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Sources and levels of endocrine disrupting compounds (EDCs) in Kuwait's coastal areas

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ABSTRACT

The sources and levels of endocrine disrupting compounds in Kuwait's coastal areas were investigated. Phthalates, alkylphenols and estrogens were measured in the inflows and outflows of three sewage treatment plants as well as in the seawater and sediments from the sewage impacted coastal areas. Phthalate levels in the inflow of the treatment plants ranged from 8.9 to 78.3 μg/l; alkylphenols from 0.7 to 279 ng/l and estrogens from 30 to 368 ng/l. On average, the treatment plants removed about 80% of these compounds. The outflows, however, contained significant levels of all three classes of compounds. The seawater from the sewage impacted area also contained detectable levels of these compounds. Sediment samples from these locations contained elevated levels of phthalates (ranging from 2145 to 15,722 μg/kg) and lower levels of alkylphenols (ranging from 2.49 to 15.14 μg/kg) and estrogens (ranging from 4.1 to 214 μg/kg, dry wt.).

The adverse effects of endocrine disrupting compounds (EDCs) in marine environment are well established (WHO, 2002). In aquatic ecosystems, these compounds can cause developmental and/or reproductive dysfunctions. Due to their hazard potential, EDCs have become worldwide concern and are considered to be one of the most serious anthropogenic threats to biodiversity and ecosystem health (Jenssen, 2006). Natural estrogens excreted by humans and animals are present in the sewage streams and consequently in the coastal areas impacted by the sewage discharge. Numerous studies have reported the presence of these compounds in aquatic environments. In addition, many industrial chemicals (e.g. phthalates and alkylphenols) have also been demonstrated to possess estrogenic activity. These chemicals are produced in huge quantities and eventually find their way to the coastal areas. The presence of estrogenic compounds in the coastal areas causes adverse biological effects e.g. feminization of male fish, abnormal development of reproductive organs. The concerns about these compounds in aquatic ecosystems resulted in many studies from all over the world (e.g. Hosokawa et al., 2003; Braga et al., 2005; Vethaak et al., 2005; Pojana et al., 2007; Tan et al., 2007; Sharma et al., 2009; Ying et al., 2009; Bjorklund et al., 2009; Zhang et al., 2009; Chang et al., 2011; Gong et al., 2011; Arditsoglou and Voutsa, 2012).

Arabian Gulf, in general, and Kuwait coastal areas, in special, has long been known to be heavily contaminated with a wide variety of pollutants (Al-Sarawi et al., 2015). Kuwait coastal areas have been reported to be contaminated with petroleum related pollution, POPs, heavy metals, and fecal matter. The major sources of these pollutants in the marine areas were the discharges from Shatt Al Arab, untreated domestic sewage, untreated industrial effluents, petroleum production, refining and transportation, in addition to atmospheric deposition (Smith et al., 2015). Discharge of untreated sewage to the coastal areas in Kuwait has been continuing for longtime due to insufficient treatment capacity until 2004. However, breakdown, accidents and downpours have caused many large discharges of sewage to the coastal areas since then. A pumping station breakdown in 2009 resulted in release of large quantity of sewage discharge which continued for almost three years (Lyons et al., 2015; Saeed et al., 2012; Saeed et al., 2015).

So far, there have been a few studies dealing with EDCs levels in the Arabian Gulf and especially in Kuwait coastal areas. Recently, Smith et al. (2015) reported the results of a screening study using ecotoxicological and chemical techniques. Low levels of phthalates and bisphenol-A were detected. In addition, hotspots with elevated estrogenic activity were identified. Apart from this, there is no information on the sources and levels of EDCs in Kuwait's coastal areas. The main objective of the present study was to assess the sources and levels of EDCs in Kuwait's coastal areas. The results of the EDCs levels in the inflows and outflows of the wastewater treatment plants as well as in seawater and sediment from the area impacted by the discharges are presented.

