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Riverbed sediments in the Apies River, South Africa: recommending the use of both *Clostridium perfringens* and *Escherichia coli* as indicators of faecal pollution

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Abstract
Purpose Sediments have been shown to contribute to the microbial quality of the water column during resuspension and serve as reservoirs for potentially pathogenic organisms. Currently, definitive guidelines regarding microbial indicators that need to be assessed in order to monitor faecal pollution in sediments do not exist. In this study, *Escherichia coli* (a well-established indicator) and *Clostridium perfringens* were monitored to determine their suitability as indicators for faecal pollution of sediments.
Materials and methods Enumeration of *E. coli* in water was performed using the Colilert™ 18/Quantitray-2000 system from IDEXX. Identification and enumeration of *C. perfringens* in water was conducted using the boil method followed by the pour plate technique. Real-time polymerase chain reaction (RT-PCR) was used to confirm isolates. *E. coli* and *C. perfringens* were enumerated in sediment by firstly using the water displacement approach to dislodge organisms from sediment and then subsequently followed by the same methods as those used for detection and enumeration of the two potential indicators in water.
Results and discussion The highest concentrations of *E. coli* and *C. perfringens* were obtained along the main stem of the Apies River which was characterised by the presence of wastewater treatment works, animal farmlands and informal settlements with inadequate sanitary facilities. The lowest concentration of both organisms was observed along the tributaries of the river, where there was minimal faecal pollution-related activity. Due to the difference in biological characteristics and survival patterns, concentrations of *E. coli* in sediments fluctuated (higher concentrations in the wet season) during the entire sampling period while concentrations of *C. perfringens* remained stable. There was a positive correlation between temperature and the presence of both organisms in the sediment, indicating the enabling environment of sediment to aid in bacterial survival.
Conclusions E. coli and *C. perfringens* are both suitable indicators of faecal pollution in riverbed sediments. However, both organisms need to be monitored together for accurate assessment of the faecal pollution of sediments. *E. coli* remains a good indicator of recent faecal pollution and provides insight into the short-term impact of faecal pollution, while *C. perfringens* gives an indication of the long-term impact of faecal deposition in riverbed sediments due to the organisms' persistence in the environment.

Keywords *Clostridium perfringens* · *Escherichia coli* · Indicator organisms · Sediment · Water

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1 Introduction

A large and diverse nature of microorganisms can be present at any given time in the aquatic environment. An attempt to enumerate all the pathogens in water and sediments can be a time-consuming and financially demanding task that would appear unrealistic to execute for monitoring purposes (Izbicki et al. 2009). The World Health Organisation (WHO) recommends the analysis of faecal indicator microorganisms for assessing microbial water quality of any water intended for drinking; though in some cases, like in disease outbreaks, analysis may also include assessment of specific pathogen densities. Several studies have often referred to these indicator organisms as 'microbial indicators (Lisle et al. 2004; Shibata et al. 2004; Costán-Longares et al. 2008; Zhang et al. 2013). Considering the ambiguity of the term microbial indicator, a reclassification was undertaken which was more reflective of the function of each indicator group (Table 1).

As recommended by the WHO, certain criteria need to be met for an organism to be considered as an indicator of faecal pollution. Some of these criteria include the universal presence of this organism in high numbers in humans and other warm-blooded animals' faeces, the simplicity of the method used for the detection of the organism and the inability of the organism to grow in natural water (WHO 2008).

There have been great advances in molecular methods allowing for the detection of the presence of pathogens in water and sediments. However, limitations such as the cost of instrumentation and the inability of most of these molecular methods to detect the viability of cells make a number of these methods unsuitable for routine monitoring purposes (Ashbolt et al. 2001; Klein 2002; Noble and Weisberg 2005). Thus, the enumeration of indicator organisms of faecal pollution using low-cost methods that can detect microbial viability remains a valuable microbial risk assessment tool (Yates 2007).

Several indicator organisms have been used in different studies around the world (Table 2). Total coliforms, faecal coliforms, *Escherichia coli*, faecal streptococci and enterococci have been the most commonly tested indicators of faecal pollution in water. However, in their review, Figueras and Borrego (2010) determined the strengths and shortcomings of indicators of faecal pollution and illustrated that none of these indicator organisms fulfilled all the required criteria necessary for assessing water quality. Thus, relying on a single indicator organism for predicting faecal pollution could be inadequate for setting protection measures of public health importance (Tyagi and Chopra 2006).

International bodies like the WHO, US Environmental Protection Agency (USEPA) and the European Union (EU) have recommended the use of *E. coli* as a suitable indicator (Figueras and Borrego 2010), despite the limitations discussed above. In addition to *E. coli*, the USEPA also recommends the use of Enterococci for marine and fresh waters. The organism (*E. coli*) has been historically and widely used, often as a singular indicator, to provide conclusive evidence of faecal pollution in water (Tallon et al. 2005; WHO 2008). The large number of *E. coli* in the human gut and the organism's absence in other environments, as well as the user-friendly nature of the detection methods for the organism, are characteristics that favour *E. coli* use over other faecal indicator bacteria (Edberg et al. 2000). However, despite these positive attributes of *E. coli* as an indicator of faecal pollution, it has been found to be a poor index organism for some bacterial pathogens (Kong et al. 2002; Voytek et al. 2005), viruses (Moresco et al. 2012; Lee et al. 2014) and protozoa (Edge et al. 2013; Xiao et al. 2013).

