Occurrence and fate of endocrine disrupting compounds in wastewater treatment plants in Israel and the Palestinian West Bank

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Highlights

- Hormone concentrations were higher in the Palestinian than in the Israeli WWTPs.
- EDC removal in this study was higher than the reported values around the world.
- Triclosan removal can be improved in secondary treatment by increasing HRT.

Abstract

Israel and its Palestinian neighbors constitute a unique venue for evaluating the treatment efficiency and potential environmental risks of endocrine disrupting compounds (EDCs) in wastewater treatment plants (WWTPs), because of their physical proximity yet contrasting societal dynamics. Israel primarily relies on advanced tertiary sewage treatment and recycles over 85% of its treated wastewater, while in the Palestinian Authority (PA), there is only secondary treatment levels at WWTPs and reuse is minimal (<1%). To evaluate the extent of EDC occurrence and treatment efficiency, we conducted four sampling campaigns over two consecutive years, and measured the concentrations of selected EDCs in raw wastewater (WW), treated WW and sludge in six WWTPs in Israel, as well as in two Palestinian plants. Low concentrations of bisphenol A, octylphenol and triclosan measured in the raw WW in the Palestinian WWTPs reflect the relatively modest industrial activity and consumption habits as compared to the westernized consumer patterns in Israel. On the other hand, hormone concentrations in raw WW were higher in the Palestinian WWTPs than those in the Israeli WWTPs, presumably because of a dilution effect associated with a higher water per capita consumption among Israelis. Despite these differences in raw WW concentrations, the removal efficiency in all advanced WWTPs was relatively high when compared to averages reported internationally.

Keywords:
Endocrine disrupting compound (EDC)
Wastewater treatment
Risk assessment
Israel
Palestinian Authority

1. Introduction

Endocrine disrupting compounds (EDCs) are a sub-group of micropollutants that may alter the hormonal functioning of the endocrine system in humans and wildlife (Cwiertny et al., 2014). Varying concentrations of EDCs have been found in different aquatic systems around the world (Vidal-Dorsch et al., 2012; Xu et al., 2011). EDCs are introduced to the aquatic environment through various pathways including the direct discharge of raw or treated wastewater (WW) from wastewater treatment plants (WWTPs), the application of treated sludge, runoff from agricultural and industrial areas, and via irrigation with treated WW (Hamid and Eskicioglu, 2012). WWTPs are of particular interest because they continuously discharge EDCs into the environment, but at the same time, can significantly reduce EDC loadings through
effective treatment (Drewes et al., 2005).

The reduction in EDC concentrations during WW treatment, without identifying specific elimination mechanisms, is often referred to as removal (Stadler et al., 2012). EDCs can be removed from wastewater by physical, chemical and biological processes, depending on the characteristics of the compounds and the conditions during the treatment (Hamid and Eskiçioğlu, 2012; Ifelebuegu, 2011). The concentrations of EDCs in raw and treated WW also depend on the socioeconomic composition of the contributing society, which is reflected, for example, by the levels of industrial development and agricultural practices (Schwarzenbach et al., 2006). Extensive WW reuse can also affect the distribution of EDCs in the environment since, in many cases, EDCs were identified in treated WW. For example, in more humid regions, treated WW is discharged into rivers, posing a risk to ecosystems (Meybeck et al., 1996; Drechsel et al., 2010). In semi-arid and arid environments, such as the Middle East, treated WW is primarily reused for irrigation and can find its way into food (Malchi et al., 2014) or can be leached toward the groundwater (Avisar et al., 2009).

Israel (IL) and the Palestinian Authority (PA) share the same geographic province in the central part of the Middle East, and most of the catchments in this area are transboundary. However, their wastewater treatment and reuse profiles differ significantly, with little cooperation taking place between the two sides, even in cases where raw or treated sewage is leached between the two territories (Al-Sa’ed and Tomaleh, 2012; Palestinian Water Authority, 2012). In Israel for example, over 90% of the WW is treated and 86% of the treated WW is reused for irrigation, while a small fraction is discharged into the aquatic environment (Israel Water Authority, 2015). In the Palestinian West Bank, there are only two advanced functioning WWTPs that serve 20-35% of the households with almost no reuse (Al-Sa’ed and Tomaleh, 2012). While the above-mentioned WWTPs in the Palestinian West Bank employ only secondary treatment, tertiary treatment is becoming standard among Israeli WWTPs due to regulatory requirements (Israel ministry of environmental protection, 2010). Despite the fact that Israel’s wastewater treatment standards are relatively new (from the year 2010), no regulation exists in Israel (nor in the world) for EDCs, and there is a clear shortage of information about EDCs and other trace organic compound occurrences in the Middle East (e.g., Aldina et al., 2014).