All of the chemicals and reagents were of analytical reagent grade and were obtained from commercial suppliers. The organic solvents used for sample extraction and cleanup were of pesticide residue grade and checked for impurities. Authentic standards and deuterated

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surrogate standards were obtained from a multitude of sources (AccuStandard, Sigma and Supelco).

Water samples from treatment plants and from the sea were collected in 2.5-liter amber glass bottles with Teflon-lined cap. As phthalates are ubiquitous, special care was needed to avoid contaminating the samples with phthalate from non-sample sources. To decontaminate, the sampling bottles were cleaned with soap, tap water, distilled water and then with acetone. The bottles were baked in a furnace at 450 °C overnight (Guo et al., 2012). Prior to the sample collection, about 0.5 g sodium azide was added to each sampling bottle to stop any biological activity during transportation and storage. Samples were transported to the laboratory and stored at 4 °C. Samples were collected over a period of 10 months, starting from October 2015 and ending in August 2016. The number of samples collected from the inflow of Sulaibiya treatment plant was eight while the six outlet samples were also collected. Similarly 12 samples (6 inlets and 6 outlets) were collected from Um Al-Hyman treatment plant. 15 samples were obtained from Kabd treatment plant. Of these 7 samples were from inlets and 8 were from outlet.

Surface sediment samples were collected with a grab sampler from the location near the discharge point from where the water samples were also collected (coordinates given in Table 1 and shown in Fig. 1). Water samples were stored at 4 °C and the sediment samples were kept at −20 °C. The sediment samples were freeze dried and were sieved through a 250 μm sieve. The sieved samples were stored in amber glass bottles. The water samples (from treatment plants and seawater) were allowed to come to room temperature. An aliquot (500 ml) was taken and filtered through a glass fiber filter (previously baked at 450 °C) with 1 μm pore size to remove the suspended particulate matter. The filtrate was spiked with 100 μl of a mixture of deuterated phthalate standard (containing 9 deuterated phthalates, 2 ppm). The sample was then passed through Oasis HLB cartridge (Waters Corporation) containing monomers of *N*-vinylpyrrolidone and divinylbenzene. Glass cartridge (6 ml) contained 200 mg of the adsorbing material (Liu et al., 2004). The cartridge was conditioned by passing 3 ml methyl *t*-butyl ether (MTBE), followed by 3 ml of methanol and finally 3 ml of ultrapure water. The sample was allowed to pass through the cartridge at about 3–4 ml/min rate. After passing the sample, the cartridge was washed with 3 ml of methanol-water mixture (5/95) and allowed to dry under slight vacuum. The cartridge was eluted with 6 ml of methanol/MTBE (10/90). The eluate was dried over anhydrous sodium sulfate and evaporated to dryness under nitrogen. The residue was taken up in 1 ml *n*-hexane.

Freeze-dried sediment samples (about 5 g) were extracted by ultrasonication with methanol. Deuterated phthalate standard mixture was added to the sample (10 μl of 2 μg/ml solution). The extraction was continued for 20 min. The sample was then centrifuged and the solvent decanted. The extraction was repeated twice with 15 ml of fresh solvent. The combined extract was then dried over anhydrous sodium sulfate and reduced to 5 ml under nitrogen. The concentrated extract was then mixed with 100 ml of ultra-pure water. The aqueous extract was then passed through the Oasis cartridge as described above.

The extract from Oasis cartridge containing phthalates, alkylphenols and estrogens was divided into two aliquots. For phthalate analysis, 100 μl were removed and transferred to a micro-vial. The remaining 900 μl were evaporated to dryness under nitrogen in a thick-walled V-shaped vial. Silylating reagent BSTFA with TMCS (100 μl) was added to the vial. The vial was heated to 70 °C on a hotplate for 30 min. After cooling to room temperature, the contents were evaporated to dryness under nitrogen. The residue was taken up in 900 μl hexane and transferred to autosampler vial.

Table 1
Locations and coordinates of seawater and sediment sample sites.

