Environment International 127 (2019) 103–113

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Retinoids and oestrogenic endocrine disrupting chemicals in saline sewage treatment plants: Removal efficiencies and ecological risks to marine organisms

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ARTICLE INFO

Handling Editor: Frederic Coulon

Keywords:
Retinoids
Endocrine disrupting chemicals
Sewage
Seawater
Risk assessment
Marine organisms

ABSTRACT

Discharge of partially treated effluent from sewage treatment plants (STPs) is a significant source of chemical contaminants, such as retinoids and oestrogenic endocrine disrupting chemicals (EDCs), which are continuously input into the marine environments of densely populated and urbanized coastal cities. In this study, we successfully developed three analytical methods to detect and qualify retinoic acids (at-RA, 13c-RA & 9c-RA), their metabolites (at-4-oxo-RA, 13c-4-oxo-RA & 9c-4-oxo-RA), and oestrogenic EDCs using high pressure liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). Using these methods, we found that the total concentrations of retinoids in the influents and effluents of three saline STPs in Hong Kong were 7.1–29 ng/L and 3.7–9.1 ng/L, respectively, and those of EDCs were 3107–5829 ng/L and 1225–2638 ng/L, respectively. Retinoids were dominated by at-4-oxo-RA or 13c-4-oxo-RA in wastewater, whereas at-RA and 13c-RA were the most abundant in sludge. Alkylphenols and bisphenol A were the dominant EDCs in wastewater, whilst alkylphenols, triclosan, and triclocarban were dominant in sludge. Overall, the sewage treatment processes in the STPs of Hong Kong were not highly efficient in the removal of retinoids and EDCs from wastewater influents, with removal efficiencies in the aqueous phase of 41–82% and 31–79%, respectively. The removals were attributed mainly to sorption and degradation. Due to such limited removal, the effluents from STPs and the adjacent seawaters (i.e., receiving water bodies) still exhibited relatively high concentrations of retinoids (2.0–4.3 ng/L in seawaters) and EDCs (71–260 ng/L in seawaters), which posed medium ecological risks to the coastal marine ecosystem of Hong Kong (i.e., hazard quotients: 0.1–1).

1. Introduction

Natural retinoids, which are the derivatives of all-*trans*-retinol (vitamin A), and in particular retinoic acids (RAs) and their metabolites (4-oxo-RAs), play crucial roles in the embryonic development, the immune response, the cellular differentiation and the proliferation processes in animals (Ross et al., 2000; Marill et al., 2003). Natural retinoids can be excreted from humans and animals through urination (Lambert and De Leenheer, 1985). In addition, all-*trans*-RA (at-RA) and 13-*cis*-RA (13c-RA) are also synthesized artificially for treating acute promyelocytic leukaemia (Huang et al., 1988) and skin diseases (Griffiths et al., 1993). Thus, these chemicals and retinoic acid receptor (RAR) agonistic activity can be found in sewage treatment plants (STPs; Zhen et al., 2009; Wu et al., 2010; Allinson et al., 2011; Inoue et al., 2011, 2013; Sawada et al., 2012; Völker et al., 2016), which may be an important source of RAs and 4-oxo-RAs in aquatic environments. Once these chemicals enter the aquatic environment, they can pose high risks to aquatic animals (Zhen et al., 2009; Wu et al., 2010). Laboratory studies showed that exposure to elevated levels of RAs could cause abnormal morphological development in vertebrates such as amphibian and zebrafish embryos (Jonas et al., 2014; Zhu et al., 2014), and imposex development (i.e., the superimposition of penis and vas deferens) in the females of the marine gastropod *Reishia clavigera* (Nishikawa et al., 2004; Horiguchi et al., 2008). Thus, understanding of the environmental levels and fate of RAs and 4-oxo-RAs in each treatment process of STPs and in the effluent is necessary for the evaluation of their ecological risks in the receiving water body.

Oestrogenic endocrine disrupting chemicals (EDCs) such as nonylphenol (NP), octylphenol (OP) and bisphenol-A (BPA) are a class of chemicals that can induce oestrogen-like responses in vertebrates. Even

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https://doi.org/10.1016/j.envint.2019.03.030
Received 13 December 2018; Received in revised form 12 March 2019; Accepted 12 March 2019
Available online 23 March 2019

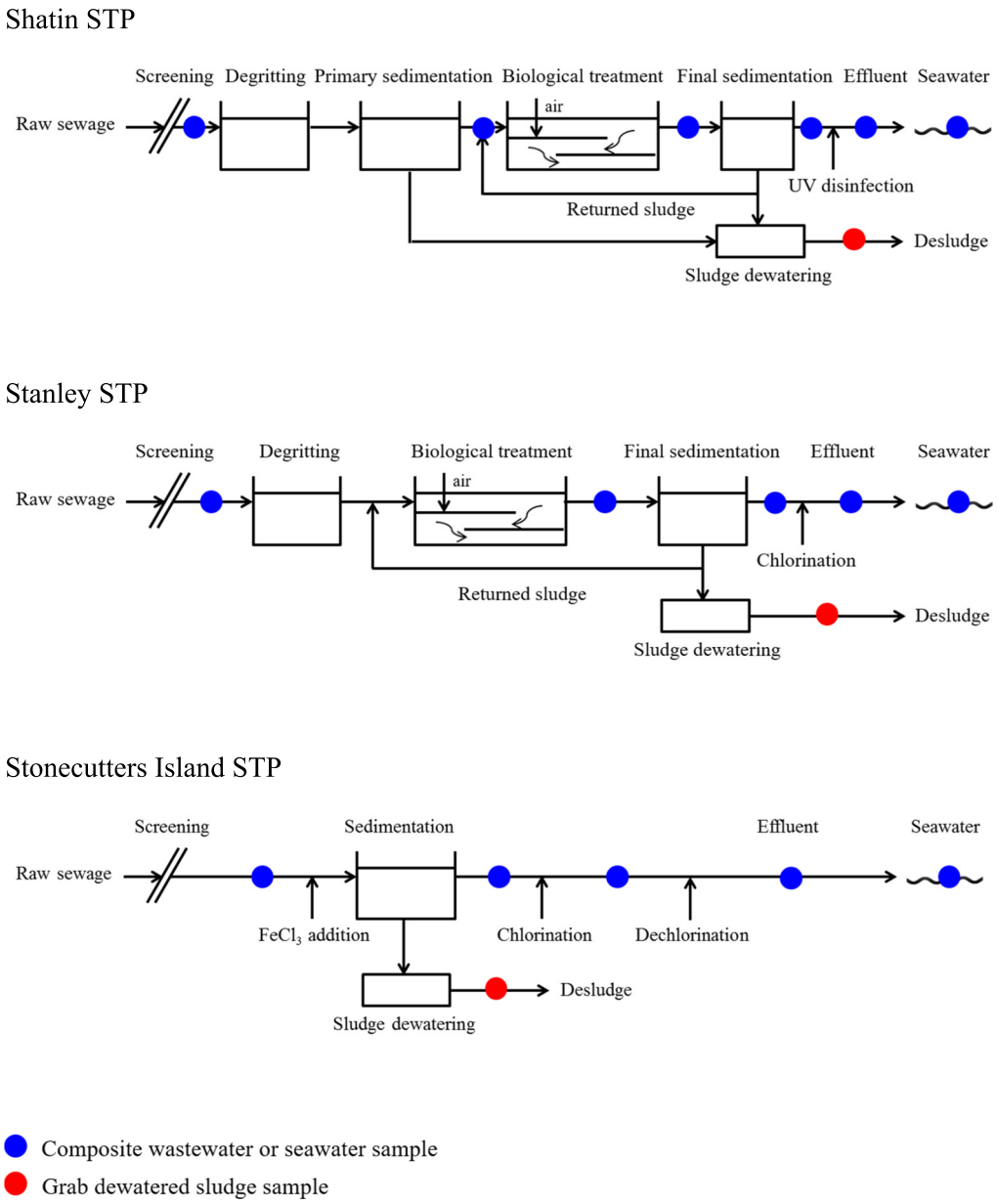

Fig. 1. Flow schemes for the three selected sewage treatment plants (STPs): Shatin STP, Stanley STP and Stonecutters Island STP.

at ng/L levels, these chemicals may disrupt the endocrine systems of animals by interacting with the oestrogen receptor, and then further affect their physiology through their impact on processes such as embryonic development, gonadal formation and sex differentiation (Johnson and Sumpter, 2001; Xu et al., 2015). In contrast to retinoids, in the past two decades, many studies have focused on the investigation of the concentrations of EDCs and their removal efficiencies in STPs (e.g., Johnson and Sumpter, 2001; Melvin and Leusch, 2016; Ahmed et al., 2017). Nonetheless, most of the reported studies were conducted in STPs with freshwater wastewater while only very few studies have been performed in coastal cities where seawater is used for flushing the toilets, leading to saline influents and different microbial communities in their STPs (Xu et al., 2014; Chiu et al., 2016).

Hong Kong is a coastal city with a population of > 7.3 million that is located on a small area of 1106 km^2 (HKCSD, 2017; HKLD, 2017). To reduce local freshwater demand, seawater has been used for toilet flushing on a large scale in Hong Kong since the 1950s (Tang et al., 2006). Seawater is supplied to > 80% of the Hong Kong population for toilet flushing (Tang et al., 2006), resulting in the saline wastewater in the STPs of Hong Kong. However, only one study has quantified the concentrations of common EDCs in the influents and effluents of three relatively small STPs that serve approximately 0.4% of the total population of Hong Kong (Xu et al., 2014). Although a recent study reported the concentrations of five EDCs (namely, 4-NP, triclosan (TCS), BPA, 17β-estradiol (E2) and 17α-ethinylestradiol (EE2)) in the semi-permeable membrane devices (SPMD) deployed at the influents and effluents of three large STPs in Hong Kong (Chiu et al., 2016), it is impossible to compare such SPMD-based data with the data collected from conventional measurements of EDCs in the influents and effluents of STPs in different regions and countries. Hence, the removal and fate of EDCs in different processes of STPs in Hong Kong with saline influents, and their risk assessments in the receiving coastal waters have not been comprehensively examined.