Other microorganisms that have been suggested as indicators of faecal pollution in the aquatic environment include *Enterococcus* spp. (Miescier and Cabelli 1982), coliphages (Payment and Franco 1993; Gantzer et al. 1998) and *Clostridium perfringens* (Payment and Franco 1993; WHO 2008). *C. perfringens* forms spores that are very resistant to conventional wastewater treatment procedures and to environmental stress. The vegetative cells of *C. perfringens* do not reproduce in the environment and the spores are largely of faecal origin (Davies and Long 1995; Venczel and Arrowood 1997). The spore-forming ability of *C. perfringens* is a positive attribute that will be required of an indicator which will be used in determining microbial sediment quality. *C. perfringens* will persist longer in the environment than conventional indicators, thus making it suitable as an indicator of both recent and previous faecal

Table 1 Classification of indicator organisms into different functional groups

Group	Definition
Process indicators	This group demonstrates how efficient a treatment process is. Examples include total heterotrophic bacteria or total coliforms in chlorine disinfection.
Faecal indicators	These organisms indicate faecal contamination. Examples are the thermotolerant coliforms or *E. coli*. They only infer the possible presence of other pathogens.
Index and model organisms	This group or species are indicative of pathogen presence and behaviour organisms respectively. For example, *E. coli* is an inex organism for *Salmonella* and F-RNA coliphages are model organisms of human enteric viruses.

Source: Ashbolt et al. (2001)

Table 2 The use of indicator organisms to determine microbial quality in fresh/marine water and sediment

Study	Location	Sample type	Indicator
Ferguson et al. 1996	Sydney, Australia	Water and sediments	Faecal coliforms, faecal streptococci, F-RNA, *Clostridium perfringens*, bacteriophage.
Luther and Fujioka 2004	Hawaii	Water, riverbed sediments, soil from agricultural farms	Male-specific RNA coliphages
Lee et al. 2006	Santa Monica Bay, USA	Beach sediments	*E. coli* and enterococci
Sinigalliano et al. 2007	New Orleans, USA	Water, shoreline sediments, deposited floodwater sediments	Enterococci, *E. coli*, FRNA coliphage, *C. perfringens*, *Bacteroidales*, and *Bifidobacterium adolescentis*
Coulliette and Noble 2008	Eastern North Carolina, USA	River water	*E. coli* and *Enterococcus* sp.
Abhirosh et al. 2010	Vembanadu Lake, India	Water and sediments	Faecal coliform bacteria and *E. coli*
Ibekwe et al. 2011	California, USA	Water and river bank sediments	*E. coli*
Turkmen et al. 2012	Dardanelles, Turkey	Coastal water	Total coliforms, Faecal coliforms and Enterococci

contamination (Davies and Long 1995; Graziano et al. 2005). Davies and Long (1995) reported that *C. perfringens* could survive for up to 85 days in marine and freshwater sediments while other indicator organisms like the faecal coliforms decreased to 10 % of their initial concentration within the same time period. Due to the characteristics of *C. perfringens* discussed earlier, the organism has previously been suggested as a possible indicator of faecal pollution in river sediments (Marcheggiani et al. 2008). However, very few studies have determined the robustness of *C. perfringens* as a suitable indicator (Marcheggiani et al. 2008). *C. perfringens* has also been identified as useful indicator for *Cryptosporidium* oocysts and *Giardia* cysts and thus could also serve as a model organism for the presence or absence of human pathogenic protozoans and viruses (Payment and Franco 1993; Tyagi and Chopra 2006).

Sediments within the aquatic ecosystem represent a more complex environment than the overlaying water column. Large numbers of pathogenic microorganisms including bacteria, protozoan cysts and viruses which are the main cause of enteric diseases to humans (Taylor et al. 2001; Leclerc et al. 2002; Ashbolt 2004; Eisenberg et al. 2006; Kumar et al. 2006) are able to survive in the aquatic environment due to attachment to suspended sediment particles (Karim et al. 2004; Rehmann and Soupir 2009; Cho et al. 2010; Taylor et al. 2011; Kunkel et al. 2013). Several studies have reported that natural events like storms and floods, and/or human activities such as recreation, may lead to the resuspension of the attached bacteria in the sediment leading to an increase in bacterial concentration in the water column (An et al. 2002; Sinigalliano et al. 2007; Turkmen et al. 2012; Campos et al. 2013; Walters et al. 2014).

In South Africa and several other developing countries, surface waters remain an alternative water source for drinking and other household uses due to a lack of access to treated pipe water (Gemmell and Schmidt 2013). In some cases, water collected is used without prior treatment (DWAF 1996). Several studies have indicated that most of these surface water bodies, especially rivers (water column and sediment), are heavily polluted with faecal matter (Khan and Khan 2012; Britz et al. 2013; Sibanda et al. 2013; Gemmell and Schmidt 2013; Teklehaimanot et al. 2014). The resuspension of bacteria from sediments into the water column could possibly lead to an increased health risk for users of these contaminated rivers. Furthermore, guidelines do not currently exist to determine the microbial quality of sediments; hence, the health risk faced by users cannot be accurately determined. The development of guidelines to determine microbial sediment quality involves identifying suitable indicators of faecal pollution. Thus, the present study was carried out to investigate the suitability of *E. coli* and *C. perfringens* as indicators of faecal pollution in the riverbed sediments of the Apies River, Gauteng, South Africa.

