Fate of estrogens in Kuwaiti municipal wastewater treatment plants

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\textbf{Abstract}

Estrogens are endocrine-disrupting chemicals that impact both human and animal health, even at very low levels. Fate of estrogens were evaluated for three municipal wastewater treatment plants (WWTPs) in Kuwait, through determination of estrogens concentrations in influent and effluent streams. The solid-phase extraction gas chromatography-mass spectrometry method was used for analysis of estrogens concentrations in wastewater. Obtained results indicated that concentration of estrogens in the influent streams ranged from 0.0 to 474 ng/L, while that in the effluent streams were between 0.0 to 233 ng/L. Both influent and effluent concentrations showed high variations around mean values. Total removal of estrogens were found to be 13%, 79%, 68%, for Kabd, Sulaibiya and Umm Al Hayman, respectively. Even with high influent loadings, Sulaibiya plant achieved the highest removal of all types of estrogens, except estrone. The obtained results demonstrated that WWTPs require upgrading/optimization to maximize estrogens' removal. The study also discussed the potential impacts of estrogens in treated wastewater reused as irrigation water and recommended that Kuwait urgently needs to develop regulations for estrogens discharges from WWTPs in order to prevent further pollution of marine environment and groundwater with estrogens.

\textbf{Keywords}: Wastewater; Municipal plants; Treatment; Estrogens; Removal efficiency

\textbf{1. Introduction}

Nowadays endocrine-disrupting chemicals (EDCs) are receiving great attention worldwide due to their potential adverse impacts on human and environmental health [1]. Estrogens hormones are a group of EDCs that are produced either naturally or synthetically. Natural estrogens are produced by humans and animals, whereas synthetic estrogens are man-made hormones. The most common types of natural estrogenic hormones are estrone (E1), estradiol (E2) and estriol (E3). Examples of the synthetic steroid are ethynylestradiol (EE2) and diethylstilbestrol (DES). EE2 and DES are commonly used in manufacturing birth control pills. Both natural and synthetic estrogens can adversely affect humans, animals or fish. Estrogens can disrupt the reproductive and sexual systems of fish, wildlife and humans [2]. According to the World Health Organization (WHO), the adverse impact of estrogens can appear at low concentrations as 1 ng/L [3].

Estrogens are often excreted as urine, and thus, they end ultimately in wastewater treatment plants (WWTPs). Since conventional WWTPs do not remove efficiently estrogens, they are considered to be the major point sources of pollution with estrogens [4]. In fact, the best control strategy for the problem of estrogens pollution is to remove estrogens efficiently during wastewater treatment [5]. This means that conventional WWTPs have to be upgraded/optimized for estrogens removal and regulations must be
issued to regulate discharges of estrogens from WWTPs. In Kuwait and many other countries, however, fate of estrogens in WWTPs is not even monitored due to absence of regulations [6].

Numerous studies have been conducted worldwide to investigate the occurrence and removal of estrogens by municipal WWTPs [7]. These studies have indicated that activated sludge WWTPs are generally effective in removing estrogens from wastewater [8,9]. However, the removal rates were found to depend on many factors such as estrogens type, estrogens load, plant design and plant operation mode [1].

Removals of estrogens in conventional activated sludge plants occur mainly during the secondary stage of treatment (biological treatment). In this stage, estrogens removals happen mainly through biodegradation and adsorption onto flocs. In fact, this stage alone was found to account for more than 80% of the removals of estrogens [10]. The removals of the primary stage (primary sedimentation) were found to be about 10%, while that of the preliminary treatment stage (bar screening and grit removal) were insignificant [11]. The operational parameters that have significant influence on the removal of estrogens in an ASPs were found to be the hydraulic retention time (HRT) and the solids retention time (SRT) [4,12]. Advanced treatment processes (e.g., membrane filtration and advanced oxidation processes) can also enhance the elimination of emerging contaminants such as estrogens [13].

The main objectives of this study were to determine the fate of estrogenic compounds in Kuwait municipal WWTPs. This information is urgently needed for assessing the environmental and health risks from estrogens pollution in Kuwait. The paper also discusses the potential adverse impacts of estrogens laden treated wastewater reused in Kuwait as irrigation water.