To evaluate the extent of EDC occurrence in WWTPs in Israel and the West Bank of the Palestinian Authority, we measured the concentrations of selected EDCs in raw WW, treated WW and sludge in eight WWTPs. The main objectives of this research were to evaluate the removal efficiency of EDCs in different treatment technologies and operating conditions (including the level of treatment), along with the associated risks from treated WW in contiguous countries with such dramatically distinct socioeconomic conditions.

2. Materials and methods

2.1. Study sites

Eight WWTPs were selected for the current study, including six in Israel and two in the PA. Despite efforts made to sample a greater number of WWTPs in the West Bank in the PA, none of the other facilities operated continuously during the study. Details on the technology, treatment levels, hydraulic retention time (HRT) during the secondary treatment, and reuse in the WWTPs are summarized in Table 1.

2.2. Water and sludge sampling

Four sampling campaigns were conducted during the winters and summers of 2013–2014. Raw, secondary and tertiary (where relevant) WW and sludge were sampled in each WWTP. Composite samples of raw and secondary-treated wastewater were obtained over a 24-h period by using automatic samplers (ISCO 3800 and global water WS1700), equipped with 4-L dark glass bottles that were kept on ice. Raw wastewater samples were taken after grit removal. Tertiary level samples were taken as grab from reservoirs, where the water is well-mixed and residence time is greater than 24 hours. Sodium sulfite was added to all water samples in order to neutralize chlorine residues, and the samples were acidified to a pH of 2 using 6 N HCl to prevent microbial activity. Sludge samples were taken in each WWTP using 120-mL glass containers. All samples were stored at 4 °C until transported to the laboratory (<24 h). Water samples were kept in the laboratory at 4 °C until extraction (<14 days), and sludge samples were frozen at −20 °C until extraction (<1 year).

2.3. Sample preparation and analysis

2.3.1. WW sample preparation and analysis

The target compounds assessed in this study included estriol, estrone, 17β-estradiol, testosterone (TST), bisphenol A (BPA), octylphenol (OP), triclosan (TCS), nonylphenol (NP) and atrazine. These compounds are either produced naturally (e.g., hormones) or are commonly used in Israel and the PA. The compounds were extracted from the WW using the solid phase extraction (SPE) technique. EDCs (except for TCS) were extracted according to EPA 539 protocol (USEPA, 2010), while TCS extraction was conducted according to EPA 525.2 protocol (Eichheilberger et al., 1995). SPE was conducted using Empore C18 extraction disks. The final extracts were stored at −20 °C until analysis (<90 days). The analysis of TCS was done by GCMS (TRACE GC2000/FINNIGAN POLARIS MS, ThermoQuest, USA) equipped with an Rtx®-5Sil MS column (Restek, Bellefonte, PA, USA), 30 m × 0.25 mm ID × 0.25 μm, and an ion trap mass spectrometer (FINNIGAN POLARIS/GCQ plus). Analysis of EDCs was conducted with ES- LCMSMS (Waters Xevo TQS, Waters Corporation, USA) and Acquity. A 1.7 μm 2.1 × 50 mm column was used for separation. The minimum quantification limit (MQL) of each compound is given in Table S1 (Supplementary information).

2.3.2. Quality Assurance/Quality Control

A Quality Assurance/Quality Control protocol was carried out during the sampling and sample preparation. Laboratory and field blanks, as well as laboratory fortification blanks and matrices (spiked blanks and samples), were used in each batch of samples. Target compounds were identified by comparing either retention times and the full mass spectrum (EPA 525.2) or 2–3 multiple reaction monitoring (MRM) transitions (EPA539) of the substance in the sample and its authentic standard, which were tested under the same conditions. Concentrations of EDCs were calculated using a standard internal calibration procedure. Internal standards were used for the following compounds: phenanthrene D10, BPA-D16, estradiol 13C6, estriol 13C6, estrone 13C6 and testosterone D5. Analytical standards were purchased from Sigma-Aldrich. Analytical grade reagents for extractions and instrumental analysis (methanol, ethyl acetate, methylene chloride, sodium sulfate, hydrochloric acid, ammonium acetate) were all purchased from either Sigma-Aldrich or J.B. Baker.

