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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 biodegradation in tropical marine environments. Recently, there has been greater interest in the use of

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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 (trimethylsilyltrifluoroacetamide) (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.

All analyses were carried out under full analytical quality control procedures that included the analysis of a Laboratory Reference Material (LRM) and a blank sample with every batch of samples analysed so that the day-to-day performance of the methods could be assessed. The LRM was an offshore UK sediment spiked with a solution containing coprostanol, epicoprostanol, coprostanone, cholesterol and cholestanol in acetone. The results obtained for the reference material were compared to specified limits for each compound determined. The limits were created by the previous analysis of the above LRM in the Cefas Lowestoft Laboratory. Warning and control limits had been defined as

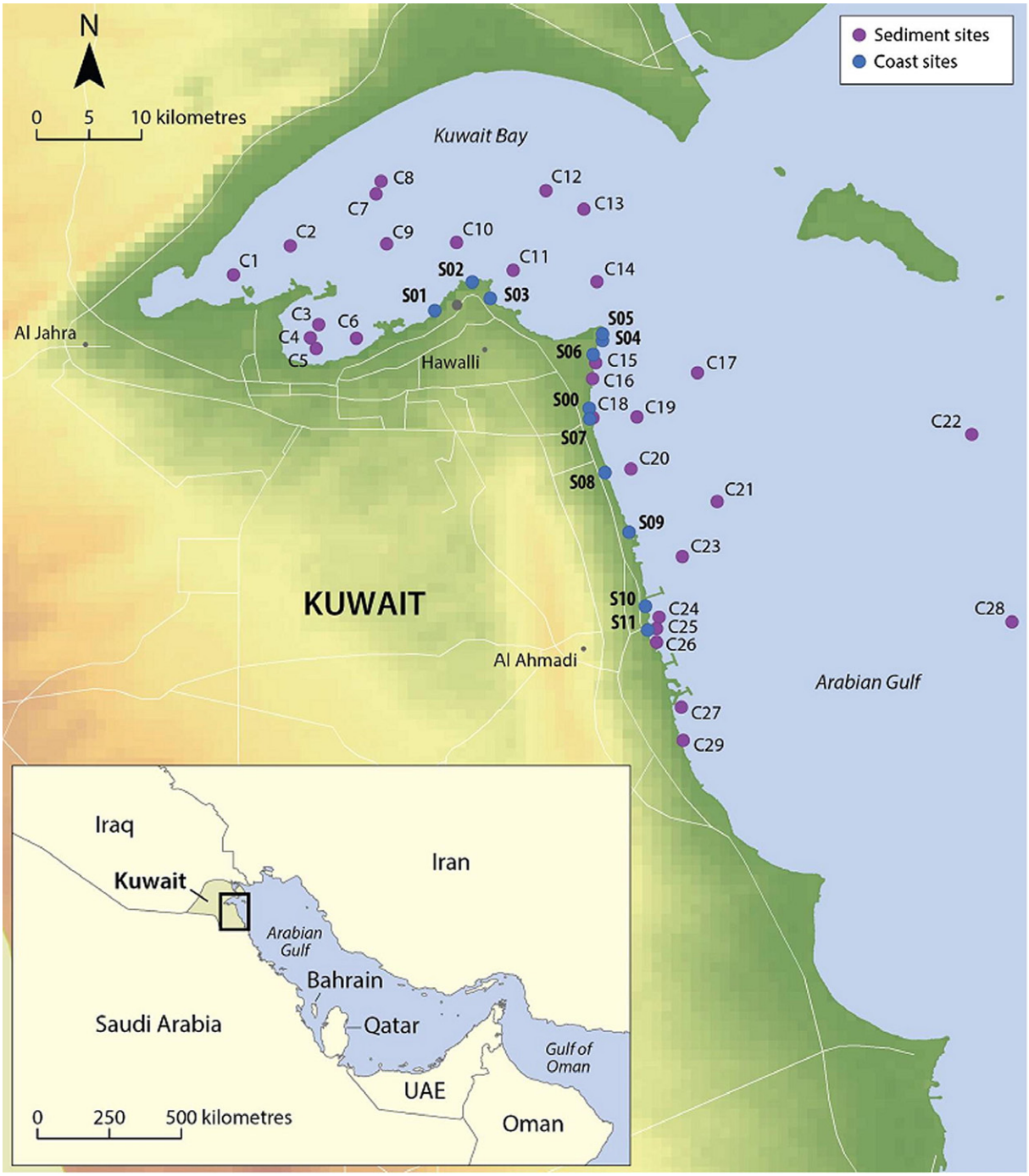


**Fig. 1.** Sediment sites (C) and shoreline microbial water quality sites (S) sampled. Outfalls associated with the Mishref crisis include S00, S06 and S07. The illegal Al-Ghazali sewer is located next to C6.

2σ and 3σ from the mean for each compound. The results obtained for all samples analysed were accepted as valid as the results for the laboratory reference materials were within the limits set by the control charts.