2. Materials and methods

Kuwait has four main activated sludge municipal WWTPs that have different design capacities and different treatment technologies. As shown in Table 1, Sulaibiya plant treats to advanced levels, using ultrafiltration (UF) and reverse osmosis (RO), while the other three plants treat to tertiary levels, using various activated sludge systems. During the study, however, estrogens concentrations were monitored for only three of these plants (Kabd, Sulaibiya and Umm Al Hayman WWTPs).

2.5-L grab samples of wastewater were collected monthly from the influent and effluent streams of the Kabd, Sulaibiya and Umm Al Hayman municipal WWTPs between October 2015 and August 2016. The samples were then transported to the laboratory for analysis in iceboxes and stored at 4°C until being analysed. Before analysis, however, the samples were allowed to come to room temperature. The sample preparation consisted of filtration through a prebaked glass fibre filter to remove suspended material. The filtered sample was then passed through Oasis HLB cartridge (Waters Corporation). The cartridge was preconditioned by passing methyl tert-butyl ether (MTBE) followed by methanol and finally ultrapure water. The sample was passed through the cartridge at 3–4 mL/min rate. The cartridge was then washed with methanol/water mixture (5/95). The dried cartridge was then eluted with methanol/MTBE to recover the retained estrogens. The eluate fraction was evaporated to dryness. The residue was transferred to a 2.5 mL V-shaped vial for derivatization (conversion of a chemical compound into a product of similar chemical structures) with BSTFA and TMCS (100 μL). After derivatization, and evaporation, the residue was dissolved in hexane for GC/MS analysis by Shimadzu GC coupled to QP2010 Plus MS. Detailed description of the analytical condition and the standard method used for quantization of estrogens are given in our previous paper [14].

3. Results and discussion

3.1. Influent concentrations

The influent concentrations of both natural (E1, E2, E3) and synthetic (EE2) estrogens are given in Table 2. This table shows that the concentration of estrogens during the sampling period ranged from 0.0 to 474 ng/L. Although this is a bit of a wide range, it falls within the range of values reported for some countries [8,15–19]. Table 2 also shows that the concentrations of both natural (E1, E2, E3) and synthetic (EE2) estrogens in the influent stream of the Sulaibiya plant were the highest compared to the other two plants. This can be attributed to the fact that the Sulaibiya plant is the largest WWTP in Kuwait (Table 1), which treats about 60% of the wastewater generated in Kuwait. Furthermore, the catchment area of this plant cover almost 80% of the highly populated urban area of Kuwait City. In this area, most of the Kuwaiti and non-Kuwaiti families live. It is expected in such areas that large amounts of both natural and synthetic estrogens will be produced by menstruating females, pregnant women and users of contraceptive pills. In contrast, Kabd plant (250,000 m³/d) and Umm Al Hayman plant are smaller and treat wastewater of suburb areas.

It is worth noting that standard deviations (STDs) of all types of estrogens concentrations, except for E1 in the

<table>
<thead>
<tr>
<th>Plant</th>
<th>Type of ASP</th>
<th>Treatment level</th>
<th>Design capacity (m³/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kabd</td>
<td>Oxidation ditch</td>
<td>Tertiary (disk filtration and chlorination)</td>
<td>250,000</td>
</tr>
<tr>
<td>Sulaibiya</td>
<td>Oxidation ditch</td>
<td>Advanced (UF and RO and chlorination)</td>
<td>600,000</td>
</tr>
<tr>
<td>Umm Al Hayman</td>
<td>Carrousel</td>
<td>Tertiary (sand filtration, chlorination and UV)</td>
<td>27,000</td>
</tr>
<tr>
<td>Riqqa</td>
<td>Extended aeration</td>
<td>Tertiary (sand filtration and chlorination)</td>
<td>180</td>
</tr>
</tbody>
</table>

Table 1
Technical information about main municipal WWTPs in Kuwait
influent of Umm Al Hayman plant (Table 2), are larger than the mean values. This indicates that variations of influent concentrations around mean values were relatively high. This is also evident from values of coefficient of variation (C.V.) being greater than 100% in most of the cases (Table 2). The high variability of estrogens concentrations in the influent streams can partly be attributed to the grab sampling method adopted. Grab sampling method often represents wastewater quality at the instant of sampling, which can be different for the same time in another day. Usually wastewater quality changes continuously. However, it often shows diurnal, weekly and seasonal patterns.

