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ORIGINAL PAPER

Prevalence of pathogenic microorganisms and their correlation with the abundance of indicator organisms in riverbed sediments

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Abstract The present study investigated the prevalence of pathogenic organisms (*Salmonella* spp, *Vibrio cholerae,* and *Shigella* spp) and their correlation to the abundance of faecal indicator organisms in water and riverbed sediments in the Apies River, South Africa. In all, 558 water and sediment samples were collected from 10 sites in the river (May 2013–February 2014) and analysed through culture and molecular (real-time PCR) techniques. Concentrations of faecal indicator organisms in sediments reached 1.39×10^5 (±standard deviation) CFU/100 mL. All three pathogens were detected in water and sediments. Pathogens were mostly detected in sediments at sites influenced either by wastewater treatment works or by informal settlements. During the wet and dry seasons (water column), a strong positive correlation was observed between *E. coli* and all pathogens; *C. perfringens* only correlated with *V. cholerae*. Within sediments, strong positive correlations were only observed between *E. coli* and *Salmonella* spp, *E. coli* and *V. cholerae* (dry season); *E. coli* and *V. cholerae* and *E. coli* and *Shigella* spp (wet season). No correlation was observed between sediments *C. perfringens* counts and all the pathogens. Thus, sediments of the Apies River harbour pathogenic organisms. Correlation between *E. coli* and pathogenic organisms in the sediments suggests that *E. coli* could also be an indicator of pathogens' presence. However, the lack of a correlation between *E. coli* and some pathogens in sediments and between *C. perfringens* and all the pathogens highlights the need to investigate for more indicators of pathogens' presence in this complex matrix.

Keywords Indicator bacteria · Microbial pathogens · Microbial sediment quality · Public health risk · Relationship · River water

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Introduction

The world's water ecosystem continues to suffer severe deterioration due to microbial pollution from urban run-off (Crowther et al. 2002; Tyrrel and Quinton 2003; Reeves et al. 2004; Signor 2005) agricultural farms (Monis and Thompson 2003; Carey et al. 2004) or more localised sources like wastewater treatment works (WWTWs) (Abraham 2011; Teklehaimanot et al. 2014). Irrespective of the source, once in the aquatic environment, the survival of these microbial pollutants depends on a number of factors, amongst which is the attachment to suspended sediment particles (Gao et al. 2011). The attached bacteria, together with the sediment particle, may then become bigger, resulting in heavier complexes that can potentially settle out onto the riverbed. Several studies carried out on the role played by riverbed sediments as reservoirs of indicator bacteria, including human pathogens, have reported higher microbial counts in the sediments than in the water column (Jamieson et al. 2004; Characklis et al. 2005; Fries et al. 2008).

Chase et al. (2012) reported that levels of faecal indicator bacteria (FIB) in sediments could be up to 100-fold higher when compared to the water column concentration.

Bacteria in riverbed sediments can be shielded from the killing effect of UV light coming from the sun (Craig et al. 2004; Stapleton et al. 2007; Koirala et al. 2008) by suspended particles. Sediments have also been found to enhance bacterial survival by providing a hiding place for the bacteria against predation from protozoa (Decamp and Warren 2000; Jamieson et al. 2005a, b). Furthermore, sediments often contain high concentrations of nutrients and other soluble organic matter essential for bacterial growth (Jamieson et al. 2005a, b; Garzio-hadzick et al. 2010). Apart from traditional indicator bacteria like *E. coli*, pathogenic bacteria, such as *Vibrio cholerae*, *Salmonella* spp and *Shigella* spp have also been reported in riverbed sediments (Santhiya et al. 2011; Vignesh et al. 2014).

Natural turbulences and/or human recreational activities could lead to the resuspension of microorganisms found in the bottom sediments (Craig et al. 2004; Budillon et al. 2006; Pandey et al. 2011). Several studies have reported higher levels of FIB in the water column of water bodies that have been associated with resuspension of riverbed sediments following disturbance of the bed sediments (Pianetti et al. 2004; Ibekwe and Papiernik 2010; Gonzalez et al. 2012) suggesting riverbed sediments could serve as an important source of microorganisms within the water column (Kinzelman and McLellan 2009; Korajkic et al. 2011). The resuspension from sediments could therefore represent a potential health hazard for populations using such untreated water for recreational purposes (Gao et al. 2011) as well as for other household activities—especially where treated pipe-borne water is not available.