2 Materials and methods

2.1 Location of study site

The Apies River located in Pretoria, Gauteng Province, South Africa, falls within the Crocodile (West) Marico Water Management Area (Fig. 1). The river falls within the Apies River basin with a total flow >500 m^3 $year^{-1}$, about 12 % of which comes from wastewater treatment works around the river (Venter 2007). The river begins in the Fountains Valley, Pretoria and follows through Gauteng, North-West and Limpopo provinces to eventually join the Limpopo River. There are a number of land use activities that occur along the river (Table 3), and these activities have an impact on the quality and quantity of the river. The flow in the Apies River is controlled by different processes such as the outflows from the four wastewater treatment works (WWTWs), the extraction of water for different usages and the total rainfall and runoff reaching the river. The

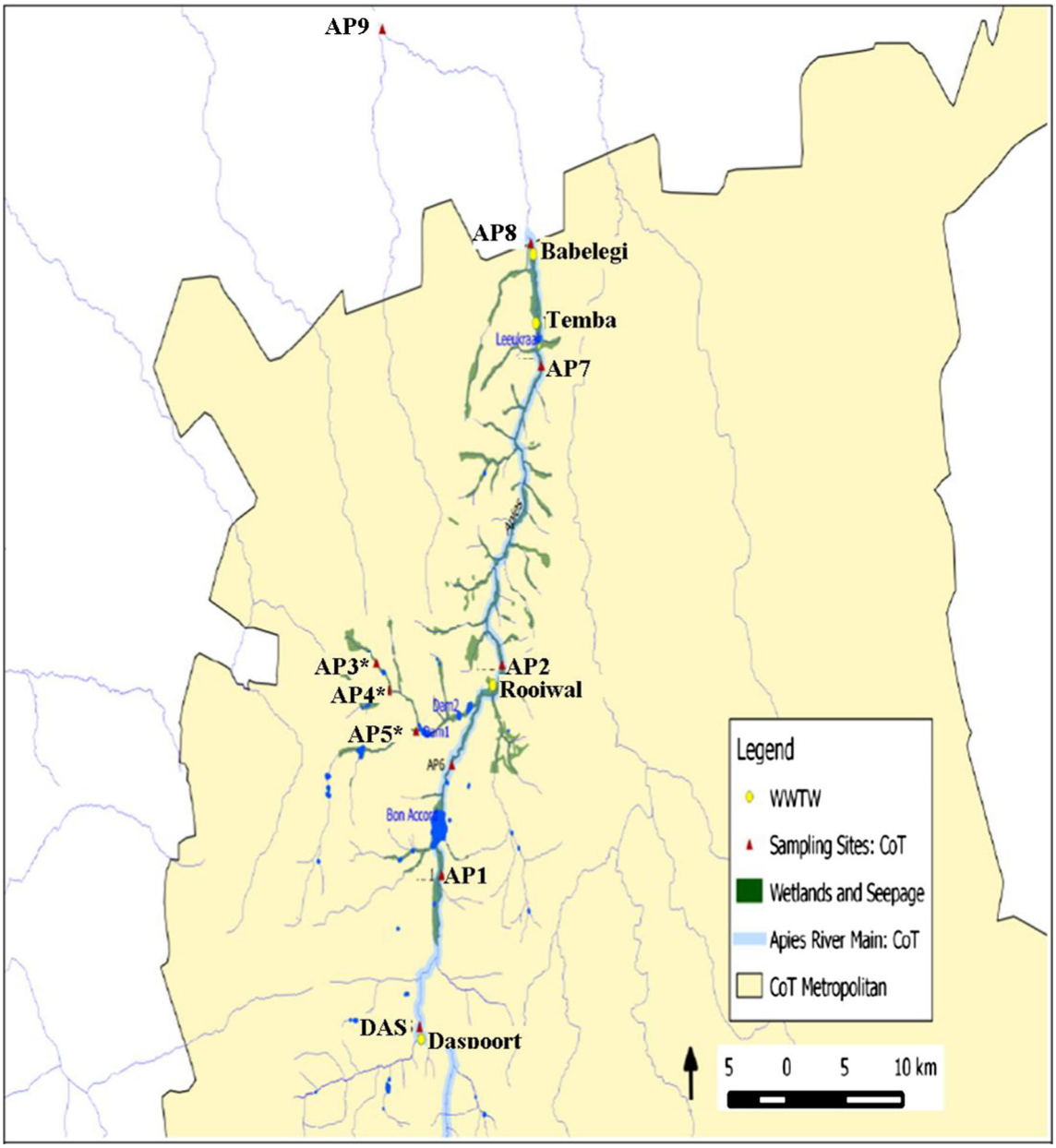


Fig. 1 Study area showing the location of the sampling sites within the City of Tshwane (*CoT*) Metropolitan area and the North West Province (**Tributaries*), South Africa

contribution of WWTWs to the total flow in the river and the extraction of water for household and agricultural usages depend on how dry (low rainfall) or wet (high rainfall) the season is.

2.2 Sample collection and treatment

A total of 10 sampling sites (Table 3) were selected over an approximate 90 km stretch of the Apies River: sites DAS and AP1 were located upstream from the Bon Accord dam inlet; sites AP6, AP2 and AP7 were between the Bon Accord dam outlet and the Babelegi dam inlet; and sites AP8 and AP9 were downstream from the Babelegi dam outlet. Three tributaries to the Apies River (AP3, AP4 and AP5) were also included in the sampling. Dry season samples (May–August 2013) and wet season samples (January–February 2014) were collected from the 10 sampling sites along the Apies River. During the entire sampling period, a total of 1116 (558 water and 558 sediment) samples were collected and analysed for *E. coli* and *C. perfringens*.

