



# Screening for contaminant hotspots in the marine environment of Kuwait using ecotoxicological and chemical screening techniques



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## ABSTRACT

Kuwait is a country with low rainfall and highly concentrated industrial and domestic effluents entering its coastal waters. These can be both treated and untreated. In this study we sampled a series of coastal and open-sea sites and used a variety of analyses to identify those sites requiring the most attention. We used a high throughput GC–MS screen to look for over 1000 chemicals in the samples. Estrogen and androgen screens assessed the potential to disrupt endocrine activity. An oyster embryo development screen was used to assess biological effect potential. The chemical screen identified sites which had high numbers of identified industrial and domestic chemicals. The oyster screen showed that these sites had also caused high levels of developmental abnormalities with 100% of embryos affected at some sites. The yeast screen showed that estrogenic chemicals were present in outfalls at 2–3 ng/l E2 equivalent, and detectable even in some open water sites.

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## 1. Introduction

Kuwait is a country with almost 500 km of coastline, which lies along the north eastern end of the Arabian Gulf and comprises Kuwait Bay, a shallow body of water to the North West. The majority of Kuwait's 3.6 million inhabitants reside in Kuwait City which is located along the southern shoreline of the bay and extends south along the Gulf coastline. Although large amounts of contaminants enter the Kuwaiti marine environment via the Shatt Al Arab, the confluence of the Tigris and Euphrates rivers in the northern Arabian Gulf (Beg and Al-Ghandban, 2003), waste effluents from industry and domestic sewage are thought to make up the key components of marine pollution around Kuwait (Al-Ghadban et al., 2002; Al-Abdulghani et al., 2013; Al-Sarawi et al., 2015). In particular, sewage contamination, both from illegal discharges or authorised releases, has been documented as being of particular concern (Al-Omran, 1998; Ghannoum et al., 1991; Lyons et al., 2015; Saeed et al., 2012). Other effluents from power stations, desalination plants and various sized industrial or commercial processes also reach the marine environment from outfalls. Due to these various inputs, the

waters of Kuwait Bay and the Gulf coast are subject to a constant stream of complex effluents varying in volume, constituents and flow. The problems associated with understanding the overall effect of these mixed effluents were recognised by the early 1990s (Matthiessen et al., 1993) and research also showed that effluents from sewage treatment plants may also, through various chemical pollutants, demonstrate endocrine disruption effects (Desbrow et al., 1998; Harries et al., 1996; Jobling et al., 1998; Harries et al., 1999). These effects can be caused by natural steroids and also by industrial chemicals acting as endocrine disruptors with variable potency (Aerni et al., 2004; Duong et al., 2010; Fawell et al., 2001; Pawlowski et al., 2004; Pothitou and Voutsas, 2008; Sohoni and Sumpter, 1998; Thomas et al., 2001). Interest in what was in these complex effluents widened and a wide range of pharmaceutical and personal care product compounds was also identified in the receiving waters (Prasse et al., 2010; Roberts and Thomas, 2004; Thomas et al., 2004). The presence of pharmaceuticals is of particular concern, as they are designed to have specific biological effects and low concentrations, and so are thought to pose high risk to organisms in receiving waters (Gaw et al., 2014). These types of effluents, and their dispersal, dilution and degradation are problematic for chemical analysis because not all the chemicals are identified in the effluent. In addition, reactions that take place within the effluents are hard to predict so reaction products and biotransformation

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products may or may not be identified or quantified. Predicting the toxicity of such complex mixtures has been one of the hot topics in environmental monitoring so far this century (Backhaus and Karlsson, 2014; Backhaus et al., 2011; Cleuvers, 2004; Flaherty and Dodson, 2005; Thorpe et al., 2001; Thorpe et al., 2003). Effective biological assays can provide data on the overall effects of a complex mixture and help to target sites with problems without any preknowledge of what chemicals might be present. Finding the right combination of suitable biological and chemical analysis can give the capability to monitor for both specific and unknown pollutants and understand how sites are changing in response to changes in climate, industry, hydrodynamics and other ecological pressures. One effective screening assay for effluent based contamination is the yeast estrogen screen (Routledge and Sumpter, 1996). This is a tool for looking at steroid and steroid mimicking chemicals in environmental samples and gives a measure of the overall effect of the sum of active steroid-like chemicals. It is a widely accepted assay and has been used in many countries to look at contamination in effluents, rivers, estuaries and marine areas (Balaam et al., 2010; Beck et al., 2006; Fernandez et al., 2007; Galluba and Oehlmann, 2012; Pawlowski et al., 2004; Thomas et al., 2001, 2002, 2004; Tollefsen et al., 2007; Viganò et al., 2008).

To date no attempt has been made to assess the potential toxicological and endocrine disrupting risks posed by effluents that are discharged into Kuwait's marine environment. To address this a series of water and effluent samples was collected from various points around Kuwait's marine coastline to establish if such a threat exists. Water samples were screened using a GC–MS target based screening approach to identify the main pollutants present and assessed for both toxic and endocrine disruption potential using bioassays.

## 2. Materials and methods

### 2.1. Sample location and collection

Over a period of a few days a series of 16 water samples was collected from four known points sources of effluent input, Al Ghazali, Salmiya, Al Bedaa and Al Messala, and from twelve other sites located offshore in Kuwait Bay and along the Gulf coastline adjacent to the city (see Fig. 1). Approximately 2.5 litres of water or effluent were collected at each site using either a stainless steel bucket or Winchester bottles to collect samples from immediately beneath the water surface. All samples were stored in 2.5 litre amber Winchester bottles prior to extraction and analysis.

The Winchesters were transported back to the EPA laboratory at Shuwaikh, Kuwait City, where they were immediately prepped for vacuum extraction.

### 2.2. Extractions

Phenomenex 200 µg 6 ml Strata X cartridges were used with an 18 ml methanol activation followed by clean seawater rinse (laboratory prepared artificial sea water using Sigma Sea Salts). Samples were extracted under a steady drip of approximately 1 ml/minute. Once the sample was extracted, the cartridges were rinsed with 18 ml deionised water to remove salt residues. Due to the nature of the samples, and the desire to extract them without filtering, it was not always possible to extract the entire sample (2.6 litres) in the time available. In this case the volume of sample that passed through the SPE was recorded for calculation of concentration factor later. It was only possible, for example,



Fig. 1. Map showing the location of the sample sites referred to in the data.

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