South Africa's water resources are negatively impacted by low average annual precipitation. Uneven distribution of surface and groundwater as a result of climate and geography makes the country water scarce (Molobela and Sinha 2011). The country entirely depends on surface water resources for most of its urban, industrial and irrigation water requirements (Basson 2011). Aquatic ecosystems and water resources on which most rural communities depend for domestic, recreational and agricultural uses are still being subject to heavy microbial pollution from different sources resulting in severe environmental, health and economic damage (Basson 2011). Several studies carried out in South Africa have focused on the quality of surface water (Kinge and Mbewe 2010; Chigor and Okoh 2012; Seanego and Moyo 2013; Sibanda and Okoh 2013; Teklehaimanot et al. 2014), and there is little information on the microbial quality of riverbed sediments. Like many countries in the world, South Africa's aquatic ecosystem monitoring bodies have not taken microbial sediment quality into consideration in the development and modification of their water quality guidelines (Republic of South Africa, Department of Environmental Affairs 2012). It has been shown recently that the sediments of the Apies River, a widely used river in the Gauteng Province of South Africa, were heavily polluted with *E. coli* (Abia et al. 2015b). The sediments were found to contain as high as 10^5 times higher concentrations of *E. coli* than the water column. However, given the complex nature of the sediment matrix within the riverbed, choosing an appropriate indicator of faecal pollution, and possibly of pathogenic organisms (POs), could be an essential step for the successful monitoring of the microbial quality of bed sediments within the aquatic ecosystem. Based on this, another recent study carried out on the Apies River suggested that *E. coli* alone, though recognised as a good indicator of microbial quality within water bodies, was not sufficient to predict the microbial quality of sediments within these ecosystems (Abia et al. 2015b). The authors of this study concluded by recommending that in order to have a better picture of both recent and past effects of microbial pollution within riverbed sediments, it was necessary to check for the presence of both *E. coli* and other potential indicators of faecal pollution such as *Clostridium perfringens* within the sediments (Abia et al. 2015a). Despite these findings regarding the microbial quality (presence of *E. coli* and *C. perfringens*) of sediments in South African water bodies, there is still no data on the possible presence of POs within the riverbed sediments. Such information could improve the understanding of the possible contribution of sediments to the overall microbial load of water catchments thus providing the necessary preliminary evidence needed to motivate the need for increased allocation of funds for water resource protection initiatives within developing countries.

With the main aim of filling up this paucity of information regarding microbial sediment quality, the present study was carried out with objectives: (1) to investigate the extent to which riverbed sediments and water in the Apies River are contaminated with pathogenic microorganisms (*Salmonella* spp, *V. cholerae* and *Shigella* spp) and (2) to investigate any correlation between the abundance of indicator organisms (*E. coli* and *C. perfringens*) and the presence of the pathogens in the sediments and the overlaying water. The study was conducted from May to August 2013 (dry season) and January to February 2014 (wet season). The river is situated in the Gauteng Province of South Africa.

Materials and methods

Study site

The present study was conducted on the Apies River, which is one of the main watercourses within the Gauteng Province of South Africa (Fig. 1). The study site and characteristics of the river as well as the various land uses