Water samples were aseptically collected using sterile 1-l containers following standard procedures. Collection was done approximately 15 to 20 cm below the water surface to

Table 3 The location and major land use surrounding sampling points along the Apies River

Sampling site	Geographical coordinates		Major land use activities occurring
	Latitude	Longitude	
DAS 1	−25.726997	28.171633	Urban area[a], 1 WWTW (Daspoort) and 1 informal settlement[b]
AP1	−25.653239	28.191207	Urban area and 1 informal settlement
AP2	−25.550772	28.243838	Rural area[c] with animal farming, and 1 WWTW (Rooiwal)
AP3	−25.549216	28.135863	Rural area
AP4	−25.562758	28.146627	
AP5	−25.582758	28.169613	
AP6	−25.599455	28.20032	
AP7	−25.404992	28.278359	Rural area, 1 Informal settlement and animal farming
AP8	−25.345428	28.269605	urban area, animal farming, 2 WWTWs (Temba and Babelegi)
AP9	−25.239979	28.143294	rural area with animal farming

[a] Structured, controlled and organised into land parcels and has services like water, electricity and waste management and formally planned and maintained roads

[b] Also known as 'squatter camps', occur on land which has not been surveyed or proclaimed as residential with informal structures and usually lacking basic sanitation and water services

[c] Areas that have the lowest level of services usually with located at long distances from the nearest service points and also characterised by the presence of large scale farming areas

Definition of each area as per Statistics South Africa 2004

avoid collection of surface debris. All bottles were hermetically closed and transported to the laboratory on ice.

Using a sterile polypropylene scooper, grab samples of approximately 250 g of sediment were collected from the top 5 cm of the riverbed at the point directly below the site where the water sample was collected. The scooper was slightly tilted to allow the collected water to drain out and grab sediments samples were then transferred into sterile 100 ml polypropylene containers and firmly closed. The sediments were transported to the laboratory in cooler boxes with ice.

All samples were collected in triplicate and were analysed within 6 h upon arrival at the laboratory. Samples that could not be analysed on the same day were kept at 4 °C and were analysed within a 24 h period of collection.

2.3 Enumeration of indicator organisms in water samples

2.3.1 Enumeration of E. coli in water samples

E. coli were enumerated using the Colilert^{TM} 18 Quanti-Tray/2000 system from IDEXX (IDEXX Laboratories (Pty) Ltd., Johannesburg, South Africa). Prior to analysis, the bottle was inverted several times, thus ensuring proper mixing of the river water. Analysis was performed as per the manufacturer's instructions. Briefly, a 100-ml portion of the river water from the sampling point was mixed with the Colilert18 reagent in a sterile container, transferred and sealed in a Quanti-Tray using the Quanti-Tray sealer and incubated at 37 °C for 18–24 h. Plates were visually examined for acid production and under UV for fluorescence. The *E. coli* concentration was then inferred from the statistical table provided with the reagent based on the number of large and small positive wells.

2.3.2 Enumeration of C. perfringens in water samples

C. perfringens were enumerated using the pour plate technique. Aliquots of 100 ml of each sample were transferred into 120 ml glass bottles and heated at 80 °C for 5 min in a water bath to kill the vegetative forms of organisms present (Araujo et al. 2004). One milliliter of the heated sample was transferred to a Petri dish, and 15 to 20 ml TSC agar (prepared following manufacturer's instructions and cooled to about 45 °C) supplemented with D-Cycloserine (Biomerieux®, France) was poured into the dish, allowed to solidify and incubated anaerobically at 37 °C. Plates were examined after 21±3 h for the presence of black or grey colonies, characteristic of *C. perfringens*. The concentration of *C. perfringens* was expressed in colony-forming units (CFU) per volume of sample.

2.4 Enumeration of *E. coli* and *C. perfringens* from sediment samples

Sediment samples were prepared for analysis using a water displacement approach as described by Abia et al. (2015). Briefly, sediments were gradually transferred into a 1-l Durham bottle containing 400 ml of 1×PBS to obtain a total of 500 ml, giving a 1:5 (vol/vol) dilution. Bottles were then vigorously shaken manually for approximately 2 min allowed to stand briefly, and appropriate volumes of the supernatant

extracted and analysed for both indicator organisms as described for the water sample.

2.5 Real-time PCR for confirmation of *C. perfringens* isolates

2.5.1 DNA extraction

Confirmation of *C. perfringens* isolates was done using real-time polymerase chain reaction (PCR). DNA was extracted as previously described (Das et al. 2012). Briefly, a single colony was peeled off a TSC plate transferred into an Eppendof tube containing 100 μl of Milli-Q water, vortexed for 10 s and boiled at 100 °C for 10 min. The tube was then centrifuged at 10,000 rpm for 5 min to remove cell debris and the top clear supernatant used as source of template DNA.

2.5.2 PCR conditions

The reaction was run targeting the *cpa* gene, and the primer sequence was as follows: Forward-GCTAATGTTACTGCCGTTGA and Reverse-CCTCTGATACATCGTGTAAG (Das et al. 2012). Primers were obtained from Inqaba Biotec, South Africa. The real-time PCR reaction was carried out on a Corbett Life Science Rotor-Gene 6000 Cycler (Qiagen, Hilden, Germany). Amplification reaction was performed in a total volume of 20 μl consisting of 10 μl of a 2× SensiFAST™ HRM Mix (final concentration, 1×) (Bioline GmbH, Germany), 1 μl (final concentration, 1 μm) of each primer (Forward and Reverse), 5 μl of DNA template and 3 μl of nuclease-free water. The PCR conditions were optimised as follows: an initial incubation step at 95 °C for 10 min, followed by a 45-cycle amplification program consisting of 95 °C for 10 s, 55 °C for 15 s, 72 °C for 20 s and a final extension step at 72 °C for 5 min. The amplification step was followed by a melting step which was carried out by slow heating from 72 to 95 °C after a 90-s hold for pre-melt conditions on the first step. Fluorescence acquisition was done at 1 °C intervals with a hold for 5 s at each increment. The reaction included three positive controls (*C. perfringens* ATCC® 13124; American Type Culture Collection (ATCC), Manassas, VA, USA) and three negative controls (reaction mixture without DNA).

