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Microbial water quality and sedimentary faecal sterols as markers of sewage contamination in Kuwait

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ABSTRACT

Microbial water quality and concentrations of faecal sterols in sediment have been used to assess the degree of sewage contamination in Kuwait's marine environment. A review of microbial (faecal coliform, faecal streptococci and *Escherichia coli*) water quality data identified temporal and spatial sources of pollution around the coastline. Results indicated that bacterial counts regularly breach regional water quality guidelines. Sediments collected from a total of 29 sites contained detectable levels of coprostanol with values ranging from 29 to 2420 ng g^{-1} (dry weight). Hot spots based on faecal sterol sediment contamination were identified in Doha Bay and Sulaibikhat Bay, which are both smaller embayments of Kuwait Bay. The ratio of epicoprostanol/coprostanol indicates that a proportion of the contamination was from raw or partially treated sewage. Sewage pollution in these areas are thought to result from illegal connections and discharges from storm drains, such as that sited at Al-Ghazali.

1. Introduction

Sewage and industrial contamination are key issues in the management of water quality in Kuwait's marine waters (Al-Ghadban et al., 2002; Al-Abdulghani et al., 2013). It is known that the organic content of sewage discharged into Kuwait's coastal waters is high and regularly septic due to long retention times, elevated ambient temperatures and concomitant anaerobicity (Ghannoum et al., 1991; El-Desouki and Abdulraheem, 1998; Al-Ghadban et al., 2002). Other pollutants, including trace metals and oil related chemicals have been detected close to known point sources of sewage effluent, which often are discharged within a few meters of the shoreline (Al-Ghadban et al., 2002; Al-Sarawi et al., this issue). Microbial water quality surveillance monitoring for the assessment of beach quality is conducted by Kuwait Environment Public Authority (KEPA), who undertake sampling at 12 coastal sites located in the vicinity of emergency sewage outfalls and recreational beaches (Al-Ghadban et al., 2002).

It is estimated that 98% of Kuwait's 3.6 million inhabitants live within the 810 km^2 that covers the Kuwait Metropolitan Area. This major population centre is currently served by 5 main Sewage Treatment Plants (STP), along with additional smaller facilities at Failaka Island, Al-Khiran and Al Wafra. The STP network is currently being upgraded and by 2016 it is expected that all of the sewage produced will receive tertiary or reverse osmosis treatment. However, until as recently as 2011 it was estimated that the treatment network was receiving up to 100,000 m^3 day^{-1} of sewage more than its design capacity, leading to frequent discharges of raw or partially treated effluent into the marine environment. In recent years environmental disasters, such as the Mishref pumping station breakdown, have also contributed to the degradation of Kuwait's marine environment (Saeed et al., 2012). The Mishref pumping station malfunctioned in August 2009, resulting in the discharge of around 150,000 m^3 day^{-1} of raw sewage directly into the sea for several years. The discharge occurred via three main outfalls at Al-Bidda, Al-Khitabi and Al-Messela, impacting beaches in a number of areas important for tourism and residential housing. Monitoring undertaken by KEPA during this period indicated that approximately 20 km of coastline was affected, with water quality and bacterial indicators greater than permitted guidelines (EPA 2001).

For a number of decades many countries have used coliform bacteria as an indicator of sewage pollution. However, this approach can suffer from major constraints, which include the temporal (often hourly) fluctuations in bacterial counts and rapid

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biodegradation in tropical marine environments. Recently, there has been greater interest in the use of biological and chemical markers to help quantify anthropogenically derived sewage pollution in coastal marine waters (González-Oreja and Saiz-Salinas, 1998; Readman et al., 2005; Adnan et al., 2012). One of these chemical markers is coprostanol (5β-cholestan-3β-ol), which comprises 40–60% of the total sterols in human faecal waste, and has been used widely around the globe as a marker of sewage contaminated (Al-Omran, 1998; Readman et al., 2005; Reeves and Patton, 2005; Adnan et al., 2012; de Abreu-Mota et al., 2014). Coprostanol like many other faecal sterols is hydrophobic, readily associating with particulate matter in sewage effluent and consequently is incorporated into bottom sediments (Tolosa et al., 2014). Studies have demonstrated that coprostanol concentrations correlate well with coliform bacteria, especially in sewage contaminated environments (Isobe et al., 2002). Under anoxic conditions coprostanol is relatively persistent and any decline will invariably be associated with sediment transport. In tropical waters coprostanol, along with other selected sterols, are considered to be a more robust and reliable marker of sewage pollution than faecal coliform enumeration (Carreira et al., 2004; Adnan et al., 2012; Tolosa et al., 2014). To gain a full understanding of sewage input and source, the analysis of coprostanol is also assessed in relation to other sterols. For example, epicoprostanol, an isomer of coprostanol, can be used as a marker to indicate the level of treatment or age of the faecal matter (Readman et al., 2005; Martins et al., 2014; Tolosa et al., 2014). This compound is a by-product of sewage treatment systems and will only occur in low concentrations if the sewage is not treated or only partially treated, increasing in anoxic environments such as sewage sludge (Martins et al., 2014). Ratios, such as the coprostanol/cholesterol index can also be used to assess the degree of sewage pollution (Leeming et al., 1996; Isobe et al., 2002).