## 3. Results and discussion

### *3.1. Temporal and spatial microbial water quality*

Monthly mean and maximum counts of faecal coliforms, faecal streptococci and *E. coli* from each location up to 2013 are presented in Table 1. The highest counts for all microbial parameters were recorded at stations S09, S00 and S07, with high counts of *E. coli* also recorded at S04. The high counts at S09 are the result of three single sampling occasions (Jan, Feb, March 2011) at Al-Fintas (S09) where counts for faecal coliforms, faecal streptococci and *E. coli* were between $10^4$ and $10^7$ over the three months. Various international microbial water quality standards are available and those such as the coastal Bathing Water Directive (cBWD) adopted in the European Union (original Directive 76/160/EEC) sets out a number of microbiological and physiochemical standards that bathing waters must either comply with ("mandatory" standards) or endeavour to meet ("guideline" standards). The two main standards used to assess the quality of bathing water are *E. coli* and faecal streptococci as they are the main bacteria commonly found in the guts of humans and other warm-blooded animals, and as such, are good indicators of sewage related pollution. KEPA have established thresholds for faecal streptococci and *E. coli* which are

compliant with the current European Bathing Water Directive (cBWD, Anon, 2006) (Table 2) and have been developed to protect bathing water sites by setting thresholds for the main microbiological organisms to avoid impact on human health. KEPA, in addition have maximum limits set against the number of total and faecal coliforms (EPA, 2001).

Fig. 2 presents a summary of the exceedances of total and faecal coliforms and faecal streptococci, noting that faecal streptococci is the most indicative of sewage pollution. Counts of exceedances are shown in four different time periods including (i) across all time periods, (ii) prior to Mishref (up to August 2009), (iii) during Mishref (August 2009–June 2012) and (iv) post Mishref (July 2012–May 2013). Pump failures during the Mishref crisis resulted in large volumes raw sewage being discharged across outlets close to S00, S06 and S07. Exceedances (measured as a percentage) are shown for poor (failures) water quality condition for total and faecal coliforms and faecal streptococci. In addition, Fig. 3 shows a shorter time series of exceedances associated with *E. coli*. The *E. coli* data is presented over only one time period (monthly counts from January 2010 to May 2013) but against three thresholds indicating "failures" (counts/100 mL >500), "mandatory" water quality (counts/100 mL between 250 and 500) and "excellent" water quality (counts/100 mL <250). Heat maps showing the degree of the exceedances for faecal coliforms and faecal streptococci (highest number of exceedances over each separate time period in relation to the area around each S-site) are provided in Fig. SD 1.

Reviewing this historic data set highlights the widespread microbial water quality failures across a number of sites around the coastline of Kuwait. Sites S00 and S07 stand out as potentially being the poorest quality from a microbial water quality viewpoint. For indicators such as faecal streptococci the highest counts were recorded at S00 and S07 during the Mishref sewage crisis (2009–2012) and S09 between January and March, 2011. It is interesting to note that for all sites, with the exception of S00 and S07 during and after the Mishref crisis, exceedances for faecal coliforms for pre (mean −48.4% ± 2.7 SE); during (mean −49.6% ± 3.6 SE); and post (mean −55.5% ± 4.2 SE) Mishref only slightly increased during and after the sewage spill. The mean exceedance for faecal streptococci across all sites (omitting S00 and S07) decreased from pre-Mishref (mean −46.8% ± 1.8 SE), during (mean −35.6% ± 5.9 SE) to post Mishref (mean −31.7% ± 4.1 SE), indicating a more permanent issue of impacted water quality from sewage outfalls across the whole monitoring area. Other sites that were directly impacted by releases of sewage related to the Mishref crisis (S04, S05 and S06) did not reflect the higher percentage exceedances associated with S07 and S00 during the period of the crisis. This is thought to be associated with the direct management action, which included dosing the raw sewage with hypochlorite during the period of the crisis. Overall, exceedances of thresholds directly associated with sewage contamination can be seen as a problem that impacts the majority of the coastline close to residential population centres. Anthropogenic pressures, such as those associated with the Mishref crisis are providing additional point sources of contamination into an already contaminated environment.

### 3.2. Spatial distribution of faecal sterols

The spatial distributions of sterols in marine sediments from Kuwait are displayed in Table 3. Detectable concentrations of coprostanol were recorded in all sediment samples analysed, with values ranging from 29 to 2420 ng $g^{-1}$ dw. A number of authors have previously attempted to set thresholds for coprostanol in sediments and it has been proposed that levels between 10 and 100 ng $g^{-1}$ dw are indicative of uncontaminated environments, values greater than 100 ng $g^{-1}$ dw indicate sewage contamination, while those in excess of 500 ng $g^{-1}$ dw signify gross sewage pollution (Grimalt et al., 1990; González-Oreja and Saiz-Salinas, 1998; Tolosa et al., 2014). Of the 29 sites sampled 3 exceeded the 500 ng $g^{-1}$ dw coprostanol threshold, indicating significant sewage pollution at these locations (Fig. 4). Sites C1, in Doha Bay (1880 ng $g^{-1}$ dw), C5 (601 ng $g^{-1}$ dw) and C6 (2420 ng $g^{-1}$ dw) in Sulaibikhat Bay are all close to known sewage inputs. In particular, C5 and C6 are located close to an area known to contain a number of illegal sewage discharges, such as the Al-Ghazali storm drain. A further 6 sites breached the 100 ng $g^{-1}$

**Table 1**
Counts of microbiological parameters averaged over each site for the duration of the monitoring period. Faecal streptococci and faecal coliforms have been collected monthly since 1988, with *E. coli* collected monthly since 2006. The high counts at S09 are the result of three single sampling occasions (Jan, Feb, March 2011) at Al-Fintas (S09) where counts for faecal coliforms, faecal streptococci and *E. coli* were between $10^4$ and $10^7$ over the three months. Counts are calculated as colonies per 100 mL for each of four to six replicate plates and reported as mean and maximum values over the total number of plates.