This study aimed to: (1) develop sensitive methods using high pressure liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) to determine the levels of retinoids (RAs and 4-oxo-RAs) and EDCs in water and sludge samples; (2) to determine the levels of retinoids and EDCs in different treatment processes, and the removal efficiencies of

retinoids and EDCs from three STPs in Hong Kong; and (3) to assess the ecological risks of retinoids and EDCs to marine organisms in Hong Kong. Here, we hypothesized that STPs with different salinities and treatment processes had different abilities for the removal of retinoids and EDCs, and the treated effluents still exhibited ecological risks to marine organisms due to the incomplete removal of these chemicals by STPs. This study will improve our understanding of the environmental fate of retinoids and EDCs, and their current ecological risks to the marine environment of Hong Kong; the results obtained in this study will also be essential for enabling the environmental authority to make informed decisions regarding the management of these chemical contaminants.

2. Materials and methods

2.1. Chemicals and sample collection

High purity standards of six retinoids, seven EDCs and seven internal standards were purchased from various suppliers. Detailed information about the chemicals and materials used in this study is provided in the Supplementary Information (Table S1). Two STPs using the activated sludge process (i.e., the Shatin STP and Stanley STP), and the Stonecutters Island STP using the chemically enhanced primary treatment (CEPT) in Hong Kong were chosen in this study to investigate the concentrations of the retinoids and EDCs in each stage of the wastewater treatment process and in their respective adjacent receiving seawaters (Fig. S1). These three STPs serve approximately 57% of the total population in Hong Kong. Based on our measurements, the salinity values for the wastewater of the Shatin STP and the Stonecutters Island STP were 20‰, while that of the Stanley STP was 5‰. Composite samples ($n = 3$) for water and grab samples ($n = 3$) for dewatered sludge were collected in November 2016 from each of the sampling points in the three STPs (Fig. 1). Further information regarding these STPs and a detailed description of the sampling are provided in the Supplementary Information (Table S2).

2.2. Sample preparation and instrumental analyses

Extraction of the samples and instrumental analyses were carried out following the methods described by Chen et al. (2010) and Wu et al. (2010) with modifications, using the method described in detail in Supplementary Information. In brief, the water samples (wastewater and seawater) were extracted by solid-phase extraction (SPE) using Waters Oasis HLB cartridges, while the sludge samples were extracted with ethyl acetate by ultrasonication. All of the extracts were further purified using silica gel columns prior to the injection to the HPLC-MS/MS for the analysis of the target chemicals. The target compounds were separated into three groups (RAs, 4-oxo-RAs and EDCs) and were analysed by HPLC-MS/MS using an Agilent 1290 HPLC coupled to a 3200 QTRAP mass spectrometer. An electrospray ionization (ESI) source was used in the negative ion mode, and the multiple reaction monitoring (MRM) mode was used to analyse the target compounds, using some isotope-labeled compounds as the internal standards. HPLC-MS/MS chromatograms of the RAs, 4-oxo-RAs and EDCs in the standard are shown in Fig. S2. More information regarding the analytical methodology is provided in the Supplementary Information (Table S3). The relative recoveries for the retinoids and EDCs spiked into the wastewater samples ranged from 65 to 160% and 100–154%, respectively, and those in the sludge samples ranged from 70 to 158% and 63–182%, respectively (Table 1).

2.3. Ecological risk assessment

The ecological risk of a chemical is assessed using the hazard quotient (HQ) that is equal to the ratio between the measured environmental concentration (MEC) and the predicted no-effect concentration (PNEC) of a chemical (i.e., HQ = MEC/PNEC) (EC, 2003). According to the risk ranking criteria, $0.01 \leq HQ < 0.1$, $0.1 \leq HQ < 1$ and $HQ \geq 1$ indicate low risk, medium risk and high risk, respectively. The proposed PNEC values for the retinoids and EDCs in seawater are as follows: 30.9 ng/L for at-RA (Wu et al., 2010), 330 ng/L for 4-NP (EU, 2002), 12.2 ng/L for 4-*t*-OP (EQS, 2005), 150 ng/L for BPA (EU, 2010), 3 ng/L for estrone (E1; Young et al., 2002), 50 ng/L for TCS (ECHA, 2015) and 58 ng/L for triclocarban (TCC; Zhao et al., 2010). For diethylstilbestrol (DES), the chronic no observed effect concentration (NOEC) value was used to calculate the PNEC value (EC, 2003). The chronic NOEC value of DES for the survival and reproduction of the marine copepod *Tisbe battagliai* was 10 µg/L (Hutchinson et al., 1999), which was divided by an assessment factor of 100 to obtain the PNEC of DES (i.e., 100 ng/L).

3. Results

3.1. Occurrence of retinoids and EDCs in three STPs

3.1.1. Retinoids and EDCs in the influents

The total concentration of the retinoids in the influents of the three STPs ranged from 7.1 to 29 ng/L, while that of the EDCs ranged from 3107 to 5829 ng/L (Fig. 2a & c). The total concentration of the retinoids in the Shatin STP was higher than that in the Stanley STP or the Stonecutters Island STP. However, the total concentration of the EDCs in Shatin STP was lower than that in the two other STPs (Fig. 2a & c). Four of six retinoids were detected in the influents of the three STPs and at-4-oxo-RA accounted for 49–77% of the total retinoid content (Figs. 2b & 3), whereas 9c-RA and 9c-4-oxo-RA were not found. EDCs were dominated by 4-NP and BPA in the three STPs, accounting for 27–87% and 5–62% of the total EDC content, respectively (Figs. 2d & 4).

3.1.2. Retinoids and EDCs in the effluents

The total concentration of the retinoids in the effluents of the three STPs ranged from 3.7 to 9.1 ng/L, while that of the EDCs ranged from 1225 to 2638 ng/L (Fig. 2a & c). Retinoids were dominated by at-4-oxo-RA and 13c-4-oxo-RA, which accounted for 52–80% of the total retinoid content (Figs. 2b & 3). Similar to the results obtained for the influents, no 9c-RA or 9c-4-oxo-RA was found in any of the effluent samples from the three STPs. EDCs were dominated by 4-NP, accounting for 47–60% of the total EDC content, followed by BPA (18–32% of the total EDC content) (Figs. 2d & 4).

3.1.3. Comparison of retinoids and EDCs between the influent and effluent

The composition profiles of the individual retinoids and EDCs in the effluents collected from the three STPs were similar to those in their corresponding influents (Figs. 2b, d, 3 & 4). A significantly positive correlation was found for the retinoids' concentrations of the influent and effluent in the Shatin STP ($r = 0.939$, $p < 0.001$) and the Stonecutters Island STP ($r = 0.966$, $p < 0.001$) (Fig. 5a). However, there was no significant correlation between the retinoid levels of the influent and effluent in the Stanley STP ($r = 0.304$, $p > 0.05$), indicating that the treatment processes or mechanisms of retinoids may be different between this relatively freshwater STP (5‰ salinity) and the other two saline STPs (20‰ salinity). A significant positive correlation was found for the EDCs' concentrations of the influents and effluents for all of the three studied STPs (Fig. 5c). High influent concentrations of EDCs usually lead to the correspondingly high effluent concentrations, regardless of the wastewater salinity and treatment technology.

3.1.4. Aqueous removal efficiencies of retinoids and EDCs

Among the three STPs, total removal efficiencies in the aqueous phases ranged from 41 to 82% for total retinoids, and 31–79% for total EDCs, respectively (Tables S4–S6). The highest total removal efficiency for retinoids was found in the Shatin STP (82% removal) and that for

Table 1
Recoveries (%), matrix effects (%), limits of detection (LOD) and quantitation (LOQ) for the investigated compounds in the influent wastewater and in the sludge.

Compound	Abbreviation	Influent[a]				Sludge[a]			
		Recovery[b]	Matrix effect	LOD[c] (ng/L)	LOQ[c] (ng/L)	Recovery[b]	Matrix effect	LOD[c] (ng/g)	LOQ[c] (ng/g)
Retinoids									
All-*trans*-retinoic acid	At-RA	65 ± 8	78 ± 5	0.32	1.1	70 ± 12	85 ± 4	0.37	1.2
13-*cis*-retinoic acid	13c-RA	107 ± 1	101 ± 8	0.40	1.3	136 ± 12	77 ± 10	0.25	0.83
9-*cis*-retinoic acid	9c-RA	80 ± 3	86 ± 10	0.83	2.8	86 ± 6	146 ± 11	0.63	2.1
All-*trans*-4-oxo-retinoic acid	At-4-oxo-RA	160 ± 21	222 ± 23	0.18	0.60	158 ± 10	181 ± 24	0.17	0.56
13-*cis*-4-oxo-retinoic acid	13c-4-oxo-RA	96 ± 9	232 ± 12	0.24	0.81	104 ± 9	174 ± 31	0.31	1.0
9-*cis*-4-oxo-retinoic acid	9c-4-oxo-RA	78 ± 8	194 ± 14	0.39	1.3	75 ± 6	202 ± 39	0.85	2.8
Endocrine disrupting chemicals									
4-Nonylphenol	4-NP	152 ± 18	70 ± 70	1.8	6.1	63 ± 19	106 ± 11	9.3	31
4-tert-Octylphenol	4-*t*-OP	109 ± 128	112 ± 159	2.3	7.7	117 ± 27	128 ± 11	3.1	10
Bisphenol-A	BPA	113 ± 32	106 ± 23	2.5	8.3	182 ± 23	97 ± 12	2.6	8.6
Estrone	E1	100 ± 14	109 ± 24	6.4	21	97 ± 25	132 ± 21	1.2	4.1
Diethylstilbestrol	DES	154 ± 15	159 ± 28	3.9	13	88 ± 7	71 ± 8	2.7	9.0
Triclosan	TCS	114 ± 68	123 ± 15	0.92	3.1	96 ± 4	79 ± 17	4.3	14
Triclocarban	TCC	101 ± 21	147 ± 10	0.085	0.28	135 ± 50	111 ± 8	0.35	1.2