Table 2 shows that the mean influent concentrations of E1 were determined to be 4, 72 and 11 ng/L for Kabd, Sulaibiya and Umm Al Hayman plants, respectively, which were the highest compared to the natural estrogens E2 and E3. This can be attributed to biodegradation and transformation of E2 and EE2 into E1 conjugates during transportation in the sewerage system [18]. In fact, Kabd, Sulaibiya and Umm Al Hayman plants are located at distances of 6, 25 and 10 km, respectively, from the catchment areas. Thus, the influent concentrations of E1 seemed to increase with the increase of the distance between the plant and its catchment area.

3.2. Effluent concentrations

Table 3 shows that the determined effluent concentrations of estrogens were between 0.0 to 233 ng/L, which fall within the range reported in literature [8,20–27]. As expected, the highest effluent concentrations of both natural estrogens (E1, E2 and E3) and synthetic estrogens (EE2) of the effluent streams were found out for the Sulaibiya plant. Sulaibiya plant experienced the highest estrogens loading during the sampling period (Table 2). Except for E1 concentrations, there were no significant difference between the average concentrations of estrogens in the effluent stream of Umm Al Hayman plant and that of Kabd plant. However, Umm Al Hayman achieved much lower concentration of E1 (1 ng/L) compared to Kabd plant (12 ng/L).

Table 3 indicates that the standard deviation (STD) of the effluent concentrations is greater than the mean effluent

<table>
<thead>
<tr>
<th>Estrogenic type</th>
<th>WWTP</th>
<th>Minimum</th>
<th>Mean</th>
<th>Maximum</th>
<th>Std. deviation</th>
<th>C.V. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>Kabd</td>
<td>0</td>
<td>4</td>
<td>14</td>
<td>5</td>
<td>147</td>
</tr>
<tr>
<td></td>
<td>Sulaibiya</td>
<td>0</td>
<td>72</td>
<td>372</td>
<td>148</td>
<td>265</td>
</tr>
<tr>
<td></td>
<td>Umm Al Hayman</td>
<td>6</td>
<td>11</td>
<td>14</td>
<td>3</td>
<td>29</td>
</tr>
<tr>
<td>E2</td>
<td>Kabd</td>
<td>0</td>
<td>3</td>
<td>9</td>
<td>4</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td>Sulaibiya</td>
<td>0</td>
<td>32</td>
<td>160</td>
<td>63</td>
<td>195</td>
</tr>
<tr>
<td></td>
<td>Umm Al Hayman</td>
<td>0</td>
<td>5</td>
<td>10</td>
<td>4</td>
<td>83</td>
</tr>
<tr>
<td>EE2</td>
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<td>19</td>
<td>50</td>
<td>15</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>Sulaibiya</td>
<td>0</td>
<td>194</td>
<td>474</td>
<td>199</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>Umm Al Hayman</td>
<td>0</td>
<td>36</td>
<td>90</td>
<td>42</td>
<td>116</td>
</tr>
<tr>
<td>E3</td>
<td>Kabd</td>
<td>0</td>
<td>3</td>
<td>20</td>
<td>8</td>
<td>265</td>
</tr>
<tr>
<td></td>
<td>Sulaibiya</td>
<td>0</td>
<td>65</td>
<td>360</td>
<td>145</td>
<td>225</td>
</tr>
<tr>
<td></td>
<td>Umm Al Hayman</td>
<td>0</td>
<td>25</td>
<td>88</td>
<td>32</td>
<td>127</td>
</tr>
</tbody>
</table>
values for most of the cases. As for the influent, this also indicates that effluent concentrations varied highly from mean values. This was also confirmed by C.V. values greater than 100%. In other countries, removals of EDCs by activated sludge WWTPs were also found to have fluctuating trends [8,28–31]. However, the very high variations (STD > mean) in effluent concentrations may point out that the operation of the Kuwaiti WWTPs need to be optimized/upgraded for the removal of estrogens.

3.3. Removal efficiencies

The average removal efficiencies of estrogens were calculated from the average influent and effluent concentrations and presented in Table 4. In spite of high loading, the Sulaibiya plant achieved the highest removal of both synthetic (93% for EE2) and natural estrogens (87% for E2 and 82% for E3), except E1. The best removal of E1 (94%) was achieved by Umm Al Hayman plant. This plant had also performed relatively better than Kabd plant in removing EE2 and E3.