| Site | Average (faecal streptococci – mean) | Average (faecal streptococci – max) | Average (faecal coliform – mean) | Average (faecal coliform –max) | Average (*E. coli* – mean) | Average (*E. coli* – max) |
|---|---|---|---|---|---|---|
| S00 | 291 | 714 | 2329 | 5014 | 14,566 | 24,185 |
| S01 | 227 | 599 | 668 | 2320 | 406 | 1999 |
| S02 | 487 | 1417 | 1986 | 5646 | 96 | 1453 |
| S03 | 83 | 231 | 217 | 685 | 68 | 1014 |
| S04 | 394 | 1182 | 990 | 3229 | 3573 | 9355 |
| S05 | 89 | 227 | 144 | 392 | 73 | 1516 |
| S06 | 127 | 326 | 191 | 576 | 108 | 1797 |
| S07 | 588 | 1491 | 128,801 | 324,056 | 974,962 | 1,901,214 |
| S08 | 144 | 431 | 184 | 614 | 120 | 1724 |
| S09 | 42,688 | 163,822 | 78,112 | 253,559 | 579,115 | 1,454,988 |
| S10 | 144 | 404 | 3102 | 11,755 | 146 | 595,925 |
| S11 | 174 | 509 | 562 | 1723 | 460 | 2769 |

**Table 2**
Thresholds for microbiological measurements set from KEPA and current thresholds within the European Bathing Water Directive (BWD). Thresholds are presented here for a guide to the degree of sewage contamination and are not intended to be fully compliant with the overall process of the revised EU rBWD.

| Parameter | Unit | Max limit KEPA | Bathing Water Directive (EU) | |
|---|---|---|---|---|
| | | | Good quality (mandatory) | Excellent quality (guide) |
| Total coliform | CFU/100 mL | 1000 | – | – |
| Faecal coliform | CFU/100 mL | 200 | – | – |
| *Escherichia coli* | CFU/100 mL | | 500 | 250 |
| Faecal streptococci | CFU/100 mL | 200 | 200 | 100 |