[a] Mean ± SD (%, $n = 3$).
[b] Spiked level. Influent: 60 ng/L each of six retinoids, 600 ng/L 4-NP, 300 ng/L each of 4-*t*-OP and BPA, 150 ng/L each of E1, DES, TCS and TCC. Sludge: 30 ng/g each of six retinoids, 15,000 ng/g 4-NP, 7500 ng/g 4-*t*-OP, 300 ng/g BPA, 75 ng/g each of E1 and DES, 1500 ng/g TCS and 3000 ng/g TCC.
[c] LOD, limit of detection; LOQ, limit of quantitation. LOD and LOQ for each investigated compound were calculated based on the signal-to-noise ratio (SNR) near the target peak. LOD is defined as three times of SNR, and LOQ is ten times of SNR.

EDCs was observed in the Stanley STP (79% removal). Removal efficiencies of the individual retinoids varied between 34% and 87% across the three STPs. However, the removal efficiencies for some species of EDCs were unexpectedly negative after the sewage treatment processes. For example, the removal efficiencies for TCC were − 131% and − 80% in Shatin STP and in Stanley STP, respectively, probably due to its resuspension from the sludge to the aqueous phase.

In the Shatin STP, only 31% removal was found for the EDCs during the entire treatment, with the exception of TCC, which showed an increase in its concentration (Table S4). The concentrations of the retinoids increased during the primary treatment, while biological treatment (75% removal) and UV treatment (55% removal) played the dominant role in the removal of the retinoids (Table S4). In the Stanley STP, biological treatment played a role in the removal of retinoids (24% removal), while it contributed little to the removal of most of the EDCs (Table S5). During final sedimentation, clear removals were found for all of the retinoids (47% removal) and EDCs (69% removal) with the exception of TCC, while chlorination was not effective for the removal

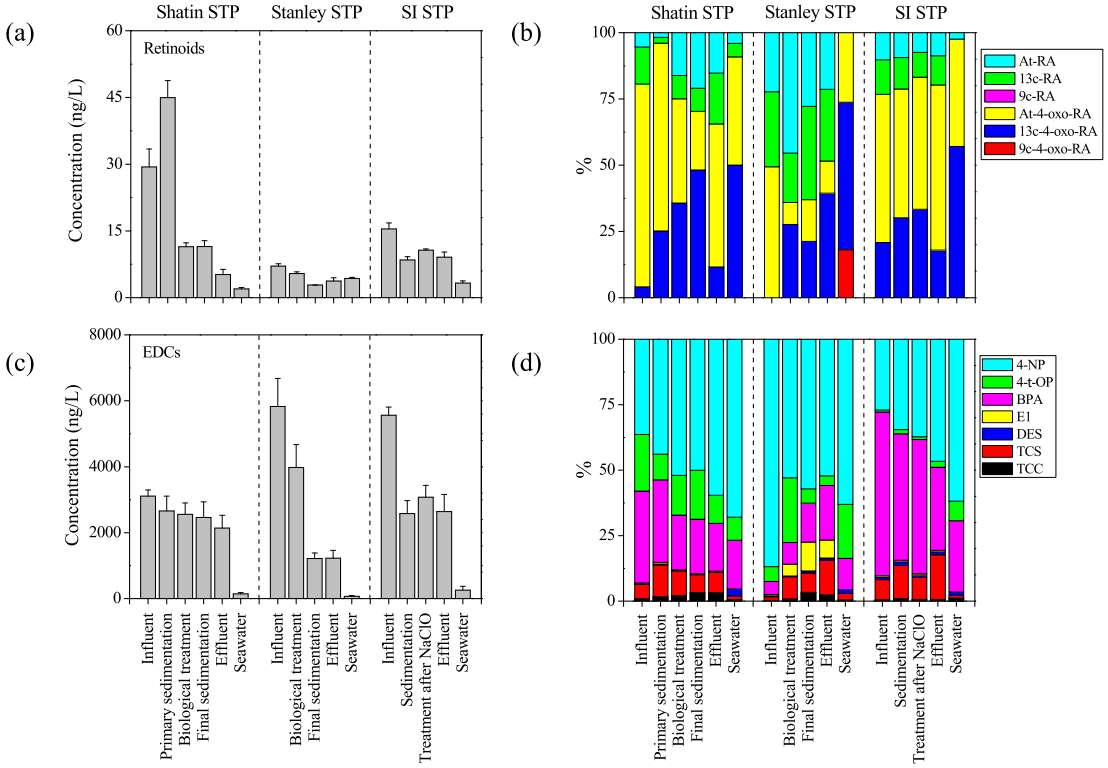

Fig. 2. Total concentrations (ng/L, $n = 3$; a&c) of the retinoids and endocrine disrupting chemicals (EDCs), and the fraction (b&d) of each of the retinoids and EDCs in the wastewater samples collected from different stages of the three sewage treatment plants (STPs): Shatin STP, Stanley STP and Stonecutters Island STP (SI STP), and in the seawater samples collected from the adjacent coastal marine environments of the three STPs in Hong Kong.

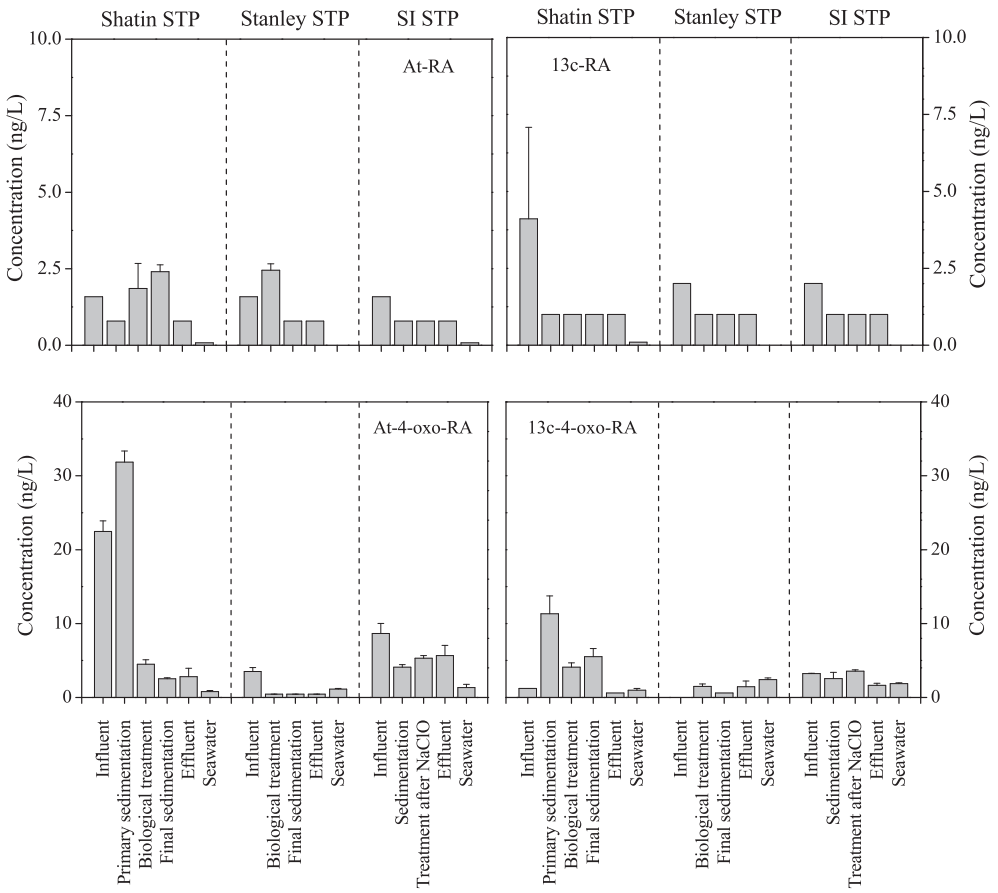

Fig. 3. Concentrations (ng/L, $n = 3$) of the four major species of retinoids in the wastewater samples collected from different stages of the three sewage treatment plants (STPs): Shatin STP, Stanley STP and Stonecutters Island STP (SI STP).

of the retinoids and EDCs (Table S5). In the Stonecutters Island STP, the chemical treatment process (i.e., CEPT) played the dominant role in the removal of all of the retinoids (45% removal) and EDCs (54% removal), while chlorination and dechlorination contributed little in this regard (Table S6).

Generally, about two-thirds of the retinoids and EDCs were removed in the three STPs (Tables S4–S6). In the two STPs (i.e., Shatin STP and Stanley STP) that use secondary biological treatment processes, most of the retinoids were clearly removed during the biological treatment, whereas most of the EDCs were not removed (Tables S4–S5). The removal of EDCs in the Stanley STP was attributed mainly to final sedimentation, while little removal of the EDCs was found in the Shatin STP during the entire treatment process. For the Stonecutters Island STP that uses the CEPT process, large proportions of the retinoids and EDCs were removed during the chemical treatment process (Table S6).