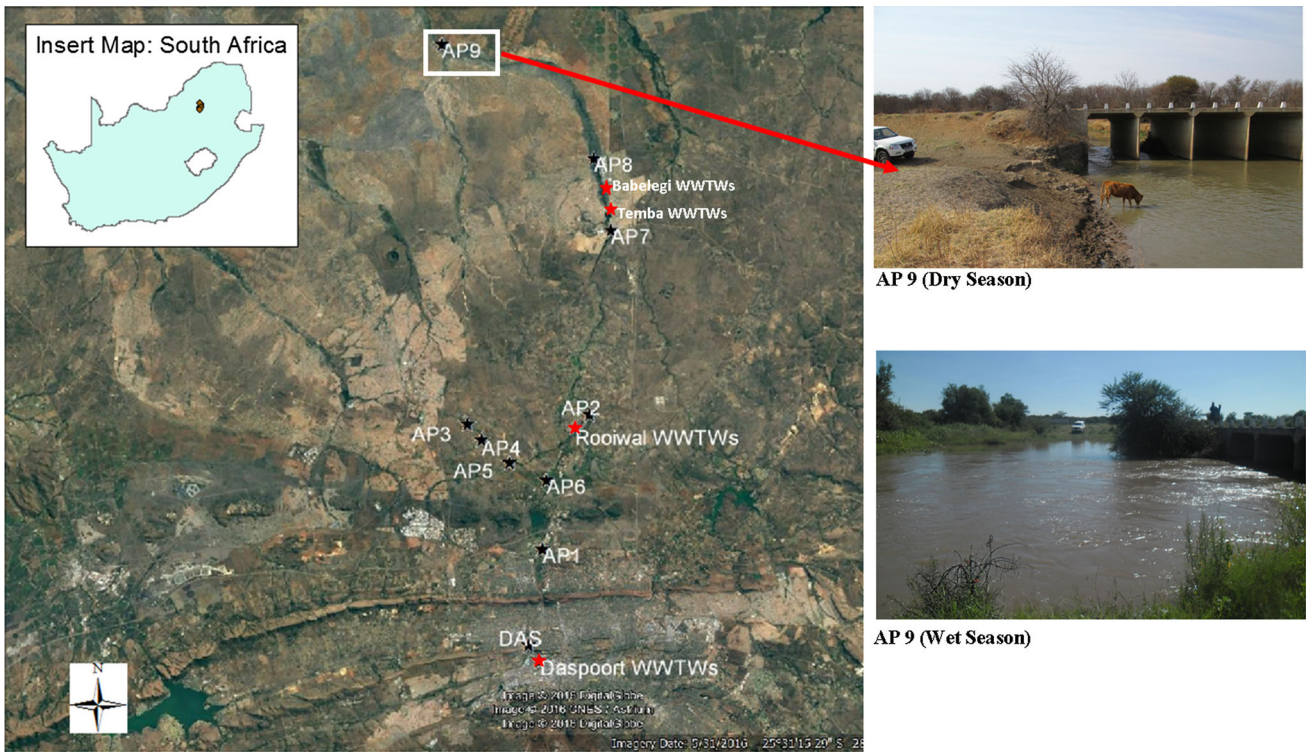

Fig. 1 Map of Apies River showing sampling sites and wastewater treatment works (*Source*: Google Earth) and pictures of site Ap 9 during the dry and the wet season

around the river have been described in previous studies (Abia et al. 2015a, b). During the dry season, most parts of the river are fully accessible by communities that use its water for activities such as laundry and bathing. During the wet season, the river usually overflows its banks, especially after heavy rainfall (attached picture, Fig. 1). During both seasons, the water and sediments of Apies River are sources of spiritual cleansing and empowerment to many traditional healers and other religious groups. Important to note is the presence of four WWTWs—Daspoort, Rooiwal, Babelegi and Temba along the river that discharge their effluents directly into the river system. These WWTWs account for about 80 % of the total river discharge during the dry season (Venter 2007). Also, parts of the river's course pass through informal settlements which completely lack sanitary facilities, thus making the river the only point of waste disposal in these areas.

Sample collection and processing

Sampling was conducted in the dry (May to August 2013) and the wet (January to February 2014) seasons. A total of 10 sampling sites were chosen for the study. In order to identify the possible sources of the pollution observed within the Apies River, study sites were selected based on the various land uses around them. This was done following an initial field visit to the river. Sites DAS, AP1, AP2, AP6, AP7, AP8 and AP9 were all located on the main Apies River. Sites AP3, AP4 and AP5 are tributaries to the Apies River. Water and sediment samples were collected during 14 sampling rounds in each season and analysed for *E. coli*, *C. perfringens*, *V. cholerae*, *Salmonella* spp and *Shigella* spp, using culture and molecular techniques. Site AP9 was only sampled during 13 rounds as the sampling site was not accessible on one of the rounds due to heavy rains that caused flooding of the river beyond it banks. Water samples were collected using clean sterile 1 L plastic containers following standard procedures (APHA/AWWA/WEF 2001). A sterile polypropylene autoclavable scooper was used to scoop the sediments samples from the top 5 cm of the riverbed. All samples were collected approximately 1 m (or further when possible) away from the river bank. This was the approximate distance the users would go during activities such as laundry or bathing. The collected sediments were transferred into sterile 100 mL polypropylene containers with lids. Both water and sediments were collected in triplicates at each sampling point and transported to the laboratory in a cooler box containing ice packs. All samples were processed and analysed within 6 h upon arrival to the laboratory. Prior to analysis, microorganisms were separated from the sediment samples using the water displacement approach as previously described (Abia et al. 2015c). The supernatant from

resuspended sediment samples was then used for the enumeration of the FIOs and for enrichment in appropriate media depending on the pathogen of interest. For the water samples, after inverting the sampling bottle several times to allow for proper mixing of the water, the samples were used directly for culture and enrichment in appropriate media.

Enumeration of indicator organisms

Escherichia coli was enumerated using the Colilert® 18/Quanti-tray® 2000 defined substrate method and confirmed by real-time PCR as previously described (Abia et al. 2015b). *C. perfringens* was enumerated using the pour plate technique on D-cycloserine supplemented TSC agar and confirmed molecularly as described by Abia et al. (2015a).

Identification of pathogens

Sample enrichment and DNA extraction

Equal volume (50 mL) of water (50 mL of supernatant in the case of the sediment samples) was added to equal volume of double-strength broth (Environment Agency 2002). Selenite broth was used for *Salmonella* spp (Chitanand et al. 2008), peptone water for *Shigella* spp (Theron et al. 2001) and alkaline peptone water for *V. cholera*e (Nandi et al. 2000; Goel et al. 2005; Akoachere et al. 2013) and incubated at 35.0 ± 0.5 °C for 24 h. All culture media were purchased from Merck (South Africa). After incubation, 1 mL of the overnight broth culture was transferred into a centrifuge tube and spun at 13,000 g for 3 min. DNA was extracted from the harvested cells using the Instagene™ Matrix (Bio-Rad Laboratories, South Africa) following the manufacturer's instruction. The supernatant from the tubes was then used as a source of template DNA for the real-time PCR reactions.

