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Impact of endocrine disrupting compounds in sewage impacted coastal area on seabream

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ABSTRACT

The pollution of coastal regions worldwide has been of a great concern due to the presence of endocrine disrupting chemicals (EDCs). These chemicals find their way to the marine environment via the sewage treatment plants (STPs). Hence, this study was designed to investigate the status and sources of EDCs and their effect on fish in Kuwait's coastal areas, from the chemical and biological perspectives. The assessment of three STPs indicated the presence of significant levels of phthalates (19 and 31 μg/l), alkylphenols (85 and 159 ng/l), and estrogens (30 and 368 ng/l) in both inflow and outflow samples. The analysis of samples from field exposure sites revealed significant levels of EDCs in seawater (phthalates: 2.1–4.6 μg/l; alkylphenols: 1.2–16.4 ng/l; estrogens: 0–36.2 ng/l) and sediment (phthalates: 2.1–15.7 mg/kg dry wt; alkyphenols: 2.5–15.1 μg/kg dry wt.; estrogens: 4.1–214.2 μg/kg dry wt.) samples. The biological perspective investigated through the exposure of fish to sewage outlets at five sites. The hepatosomatic index (HSI) revealed a higher level in winter samples 0.48–0.79%) in comparison to summer samples 1–1.5%). Histological observation of hepatic tissue of fish exposed during winter months in all sites, showed much less necrotic changes and hepatic vacuolation in the hepatic tissue of summer exposed fish. Imunnohistochemistry evidences revealed a significant level of positive signals and Vtg localization in the hepatic tissue as the results support the histopathological alterations observed. Results of enzyme-linked immunosorbent assay (ELISA) showed no significant difference between the plasma protein content of winter and summer samples. Overall, the study suggest that there is possible local source or a chronic input of untreated and/or partially treated water due to the significant levels of phthalates, alkyphenols, and estrogens detected in the Kuwait Bay. These levels were enough to initiate alteration in the hepatic tissue of fish exposed to the sewage outlets in Kuwait for two weeks.

1. Introduction

Endocrine disrupting compounds, (EDCs) are of global concern and generally known as chemicals that interfere with the normal functioning of the endocrine systems in wildlife and humans (Burkhardt-Holm, 2010). EDCs are exogenous agents that interfere or disrupt the normal synthesis, secretion, transportation, binding, and metabolism of natural hormones eventually affecting homeostatic mechanisms, reproduction, and development. Several laboratory studies indicated the negative effect of EDCs on fish reproduction, osmoregulation, growth, behaviour, and immune function via disrupting the endocrine system (Mills and Chichester, 2005; Al-Jandal et al., 2011). Most EDCs are man-made organic chemicals, which can reach the marine environment through contaminated sewage effluents (Xu et al., 2014). A growing body of research has indicated that the municipal sewage treatment plants (STPs) are important pollution sources of EDCs released into the environment (Ternes et al., 1999; Baronti et al., 2000). One of the main reasons for the presence of these compounds in aquatic environments is; the discharge of untreated wastewater (sewage), incomplete removal of these compounds in STPs, and subsequent disposal of treated water (effluent) to the coastal areas (Liu et al., 2009; Marfil-Vega et al., 2010). Pollutants with limited solubility in water, such as hydrocarbons, will ultimately settle in the sediment (Peterson et al., 2003). Generally, sediments regarded as the ultimate sink of the pollutants entering the marine ecosystem including EDCs.

The marine environment has a critical socioeconomic importance for Kuwait, where sea is an important source of food and potable water by desalination (Price et al., 1993). Sewage and industrial discharges are crucial in the management of the water quality of Kuwait's marine waters (Al-Ghadban et al., 2002; Al-Abdulghani et al., 2013). Kuwait's

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coastline had a remarkable environmental degradation due to human activities such as dumping of sewage that led to deteriorating the quality of seawater and sediment in Kuwait. Kuwait has a long history of discharging untreated wastewater to the coastal areas. A study carried out on the spatial assessment of sewage in the coastal areas of Kuwait. Fecal sterols were determined as indicators of sewage contamination in the marine sediments collected from 112 locations throughout Kuwait's marine areas. The results showed that areas near the sewage outfalls were heavily contaminated, especially the western part of the Kuwait Bay, whereas the southern coastal areas were less severely contaminated (Saeed et al., 2015).