Location	Coordinates	Comments
Fintas	N 29.195489 E 48.119061	Sewage outlet, impacted
Fahaheel	N 29.094903 E 48.140030	Sewage outlet, close to refinery discharge
Shuwaikh Port	N 29.351787 E 47.919172	Commercial port
Ghazali	N 29.350296 E 47.900964	Sewage discharge
Sulaibekhat	N 29.362188 E 47.863953	Offshore, impacted by sewage

The aliquot for phthalate analysis (100 μl) was analyzed by Agilent GC/MS instrument consisting of 6890N GC, 5975 MSD and 7683B auto sampler. The operating conditions employed for the analysis of phthalates were: splitless injection port temperature, 280 °C; fused silica column (30 m × 0.25 mm) coated with SE-54 (HP-5MS), initial column temperature 45 °C; 1 min hold; programmed to 290 °C at 10 °C/min; final hold time 10 min; interface temperature 280 °C; ion source temperature 230 °C and quadruple temperature 150 °C. Selected ion monitoring (SIM) mode was used to identify and quantify the target compounds. The target ions used for the surrogate (deuterated) standard phthalates included 167 and 153; and ions 163 and 149 for phthalates.

Alkylphenols and estrogens in the 900 μl aliquot were analyzed by Shimadzu 2010 GC coupled to QP2010 Plus MS. Shimadzu AOC-20i autosampler was also used. The analytical condition used for this analysis were: injection port temperature 250 °C; interface temperature 280 °C; ion source temperature 250 °C; column oven program: initial temperature 50 °C, held for 1 min; programed to 200 °C at 20 °C/min, held for 3 min and then heated to 280 °C at 3°/min; finally heated to 290 °C at 30 °C/min and held for 3 min. The target ion used for alkyl phenols were 179 and 207. The ions used for estrogens were 357, 412, 342, 416 and 504.

For quantitation of phthalates; deuterated phthalates were added to the sample to act as recovery and internal standards. External standard method was used for alkylphenols and estrogens. For phthalates, the instrument was calibrated by using a series of quantitative mixtures. The concentration of the calibration mixtures were 0.1, 0.5, 1.0, 2.5 and 5.0 ppm. The calibration mixture contained all 9-phthalates. Alkyl phenols and estrogens were quantified by calibrating the instrument with standard mixtures containing 4 alkyl phenols, bisphenol-A and 5 estrogens (DES, E1, E2, EE2 and E3) at 1.0, 2.5, 5.0, 7.5 and 10.0 ppm.

A number of measures were employed to control and ensure the quality of the data produced. About 10% of the samples were duplicated in the analytical scheme. The incorporation of recovery standards helped monitoring of the efficiency of the sample preparation procedure for phthalates. For alkylphenols and estrogen, the samples were spiked and the recovery of the spike monitored (recovery given in Table S1).

Most of the EDCs reaching the marine ecosystem originate on land. The presence of natural and synthetic estrogenic compounds is a marker of inputs from land based sources. Discharge of treated and untreated wastewater is generally the main reason for the presence of EDCs in coastal areas. Kuwait has 4 wastewater treatment plants which treat about 800,000 m^3/day. Sulaibiya water treatment plant is the largest of the treatment plants in Kuwait. Bulk of Kuwait's domestic sewage is treated by this treatment plant (450,000 m^3/day). The treated water is then piped to agricultural farms in Wafra and Abdali. In case of malfunction or downpour, the water may be discharged to the coastal areas.

The summary of the results of the analysis of phthalate in the water samples collected from this plant are shown in Table 2 (detailed results given in Table S2). Significant levels of phthalates (ranging from 12.16 to 28.09 μg/l) were present in the inflow stream. The average levels during the sampling period were 19.71 μg/l in the inflows. Two compounds contributed highly towards the total phthalate burden of the inflow streams. DEP and DEHP together made up about 80% of the total phthalates. The probable reason for relatively high levels of DEP and DEHP in sewage is their use in the production of articles of everyday use. DEP is used in the production of cosmetics, perfumes, detergents, aerosol sprays in addition to other uses. DEHP is also used in the manufacture of many items of everyday use (e.g. PVC pipes).

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