3.1.5. Retinoids and EDCs in the dewatered sludge

Total concentrations of the retinoids in the dewatered sludge samples of the three STPs ranged from 14 to 26 ng/g, and those of EDCs ranged from 8009 to 37,071 ng/g (Table 2). Only two (at-RA and 13c-RA) of the six retinoids and all of the seven EDCs were detected in the dewatered sludge samples of the three STPs (Table 2). EDCs were dominated by 4-NP in the Shatin STP and Stanley STP, accounting for 62% and 46% of the total EDC content, respectively. However, TCC and TCS were the two predominant EDCs in the Stonecutters Island STP, and these two EDCs contributed 48% and 36% of the total EDC content, respectively (Table 2).

3.1.6. Mass balance analysis

Total mass loads of the retinoids in the influents of the Shatin STP, Stanley STP and Stonecutters Island STP were 7.4 g/d, 0.058 g/d and 30 g/d, respectively, and those of the EDCs in the influents were 786 g/d, 48 g/d and 10,906 g/d, respectively. The mass loss percentage for the total retinoid content accounted for 77% of the total mass loads in the Shatin STP, while it accounted for only 21% and 16% in the Stanley STP and Stonecutters Island STP, respectively (Table S7). These results indicated that the removal of the retinoids in the Shatin STP was mainly attributed to degradation, whereas those in the two other STPs were attributed to sorption in the sludge (25–27%), followed by degradation (16–21%) (Table S7). For the saline Shatin STP and relatively freshwater Stanley STP using the activated sludge process, the difference in the removal of the retinoids between these two STPs may be due to the different microbial communities present in the activated sludge that are significantly influenced by the salinity. The mass loss percentage for the total EDC content was −99% in the Shatin STP, suggesting the release of EDCs from the sludge or their transformation from the chemicals with similar structures. The mass loss percentages for the total EDC content were 67% and 18% in the Stanley STP and Stonecutters Island STP, respectively, while their sludge fractions were 12% and 34%, respectively (Table S7). These results showed that the removal of EDCs was attributed mainly to degradation for the Stanley STP and to sorption for the Stonecutters Island STP.

3.2. Occurrence of retinoids and EDCs in seawater

3.2.1. Retinoids and EDCs in seawater

The total concentrations of the retinoids in the seawaters of the receiving water bodies adjacent to the three STPs ranged from 2.0 to 4.3 ng/L, and those of the EDCs ranged from 71 to 260 ng/L (Fig. 2a & c). Five of the six retinoids were detected in the seawater samples, and

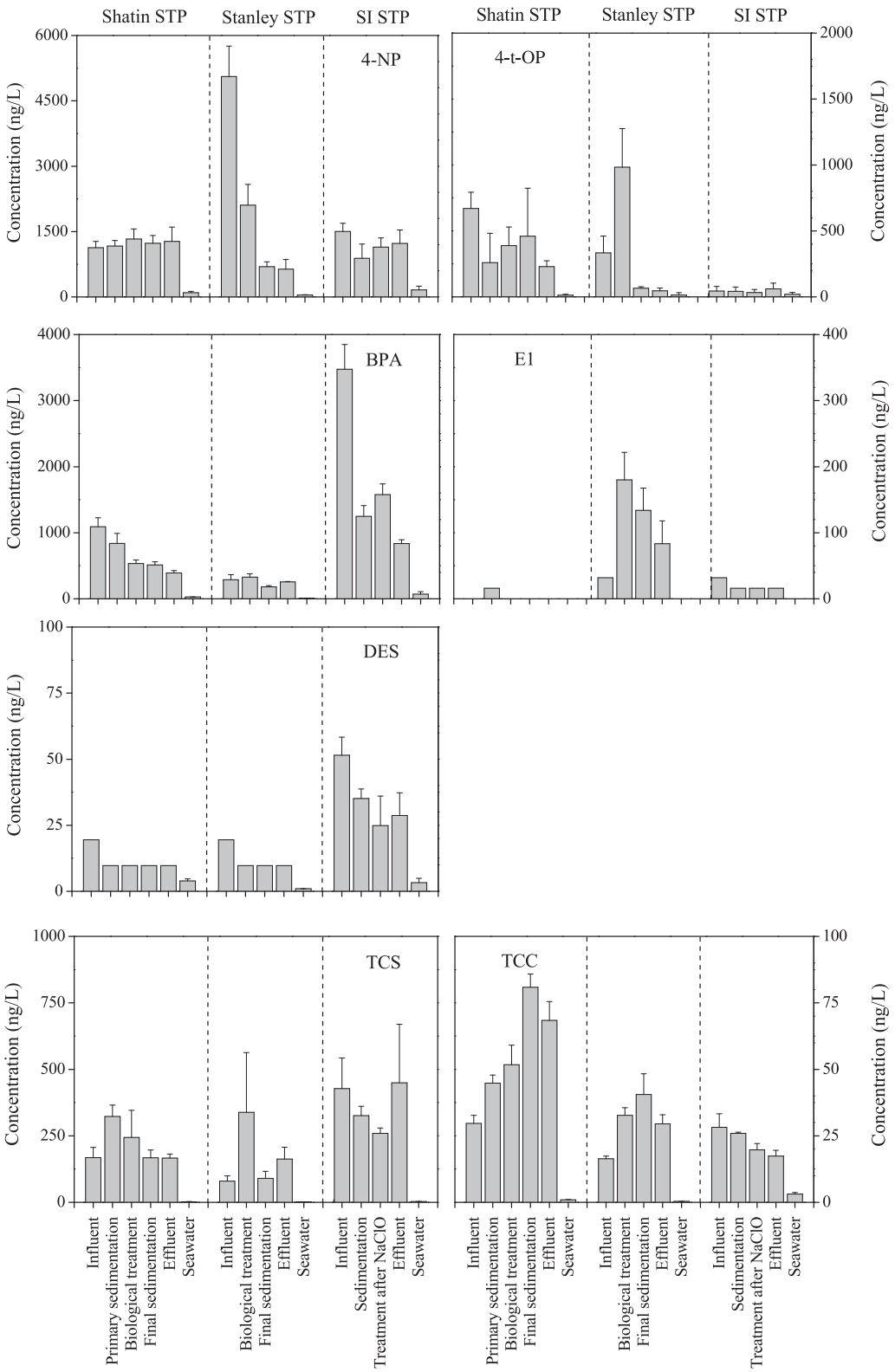

Fig. 4. Concentrations (ng/L, $n = 3$) of each of the endocrine disrupting chemicals (EDCs) in the wastewater samples collected from different stages of the three sewage treatment plants (STPs): Shatin STP, Stanley STP and Stonecutters Island STP (SI STP).

the retinoids were dominated by 13c-4-oxo-RA that accounted for 50–57% of the total retinoid content, followed by at-4-oxo-RA (26–41%; Figs. 2b & 3). No 9c-RA was found in any seawater samples. EDCs were dominated by 4-NP that accounted for 62–68% of the total EDC content, followed by either BPA (12–27%) or 4-*t*-OP (8–21%) (Figs. 2d & 4). E1 was not found in any of the seawater samples.

3.2.2. Comparison of retinoids and EDCs between effluent and seawater

Across all three STPs, concentrations of both groups of chemicals in the seawaters increased with those in the corresponding effluents

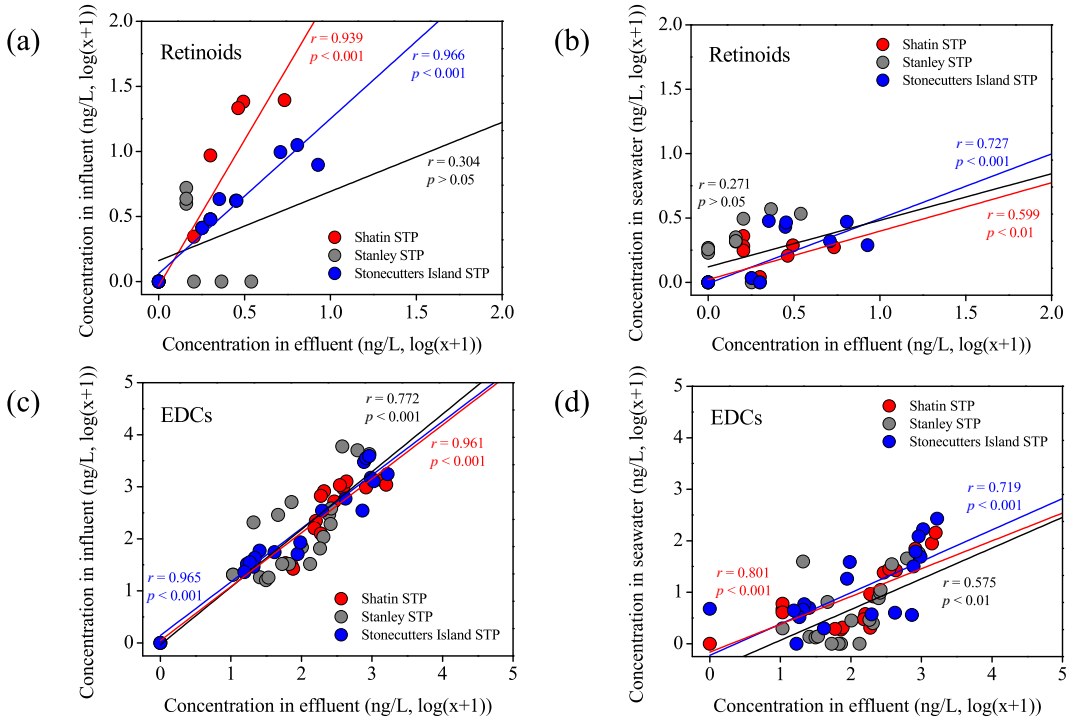

Fig. 5. Comparison of the concentrations of the retinoids or endocrine disrupting chemicals (EDCs) between the effluents and influents (a&c) or the receiving seawaters (b&d) adjacent to the three sewage treatment plants (STPs): Shatin STP, Stanley STP and Stonecutters Island STP.