Prior to 2004, Kuwait did not have enough capacity to treat all of the wastewater produced. In 2004, after the commissioning of the Sulaibiya plant, there was enough capacity to treat all of the domestic wastewater generated. However, in August 2009, there was a catastrophic failure of a pumping station resulting in the discharge of 70 million cubic meter of raw sewage via the emergency outlets to the southern marine coastal areas for three years (Saeed et al., 2012). The water quality of Kuwait's marine waters may have improved after the repair of pumping station in 2012. The influence of other discharges, mainly those in the southwestern parts of the Kuwait Bay (Sulaibikhat Bay), needs to be considered in the long-term ecological health assessment (Devlin et al., 2015). There are multiple sewage and industrial discharge sources in Kuwait, particularly sewage outfalls around the Al-Doha area, Sulaibikhat Bay in Kuwait Bay, Mishref area, and the Shuaiba industrial area at the southern part of Kuwait. Sewage flows into Kuwait Bay are often septic due to low flows, extended retention times, high ambient temperatures, and oxygen depletion (Al-Ghadban et al., 2001). Sewage effluents can also introduce estrogen-like effects on fish as ECDs are detected in sewage effluents worldwide (Baronti et al., 2000; Gibson et al., 2005; Servos et al., 2005). Exposure to estrogen stimulates the synthesis of vitellogenin (Vtg), which is an egg yolk protein and considered as a well-known biomarker for estrogen exposure (Gimeno et al., 1998).

The primary objective of this study was to assess the availability and status of EDCs in Kuwait marine environment (chemicals perspective) and possible effects of EDCs exposure on local fish (biological perspective).

2. Materials and methods

Fish cages were designed in-house (size 80 × 80 × 80 cm) for fish exposure in the field. The experiments were carried out during two different seasons, summer and winter, to study the effects of season (if any) on fish after exposure to the sewage outlet. Yellowfin sea bream (*Acanthopagrus latus*); locally called shaem were obtained from the Aquaculture Program's hatchery at the Kuwait Institute for Scientific Research (KISR). Juvenile male fish selected for the experiment with an average size of 23.3 cm (total length) and 291.5 g (body weight).

2.1. Exposure scenario

Two fish cages were used for every exposure experiment with 15 fish in each cage (total of 30 fish per exposure). The field exposure was carried out for two weeks before reclaiming the cages for terminal sampling. The selected experimental sites were inside Kuwait Bay (Sulaibikhat Bay, Al-Ghazali outlet, and Shuwaikh Port); and outside Kuwait Bay (Fintas and Fahaheel). The main reasons for selecting these locations were the availability of active sewage outlet in the area, feasibility of fixing the fish cages in the water for two weeks without any risk of cage loss or theft, and choosing a suitable distance from the outlet to avoid the low tide.

2.2. Seawater and sediment

Sampling from the Exposure Sites. Water and sediment samples were collected during the summer and winter exposures. Surface sediment samples were collected with a grab sampler from the location near the discharge point from where the water samples were also collected. All collected samples were transferred to the laboratory for analysis of the three major classes of ECDs (phthalates, alkylphenols and estrogens).

The sediment samples were freeze dried. The freeze-dried samples were sieved through a 250-μm sieve. The sieved samples were stored in amber glass bottles. The total organic carbon (TOC) contents of the sieved sample were determined by a standard procedure (ROPME, 2010). Freeze-dried samples (about 5 g) were extracted by ultrasonication with methanol. Deutrated 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 was 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 ultrapure water. The aqueous extract was then passed through the Oasis cartridge as described above.

The collected water samples were kept to reach to the 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 deutrated phthalate standard (containing nine deutrated phthalates, 2 ppm). The sample was then passed through Oasis HLB cartridge (Waters Corporation) containing monomers of N-vinylpyrrolidone and divinylbenzene. The 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/diethyl ether mixture (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.

2.3. Statistical analysis

Significant differences between control and exposed fish was determined by using One Way Analysis of Variance (ANOVA), and differences was considered to be significant when $P < 0.05$. All statistical analysis was conducted using Sigmastat 3.5 (Systat Software, Inc.).

3. Terminal sampling

After two weeks of exposure, fish cages reclaimed back to the laboratory for terminal sampling and analysis. According to the literature, the exposure duration (two weeks) were selected (Verslycke et al., 2002; Al-Jandal et al., 2011). Prior to sampling, fish were anesthetized with clove oil (20 ppm), body measurements (weight and length) were taken, and blood sampling via caudal puncture was carried out. Blood samples were transferred to 1.5 ml Eppendorf tubes with the anticoagulant, ethylene diamine tetra acetic acid (EDTA). Samples were kept on ice throughout the sampling before centrifugation to separate the plasma. Liver samples were dissected for histological examination.

3.1. Hepatosomatic index (HSI)

The total weight of each dissected liver from the control and exposed fish was taken for the HSI, and calculated as follows:

$$\text{HSI} = (\text{Liver weight (g)} / \text{Fish weight (g)}) \times 100.$$