The MQL was evaluated separately for each compound during initial validation of the analytical methods. Spiked samples were treated according to the procedure described above, and were used to create the calibration curves. The accuracy and precision of the
results were calculated with criteria of 50–150% recovery and ≤50% precision. When concentrations were not close to the MQL, measurement precision was found to be ±30% and recovery of the tested compounds was 70–130%.

2.4. Data analysis

Removal of compounds during the treatment was calculated following Eq. (1):

\[
\text{Removal} (\%) = \frac{C_i - C_f}{C_0} \times 100
\]

where \(C_i\) is the concentration before the treatment, \(C_f\) is the concentration after the treatment, and \(C_0\) is the concentration in raw WW.

Comparisons between the concentrations of EDCs in IL, the PA, and selected studies around the world were done using the Kruskal-Wallis test since the data did not meet the normality assumption by using STATISTICA v10.0 (StatSoft, Ltd., Tulsa, OK, USA). Removal data was analyzed by a two-way ANOVA using SPSS v22 (IBM Corp., Armonk, NY, USA). The level of significance utilized in evaluating data was determined as \(p < 0.05\).

3. Results and discussion

3.1. Occurrence and levels of EDCs in wastewater

Concentrations of EDCs were highly variable as seen by the relatively large standard deviation in Fig. 1 and Table S1 (Supplementary information). The detection frequency of the tested compounds appears in Fig. S1 (Supplementary information 1). Only the samples that passed the rigorous Quality Assurance/Quality Control procedures (Section 2.3.2) were included in the analysis. This includes 24 samples of raw WW, 24 samples of secondary-level-treated WW, and 18 of tertiary-level-treated WW samples in the Israeli WWTPs. For the Palestinian WWTPs, it included 6 samples of raw and 6 samples of secondary-level-treated WW (no tertiary level treatment exists in the Palestinian WWTPs) (Table 1).

Despite the small number of samples from the PA WWTPs, the variations in concentrations were similar to those from IL and from the selected studies around the world. In general, the maximum concentrations of TCS, BPA and OP in raw WW were 10–100 times higher than the hormone concentrations (Fig. 1a). In order to evaluate the local magnitude and patterns of EDCs levels, the concentrations that were found in IL and in the PA were compared to data from selected studies around the world. For this comparison, we selected 47 studies from four continents (Europe, America, 

![Fig. 1. Concentrations of EDCs in raw wastewater (a) and treated WW (b) in WWTPs in IL (black bars), the PA (gray bars), and selected countries around the world (hatched gray bars), which were taken from the publications that are listed in Supplementary information 3.](image-url)
Asia and Australia) that reported a total of 170 samples of raw WW and 174 of treated WW (Fig. 1a and Supplementary information 3). The concentrations of TCS, BPA and OP in raw WW in IL WWTPs were not significantly different than those reported in various studies around the developed world (p > 0.05). However, the concentrations of TCS, BPA and OP were significantly lower in the PA WWTPs than the concentration in IL WWTPs and in selected countries around the world (p < 0.05). BPA and OP are highly associated with industrial activity, while TCS is used as an antimicrobial agent in various personal care products (PCPs), and their relative absence in the PA is attributed to the lower level of industrial activity and to the differences in the PCP market (Al-Sa‘ed and Hithnawi, 2006).