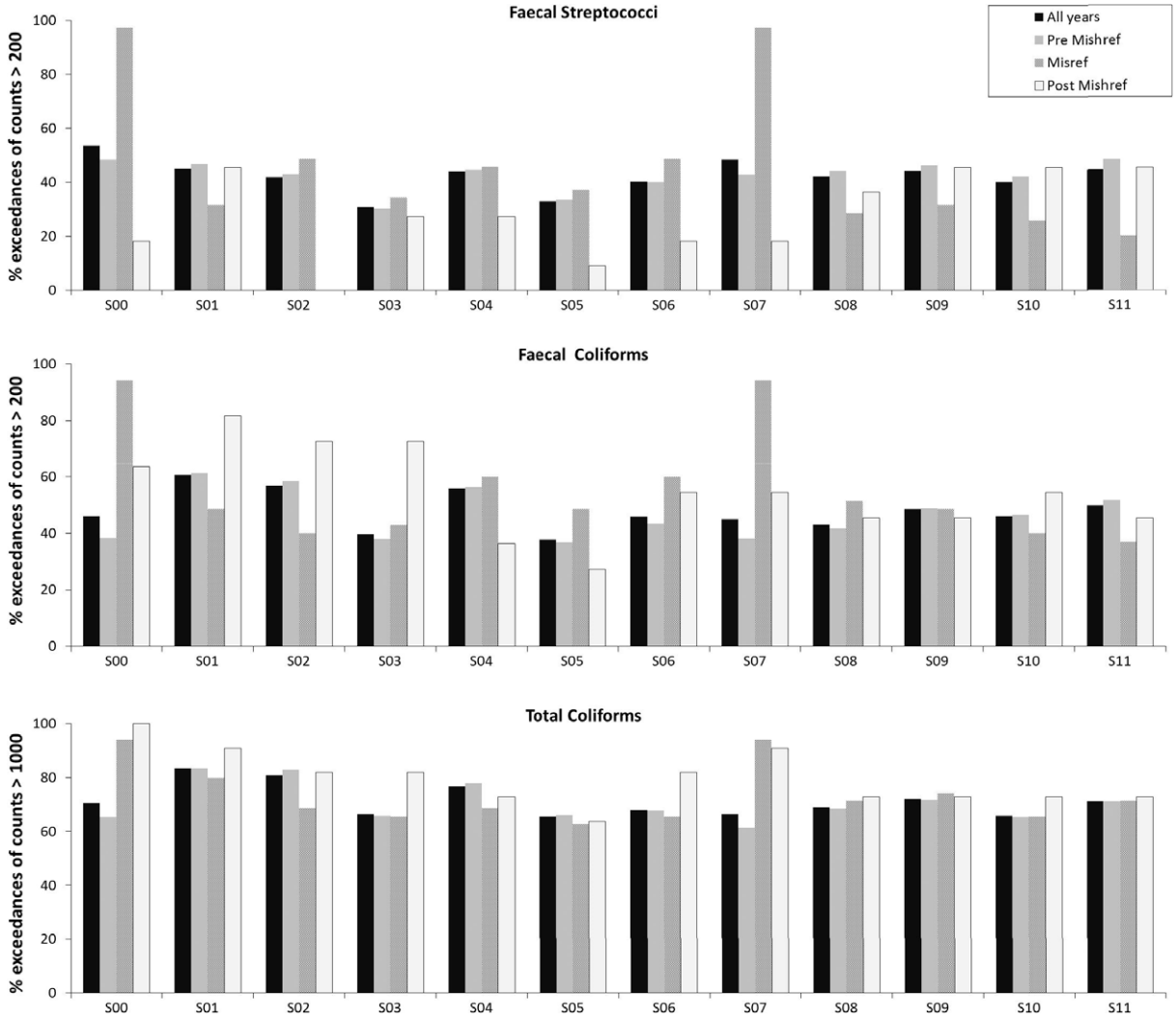


**Fig. 2.** Exceedances (expressed as a percentage) of faecal streptococci, faecal coliforms and total coliforms as assessed against current KEPA guidelines (EPA, 2001). Thresholds for faecal streptococci are also set against the mandatory guidelines for the European Union Bathing Water Directive (76/160/EEU). The percentage exceedances are calculated from the number of data points, collected monthly between 1988 and 2013, that have exceeded either a threshold for poor water quality for faecal streptococci (counts/100 ml >200), faecal coliforms (counts/100 ml >200) and total coliforms (counts/100 ml >1000). Exceedances are presented for four time periods including all years (1988–2013), pre Mishref (1988–2008), Mishref (2009–2012) and post Mishref (2013–2014).

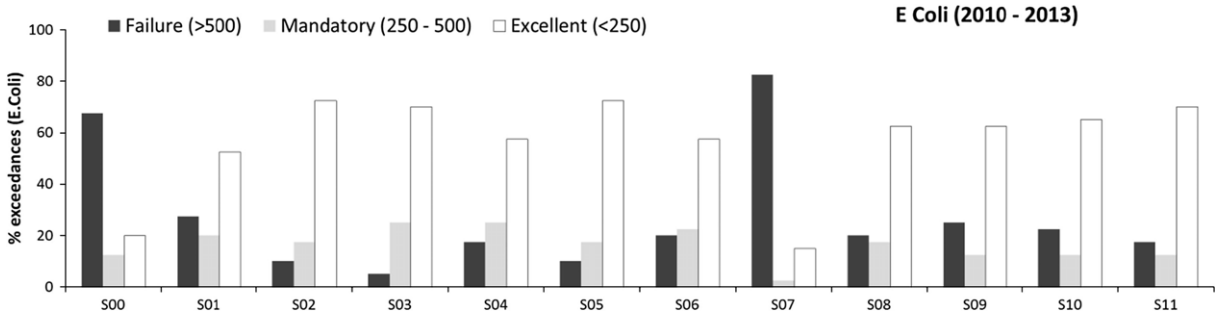


**Fig. 3.** Exceedances (expressed as a percentage) of *E. coli* as assessed against current KEPA guidelines (EPA, 2001) Thresholds for *E. coli* are also set against the mandatory and excellent quality guide as set in the European Union Bathing Water Directive (76/160/EEU). The percentage exceedances are calculated from the number of data points, collected monthly between 2010 and 2013, that have exceeded either a threshold for poor water quality (counts/100 ml >500), moderate water quality (counts/100 ml between 250 and 500) and good water quality (counts/100 ml <250).

dw threshold (105–329 ng $g^{-1}$ dw), including C3 and C4 also situated in Sulaibikhat Bay. The sites breaching the 100 ng $g^{-1}$ threshold in Kuwait Bay (C9, C10, C11), were up to 10 km offshore and are far from any obvious point sources of pollution, while C24 was close inshore adjacent to known outfalls (close to S10 and S11).

Levels of coprostanol in the present study are generally lower than the 50–45,000 ng $g^{-1}$ dw (Al-Omran, 1998), 108–45,228 ng $g^{-1}$ dw (Saeed et al., 2012) and <LOD – 39,428 ng $g^{-1}$ dw (Saeed et al., 2015) previously published for Kuwaiti sediments. However, it should be noted that the extremely high concentrations of coprostanol reported

**Table 3**
Concentration of sterols analysed in Kuwait coastline (ng $g^{-1}$ all dry weight); cop: coprostanol (5β-cholestan-3β-ol); e-cop: epicoprostanol (5β-cholestan-3α-ol); cope: Coprostanone (5β-cholestan-3β-one); chol-e: cholesterol (Cholest-5en-3β-ol); chol-a: cholestanol (5α-cholestan-3β-ol); cop/chol-e: coprostanol/cholesterol; e-cop/cop: Epicoprostanol/Coprostanol; 5β/(5β + 5α): (Coprostanol)/(Coprostanol + Cholestanol).