(Fig. 5b & d). The compositions and ratios of the detected retinoids or EDCs in the receiving seawaters of the three STPs were similar to those of the respective effluents of the three STPs with the exception of the 9c-4-oxo-RA that was only found in the receiving seawater of the Stanley STP (Figs. 2b, d, 3 & 4). A significant positive correlation was found for the retinoids' concentrations of the seawaters and the effluents in the Shatin STP ($r = 0.599$, $p < 0.01$) and Stonecutters Island STP ($r = 0.727$, $p < 0.001$) (Fig. 5b). No significant correlation was found for the Stanley STP ($r = 0.271$, $p > 0.05$), mostly likely due to the dilution by seawater, and either adsorption or degradation by marine organisms in the seawater adjacent to this relatively freshwater STP. A significant positive correlation was found for the EDCs' concentrations of the seawaters and the effluents for all of the three studied STPs (Fig. 5d), suggesting that sewage effluents of the STPs were an important source of EDCs for the coastal marine waters in Hong Kong.

3.3. Assessment of the ecological risk posed by retinoids and EDCs to marine organisms

The hazard quotient (HQ = MEC/PNEC) is usually used to assess the ecological risk of a chemical. A HQ greater than one implies that the chemical poses a high risk so that further risk management action is required. The results of the ecological risk assessment of these two groups of chemicals based on the HQ are shown in Fig. 6. Four EDCs (i.e., 4-NP, 4-*t*-OP, BPA and TCS) exhibited high risks (HQ > 1) to marine organisms in the effluents of all three STPs. In seawaters, retinoids, 4-NP, 4-*t*-OP, and in some cases BPA exhibited medium or high risks to marine organisms. However, other chemicals (i.e., E1, DES, TCS and TCC) posed low or no risks to marine organisms in seawater (Fig. 6).

Table 2
Concentration (ng/g, mean ± SD, $n = 3$) and respective ratio (%, in parentheses) of the retinoids and endocrine disrupting chemicals (EDCs) in the sludge samples collected from three sewage treatment plants (STPs). N.D.: not detected.

Compound	Shatin STP	Stanley STP	Stonecutters Island STP
Retinoids			
At-RA	4.8 ± 2.0 (35% ± 7%)	10 ± 0.68 (48% ± 4%)	17 ± 0.92 (65% ± 0%)
13c-RA	9.4 ± 4.5 (65% ± 7%)	11 ± 1.3 (52% ± 4%)	9.0 ± 0.38 (35% ± 0%)
9c-RA	N.D. (0%)	N.D. (0%)	N.D. (0%)
At-4-oxo-RA	N.D. (0%)	N.D. (0%)	N.D. (0%)
13c-4-oxo-RA	N.D. (0%)	N.D. (0%)	N.D. (0%)
9c-4-oxo-RA	N.D. (0%)	N.D. (0%)	N.D. (0%)
Total retinoids	14 ± 6.4	21 ± 1.2	26 ± 1.3
Endocrine disrupting chemicals			
4-NP	22,900 ± 4643 (62% ± 3%)	3705 ± 724 (46% ± 9%)	1045 ± 165 (9% ± 1%)
4-*t*-OP	9318 ± 3252 (25% ± 5%)	198 ± 50 (2% ± 1%)	134 ± 19 (1% ± 0%)
BPA	230 ± 152 (1% ± 1%)	111 ± 41 (1% ± 0%)	683 ± 86 (6% ± 0%)
E1	7.5 ± 6.0 (0% ± 0%)	19 ± 7.4 (0% ± 0%)	14 ± 5.6 (0% ± 0%)
DES	N.D. (0%)	283 ± 152 (3% ± 2%)	13 ± 6.7 (0% ± 0%)
TCS	1201 ± 841 (3% ± 2%)	1852 ± 556 (23% ± 7%)	4610 ± 1241 (36% ± 3%)
TCC	3415 ± 461 (10% ± 3%)	1841 ± 404 (23% ± 5%)	6050 ± 1084 (48% ± 1%)
Total EDCs	37,071 ± 6967	8009 ± 163	12,548 ± 2514

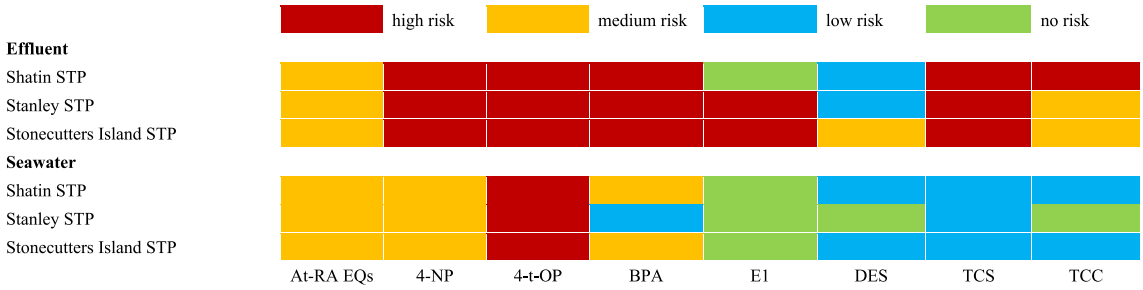

Fig. 6. Calculated hazard quotients (HQs) of the retinoids and endocrine disrupting chemicals (EDCs) detected in the effluent and seawater samples. $0.01 \leq HQ < 0.1$, $0.1 \leq HQ < 1$ and $HQ \geq 1$ indicate low risk, median risk and high risk, respectively. At-RA EQs: at-RA equivalents.

4. Discussion

4.1. Occurrence and fate of retinoids and EDCs in STPs

4.1.1. Retinoids

In this study, retinoic acids (at-RA and 13c-RA) and their metabolites (at-4-oxo-RA and 13c-4-oxo-RA) were found in the wastewaters (7.1–29 ng/L in influents, and 3.7–9.1 ng/L in effluents) of three STPs in Hong Kong. Our results were comparable with those reported in the previous studies on the identification of these compounds in China and Japan (Zhen et al., 2009; Wu et al., 2010; Sawada et al., 2012). In some cases, the highest retinoid levels were observed in the middle treatment stages (e.g., the primary sedimentation process in the Shatin STP), which may be due to the deconjugation of their glucuronide conjugates caused by the microbial activities in the STPs (Zhen et al., 2009). We observed variable removal efficiencies (41–82% removal) in the aqueous phase for the total retinoid content during the wastewater treatment in the three STPs, indicating that these chemicals could be partially removed by microbial activities or chemical treatment. For the Shatin STP, the removal of the retinoids was attributed mainly to degradation, while it was attributed to sorption by sludge or degradation for the other two STPs (Table S7); these results are similar to the results reported by Inoue et al. (2013).

In the present study, retinoids were dominated by at-4-oxo-RA and 13c-4-oxo-RA in wastewaters, but only at-RA and 13c-RA were found in the three STP sludges, implying that at-4-oxo-RA and 13c-4-oxo-RA were relatively unstable in the anaerobic sludge, and/or the oxidation of retinoic acids was inhibited in the anaerobic sludge. The amounts of the retinoids in the sludge accounted for 25% of their total content in the Stonecutters Island STP but only for 5% of the total retinoid content in the Shatin STP, indicating that the retinoids were more readily accumulated in the CEPT sludge than in the sludge from the activated sludge process, most likely due to the greater degradation provided by the microbial activity during the activated sludge process. This study represents the first study to investigate the fate and removal of the retinoids in the STPs that receive saline wastewaters from toilet flushing with seawaters. Our results clearly showed the potential difference in the levels and removal of the retinoids among the STPs that employ different wastewater treatment technologies.

4.1.2. EDCs

Our results showed that the concentrations of each of the EDCs in the wastewaters of the three STPs were highly variable, ranging from ng/L to μg/L levels. Such high variabilities were noted in previous studies conducted in different parts of the world (Johnson and Sumpter, 2001; Melvin and Leusch, 2016; Ahmed et al., 2017). In good agreement with our results (Fig. 2d), alkylphenols (NP and OP) and BPA were often found to be the most abundant EDCs and were the most frequently detected EDCs in wastewater of STPs (Nakada et al., 2006; Xu et al., 2014). Although NP and BPA have weaker oestrogen-mimicking potentials than the natural oestrogens, these chemicals may be the most important endocrine disrupters in the aquatic environments where their total concentrations are > 1 μg/L (Johnson and Sumpter, 2001). In addition to alkylphenols and BPA, in this study, TCC and TCS were found at higher concentrations in the various stages of the wastewater treatment. In the following discussion, we will focus on these five dominant EDCs (i.e., 4-NP, 4-*t*-OP, BPA, TCC and TCS).

As shown in this study, the removal efficiencies of alkylphenols varied between the three STPs, and the levels of alkylphenols in some cases even increased after the wastewater treatment process, most likely due to the degradation of their alkylphenol ethoxylates (APEs) parent compounds (Johnson and Sumpter, 2001). According to mass balance analysis (Table S7), alkylphenols were mainly removed by degradation in the Stanley STP, whereas they were removed by accumulation in the sludge in the other two STPs. Our results are in agreement those reported in other studies in the literature (Johnson and Sumpter, 2001; Ahmed et al., 2017). Although the concentrations of the alkylphenols in wastewaters were comparable among the three STPs in this study (Fig. 4), their concentrations in the sludge of the Shatin STP were much higher than those values from the other two STPs. The low levels of alkylphenols in the sludge of the Stonecutters Island STP may be attributed to the strong chemical degradation in the presence of flocculant (i.e., ferric chloride).