The concentrations of the estrogenic hormones, estrone, estriol, and 17β-estradiol, followed a more complex pattern. For example, concentrations of estrone in IL and the PA were significantly higher than in the selected studies around the world (p < 0.05). In addition, the concentrations of estrone and estriol were significantly higher in the raw WW in the PA than those reaching Israeli WWTPs (p < 0.05). The selected WWTPs do not receive significant discharges from livestock agriculture with the majority of hormone contributions coming from humans. Thus, it is postulated that the hormone concentrations in raw WW are heavily influenced by the amount of water consumption in the different regions. The high concentrations in the PA WWTPs (Fig. 1a) can be partly attributed to the modest amount of water consumed in the PA (i.e., less dilution), which is lower than in Israel and other developed countries. Accordingly, the average water supply levels per capita in the PA, IL and OECD countries are 104, 281.9 and 602.3 m³/yr, respectively (ChartsBin, 2011). The fact that the differences in hormone concentrations do not exactly match the expected level of dilution due to water consumption suggests that this is not the sole determining parameter, and other processes, such as contribution from runoff and dilution from the minor industrial activity, also influence the hormone concentrations in raw WW.

The EDCs concentrations in treated wastewater displayed different patterns than those in raw wastewater: TCS, BPA and OP were significantly lower than the concentrations measured around the world (p < 0.05, Fig. 1b). The low concentrations in the PA's WWTPs presumably can be attributed to the low levels in the raw WW. In IL, they can be attributed to the high efficiency of the treatment, which is driven by the rigorous regulations promulgated given the country’s extensive wastewater reuse. In addition, the concentrations of all hormones measured in WWTPs around the world were significantly higher than the concentrations found in IL and the PA (p < 0.05).

3.2. Removal of EDCs in WWTPs

The EDC removal efficiencies in this study varied from 27 to 100% (Fig. 2). This wide range of values was typically observed for these kinds of chemicals in different WWTPs around the world (e.g., Luo et al., 2014). These differences are attributed to the physicochemical properties of the different compounds and to the differences in the operational conditions and temperatures (Luo et al., 2014; Ternes et al., 1999; Thompson et al., 2011).

The removal efficiencies of TCS ranged from 27 to 84% and were much lower than the rest of the EDCs. Heidler and Halden (2007) claimed that the elimination of TCS from WW by activated sludge processes is equally dependent on sorption and biodegradation. It was also shown that primary clarifiers decrease a major part of TCS (Stasinakis et al., 2010). The data in our study clearly display a link between TCS removal by AS technology and the HRT (Fig. 3), which is associated with biodegradation and sorption processes as TCS was also detected in the sludge (Table S4, Supplementary information). The removal of TCS in AP, on the contrary, was not affected by the long HRT and usually was lower than the removal in AS facilities, probably due to the lower levels of the mixture of WW and sludge, which reduces the chances for sorption and biodegradation.

The removal efficiencies of estradiol, estrone and TST by secondary treatment were, in most cases, higher than 90% (with the exception of estrone in YH), similar to the results found, for example, by Drewes et al. (2005). TST was completely eliminated in the AS WWTPs (Fig. 2) and was not detected in the sludge (Table S4, Supplementary information); therefore, its removal was associated with biodegradation. Estrone was detected only in the sludge of one WWTP (ELB), and thus its removal is also attributed mostly to biodegradation. Estrone showed a more complex behavior, exhibiting high removal efficiency but with consistent detection in the sludge (Table S4, Supplementary information). With a $K_{ow}$ of 3.43, it is expected that estrone will show affinity to the solids as observed here, but its overall removal is also attributed to biodegradation as shown by various studies that focused on the removal mechanism (Silva et al., 2012). Since we did not conduct a complete mass balance estimation in this study, the relative contributions of biodegradation and sorption to the removal efficiency of estrone cannot be determined. In general, high removal efficiencies of hormones were also shown by previous studies in AS WWTPs (Hamid and Eskiçioğlu, 2012; Auriol et al., 2006; Joss et al., 2004). Since the highest biodegradation of estrogens occurs under aerobic conditions, aeration, combined with high HRT (Table 1), was considered to be a major cause of the high efficiencies (Manickum and John, 2013; Silva et al., 2012). However, other operational conditions that have been shown to influence EDCs removal may have influenced the performance of the WWTPs in this study. For example, solids retention time (SRT), which was shown to be as important as HRT (Drewes et al., 2005; Johnson et al., 2005; Servos et al., 2005), could not be evaluated in this study due to the lack of information on its values.