Primers and real-time PCR conditions

Genes targeted for the identification of the various pathogens were the outer membrane protein (*ompW*) and the cholera toxin (*ctxAB*) genes for *V. cholerae*, the invasive gene A (*invA*) for *Salmonella* spp and the invasive plasmid antigen H (*ipaH*) gene for *Shigella* spp (Table 1).

PCR reactions were carried out on a Corbett Life Science Rotor-Gene 6000 Cycler (Qiagen, Hilden, Germany). Amplification reactions were 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 (1 µl of nuclease-free water in the case of *V. cholerae*). The PCR conditions for *V. cholerae* were optimised as described by le Roux and van Blerk (2011). This included an initial activation at 95 °C for 10 min followed by 50 cycles involving denaturation at 95 °C (10 s), annealing at 64 °C (15 s), extension at 72 °C (25 s) and acquiring after each cycle and a final extension at 72 °C for 5 min. Melting was done by ramping from 72 to 90 °C, with a 0.1 °C rise at each step, a pre-melt hold for 90 s on the first step followed by a hold for 2 s on the next steps. The same conditions were applied for *Salmonella* and *Shigella*. However, the PCR for *Shigella* was semi-nested as described by Theron et al. (2001). Melt curve analysis of the PCR product was carried out using the Rotor-Gene Q Series Software version 6.1 (Qiagen, Hilden, Germany) to detect the presence of the genes of interest for each organism. Samples for the POs were analysed in duplicate.

All PCR reactions included a positive (genomic DNA of a reference strain) and a negative control (made of the PCR reaction mixture without the template DNA). Reference strains used were *Salmonella* ser Typhimurium (ATCC® 14028) (American Type Culture Collection, Manassas, VA, USA), *Shigella flexneri* (ATCC® 12022) and *V. cholerae* O1 (NCTC 5941) (National Collection of Type

Table 1 Primers used for the real-time PCR reactions

Organism	Gene	Primer[a] sequence	References
V. cholera	*ompW*	F-CACCAAGAAGGTGACTTTATTGTG	Nandi et al. (2000)
		R- GAACTTATAACCACCCGCG	
	ctxAB	F-GCCGGGTTGTGGGAATGCTCCAAG	Goel et al. (2005)
		R- GCCATACTAATTGCGGCAATCGCATG	
Salmonella spp	*invA*	F-GTGAAATTATCGCCACGTTCGGGCAA	Malorny et al. (2003)
		R- TCATCGCACCGTCAAAGGAACC	
Shigella spp	*ipaH*	H8-GTTCCTTGACCGCCTTTCCGATAC	Theron et al. (2001)
		H15-GCCGGTCAGCCACCCTC	
		H10-CATTTCCTTCACGGCAGTGGA	

[a] All primers were obtained from Inqaba Biotechnologies Ltd, Pretoria, South Africa

Cultures, London, UK). All positive strains were obtained from the microbiology laboratory of the Natural Resources and the Environment Department of the CSIR (Council for Scientific and Industrial Research), Pretoria, South Africa.

Statistical analysis

Data analysis was performed using Microsoft Excel and SPSS statistical analysis software version 20 (IBM Corporation, Armonk, New York, USA). Concentrations of microorganisms were expressed as Log_{10} Geometric mean. For the calculation of the geometric means, all MPN values of *E. coli* below the detection limit were assumed to be 1. The Spearman's rank correlation was used to determine the correlation between the abundance of each FIO and the presence of the various pathogens. For the pathogens, a sampling point was considered positive for any of the organism of interest when both duplicate samples from that site were positive at any given time. The one-way analysis of variance (ANOVA) was used to check for any statistical differences between data sets. All analyses were undertaken at a level of significance of $\alpha = 0.05$.

Results and discussion

Abundance of indicator organisms and prevalence of pathogenic organisms in water and sediments

The importance of sediments as a reservoir and as a possible source of FIOs and POs within the water column in aquatic ecosystems has been studied for many years and still represents an issue of concern in the present day (Labelle et al. 1980; Burton et al. 1987; Santhiya et al. 2011; Walters et al. 2014). This is particularly a major problem in most developing countries that still rely on untreated water from polluted surface water bodies for their personal, domestic and recreational activities.