Removals of BPA in wastewaters were comparatively efficient (64–76%) in the Shatin STP and Stonecutters Island STP, whereas only 11% removal of BPA was found in the wastewater of the Stanley STP (Tables S4–S6). Sorption of BPA by sludge contributed only 2–3%, and the removal of BPA was attributed mainly to degradation (Table S7). This result was supported by the study of Nakada et al. (2006) who found that the high removal efficiency (> 92%) of BPA was due to aerobic biodegradation rather than adsorption onto solids. Moreover, Fernandez et al. (2009) reported that 52–100% of BPA was removed in a STP in Spain that used the activated sludge process, while no adsorption or accumulation was found in the sludge, demonstrating the great water solubility of BPA with no partitioning to the sludge. BPA levels (mean 530 and median 280 ng/g dw) similar to those found in the current study were found in the sludge of several STPs in Greece that used the activated sludge process (Stasinakis et al., 2008). A rapid sorption of BPA on the activated sludge and subsequent biodegradation are the key removal mechanisms (Zhao et al., 2008). BPA (111–230 ng/g dw) accounted for only 1% of the total EDCs accumulated in the sludge in the two STPs that used the activated sludge process, while BPA (683 ng/g dw) represented 6% of the total EDC content in the Stonecutters Island STP that used the CEPT process (Table 2), implying that BPA could be more easily degraded by the microorganisms in the activated sludge.

The levels of TCS (80–428 ng/L, Fig. 4) in the influents of the three STPs in Hong Kong were comparable to those found in the STPs in other countries including Germany (Bester, 2003), Switzerland (Singer et al., 2002) and USA (Heidler and Halden, 2007; Lozano et al., 2013). However, the removal efficiencies (no or negative removal) of TCS in the three STPs of Hong Kong were far lower than those in other jurisdictions. Previous studies indicated that over 90% of TCS can be removed in the STPs with the activated sludge process (Bester, 2003;

Heidler and Halden, 2007; Lozano et al., 2013). The removal of TCS was mainly due to sorption by the sludge (Singer et al., 2002; Heidler and Halden, 2007; Lozano et al., 2013), and its subsequent degradation or transformation by microbial activity to its derivatives such as methyltriclosan (Lozano et al., 2013), and mineralization to CO_2 (Singer et al., 2002). In this study, no TCS was removed in the three STPs, while TCS was found with high concentrations (1200–4610 ng/g) in the sludge. These concentrations were similar to those reported in other studies (Bester, 2003; Ying and Kookana, 2007). Apparently, TCS can be easily accumulated in the STPs with low degradation efficiencies, while the TCS in the sludge during the treatments may be released to the aqueous phase; this contributes to the high concentrations of TCS in the effluents. Bester (2003) reported that 30% of TCS was adsorbed to the sludge, and 5% was dissolved in the effluent. Heidler and Halden (2007) reported that 50% of TCS was accumulated in the sludge, and 2% of TCS was found in the effluent. According to mass balance analysis (Table S7), the amounts of TCS in the effluent were close to those in the sludge, suggesting a low removal efficiency of this compound for the three STPs in Hong Kong. Such a low removal efficiency was most likely related to the overall design and daily operation of the STPs, the input loading of the pollutants and the hydraulic retention time (Ying and Kookana, 2007; Lozano et al., 2013).

The levels of TCC in the influents (16–30 ng/L) and effluents (17–68 ng/L) of the three STPs of Hong Kong were far lower than those reported in the STPs in the USA (4.9–6.1 μg/L in influents, and 0.12–0.17 μg/L in effluents) (Heidler et al., 2006; Lozano et al., 2013) but are comparable to the values obtained for a STP in mainland China (the TCC levels in the influent and effluent were 57 ng/L and 30 ng/L, respectively; Zhou et al., 2014). The TCC removal values for the three STPs in Hong Kong were similar to their TCS removal values (negative removal) with the exception of 38% of TCC removed by the Stonecutters Island STP (Tables S4–S6). The TCC removal efficiencies found in this study were lower than those reported in the STPs in the USA (approximately 97%) and in mainland China (48%) (Heidler et al., 2006; Lozano et al., 2013; Zhou et al., 2014). However, we found that the amounts of TCC in the sludge were much higher than those in the effluents (Table S7), which was not consistent with the results for TCS. These results indicated that TCC was easily accumulated in the sludge of the STPs, as was also suggested in the previous studies (Heidler et al., 2006; Lozano et al., 2013).

4.1.3. Comparison among the STPs with different treatment processes and salinities

Conventional activated sludge process is commonly used to treat sewage in STPs worldwide. Our study indicated that not all of the retinoids and EDCs were removed in the two STPs (i.e., the Shatin STP and Stanley STP) using the activated sludge process, which is consistent with the results of the previous studies (Johnson and Sumpter, 2001; Inoue et al., 2013). It was reported that the conventional activated sludge process showed a comparatively poor overall removal for EDCs (Melvin and Leusch, 2016), but appeared to be more efficient in removing the retinoic acid activity than for the other STPs (i.e., lagoon-based STPs) (Allinson et al., 2011). Based on this study, better removal of the retinoids and alkylphenols was obtained in the aqueous phase of the two STPs using the activated sludge process (i.e., the Shatin and Stanley STPs) than in the Stonecutters Island STP that uses the CEPT process, while the latter STP was more advantageous for the removal of BPA, TCS and TCC than the two former STPs. In particular, degradation contributed to the removal of BPA, while sorption contributed to the removal of TCS and TCC. Both sorption and degradation were essential for the overall removal of other EDCs from the STPs.

Salinity can affect the composition of the microbial community and the pollutants removal performance during the wastewater treatment process (Wu et al., 2008; Jang et al., 2013). Both the Shatin and Staley STPs employ the activated sludge process, but the salinity of the wastewater in the Shatin and Staley STPs of with 20‰ and 5‰, respectively, are significantly different. Such a salinity difference most likely lead to the markedly different microbial communities present in the activated sludge and thus may have influenced the degradation or accumulation efficiency of retinoids and EDCs in the wastewater and sludge. It was reported that *Proteobacteria* was dominated by α-*Proteobacteria* that accounted for 18.5–25.7% of the total bacterial effective sequences in the activated sludge of the saline Shatin STP (Zhang et al., 2012). However, in the freshwater Stanley STP, the δ-*Proteobacteria* was the most dominant class in the sludge, accounting for 12.9% of the total (Zhang et al., 2012). In this study, the Shatin STP showed better removal of retinoids in the aqueous phase but less removal of EDCs than the Stanley STP, indicating a potential difference in the removal of the contaminants between the saline and freshwater STPs.

Generally, the removal efficiency of contaminants in wastewater increased with increased retention time (Ejhed et al., 2018). The hydraulic retention time (HRT) values were 20 h and 13.6 h for the Shatin and Stanley STPs, respectively (Table S2). Compared to these two STPs that use the activated sludge process, the Stonecutters Island STP that uses the CEPT process did not show any obvious advantage for the removal of the contaminants. On the one hand, as the world's largest STP that uses the CEPT process, the Stonecutters Island STP had an HRT of only 1.5 h, consuming less energy and saving capital cost during wastewater treatment. On the other hand, the CEPT sludge contains a large amount of organics and phosphorus that are valuable resources that can be recovered. Thus, a novel technology integrating wastewater treatment with the CEPT process and resource recovery is expected to be developed and used in the future.

4.2. Assessment of the risk posed by retinoids and EDCs to marine organisms

4.2.1. Retinoids

Herrmann (1995) reported that a short-term exposure of zebrafish *Brachydanio rerio* embryos at 900 ng/L at-RA from stage 13 to 23 (i.e., 5–28 h) led to deformities of their brain and tail. Degitz et al. (2003) reported that exposure of 600 ng/L at-RA for three days (stages 8–41) caused craniofacial deformities in embryos of *Xenopus laevis* frogs. The levels of retinoids in the effluents of STPs were usually found to be much lower than the concentration that triggered the toxicological effects, and thus the researchers declared that retinoids in STP effluents were unlikely to give rise to deleterious effects on aquatic organisms (Inoue et al., 2011; Sawada et al., 2012). However, toxicity may be underestimated by these short-term laboratory studies because long-term continuous exposure of such contaminants is expected in field conditions. The value of the benchmark dose low (BMDL) of mortality occurrence induced by at-RA was calculated as 30.9 ng/L (Wu et al., 2010) based on the exposure experiment of at-RA to the *X. laevis* embryos (Degitz et al., 2003). The present study adopted this value as PNEC to perform the risk assessment of at-RA for marine organisms. Considering that RAs and 4-oxo-RAs have roughly equivalent RAR agonistic activity (Zhen et al., 2009; Wu et al., 2010; Sawada et al., 2012) and similar mode of action (Herrmann, 1995), we assessed the total ecological risk posed by these retinoids in the effluents and in seawaters to the marine ecosystems of Hong Kong. The at-RA equivalents of the samples were calculated by summing the concentration of each of the detected retinoids multiplied by their respective at-RA equivalency factor (0.04 for 13c-RA, 0.15 for 9c-RA, 3.87 for at-4-oxo-RA, 0.46 for 13c-4-oxo-RA, 0.46 for 9c-4-oxo-RA; Zhen et al., 2009; Wu et al., 2010). Overall, only medium risk of retinoids to marine organisms (HQ: 0.1–1) was found in the effluents and seawaters.