| Station | <2 mm: Organic carbon (% m/m) | EUNIS sediment classification | Cop ng $g^{-1}$ | E-cop ng $g^{-1}$ | Cope ng $g^{-1}$ | Chol-e ng $g^{-1}$ | Chol-a ng $g^{-1}$ | Cop/Chol-e | E-cop/Cop | (5β/(5β + 5α) |
|---|---|---|---|---|---|---|---|---|---|---|
| C1 | 1.47 | Mud and sandy mud | 1880 | 511 | 663 | 2670 | 3530 | 0.70 | 0.27 | 0.35 |
| C2 | 0.96 | Mixed sediments | 91.4 | 50.4 | 88.6 | 845 | 623 | 0.11 | 0.55 | 0.13 |
| C3 | 0.69 | Mixed sediments | 209 | 81.5 | 179 | 1510 | 468 | 0.14 | 0.39 | 0.31 |
| C4 | 0.66 | Mud and sandy mud | 239 | 68.3 | 127 | 2850 | 404 | 0.08 | 0.29 | 0.37 |
| C5 | 0.71 | Mud and sandy mud | 601 | 102 | 230 | 1080 | 994 | 0.56 | 0.17 | 0.38 |
| C6 | 0.62 | Sand and muddy sand | 2420 | 183 | 446 | 1530 | 785 | 1.58 | 0.08 | 0.76 |
| C7 | 0.58 | Mud and sandy mud | 37.5 | 18.7 | 68.4 | 449 | 211 | 0.08 | 0.50 | 0.15 |
| C8 | 0.73 | Mud and sandy mud | 55.9 | 23.9 | 17.8 | 602 | 641 | 0.09 | 0.43 | 0.08 |
| C9 | 0.97 | Mud and sandy mud | 124 | 52.3 | 89.4 | 645 | 988 | 0.19 | 0.42 | 0.11 |
| C10 | 0.69 | Mixed sediments | 112 | 42.9 | 101 | 1320 | 531 | 0.08 | 0.38 | 0.17 |
| C11 | 0.68 | Mixed sediments | 105 | 44.8 | 140 | 1350 | 437 | 0.08 | 0.43 | 0.19 |
| C12 | 0.67 | Mud and sandy mud | 62.4 | 27.3 | 428 | 1960 | 418 | 0.03 | 0.44 | 0.13 |
| C13 | 0.57 | Mud and sandy mud | 45.3 | 18.3 | 195 | 1230 | 285 | 0.04 | 0.40 | 0.14 |
| C14 | 0.64 | Mud and sandy mud | 54.4 | 22.7 | 56.9 | 582 | 446 | 0.09 | 0.42 | 0.11 |
| C15 | 0.20 | Sand and muddy sand | 29.0 | 5.37 | 1.93 | 107 | 34.3 | 0.27 | 0.19 | 0.46 |
| C16 | 0.22 | Sand and muddy sand | 70.3 | 12.4 | 26.8 | 204 | 36.1 | 0.34 | 0.18 | 0.66 |
| C17 | 0.61 | Mud and sandy mud | 41.9 | 17.1 | 19.6 | 440 | 357 | 0.10 | 0.41 | 0.11 |
| C18 | 0.66 | Mixed sediments | 96.8 | 26.6 | 54.8 | 3250 | 160 | 0.03 | 0.27 | 0.38 |
| C19 | 0.63 | Mixed sediments | 61.2 | 23.8 | 165 | 993 | 396 | 0.06 | 0.39 | 0.13 |
| C20 | 0.63 | Mixed sediments | 59.4 | 14.7 | 34.8 | 609 | 210 | 0.10 | 0.25 | 0.22 |
| C21 | 0.55 | Mud and sandy mud | 38.3 | 14.2 | 18.5 | 349 | 252 | 0.11 | 0.37 | 0.13 |
| C22 | 0.78 | Mud and sandy mud | 50.5 | 22.0 | 18.9 | 416 | 506 | 0.12 | 0.44 | 0.09 |
| C23 | 0.80 | Mud and sandy mud | 79.0 | 30.0 | 51.9 | 889 | 512 | 0.09 | 0.38 | 0.13 |
| C24 | 1.47 | Mixed sediments | 329 | 82.0 | 264 | 1540 | 298 | 0.21 | 0.25 | 0.52 |
| C25 | 0.20 | Coarse sediment | 31.9 | 9.18 | 17.2 | 1370 | 119 | 0.02 | 0.29 | 0.21 |
| C26 | 1.25 | Mud and sandy mud | 97.1 | 38.6 | 79.9 | 963 | 514 | 0.10 | 0.40 | 0.16 |
| C27 | 1.73 | Coarse sediment | 33.2 | 9.00 | 7.97 | 1410 | 102 | 0.02 | 0.27 | 0.25 |
| C28 | 0.92 | Mud and sandy mud | 45.0 | 20.7 | 37.4 | 747 | 410 | 0.06 | 0.46 | 0.10 |
| C29 | 2.85 | Coarse sediment | 29.2 | 6.81 | 7.03 | 1560 | 69.4 | 0.02 | 0.23 | 0.30 |

by these authors were restriced to shoreline monitoring close to known sewage outfalls (50–200 m from point source) and intertidal sediments, whereas the present study sampled coastal sediments ranging from 100 m to >30 km from shore. The earlier work of Al-Omran (1998) reported high levels of coprostanol from inter-tidal sediments all around the coast of Kuwait (>500 ng $g^{-1}$ dw). Significantly, the highest concentrations (4080–45,060 ng $g^{-1}$ dw) were detected in the region of Sulaibikhat Bay, next to the illegal Al-Ghazali storm drain (close to sites C3–C6 in the present study). This was also supported by the recent finding of Saeed et al., 2015, who also recorded concentrations of coprostanol upto 23,803 ng $g^{-1}$ dw in Sulaibikhat Bay. This highlights the long-term, chronic nature of this outfall, which has been contributing significant amounts of raw sewage into the south-western corner of Kuwait Bay for several decades. The levels of contamination detected in Sulaibikhat Bay during the study of Al-Omran (1998) actually matched or exceeded the levels of coprostanol reported in sediments collected from beaches during the 2009–2011 Mishref sewage crisis (Saeed et al., 2012). It is also worthy to note that Saeed et al. (2012), reported the highest values of coprostanol (41,228 ng $g^{-1}$ dw) at Al-Fintas (S09 in this current study), which is also where a review of the EPA microbial water quality data recorded extremely high episodic pulses of microbial contamination. In comparison to studies conducted elsewhere the levels of coprostanol measured were similar to surface sediments from Venice Lagoon, Italy (40–4410 ng $g^{-1}$; Fattore et al., 1997); Sochi, Black Sea Russia (54–5400 ng $g^{-1}$; Readman et al., 2005), the Danube coastline, Black Sea Ukraine (170–2600 ng $g^{-1}$; Readman et al., 2005), Babitonga Bay, Brazil (30–6080 ng $g^{-1}$; Martins et al., 2014) and Cienfuegos Bay, Cuba (10–5400 ng $g^{-1}$; Tolosa et al., 2014).