2.6 Physical and chemical water parameters

Physical parameters were measured in situ during sampling. Water temperature (°C), dissolved oxygen (DO, mg l^{-1}), electrical conductivity (EC, μs cm^{-1}) and pH were measured using an HQ40d Portable Multi-Parameter Meter (Hach, USA). Turbidity (Nephelometric Turbidity Units or NTUs) was measured using a T100 portable turbidly meter (EUTECH Instruments, Germany).

2.7 Statistical analysis

Statistical Package for the Social Sciences (SPSS) Version 20 (IBM Corporation, Armonk, New York, USA) and Microsoft Excel 2010 were used for data analysis. The Mann–Whitney rank sum test was used to compare seasonal occurrence of indicator organisms in water and sediment. The means of the different environmental parameters for both seasons were compared using a one-way analysis of variance (ANOVA). The correlation between the environmental parameters studied and the abundance of each indicator organism were determined using the nonparametric Spearman's rank correlation. All statistical tests were considered significant at a 95 % confidence limit.

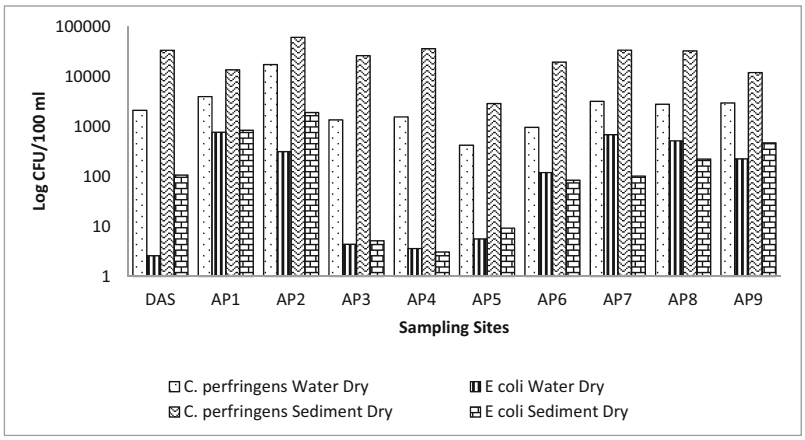
3 Results

3.1 Concentrations of *E. coli* and *C. perfringens* in water and sediments

Both organisms were detected at varying concentrations at all sampling sites in both the water column and sediments during the entire sampling period. All the *C. perfringens* isolates were confirmed positive for the *cpa* gene using real-time PCR (Appendix A – Electronic Supplementary Material). The mean *E. coli* concentration at AP2 was 1.47E+03 MPN/100 ml in the dry season and 3.37E+04 MPN/100 ml in the wet season, while for *C. perfringens* it was 1.72E+04 CFU/100 ml and 6.02E+04 CFU/100 ml for the dry and wet seasons, respectively (Appendix B – Electronic Supplementary Material). Relatively high concentrations of *E. coli* (2.30E+02 CFU/100 ml) and *C. perfringens* (3.09E+04 CFU/100 ml) (Appendix B – Electronic Supplementary Material) in the sediments were also recorded at AP8 which is situated downstream from the Babelegi and Temba WWTWs.

3.2 Comparison of *E. coli* and *C. perfringens* concentrations and detection rates in sediments

For all sampling rounds, the mean sediment concentrations of *C. perfringens* were higher than those of *E. coli*. The same trend was recorded in the overlaying water (Fig. 2). While *C. perfringens* showed a 100 % detection rate throughout the study, there were days where *E. coli* was not detected in water and sediment



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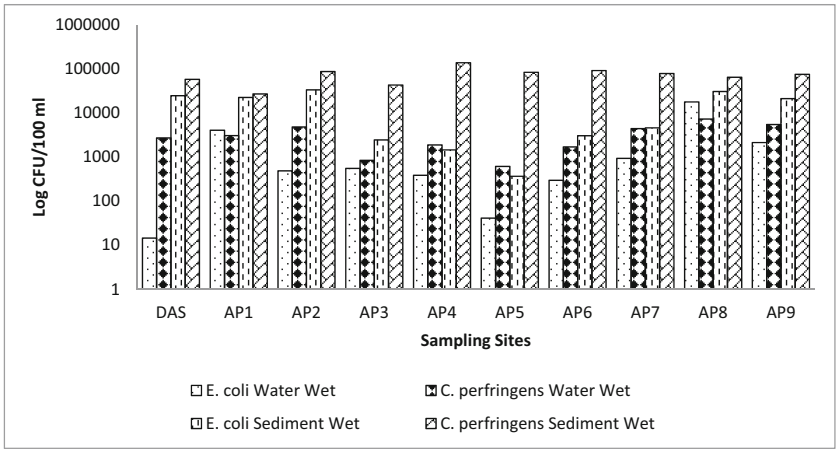

b

Fig. 2 Comparative abundance of mean *E. coli* and *C. perfringens* counts in water and sediment for the dry season (**a**) and wet season (**b**) sampling periods

for various sampling sites (Appendix B – Electronic Supplementary Material). The highest number of days in which *E. coli* was not detected occurred at the DAS sampling point. A statistically significant difference ($p<0.05$) was observed between the mean water count and the mean sediment count for both indicator organisms. Of the two indicator organisms, *C. perfringens* counts were higher in the sediments than in the water for the entire study period. On the other hand, higher *E. coli* concentrations in the sediments were only observed at some sites (AP1, AP2, AP5 and AP9) during the dry season (Table 4).

