRA (carboxytetramethylrhodamine; quencher).

Figure S1. Total dissolved solids (lines) and dissolved oxygen levels (bars) in the Almendares River water column during the dry season sampling program in 2007. Errors bars refer to concentration ranges based on n = 4.



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