A number of previous studies have developed and applied diagnostic indexes of faecal contamination, such as the ratio of coprostanol/cholesterol, where values >0.2 are considered sewage contaminated while those >1.0 are considered highly contaminated (Readman et al., 2005; Saeed et al., 2012; Tolosa et al., 2014). When applying these criteria to the present dataset we can conclude that C6 was highly contaminated, C1, C5, C9, C15–16 and C24 were moderately contaminated and C2–4, C7–14, C17–23 and C25–29 were relatively uncontaminated (Fig. 5). By comparison the earlier work of Saeed et al. (2012) documented gross sewage pollution at all sites studied around Kuwait with coprostanol/cholesterol ratios at all sites, except Failaka Island (0.07–0.12), ranging between 0.31 and 5.41. Interestingly, Saeed et al. (2012) documented gross sewage contamination using the coprostanol/cholesterol ratio at sites directly impacted Mishref sewage crisis (Al Bedda 0.84–5.41) and Al-Messala (1.27–4.11). These sites are close to C15 and C16 in the present study, suggesting that sewage contamination is still present, several years after the Mishref sewage discharge had stopped. Such assumptions are supported by the continued exceedance in microbial water quality parameters at these locations.

The concentration of epicoprostanol has also been used as an indicator of the level of sewage treatment of the faecal material (Mudge and Seguel, 1999; Readman et al., 2005; Tolosa et al., 2014). Epicoprostanol is usually only present in trace amounts in human sewage, being formed in sewage treatment works (McCalley et al., 1981). Concentrations in the present study ranged from 5.37 to 511 ng $g^{-1}$ dw (Table 3). It has been proposed that sediments containing untreated sewage display an epicoprostanol/coprostanol ratio <0.2, while values >0.8 are related to treated sewage (primary or secondary) (Mudge and Seguel, 1999). The lowest epicoprostanol/coprostanol ratio were detected at C6 (0.08), C5 (0.17), C16 (0.18) and C15 (0.19), indicating that raw sewage predominated at these locations. All other samples had a ratio <0.8 indicating that the majority of sediments received untreated or partially treated sewage, which is supported by the number of S-sites continuing to fail faecal coliform, faecal streptococci and *E. coli* water quality assessment criteria. The plot of coprostanol/cholesterol versus epicoprostanol/coprostanol demonstrates that sites closest to the Al-Gahzali storm drain (C5 and C6) clearly show high levels of