Here we present a survey using historic microbial water quality (faecal coliform, faecal streptococci and *Escherichia coli*) data to identify temporal and spatial point sources of pollution around the coastline of Kuwait. This is augmented with the analysis of sediment faecal sterols to establish the spatial extent of sewage contamination at a number of coastal and offshore sites in Kuwait Bay and along the Gulf coast.

2. Material and methods

2.1. Study location and field sampling

KEPA has been collecting water samples for microbial analysis at coastal sites (S00 to S11) over a period of almost 30 years (Fig. 1). The 12 coastal sites have been sampled monthly from 1987 to 2013. Additionally, a total of 29 sites were sampled for an evaluation of sediment contamination during the winter of 2013 and spring of 2014 (Fig. 1). Sediment was collected using a hand held van veen grab deployed from research vessels provided by KEPA, Kuwait Institute of Scientific Research (KISR) and Public Authority for Agriculture and Fish Resources (PAAFR). A stainless steel spoon was used to collect the top layer of each grab sample, which was then immediately transferred to a hexane rinsed 500 mL glass jar. Samples were kept on ice before transferring to a −20 °C freezer for storage prior to analysis. Sediment was characterised based on particle size analysis and total organic carbon content (TOC), a full characterisation of the sediment is provided in Lyons et al. (this issue).

2.2. Microbial water quality analysis

Historic datasets detailing total and faecal coliforms, faecal streptococci and *Escherichia coli* (*E. coli*) concentrations at S-site location around Kuwait were made available by KEPA. All analysis was undertaken according to membrane filtration methods as outlined in Standard Methods for the Examination of Water and Wastewater (2012). Briefly, replicate water samples (4-6 per site) were taken from each S-site (Fig. 1) and stored on ice for no longer than 6 h, before being returned to the laboratory for analysis. Samples (volume governed by degree of contamination) were filtered, from the highest dilution in order to avoid contamination, through sterile membranes (0.45 μm pore size) using aseptic techniques. The membrane filter was removed with flamed sterilized forceps and placed in Petri dishes containing agar and appropriate media. Petri dishes containing the membrane filters were sealed and incubated immediately for 24 h at 36 °C (total coliform media), 48 h at 36 °C (faecal streptococci) or 24 h at 44.5 °C for (faecal coliform). Blank and positive control samples were analysed in parallel with those collected from the field. Counts were adjusted to the number of colonies per 100/mL of sample filtered and presented as minimum, mean and maximum number of colonies per set of replicates (triplicate).

2.3. Sterol analysis

Sediment samples were air dried and sieved (<2 mm) in a controlled environment. 10 g of dried sediment were mixed with sodium sulphate, transferred to a glass Soxhlet thimble on top of a 1 cm layer of sodium sulphate. Prior to extraction, the samples were spiked with an internal standard (IS) solution containing 2 μg (each) of coprostanol-d5, epicoprostanol-d5, cholesterol-d4, and cholestanol-d5. The samples were subjected to Soxhlet extraction using 120 mL of acetone: n-hexane 1:1 (v:v) for ~6 h. Sulphur residues were removed at this stage with activated copper filings. A 10 mL aliquot of the 100 mL sediment extracts was taken to dryness and derivatised by the addition of 100 μL of N,O-bis (trimethyl silyltrifluoroacetamide) (BSTFA) and heating at 60 °C for 1 h. The final GC-ready fractions were spiked with injection standard PCB53 and made up to a final volume of 1 mL with iso-octane. Sterols were quantified using gas chromatography-tandem mass spectrometry (GC-MS/MS) in the electron impact (EI) mode at 70 eV. Multiple Reaction Monitoring (MRM) analysis was conducted using an Agilent 7890A gas chromatograph coupled to a 7000B triple quadrupole mass spectrometer (Agilent Technologies, Waldbronn, Germany). The separation of analytes was performed on a 15 m × 250 μm, 0.25-μm-film-thickness DB-5 capillary column (J&W) connected in series with a 25 m × 250 μm, 0.25-μm-film-thickness DB-5 capillary column (J&W). The carrier gas was helium (constant flow 2 mL min^{-1}) and the collision gas was nitrogen. The initial oven temperature was 80 °C, held for 1.5 min, then increased to 265 °C at 12 °C/min, held for 5 min, increased to 278 °C at 0.8 °C/min, increased to 300 °C at 10 °C/min, and finally held for 22 min. The injector, transfer line and source temperatures were 290 °C, 300 °C and 250 °C respectively. A 1 μL extract was injected in pulsed splitless mode with a purge time of 2.5 min. Quantitation for sterols was performed using internal standards and 6 calibration levels (range 6–12,000 ng mL^{-1} for epicoprostanol, cholestanol, coprostanone; range 25–50,000 ng mL^{-1} for cholesterol and coprostanol, 200 ng mL^{-1} all labelled compounds). The sterols standard solutions contained the following 9 compounds in acetone: Coprostanol, coprostanol-d5, epicoprostanol, epicoprostanol-d5, coprostanone, cholesterol, cholesterol-d4, cholestanol and cholestanol-d5. No labelled analogue was available for coprostanone so coprostanol-d5 was used as IS for this chemical. Standards were derivatised following the same process as for the samples, spiked with PCB53 and made up to a final volume of 1 mL with iso-octane. Details of the MS/MS transitions used and instrumental limits of quantitation are listed in Table SD1.

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