In the current study, the mean concentration of *E. coli* in the water ranged between 3.80 and 7.03 $\times$ 10^2 $\pm$ SD (Standard deviation) MPN/100 mL during the dry season and between 1.65 $\times$ 10^1 and 4.05 $\times$ 10^3 $\pm$ SD MPN/100 mL in the wet season as previously described by (Abia et al. 2015a). Sediment concentrations ranged between 3.90 and 1.47 $\times$ 10^3 $\pm$ SD MPN/100 mL and between 3.65 $\times$ 10^2 and 3.37 $\times$ 10^4 $\pm$ SD MPN/100 mL in the dry and the wet season, respectively. For *C. perfringens*, mean water concentrations for the dry season and wet season ranged from 4.18 $\times$ 10^2 to 1.72 $\times$ 10^4 $\pm$ SD CFU/100 mL and from 4.70 $\times$ 10^2 to 7.32 $\times$ 10^3 $\pm$ SD CFU/100 mL, respectively. The sediment concentrations ranged from 2.86 $\times$ 10^3 to 6.02 $\times$ 10^4 $\pm$ SD CFU/100 mL and 2.72 $\times$ 10^4 to 1.39 $\times$ 10^5 $\pm$ SD CFU/100 mL for the dry and wet season, respectively. There was a statistically significant difference ($p < 0.05$) between the mean water and the mean sediment counts for both FIOs and during both seasons. The wet season also recorded an overall statistically significant higher count ($p < 0.05$) than the dry season for each FIO. The pathogens were detected on a presence/absence basis targeting the *ompW* and *ctxAB* of *V. cholerae*, *invA* gene of *Salmonella* spp and the *ipaH* of *Shigella*. The overall prevalence of all the pathogens is shown in Table 2. The most detected pathogen during the entire sampling period (water and sediment) was *V. cholerae* (58.8 %) while *Shigella* spp recorded the lowest prevalence (10.1 %). Site AP1 recorded the highest prevalence of *V. cholerae* (52/56; 92.9 %), while site AP2 recorded the highest prevalence for *Salmonella* spp (24/56; 42.7 %), and site AP8 recorded the highest prevalence for *Shigella* spp (28/56; 49.6 %). Figure 2 shows the contribution of water and sediments to the overall observed prevalence of each pathogen at the individual sampling sites during the dry and the wet season. The pathogens were more detected at sampling sites during the wet season (January–February 2014) than during the dry season (May–August 2013) (Fig. 2). None of the pathogens was detected at site DAS and AP5 during the dry season.

All the sites that recorded the highest abundance of FIOs and prevalence of the POs were located along the main river course. The sites on the main river were either characterised by informal settlements (AP1) or located downstream a WWTW (DAS, AP2) or had a combination of both (AP8). The highest prevalence for *V. cholerae* was at site AP1 (Table 2) which is characterised by an informal settlement. Site AP8 is located downstream from two WWTWs (Babelegi and Temba) and also around agricultural areas; this site also demonstrated a similarly high *V. cholerae* prevalence as was observed at site AP1. These sites that recorded high prevalence of the POs equally recorded high abundance of the FIOs. The negative impact of informal settlements (Paulse et al. 2009; Khan and Khan 2012; Ndlovu et al. 2015) and agriculture (Kay et al. 2008; Páll et al. 2013) on the microbial quality of surface water bodies has been reported. However, considering that none of the *V. cholerae* isolated from the sediments and water of the Apies River carried the gene for cholera toxin production (*ctx*), it could be unlikely that this high prevalence (58.8 %) was solely due to human influence. Non-pathogenic strains of *V. cholerae* are widely distributed in the aquatic environment (Finkelstein 1996). Studies have shown that *V. cholerae* forms an integral part of the aquatic ecosystem and is usually associated with zooplanktons (copepods) that can contain up to 10^5 *V. cholerae* cells on their carapace and in their gut (Rawlings et al. 2007; de Magny et al. 2011; Kirschner et al. 2011). Although the *ctx* gene of *V. cholerae* was not isolated in this study, the

Table 2 Number of positive samples for each pathogen at each sampling site during the entire sampling period

Sampling site	Number of samples collected	Number of samples positive*		
		V. cholerae	*Salmonella* spp	*Shigella* spp
DAS	56	19 (33.9 %)	6 (10.9 %)	7 (12.9 %)
AP1	56	52 (92.9 %)	15 (27.8 %)	4 (7.0 %)
AP2	56	40 (71.4 %)	24 (42.7 %)	18 (32.1 %)
AP3	56	21 (37.5 %)	15 (26.8 %)	0 (0.0 %)
AP4	56	18 (32.1 %)	18 (31.8 %)	0 (0.0 %)
AP5	56	11 (19.6 %)	3 (5.0 %)	0 (0.0 %)
AP6	56	40 (71.4 %)	7 (12.9 %)	2 (4.0 %)
AP7	56	34 (60.7 %)	22 (39.7 %)	2 (4.0 %)
AP8	56	50 (89.3 %)	13 (22.8 %)	28 (49.6 %)
AP9	52	42 (75.0 %)	14 (24.8 %)	2 (4.0 %)
Overall	556	327 (58.8 %)	133 (23.5 %)	56 (10.1 %)

* Values are a summation of the water and sediment positive samples for both dry and wet season

detection of the *ompW* still represents a possible health threat as it has been shown that environmental stains of *V. cholerae* could contain other virulence factors that would allow them to induce infection under appropriate conditions within the intestines of rabbits (Faruque et al. 2004). Similarly, Bag et al. (2008) demonstrated that non-O1 and non-O139 strains of *V. cholerae* isolated from surface water in India showed haemolytic activity when exposed to human erythrocytes. Thus, these strains might have the potential of causing disease once in the human system, especially in immune-compromised individuals.