Table 4 The number of days during the sampling period when *E. coli and C. perfringens* were not detected in water and/or sediments at each sampling site

		Sample type	DAS	AP1	AP2	AP3	AP4	AP5	AP6	AP7	AP8	AP9
Dry season	*E. coli*	Water	12	0	1	4	3	0	0	0	0	0
		Sediment	0	0	0	2	2	0	0	0	0	0
	C.perfringens	Water	0	0	0	0	0	0	0	0	0	0
		Sediment	0	0	0	0	0	0	0	0	0	0
Wet season	*E. coli*	Water	4	0	1	0	0	0	0	0	0	0
		Sediment	0	0	0	0	0	0	0	0	0	0
	C.perfringens	Water	0	0	0	0	0	0	0	0	0	0
		Sediment	0	0	0	0	0	0	0	0	0	0

3.3 Seasonal variation in the concentration of *E. coli* and *C. perfringens* in sediments

The concentrations of *E. coli* and *C. perfringens* were also influenced by the change in season with the wet season recording higher concentrations than the dry season for both indicator organisms in the sediments. Although the difference between the dry season and the wet season mean concentrations for both indicator organisms was statistically significant ($p<0.05$), *E. coli* showed a higher increase in concentration levels during the wet season as compared to *C. perfringens* (Table 5).

3.4 Correlation between indicator organisms and physico-chemical parameters

A statistically significant difference ($p<0.05$) was observed between the dry and wet season measurements. A positive correlation was observed between temperature and concentrations of both indicator organisms in water and sediments ($p<0.05$). A negative correlation was observed between the concentration of both indicator organisms in sediments and the water turbidity (Table 6). No correlation was observed between the concentration of *C. perfringens* in sediment and pH. A summary of the physic-chemical parameters is given in Appendix C (Electronic Supplementary Material)

4 Discussion

4.1 Concentrations of *E. coli* and *C. perfringens* in water and sediments

In order to assess the suitability of *E. coli* and *C. perfringens* as possible indicators of faecal pollution in riverbed sediment, it was essential to first determine if both organisms were present in the water column and the sediments, and secondly, to determine if the observed concentrations of the organisms obtained at the different sampling sites along the Apies River was reflective of the faecal pollution that might be occurring at each site. Throughout the entire study, the highest mean concentration of *E. coli* and *C. perfringens* in the sediments was recorded at site AP2 which is situated immediately downstream from Rooiwal WWTW.

The Apies River has four WWTWs situated along it (Fig. 1). Like several water bodies in South Africa, the river is highly likely to experience a deterioration in microbial quality due the presence of these WWTWs (Kinge et al. 2010; Britz et al. 2013; Gemmell and Schmidt 2013; Sibanda et al. 2013; Teklehaimanot et al. 2014). In 2008, South Africa initiated and finally adopted an incentive-based regulation to identify, reward, ensure and promote excellence regarding wastewater management. According to the 2012 report (The Green Drop Report) of this initiative, most of the country's WWTWs were functioning above their design and/or operational capacity with 72.9 % of the 831 WWTWs assessed falling within the medium-to-critical risk categories (DWA 2012). Non-functional or sub-optimally functioning WWTWs result in the discharge of incompletely treated waste or, at times, untreated waste during plant failure, directly into surrounding water bodies. Both *E. coli* and *C. perfringens* serve as suitable indicators of the contribution that WWTWs might be adding to the faecal pollution load in sediment as both organisms were present in high concentrations in faecally polluted water. However, each organism provides a different timeline with regard to the pollution occurring. *E. coli* is generally very susceptible to most wastewater treatment processes and does not survive outside the gut for long periods of time. A well-functioning treatment plant will have the ability to completely reduce *E. coli* loads in raw waste resulting in effluent that can be safely discharged into the river with negligible impact on the river (van Der Drift et al. 1977; Olańczuk-Neyman 2001; George et al. 2002). The high *E. coli* concentrations recorded at the sites located downstream of WWTWs therefore suggest the discharge of untreated or poorly treated waste into the river, thus indicating recent faecal pollution. Unlike *E. coli*, *C. perfringens* is more resistant to most WWTWs processes and is usually discharged together with the final effluent (Bisson and Cabelli 1980; Fujioka et al. 1985; Skanavis and Yanko 2001; Teklehaimanot et al. 2014). Considering that the organism does not grow in natural water, the presence of *C. perfringens* in high concentrations at these sites is therefore indicative of faecal pollution that has occurred over a period of time and not necessarily in one pollution event.