Removal by secondary treatment was extremely efficient for BPA and OP as well, with more than 90% eliminated in all cases with significant impact of sorption to the sludge (Table S4, Supplementary information). Similar to estrone, it was not possible to quantify exactly the contribution of sorption versus biodegradation. It is known that BPA is less hydrophobic and has a low sorption coefficient as compared to OP (Gómez et al., 2007; Ying and Kookana, 2005). However, OP was not detected in most of the sludge samples (Table S4, Supplementary information); therefore, it is likely that biodegradation was the most influential process, while BPA was removed by a combination of sorption and biodegradation.

The data shown here suggest that on average, the removal efficiencies in this study are higher than those reported elsewhere (Fig. 1). This is manifested in the low concentrations in the treated WW (Fig. 1b). Although various removal efficiencies were reported in the literature, it is still not entirely clear what factors affect the removal of EDCs in WWTPs. It is definitely linked to the operational features and the environmental conditions during treatment. For example, HRT is considered a major factor that influences the removal efficiency (e.g., Thompson et al., 2011). This is shown here for TCS by plotting individual measurements from the different campaigns from those WWTPs that utilize AS technology (Fig. 3 and Table 1). For comparison, the average HRT of all the WWTPs in this study was 24 h, while the average HRT in European activated sludge WWTPs is commonly in the range of 4–14 h (Johnson et al., 2001). Nevertheless, no correlation was found between HRT and the other EDCs that were measured in this study. The higher efficiencies reported in this study could also be partly attributed to elevated temperatures in the study area (the Middle East), which on average are higher than temperatures in most WWTP locations.
reported in the literature (from Europe and North America). However, for most compounds, the effect of temperature on removal is expected to be relatively small (Thompson et al., 2011).

The tertiary treatment was shown to effectively improve the removal gained by the secondary treatment (Fig. 2). Trace amounts of hormones were removed by all types of tertiary treatments, including sand filtration, chlorination, UV, MBR, and SAT. Even in cases where the secondary treatment was less efficient (e.g., SDAN), the efficient removal by the SAT technology resulted in a complete removal of most EDCs (Fig. 2). Finally, despite the significant contribution of all tertiary treatment to TCS removal, the overall elimination efficiency was lowest of all EDCs measured, and only ranged from 55 to 91% removal. Despite the fact that tertiary treatment’s contribution to the removal of EDCs was modest, it should not be dismissed, as even de minimis concentrations of EDCs can still impose negative impacts on aquatic ecosystem.

3.3. Environmental implications

Recent studies indicate that although the concentrations of most EDCs in treated WW are generally low, their potential negative effect on the environment is still an open question (e.g., Cwiertny et al., 2014). This is especially relevant in semi-arid environments, such as the study region, where the receiving aquatic environment is characterized by low natural flow and, in many cases, dilution is insignificant. In the PA for example, no extensive reuse takes place, with treated WW released into the environment, creating perennial streams in what were previously ephemeral channels. In Israel, most of the treated WW is reused for irrigation. However, there are cases in which treated WW is used for streamflow augmentation...
(e.g., Arnon et al., 2015). Treated WW from HH into the Yarqon Stream triples the discharge, with freshwater composing about one third of the flow. In order to evaluate the potential risk to ecosystems from the release of treated WW, we followed the approach recommended by the EU, which uses Risk Quotient (RQ) as a fundamental tool to evaluate chemical risk (Salgot and Huertas, 2006).

The RQ is the ratio between the measured environmental concentrations and the predicted no-effect concentration (PNEC). To test whether a risk to aquatic ecosystems from treated wastewater reuse exists in our region, we considered concentrations in the treated WW without any dilution, which represents the worst case scenario and the most common concentrations in the study sites. The PNEC value is based on toxicity assays on different organisms (Xu et al., 2011), while we used data that is based on the risks inferred for aquatic organisms.

The RQs of the treated WW are summarized in Table 2. The risks posed by the EDCs were mostly “low” to “medium”. The most potent compound was estrone, with an RQ ranging from moderate to high risk in all WWTPs. The aeration pond in YH had, on average, higher RQs relative to the more advanced treatment technologies utilized in the other IL WWTPs. The data used to calculate the RQs suggest that despite the high removal efficiencies shown in this study, a significant risk still exists to both aquatic ecosystems and to human health, which cannot be disregarded.