Sites AP2 and AP8 that recorded highest prevalence of *Salmonella* and *Shigella*, respectively, are located downstream from WWTWs. This site (AP2) also recorded the highest concentration of both FIOs in the sediments. Site AP2 is located downstream from the Rooiwal WWTW which has been reported to function above its operational capacity (South African Department of Water Affairs 2012). Teklehaimanot et al. (2014) earlier reported high prevalence of *Salmonella* spp in effluents from three WWTWs in South Africa and their respective receiving water bodies. However, just like *V. cholerae*, *Salmonella* spp have been found to survive in the environment for long periods and have also been found to infect other domestic animals and birds (Jeong et al. 2010). Some water amphibians and reptiles have also been reported to be carriers of *Salmonella* spp (CDC 2013) although humans are the only known natural hosts and reservoir for some strains like *Salmonella enterica* serovar Typhi (*S.* Typhi) (Kaur and Jain 2012). Considering that the detection of this pathogen was done through the identification of the *invA* gene which is highly specific for all members of the genus *Salmonella*, it would be impossible to determine whether the genes detected were of animal or human origin. Nevertheless, studies have reported human infections originating from *Salmonella* spp of animal origin (Olsvik et al. 1985; Zhao et al. 2003; Hendriksen et al. 2004). Thus, the detection of *Salmonella* in the sediments and water of the Apies river still represents a health threat if these waters are used untreated by surrounding communities.

Unlike *V. cholerae* and *Samonella* spp, the *ipaH* gene of *Shigella* spp was only detected at sites located on the main river course. Samples from the tributaries (AP3, AP4 and AP5) were all negative for the *ipaH* gene. Again, these tributary sites had little or no direct human influence suggesting that human activities around the Apies River may contribute to the pollution observed in the river. *Shigella* has been reported in other hosts such as monkeys, rabbits, calves, piglets and even chickens (Jiang et al. 2005; Pan et al. 2006; Shi et al. 2014). However, several studies using animal models have demonstrated that *Shigella* acquired through the oral route in these animals would hardly result in a disease condition in immuno-competent hosts due to immune clearance by the animals' defence systems. The organism could only cause disease in immuno-suppressed experimental animals (Rabbani et al. 1995; Jeong et al. 2010; Mostowy et al. 2013; Shi et al. 2014). In humans, this is not the case as the organism can bypass the human immune system and invade the large intestine thus establishing an infection (Ashida et al. 2011). Therefore, considering that the faecal–oral route is the main mode of transmission of *Shigella* and that the organism has minimal ability of evading the immune system of non-human hosts, it is likely that the presence of this pathogen in the aquatic environment could be predominantly of human origin. Shi et al. (2014) also demonstrated that *Shigella* of human and chicken origins shared similar pathogenicity and that there was the possibility of human–poultry cross-infection. This means that even if the *ipaH* genes of *Shigella* isolated in the current study were of animal origin, they could still