The overall average removal of estrogens achieved by Sulaibiya, Umm Al Hayman, and Kabd plants were calculated to be 79%, 68% and 13%, respectively. The removal rates of Sulaibiya, Umm Al Hayman were consistent with the rates reported in literature for activated sludge systems [1,18]. As given in Table 1, Kabd and Umm Al Hayman treat up to tertiary level, whereas Sulaibiya plant treats up to advanced level that consist of UF and RO. Thus, the highest removal of estrogens at the Sulaibiya plant may be partly attributed to the advanced treatment level used there. However, this study did not investigate the contributions of the different stages of wastewater treatment (preliminary, secondary, tertiary or advanced) on estrogens removals nor monitored the operational variables of the plants. Thus, it is difficult to attribute the superior performance of the Sulaibiya plant to only advanced treatment nor give scientific explanations of the relatively poor performance of the Kabd plant.

3.4. Potential impacts of treated wastewater reused

As shown above, the final effluents of the three plants studied in Kuwait contain significant and highly fluctuating concentrations of estrogens that can adversely impact the human and environmental health. In a very recent study, it had been found that concentrations of EDCs, including estrogens, near the sewage outlets in the coastal area of Kuwait were high enough to initiate alterations in the hepatic tissue of fish in a period of two weeks [32].

On the other hand, threats to human health arises from ingesting estrogens via drinking water (un)intentionally mixed with treated wastewater or eating food irrigated by treated wastewater [33–36]. The likelihood of mixing treated wastewater with drinking water, intentionally or unintentionally, is zero in Kuwait as treated wastewater is not reused in any potable applications. Also treated wastewater is not allowed to be used for irrigating edible crops. In Kuwait treated wastewater is reused mainly for irrigating fodder crops. Nonetheless, there is a probability of indirectly ingesting estrogens via eating meat of animals fed on grass irrigated with treated wastewater. Although this risk seems to be very low, it needs to be carefully assessed in order to help in developing appropriate regulations for estrogens in effluents reused as irrigation water in Kuwait. Such regulations are urgently needed because estrogens may not only contaminate the food indirectly, but they may also pollute the groundwater [37]. Development of regulations for the discharges of estrogens into the environment has recently started in EU, USA, Japan and Canada. So far, however, only the EU has proposed some regulations for estrogens in agriculture waters [38].

4. Conclusions and recommendations

To assess the fate of estrogens in municipal WWTPs of Kuwait, influent and effluent concentrations of three plants (Kabd, Sulaibiya and Umm Al Hayman) were determined through solid-phase extraction gas chromatography-mass spectrometry method. Accordingly, the following conclusions and recommendations were drawn:

- Concentration of estrogens in the influent streams ranged from 0.0 to 474 ng/L, while that in the effluent streams ranged between 0.0 and 233 ng/L.
- Estrogens concentrations in both influent and effluent streams of the three plants varied highly from mean values.
- Influent concentration of E1 seemed to increase with the distance of plant from catchment area.
- The average removal of total estrogens were 13%, 79% and 68% for Kabd, Sulaibiya and Umm Al Hayman, respectively.
- Despite high influent loading, the Sulaibiya plant achieved the highest removal of EE2, E2 and E3.
- Umm Al Hayman plant accomplished the best removal of E1.
- Operations of WWTPs in Kuwait need to be upgraded/optimized in order to maximize estrogens’ elimination.
- Kuwait needs to regulate estrogens discharges from WWTPs in order to prevent further pollution of marine areas and ground water by estrogens.

<table>
<thead>
<tr>
<th></th>
<th>E1</th>
<th>E2</th>
<th>EE2</th>
<th>E3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kabd</td>
<td>-234</td>
<td>66</td>
<td>49</td>
<td>-131</td>
<td>13</td>
</tr>
<tr>
<td>Sulaibiya</td>
<td>37</td>
<td>87</td>
<td>93</td>
<td>82</td>
<td>79</td>
</tr>
<tr>
<td>Umm Al Hayman</td>
<td>94</td>
<td>52</td>
<td>56</td>
<td>74</td>
<td>68</td>
</tr>
</tbody>
</table>
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References