Table 5 Increase in indicator organisms' concentration due to change in season (dry to wet) expressed as the order of magnitude increases in cell counts

	Sample type	DAS	AP1	AP2	AP3	AP4	AP5	AP6	AP7	AP8	AP9
E. coli	Water	10^0	10^2	10^1	10^2	10^2	10^1	10^0	10^1	10^1	10^1
	Sediments	10^1	10^1	10^{-1}	10^2	10^2	10^2	10^2	10^3	10^3	10^0
C.perfringens	Water	10^0	10^0	10^{-1}	10^0	10^0	10^1	10^0	10^0	10^1	10^0
	Sediments	10^0	10^0	10^1	10^0	10^1	10^2	10^1	10^1	10^1	10^1

Table 6 Correlation between faecal indicator organisms and physicochemical parameters for the entire sampling period in sediments

Physicochemical parameter	*E. coli*		*C. perfringens*	
	r_s	*p*-values	r_s	*p*-values
Temperature	.528[a]	0	.468[a]	0
Turbidity	−.258[a]	0	−.166[a]	0.015
Electrical conductivity	.588[a]	0	.196[a]	0.004
Dissolved oxygen	.401[a]	0	.322[a]	0
pH	.378[a]	0	0.133	0.053

[a] Correlation is significant at the 0.05 level (2-tailed)

High concentrations of *E. coli* and *C. perfringens* were also observed at sites (AP1, AP7, AP8 and AP9) along the river that were not directly downstream of WWTWs. At these sites, with the presence of informal settlements and the use of the land for agricultural purposes, the microbial quality of the river is expected to be compromised. Informal settlements often lack sanitary facilities and hence the river is sometimes used as a dumping site for human waste. Also, runoffs from agricultural farms have been identified as important sources of faecal indicator organism (Walters et al. 2010; Liang et al. 2013). The possible contribution of agricultural practice to the microbial load in sediment was observed by the presence of *E. coli* and *C. perfringens* in high concentrations especially at site AP9.

The lowest mean *E. coli* and *C. perfringens* concentrations were recorded at sites AP3, AP4 and AP5. These sites are all located on the tributaries of the Apies River and had very little land use activity and no WWTWs. Very little to no faecal pollution occurred at these sites. As expected, very low concentrations of both indicator organisms at the tributary sites were recorded, demonstrating their suitability as indicators of sediment faecal pollution even when pollution levels are very low. The suitability of both organisms as indicator organisms was further highlighted when during the wet (rainy) season samples obtained from these same tributary sites now had elevated levels of organisms. During the wet (rainy) season, these tributary sites receive large amounts of run-off from a neighbouring informal settlement characterised by high population density and inadequate sanitation facilities.

4.2 Comparison of *E. coli* and *C. perfringens* concentrations and detection rates in sediments

The location of site DAS downstream from the outlet of the Daspoort WWTW suggests that the absence of *E. coli* could be due to the inability of the cells to grow in the culture media because of injury from the treatment process. Thus, drawing conclusions based on the *E. coli* concentration alone could be misleading. *C. perfringens* forms stress-resistant spores that can survive for long periods of time in the environment. Also, *C. perfringens* has been used to check for the efficiency of water treatment processes as the organism's spore-forming ability makes it resistant to conventional treatment methods (Bisson and Cabelli 1980; Hill et al. 1996; Skanavis and Yanko 2001; Wéry et al. 2008). As a result, it would be necessary to include *C. perfringens* as an indicator of faecal pollution in the sediments alongside *E. coli* especially at sites influenced by WWTWs. Furthermore, *E. coli* has been found to survive in sediments for shorter periods than many human enteric pathogens like *Cryptosporidium* spp. *and Giardia* spp. (Harwood et al. 2005). As such, the absence of *E. coli* is not conclusive of the absence of faecal pollution. However, this limitation could be overcome by including *C. perfringens* whose spore-forming ability allows it to survive longer than *E. coli* and also makes it a better indicator of long-term accumulation of faecal pollution (Desmarais 2002).

The observed high concentration of *E. coli* at sites AP1, AP2, AP5 and AP9 during the dry season could be mainly due to the various land uses around these areas. In AP9, for example, during the dry season, farmers water their cows directly in the river. During this process, the animals release waste directly into the water and because of the slow flowing conditions of the river during this period, the faeces rapidly settle into the sediments, thus polluting it. At site AP2, the WWTW could have contributed to the sediment *E. coli* during the dry season. Considering that the river receives little or no runoff during the dry season, the WWTW upstream contributes a greater percentage of the total river flow at this point and the presence of *E. coli* at these sites could indicate recent faecal pollution.

However, the overall higher concentrations of *C. perfringens* in the sediments compared to that of *E. coli* observed in this study (Fig. 3) could be due to the higher ability of *C. perfringens* to survive in the cold winter periods of the dry season. *C. perfringens* has been reported to survive in sediments long after pollution has occurred (Figueras and Borrego 2010; Devane et al. 2014). In such case, relying on *C. perfringens* alone in the absence of *E. coli* may lead to a false alarm of recent faecal pollution. Although clay has been demonstrated to aid survival of microorganisms (Santamarı and Gary 2003; Brennan et al. 2014), the higher counts of both indicator organisms in the sediments of the Apies River were not influenced by the sediment type, as other factors such as nutrient availability and available pollution source could affect the presence of the organisms.

4.3 Seasonal variation in the concentration of *E. coli* and *C. perfringens* in sediments

Rainfall increases the concentration of indicator organisms within water bodies through processes like surface runoff from surrounding areas (Guber et al. 2006; Walters et al.