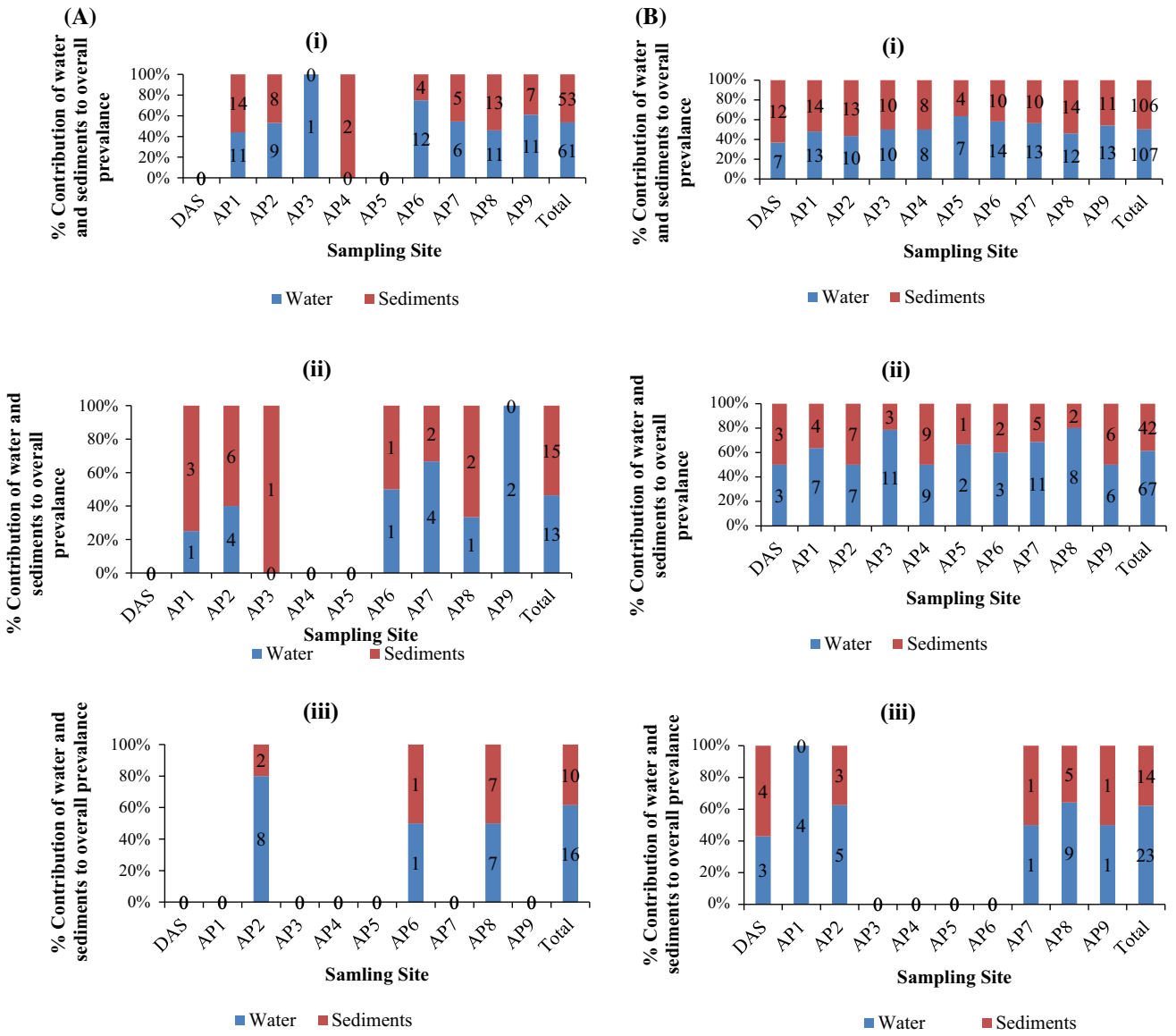

Fig. 2 Contribution of water and sediments to the overall prevalence of (i) *V.* cholerae, (ii) *Salmonella* spp and (iii) *Shigella* spp at individual sampling sites during the (**a**) dry and (**b**) wet season (*Data labels* represent overall number of positive samples for each sample type)

imply a possible threat to human. When compared to *Salmonella* (>10^5 CFU) and *V. cholerae* (~10^4 CFU) the infective dose of *Shigella* is relatively very low (<10–100 CFU) (Kothary and Babu 2001; Lemarchand et al. 2004; WHO 2005; Al-bashan 2012). Thus, the presence of *Shigella* in the aquatic environment even at low prevalence still represents a threat of public health importance, especially in areas where such surface water is used for drinking without prior treatment (Mulamattathil et al. 2014). Studies have also shown that *Shigella* is quickly inactivated when out of its host and does not survive for longer than 7 days in the environment when compared to other enteric pathogens (Mcfeters et al. 1974; Islam et al. 2001; Al-bashan 2012). This could therefore imply that the presence of the organisms in the environment may be indicative of recent faecal pollution. However, this could not be confirmed in this study considering that the detection of pathogens was carried out using PCR and not isolation of viable cells through culture. Although the *ipaH* gene detected in the present study is also carried by enteroinvasive *E. coli* strains (EIEC) (Theron et al. 2001), *Shigella* and EIEC are both causative agents of bacillary dysentery in humans (Hsu et al. 2010) and thus the presence of the *ipaH* gene in riverbed sediments is still indicative of possible negative health implications to users of the untreated river water.