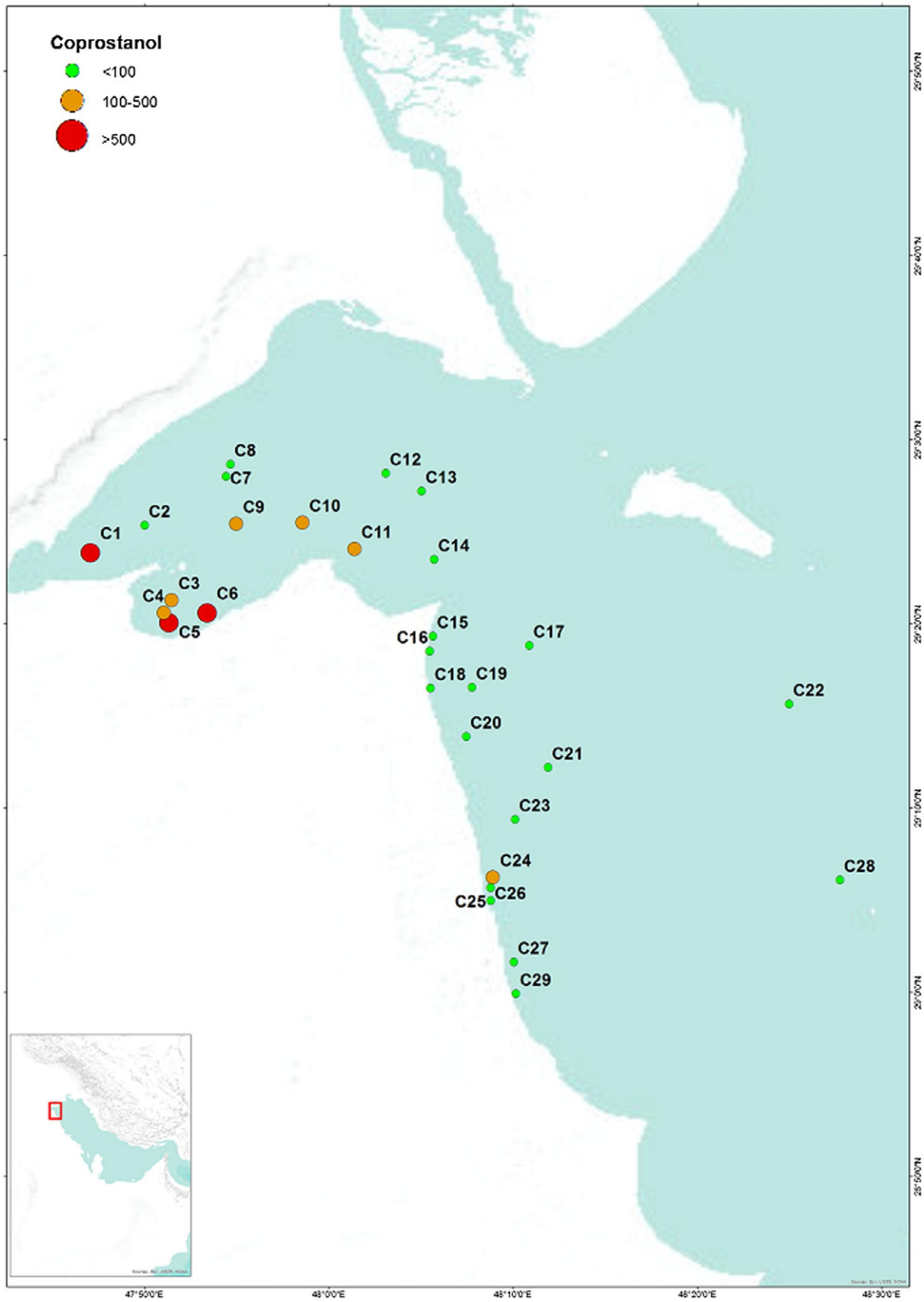


**Fig. 4.** Concentrations of coprostanol in marine sediments from Kuwait. Assessed against thresholds <100 ng $g^{-1}$: uncontaminated; >100 to <500 ng $g^{-1}$: sewage contaminated; >500 ng $g^{-1}$: gross sewage contamination (all dry weight).

contamination dominated by raw sewage (Fig. 6). The detection of low epi-coprostanol/coprostanol ratios at sites close to the Mishref outfalls (C15 and C16), supports the analysis of coprostanol/cholesterol ratios, indicating that untreated sewage may still be entering the marine environment at these locations.

A number of authors have applied the 5β/(5β + 5α) colestan-3β-ol index (coprostanol/(coprostanol + cholestanol)) ratio, where it has been proposed that values <0.3 represent un-contaminated areas, whereas >0.7 indicates sewage contamination (Grimalt et al., 1990; Readman et al., 2005; Reeves and Patton, 2005). However, a number