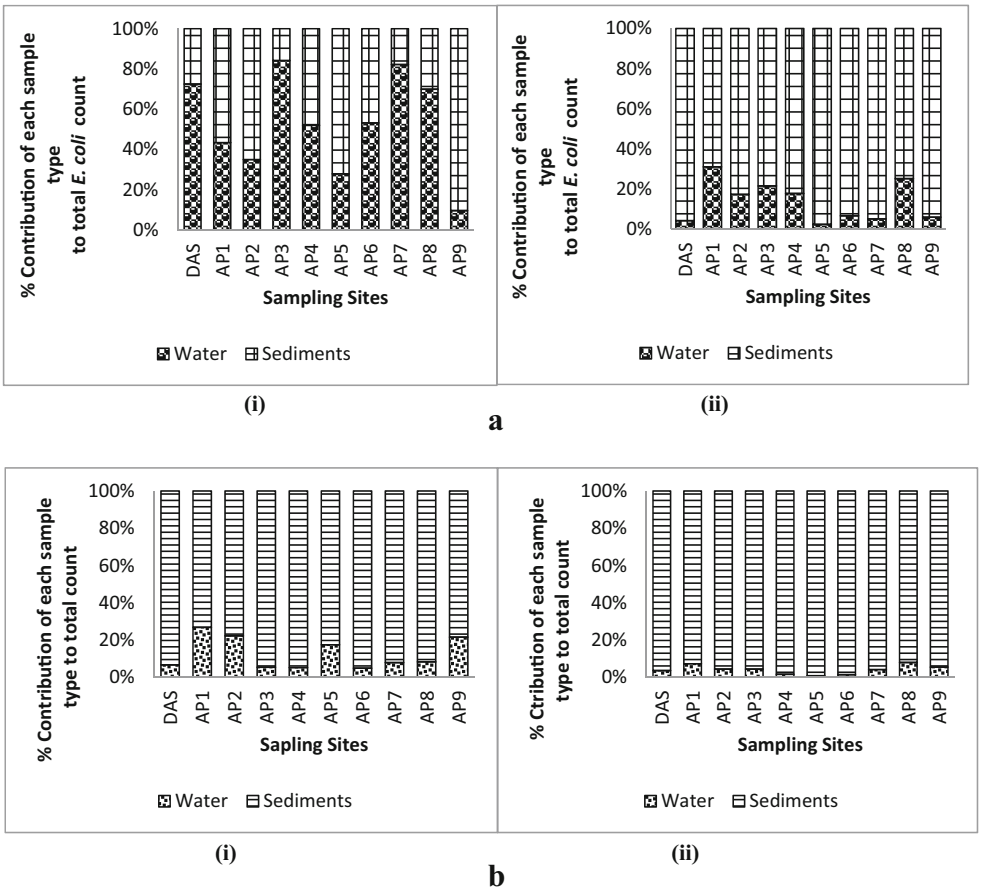

Fig. 3 Percentage contribution of water and sediments to the total *E. coli* (**a**) and mean *C. perfringens* (**b**) count at each site during the entire dry season (*i*) and wet season (*ii*)

2010; Liang et al. 2013; Martinez et al. 2014). The higher increase in the concentration of *E. coli* compared to that of *C. perfringens* could be due to the fact that *E. coli* is found in the faeces of many warm-blooded animals including ruminants and birds (Schierack et al. 2007; Figueras and Borrego 2010). Even though the high increase in the concentration of *E. coli* due to seasonal changes observed in this study could indicate faecal pollution, it does not necessarily mean pollution of human origin. Also, a good faecal indicator organism is not supposed to grow in unpolluted environments. However, *E. coli* has been reported to survive and grow with very low die-off rates even in unpolluted environments, meaning that its presence in the environment could also be of non-faecal origin (Martinez 2009; Figueras and Borrego 2010). These shortcomings could therefore limit the use of *E. coli* as the sole indicator of faecal pollution in the sediments of the Apies River during the wet season. On the other hand, *C. perfringens* has been found in the faeces of carnivorous animals and humans, but not herbivores (Vierheilig et al. 2013). However, due to the low number of carnivores, it is unlikely that these predators could be a source of marked faecal pollution in the aquatic environment due to runoff during the wet season (Mueller-spitz et al. 2010; Vierheilig et al. 2013). Therefore, the lower influence of the wet season on the concentration of *C. perfringens* compared to that of *E. coli* could imply that human sources might be the potential explanation for the increased *C. perfringens* counts observed during the wet season, especially at the tributaries (AP4 and AP5) and areas with agriculture (AP9) and informal settings (AP7 and AP8). As such, including *C. perfringens* together with *E. coli* in sediment monitoring during the wet season could give a better indication of the presence or absence of faecal pollution.

4.4 Correlation between indicator organisms and physico-chemical parameters

Several factors have been reported to affect the abundance of microorganisms in sediments. Temperature has been found to be one of the most important physical factors influencing growth and survival of microorganisms in the environment (Ross et al. 2003; Blaustein et al. 2013; Pachepsky et al. 2014). The negative correlation observed between the abundance of the indicator organisms in the sediments and turbidity of the water column could suggest that sediment disturbance would lead to the resuspension of organisms from the sediments to the water column.

5 Conclusions

The detection of both *E. coli* and *C. perfringens* at all the sampling sites (water and sediment) indicates possible faecal pollution in the Apies River. Sediments might enable *E. coli* to thrive for a longer period than in the water column, thus making it a suitable indicator for monitoring riverbed sediments. However, *E. coli* is still only an indicator of recent faecal pollution in sediment, as the organism is susceptible to environmental conditions and may not survive long enough to indicate a faecal pollution event has occurred. *C. perfringens* persists longer in the environment as compared to *E. coli* and was found to be present when faecal pollution had occurred even in the absence of *E. coli*. However, because *C. perfringens* can survive in sediments long after pollution has occurred, its presence alone cannot be used to predict a recent faecal pollution. Both organisms need to be monitored together in order to obtain an accurate assessment of the impact of faecal pollution in the sediments.

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