Wu et al. (2010) investigated the occurrence of retinoids in Liaodong Bay and its adjacent rivers. They found that the retinoids were widely distributed in the rivers, but they did not find any retinoids in their seawater samples (Wu et al., 2010). Our study is the first report on the measurable ambient concentrations of retinoids (i.e., retinoic acids and their metabolites) in coastal seawater samples. Conventionally,

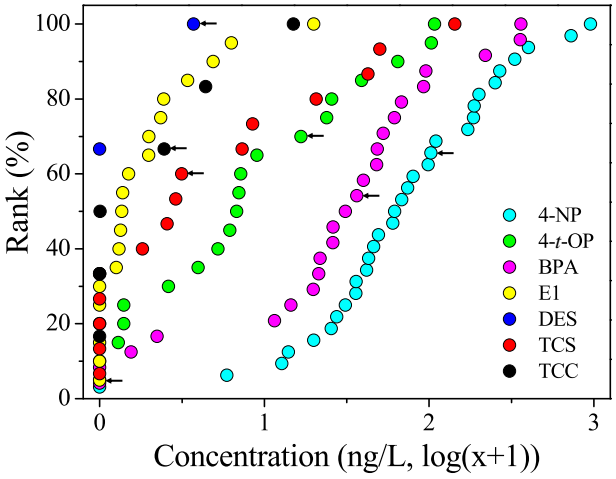

Fig. 7. Average concentrations (ng/L, log(x + 1)) of endocrine disrupting chemicals (EDCs) in surface seawaters around the world. Arrows indicate the values derived from this study. See Table S8 for detailed information.

development of imposex in marine female gastropods has been linked solely to exposure to synthetic organotin compounds in the marine environment (e.g., Leung et al., 2006) by binding with the retinoid X receptor (Nishikawa et al., 2004). In this study, the direct finding of the presence of retinoids in natural seawater challenges this simplistic view of organotin-induced imposex because laboratory-based exposure experiments have shown that pure retinoic acids can also induce the development of imposex in female gastropods (Nishikawa et al., 2004; Horiguchi et al., 2008). Thus, retinoids originating from the effluents of STPs may also contribute to the imposex development in female gastropods in the marine environment of Hong Kong where 100% of the females of the whelk *R. clavigera* showed imposex. The results of this study can improve our understanding on the source, level, risk and management of retinoids in coastal seawaters, and open up a new area of research in ecotoxicology of retinoids.

4.2.2. EDCs

In the past several years, EDCs have been detected in the seawaters around the world (Figs. 7 & S3; Table S8). Generally, 4-NP and BPA had high environmental concentrations in seawaters, 4-*t*-OP and TCS were found in medium concentrations, while other EDCs (i.e., E1, DES and TCC) were found in low concentrations in seawaters worldwide (Fig. 7; Table S8). Overall, the concentrations of each EDCs in seawaters of Hong Kong derived from this study was average compared to the corresponding levels in the seawaters worldwide (Fig. 7; Table S8), even though the EDCs still posed risks to local marine organisms (Fig. 6). Alkylphenols and BPA usually exhibited medium or high risks to marine organisms in seawaters, but they exhibited high risks in the effluents from STPs. Other chemicals (i.e., E1, DES, TCS and TCC) had low or no risks to the marine organisms in seawaters, even though their risks were still present in the effluents from STPs (Fig. 6). These data were also supported by previous studies that found the effluents of STPs and/or seawaters in the protected marine areas of Hong Kong exhibited oestrogenic activities (Xu et al., 2015, 2016) and elicited a transcriptional response of the genes related to the endocrine disruption pathways in the marine medaka fish *Oryzias melastigma* (Xu et al., 2014, 2015). In those studies, the protected marine areas of Hong Kong exhibited high concentrations of 4-NP (13–497 ng/L), BPA (3.4–408 ng/L), and 4-*t*-OP (1.3–18 ng/L) (Table S8) (Xu et al., 2014, 2015, 2016) that were comparable with the results of the present study.

5. Conclusions

In the present study, the HPLC-MS/MS methods were successfully developed and applied for the determination of retinoids and oestrogenic EDCs in wastewater, sludge and seawater samples. Globally, the current study represents the first comprehensive examination of retinoids and EDCs in saline wastewater and sludge collected from each treatment process of the STPs and their adjacent seawaters. High concentrations of retinoids and EDCs were found in the wastewater and sludge samples collected from three STPs of Hong Kong which is one of the most highly populated coastal cities in the world. The retinoids were dominated by their oxidative metabolites (i.e., at-4-oxo-RA or 13c-4-oxo-RA) in the wastewater, whereas at-RA and 13c-RA were the most abundant in the sludge (i.e., without oxidation). EDCs were dominated by alkylphenols and BPA in the wastewater, and by alkylphenols, TCS or TCC in the sludge.

The present results indicated that the sewage treatment processes of the three STPs in Hong Kong could remove the retinoids and EDCs from wastewater by 41–82% and 31–79%, respectively. Hence, the treated effluents from the STPs and the adjacent seawaters still exhibited relatively high concentrations of these two groups of chemicals, posing medium risks to marine organisms living in the coastal marine environment of Hong Kong. Considering the additive or synergistic effects of these chemicals, and the occurrence of other undetected retinoids (e.g., retinal) and EDCs in seawaters, the risks of these chemicals to marine organisms were likely to have been underestimated by this study. Thus, future studies should focus on the combined risk assessments of these chemicals to marine organisms. Moreover, more extensive samplings of wastewater and seawater throughout the year are needed to obtain a better understanding of the distribution and fate of the retinoids and EDCs of concern in different seasons. Bioaccumulation and biodegradation of the retinoids and dominant EDCs by marine organisms should also be investigated to better reveal their impact on the marine ecosystem.

Acknowledgements

This research is funded by the Research Grants Council of the Hong Kong SAR Government via the General Research Fund (project no.: 17126517) to KMYL; the Collaborative Research Fund (project no.: C7044-14G) and Theme-based Research Scheme (project no. T21-711/16-R) to XYL, and the Hong Kong Scholars Program (Project no.: XJ2012050) to GJZ. The authors are grateful to the Drainage Services Department for granting us permissions to collect wastewater and sludge samples for this study. The authors would like to thank Jian-Liang Zhao (Chinese Academy of Sciences) for his assistance in supplying electronic background world map; Katie Yeung and Wa-Tat Yan for their assistances with sampling; Helen Leung and Apple Chu for technical support. The authors are thankful to the anonymous reviewers and the editor for their constructive and useful comments.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.03.030.

References

Ahmed, M.B., Zhou, J.L., Ngo, H.H., Guo, W., Thomaidis, N.S., Xu, J., 2017. Progress in the biological and chemical treatment technologies for emerging contaminant removal from wastewater: a critical review. J. Hazard. Mater. 323, 274–298.
Allinson, M., Shiraishi, F., Salzman, S.A., Allinson, G., 2011. In vitro assessment of retinoic acid and aryl hydrocarbon receptor activity of treated effluent from 39 wastewater-treatment plants in Victoria, Australia. Arch. Environ. Contam. Toxicol. 61, 539–546.
Bester, K., 2003. Triclosan in a sewage treatment process–balances and monitoring data. Water Res. 37, 3891–3896.
Chen, F., Ying, G.G., Yang, J.F., Zhao, J.L., Wang, L., 2010. Rapid resolution liquid chromatography-tandem mass spectrometry method for the determination of endocrine disrupting chemicals (EDCs), pharmaceuticals and personal care products (PPCPs) in wastewater irrigated soils. J. Environ. Sci. Health B 45, 682–693.
Chiu, J.M.Y., Degger, N., Leung, J.Y.S., Po, B.H.K., Zheng, G.J., Richardson, B.J., Lau,