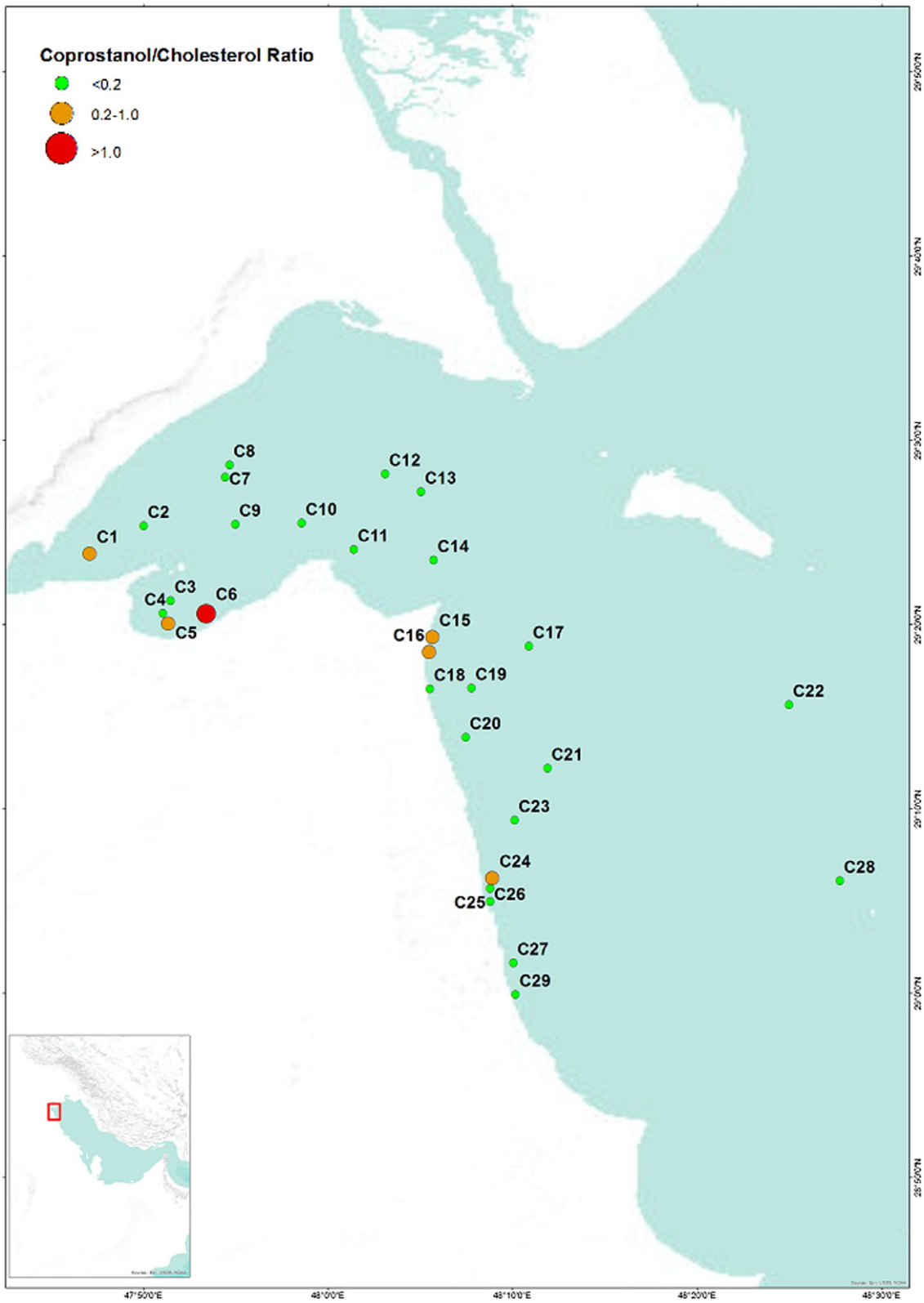


**Fig. 5.** Spatial distribution of coprostanol/cholesterol index in marine sediments. Values >0.2 are considered sewage contaminated while those >1.0 are considered highly contaminated.

of studies have suggested that a 5β/(β + 5α) cholestan-3β-ol index ratio of >0.7 for sewage contaminated sediments may be too high when applied to tropical environments, due to the faster bioconversion of cholesterol to stanols in these climatic zones (Isobe et al., 2002; Tolosa et al., 2014). It is also known that the 5β/(5β + 5α) cholestan-3β-ol ratio can be influenced by direct inputs of cholestanol from phytoplankton and zooplankton, leading to intermediate 5β/(5β + 5α) cholestan-3β-ol ratios (<0.7), being masked by the high presence of phytosterols (Carreira et al., 2004; Tolosa et al., 2014). This also appears to be a factor when reviewing the 5β/(5β + 5α) cholestan-3β-ol index

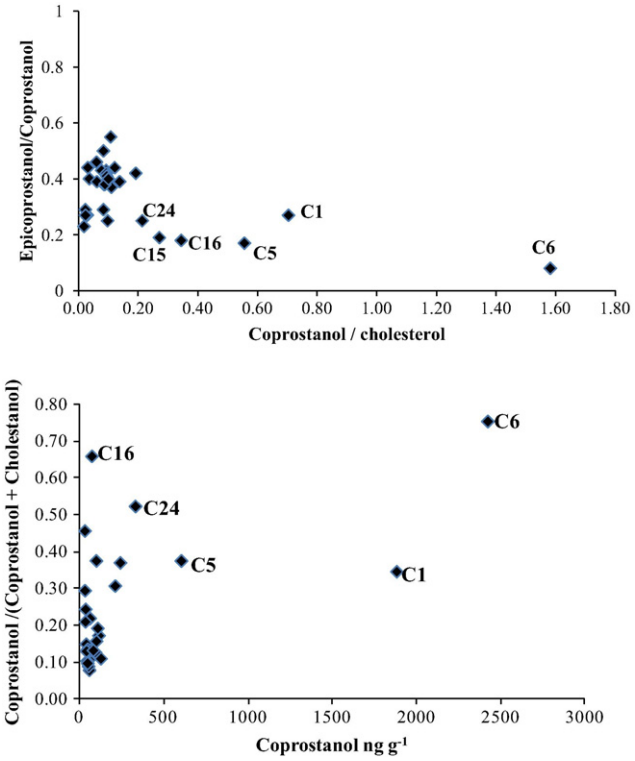


**Fig. 6.** A scatter plots of coprostanol/cholesterol ratio with the corresponding epicoprostanol/coprostanol ratio and 5β/(5β + 5α) colestan-3β-ol and corresponding coprostanol concentrations (dry weight).

ratio for the current study where only one sample (C6) has a value >0.7, thus indicating sewage contamination (Table 3; Fig. 6)

## 4. Conclusions

The review of water microbiological data demonstrates that sewage contamination has been a chronic problem for many years in Kuwait. While the highest number of exceedances were observed around S00 and S07 during the Mishref crisis, it is clear to see that the problem is wide spread and covers all of the locations monitored (S00–S11). This is attributed to the failure of the sewage treatment network to keep pace with demands for capacity driven by rapid population growth that has almost tripled since 1975 (Al-Zaidan et al., 2013). This assumption is supported by the other faecal sterol contamination data available for Kuwait, which suggests wastewater discharge regimes did not significantly change between 1998 and 2012 (Al-Omran, 1998; Saeed et al., 2012; Saeed et al., 2015). Major improvements in the sewage treatment network are underway and the Ministry of Public Works expects capacity to have increased by 60% (capable of treating 1.35 million $m^3$ effluent per day) by 2016. This should ultimately lead to improved seawater quality at the current S-site monitoring locations. However, the data presented for faecal sterol contamination of sediments indicates that there are several areas still receiving large quantities of sewage contamination, which should be of concern. In particular, sites located around Sulaibikhat Bay are particularly impacted, with data indicating untreated sewage is being discharged in significant quantities. It is thought that the illegal connections and discharges via the Al-Ghazali storm drain may account for a considerable amount of this pollution. Therefore, the planned improvements in the wider sewage treatment system may not result in an improvement in these locations where discharges by-pass the official treatment network. Work detailed in this study is now being used by KEPA to update and redesign its water quality monitoring programme to take into account areas identified by sedimentary faecal sterol markers as being impacted by sewage.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.marpolbul.2015.07.043.

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