The risks shown here represent an underestimation of the full risk portfolio from WW release since the evaluation was done based on selected compounds. Risks from other compounds or risks associated with a mixture of compounds are not included in the present analysis. For example, Backhaus and Karlsson (2014) calculated the total expected risk of the analytically determined mixtures of various pharmaceuticals and PCPs. They found that the RQ of a single, randomly selected pharmaceutical is often more than a factor of 1000 lower than the mixture risk. This strongly indicates the need to systematically analyze the risk from treated WW reuse by a combination of direct measurements, and by evaluating the overall toxicity of a water sample by using model organisms (Cleuvers, 2005; Schnell et al., 2009). Finally, the risk quotient analysis in this study was based on concentrations in water only, while concentrations in the sediment were not evaluated and may add additional risk.

The contrasting economic and physical realities faced by Israeli and Palestinian water managers reflect the asymmetrical dynamics found in several areas where developed countries border developing countries (e.g., U.S./Mexico; North/South Korea; and even Greek and Turkish Cyprus). As recycled wastewater is a growing resource in water scarce regions, EDCs are an issue that should concern all countries who release effluents into the environment, regardless of socioeconomic status. Our findings do not support the common assumption that EDCs in wastewater are a problem that can only be addressed by countries after they have established an advanced sewage infrastructure (i.e., tertiary treatment, advanced oxidation technology, etc.). The reason for the relative high efficiency of EDCs removal in this study (versus other locations around the world) is not entirely clear. However, it is suggested that WWTP designers in developing countries with hot climates (such as in the Middle East) could at least refer to the results from this study to include basic secondary levels of treatment with a relatively high HRT to significantly reduce the occurrence of the vast majority of EDCs in wastewater reuse, thus also reducing the associated human health and ecological risks. At the same time, further study is needed, especially in developing countries, to optimize the performance of AS plants with regards to other operating parameters, such as SRT.

4. Conclusions

This study is the first to report comprehensive information about the occurrence and fate of EDCs in Israeli and Palestinian

<table>
<thead>
<tr>
<th>PNEC [ng/L] (source)</th>
<th>TCS</th>
<th>Estrone</th>
<th>Estriol</th>
<th>BPA</th>
<th>OP</th>
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<td></td>
<td></td>
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<td></td>
<td>Mean</td>
<td>Max</td>
<td>Mean</td>
<td>Max</td>
<td>Mean</td>
</tr>
<tr>
<td>YH</td>
<td>0.12 (M)</td>
<td>0.26 (M)</td>
<td>1.15 (H)</td>
<td>2.17 (H)</td>
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<tr>
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<td>0 (L)</td>
<td>0.24 (M)</td>
<td>0.57 (M)</td>
<td>0.06 (L)</td>
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<tr>
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<td>0.52 (M)</td>
<td>1.4 (H)</td>
<td>0.07 (L)</td>
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<tr>
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<td>0.98 (M)</td>
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<td>0 (L)</td>
<td>0.83 (M)</td>
<td>1.15 (H)</td>
<td>0 (L)</td>
</tr>
</tbody>
</table>

n.d.: not detected.
WWTPs. While contributing valuable information about the fate of EDCs following treatment in a Mediterranean climate, it also provides important insights for water resources management in a region with extreme cases of wastewater reuse. While Israel reuses >85% of its WW, the PA WWTPs recycle <1% of their effluents. Although natural estrogens are found in both populations, societal differences result in higher concentrations in raw WW in the PA than in IL (mainly due to water consumption differences and WW dilution).

In addition, differences in the levels of industrial activity and PCP consumption are reflected in the type of EDCs in the WWTPs (more diverse compounds were found in IL than in the PA). The removal efficiency in all advanced WWTPs was relatively high when compared to averages reported around the world. It was postulated that an elevated HRT yielded high removal rates in the Palestinian WWTPs as well, even though they only employ secondary treatment. However, this could not be directly verified or compared to other important operating parameters such as SRT. In cases in which the secondary treatment was not sufficient to produce large removal efficiencies, the most effective tertiary treatment was the SAT. Despite the relative high removal of EDCs in WWTPs, there is still a risk from EDCs when recycling treated wastewater for agricultural uses, as well as for streamflow augmentation.

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Appendix A. Supplementary data
Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2016.04.027.

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