T.C., Wu, R.S.S., 2016. A novel approach for estimating the removal efficiencies of endocrine disrupting chemicals and heavy metals in wastewater treatment processes. Mar. Pollut. Bull. 112, 53–57.
Degitz, S.J., Holcombe, G.W., Kosian, P.A., Tietge, J.E., Durhan, E.J., Ankley, G.T., 2003. Comparing the effects of stage and duration of retinoic acid exposure on amphibian limb development: chronic exposure results in mortality, not limb malformations. Toxicol. Sci. 74, 139–146.
Ejhed, H., Fång, J., Hansen, K., Graae, L., Rahmberg, M., Magnér, J., Dorgeloh, E., Plaza, G., 2018. The effect of hydraulic retention time in onsite wastewater treatment and removal of pharmaceuticals, hormones and phenolic utility substances. Sci. Total Environ. 618, 250–261.
Environmental Quality Standards (EQS), 2005. Common Implementation Strategy for the Water Framework Directive. Priority Substance No. 25. Octylphenols (Para-Tert-Octylphenol) CAS-No. 1806-26-4 and 140-66-9. (Brussels).
European Chemicals Agency (ECHA), 2015. Biocidal Products Committee (BPC). Opinion on the application for approval of the active substance: Triclosan product-type: 1. In: ECHA/BPC/066/2015.
European Commission (EC), 2003. Technical Guidance Document on Risk Assessment in Support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances and Commission Regulation (EC) No. 1488/94 on Risk Assessment for Existing Substances and Directive 98/8/EC of the European Parliament and of the Council Concerning the Placing of Biocidal Products on the Market. Part II. Official Publications of the European Communities. pp. 1–337.
European Union (EU), 2002. European Union risk assessment report. 4-nonylphenol (branched) and nonylphenol. In: EUR 20387 EN. European Commission.
European Union (EU), 2010. Updated European Union risk assessment report. 4,4'–Isopropylidenediphenol (Bisphenol–A). European Commission, EUR 24588 EN.
Fernandez, M.P., Noguerol, T.N., Lacorte, S., Buchanan, I., Piña, B., 2009. Toxicity identification fractionation of environmental estrogens in waste water and sludge using gas and liquid chromatography coupled to mass spectrometry and recombinant yeast assay. Anal. Bioanal. Chem. 393, 957–968.
Griffiths, C.E.M., Russman, A.N., Majmudar, G., Singer, R.S., Hamilton, T.A., Voorhees, J.J., 1993. Restoration of collagen formation in photodamaged human skin by tretinoin (retinoic acid). New Engl. J. Med. 329, 530–535.
Heidler, J., Halden, R.U., 2007. Mass balance assessment of triclosan removal during conventional sewage treatment. Chemosphere 66, 362–369.
Heidler, J., Sapkota, A., Halden, R.U., 2006. Partitioning, persistence, and accumulation in digested sludge of the topical antiseptic triclocarban during wastewater treatment. Environ. Sci. Technol. 40, 3634–3639.
Herrmann, K., 1995. Teratogenic effects of retinoic acid and related substances on the early development of the zebrafish (*Brachydanio rerio*) as assessed by a novel scoring system. Toxicol. in Vitro 9, 267–283.
Hong Kong Census and Statistics Department (HKCSD), 2017. http://www.censtatd.gov.hk/home.html.
Hong Kong Lands Department (HKLD), 2017. https://www.landsd.gov.hk/.
Horiguchi, T., Ohta, Y., Nishikawa, T., Shiraishi, F., Shiraishi, H., Morita, M., 2008. Exposure to 9-*cis* retinoic acid induces penis and vas deferens development in the female rock shell, *Thais clavigera*. Cell Biol. Toxicol. 24, 553–562.
Huang, M.E., Ye, Y.C., Chen, S.R., Chai, J.R., Lu, J.X., Zhao, L., Gu, L.J., Wang, Z.Y., 1988. Use of all-*trans* retinoic acid in the treatment of acute promyelocytic leukemia. Blood 72, 567–572.
Hutchinson, T.H., Pounds, N.A., Hampel, M., Williams, T.D., 1999. Life-cycle studies with marine copepods (*Tisbe battagliai*) exposed to 20-hydroxyecdysone and diethylstilbestrol. Environ. Toxicol. Chem. 18, 2914–2920.
Inoue, D., Nakama, K., Sawada, K., Watanabe, T., Matsui, H., Sei, K., Nakanishi, T., Ike, M., 2011. Screening of agonistic activities against four nuclear receptors in wastewater treatment plants in Japan using a yeast two-hybrid assay. J. Environ. Sci. 23, 125–132.
Inoue, D., Sawada, K., Wada, Y., Sei, K., Ike, M., 2013. Removal characteristics of retinoic acids and 4-oxo-retinoic acids in wastewater by activated sludge treatment. Water Sci. Technol. 67, 2868–2874.
Jang, D., Hwang, Y., Shin, H., Lee, W., 2013. Effects of salinity on the characteristics of biomass and membrane fouling in membrane bioreactors. Bioresour. Technol. 141, 50–56.
Johnson, A.C., Sumpter, J.P., 2001. Removal of endocrine-disrupting chemicals in activated sludge treatment works. Environ. Sci. Technol. 35, 4697–4703.
Jonas, A., Buranova, V., Scholz, S., Fetter, E., Novakova, K., Kohoutek, J., Hilscherova, K., 2014. Retinoid-like activity and teratogenic effects of cyanobacterial exudates. Aquat. Toxicol. 155, 283–290.
Lambert, W.E., De Leenheer, A.P., 1985. Demonstration of retinoic acid isomers in human urine under physiological conditions. Experientia 41, 359–360.
Leung, K.M.Y., Kwong, R.P.Y., Ng, W.C., Horiguchi, T., Qiu, J.W., Yang, R., Song, M., Jiang, G., Zheng, G.J., Lam, P.K.S., 2006. Ecological risk assessments of endocrine disrupting organotin compounds using marine neogastropods in Hong Kong. Chemosphere 65, 922–938.
Lozano, N., Rice, C.P., Ramirez, M., Torrents, A., 2013. Fate of Triclocarban, Triclosan and Methyltriclosan during wastewater and biosolids treatment processes. Water Res. 47, 4519–4527.
Marill, J., Idres, N., Capron, C.C., Nguyen, E., Chabot, G.G., 2003. Retinoic acid metabolism and mechanism of action: a review. Curr. Drug Metab. 4, 1–10.
Melvin, S.D., Leusch, F.D.L., 2016. Removal of trace organic contaminants from domestic wastewater: a meta-analysis comparison of sewage treatment technologies. Environ. Int. 92–93, 183–188.
Nakada, N., Tanishima, T., Shinohara, H., Kiri, K., Takada, H., 2006. Pharmaceutical chemicals and endocrine disrupters in municipal wastewater in Tokyo and their removal during activated sludge treatment. Water Res. 40, 3297–3303.
Nishikawa, J.I., Mamiya, S., Kanayama, T., Nishikawa, T., Shiraishi, F., Horiguchi, T., 2004. Involvement of the retinoid X receptor in the development of imposex caused by organotins in gastropods. Environ. Sci. Technol. 38, 6271–6276.
Ross, S.A., McCaffery, P.J., Draguer, U.C., De Luca, L.M., 2000. Retinoids in embryonal development. Physiol. Rev. 80, 1021–1054.
Sawada, K., Inoue, D., Wada, Y., Sei, K., Nakanishi, T., Ike, M., 2012. Detection of retinoic acid receptor agonistic activity and identification of causative compounds in municipal wastewater treatment plants in Japan. Environ. Toxicol. Chem. 31, 307–315.
Singer, H., Müller, S., Tixier, C., Pillonel, L., 2002. Triclosan: occurrence and fate of a widely used biocide in the aquatic environment: field measurements in wastewater treatment plants, surface waters, and lake sediments. Environ. Sci. Technol. 36, 4998–5004.
Stasinakis, A.S., Gatidou, G., Mamais, D., Thomaidis, N.S., Lekkas, T.D., 2008. Occurrence and fate of endocrine disrupters in Greek sewage treatment plants. Water Res. 42, 1796–1804.
Tang, S.L., Yue, D.P.T., Li, X.Z., 2006. Comparison of engineering costs of raw freshwater, reclaimed water and seawater for toilet flushing in Hong Kong. Water Environ. J. 20, 240–247.
Völker, J., Castronovo, S., Wick, A., Ternes, T.A., Joss, A., Oehlmann, J., Wagner, M., 2016. Advancing biological wastewater treatment: extended anaerobic conditions enhance the removal of endocrine and dioxin-like activities. Environ. Sci. Technol. 50, 10606–10615.
Wu, G., Guan, Y., Zhan, X., 2008. Effect of salinity on the activity, settling and microbial community of activated sludge in sequencing batch reactors treating synthetic saline wastewater. Water Sci. Technol. 58, 351–358.
Wu, X., Hu, J., Jia, A., Peng, H., Wu, S., Dong, Z., 2010. Determination and occurrence of retinoic acids and their 4-oxo metabolites in Liaodong Bay, China, and its adjacent rivers. Environ. Toxicol. Chem. 29, 2491–2497.
Xu, E.G.B., Liu, S., Ying, G.G., Zheng, G.J.S., Lee, J.H.W., Leung, K.M.Y., 2014. The occurrence and ecological risks of endocrine disrupting chemicals in sewage effluents from three different sewage treatment plants, and in natural seawater from a marine reserve of Hong Kong. Mar. Pollut. Bull. 85, 352–362.
Xu, E.G.B., Morton, B., Lee, J.H.W., Leung, K.M.Y., 2015. Environmental fate and ecological risks of nonylphenols and bisphenol A in the Cape D'Aguilar Marine Reserve, Hong Kong. Mar. Pollut. Bull. 91, 128–138.
Xu, E.G.B., Ho, P.W.L., Tse, Z., Ho, S.L., Leung, K.M.Y., 2016. Revealing ecological risks of priority endocrine disrupting chemicals in four marine protected areas in Hong Kong through an integrative approach. Environ. Pollut. 215, 103–112.
Ying, G.G., Kookana, R.S., 2007. Triclosan in wastewaters and biosolids from Australian wastewater treatment plants. Environ. Int. 33, 199–205.
Young, W.F., Whitehouse, P., Johnson, I., Sorokin, N., 2002. Proposed Predicted-No-Effect-Concentrations (PNECs) for Natural and Synthetic Steroid Oestrogens in Surface Waters. Environment Agency, Bristol. Research and Development (Technical Report P2-T04/1).
Zhang, T., Shao, M.F., Ye, L., 2012. 454 pyrosequencing reveals bacterial diversity of activated sludge from 14 sewage treatment plants. ISME J 6, 1137–1147.
Zhao, J., Li, Y., Zhang, C., Zeng, Q., Zhou, Q., 2008. Sorption and degradation of bisphenol A by aerobic activated sludge. J. Hazard. Mater. 155, 305–311.
Zhao, J.L., Ying, G.G., Liu, Y.S., Chen, F., Yang, J.F., Wang, L., 2010. Occurrence and risks of triclosan and triclocarban in the Pearl River system, South China: from source to the receiving environment. J. Hazard. Mater. 179, 215–222.
Zhen, H.J., Wu, X.Q., Hu, J.Y., Xiao, Y., Yang, M., Hirotsuji, J., Nishikawa, J., Nakanishi, T., Ike, M., 2009. Identification of retinoic acid receptor agonists in sewage treatment plants. Environ. Sci. Technol. 43, 6611–6616.
Zhou, G.J., Ying, G.G., Liu, S., Zhou, L.J., Chen, Z.F., Peng, F.Q., 2014. Simultaneous removal of inorganic and organic compounds in wastewater by freshwater green microalgae. Environ. Sci.: Processes Impact 16, 2018–2027.
Zhu, J., Yu, L., Wu, L., Hu, L., Shi, H., 2014. Unexpected phenotypes of malformations induced in *Xenopus tropicalis* embryos by combined exposure to triphenyltin and 9-*cis*-retinoic acid. J. Environ. Sci. 26, 643–649.