The overall (water and sediments) seasonal pattern in the detection of all the three pathogens (*V. cholerae*,

Salmonella spp and *Shigella* spp) was similar to that observed with the FIOs, with the wet season recording higher prevalence than the dry season. This observation strengthens the role played by seasonal variation on pollution in the aquatic environment. The higher prevalence of POs in the river system during the summer (wet) months observed in this study contradicts those of Kinge and Mbewe (2010). In their study, the authors recorded higher prevalence of *Shigella* spp during the winter (dry months) compared to the summer months in river catchments of the Northwest Province of South Africa. On the other hand, findings of this study agree with those of Mulamattathil et al. (2014), who reported a higher prevalence of *Shigella* in surface water in the Mafikeng area in the Northwest Province of South Africa during the summer months. However, results of the studies by Kinge and Mbewe (2010) and by Mulamattathil et al. (2014) were only based on surface water and did not include the detection of the pathogens in the bed sediments in these catchments. As opposed to the FIOs, the pathogens were more detected in the water column than in the riverbed sediments. However, a strong positive correlation ($p < 0.01$) was observed between the water prevalence and the sediment prevalence for all three pathogens. This indicates that there could be vertical movement of the organisms between the water column and the sediments with the sediments either acting as a sink for or a source (or both) of microorganisms within the aquatic environment.

Correlation between indicator organisms and pathogens in water and sediments

While several studies have investigated the correlation between FIOs and POs within the water column in aquatic environments, data demonstrating strong correlation between FIOs and pathogens in the sediments are rare (Weaver and Sinton 2009). Also, long debates surrounding the use of the term "indicator organisms" and the ambiguous use of the term "microbial indicator" led to the reclassification of indicator organism into *process indicators* (demonstrates how efficient a treatment process is—e.g. total coliforms), *faecal indicators* (indicate faecal contamination—e.g. *E. coli*) and *index and model organisms* (indicative of pathogen presence and behaviour, respectively—e.g. F-RNA coliphages) (Ashbolt et al. 2001).

In the present study, we investigated whether the abundance of *E. coli* and *C. perfringens* correlated with the detection of *V. cholerae*, *Salmonella* spp and *Shigella* spp, especially within the sediments. The nonparametric Spearman rank correlation coefficient was used to establish any relationship between the FIOs and the POs during the dry season (Table 3) and during the wet season (Table 4). During both seasons within the water column, a strong positive correlation was observed between *E. coli* and each of the pathogens while *C. perfringens* only correlated with *V. cholerae* (Tables 3, 4). Within the sediments, strong correlations were only observed between *E. coli* and *Salmonella* spp, *E. coli* and *V. cholerae* (dry season) and *E. coli* and *V. cholerae* and *E. coli* and *Shigella* spp (wet season) (Tables 3, 4). These results corroborate with findings of Abdallah et al. (2005). In their study, the authors reported that faecal coliforms were strongly correlated to *Salmonella* and *V. cholerae* in beach sand of the Gaza Strip. On the other hand, no correlation was observed between *C. perfringens* and any of the pathogens within the sediments. This could be due to the ability of *C. perfringens* to survive for very long periods in the environment even after a pollution event has occurred (Gemmell and Schmidt 2013; Shibata et al. 2004). Most bacterial pathogens survive for shorter periods in the environment than *C. perfringens* (WHO 2008). In a study by Tyagi and Chopra (2006), the authors also reported a lack of correlation between *C. perfringens* and other POs and suggested that while *C. perfringens* is not a good indicator of bacterial pathogens in the aquatic environment, it is a good index organism for viruses and some parasites that survive in the environment for longer periods.

Within the water column, however, *E coli* demonstrated a strong positive correlation with all three pathogens during both seasons while *C. perfringens* only correlated with *V. cholerae*. *V. cholerae* has been reported to survive longer in the environment than many other bacterial pathogens like *Salmonella* (Djaouda et al. 2013). This could possibly explain its correlation with *C. perfringens* as observed in our study. In a recent study by Gemmell and Schmidt (2013) on the microbiological quality of the Msunduzi River in KwaZulu-Natal, South Africa, the authors reported that *E. coli* was a suitable indicator of POs, especially *Salmonella* spp in river water. The level of detection of *Salmonella* dropped with a corresponding drop in the *E. coli* counts within the river. In their study, they also reported the lack of correlation between *C. perfringens* and pathogenic bacteria. Several other studies have reported a correlation between *E. coli* and other pathogenic bacteria (Abdallah et al. 2005; Devane et al. 2014; Leclerc et al. 2001).

Thus, the findings of the present study together with previous studies suggest that although *E. coli* is considered a faecal indicator organism, it could also be a good indicator of other bacterial pathogens, especially in the water column. Sediments represent a more complex environment than the water column in terms of microbial diversity and chemical composition. The fact that *E. coli* only correlated with some organisms in the sediments during the dry and wet season further emphasises the need for better indicators

Table 3 Correlation between indicator and pathogenic organisms in water and sediments during the dry season

Organism		*V. cholerae*	*Shigella* spp	*Salmonella* spp
Correlation between indicator and pathogenic organisms in the water column during the dry season				
E. coli	r_s	.580	.295	.344
	p	***.000***	***.000***	***.000***
C. perfringens	r_s	.268	.043	.067
	p	***.003***	.638	.466
Correlation between indicator and pathogenic organisms in the sediments during the dry season				
E. coli	r_s	.515	.282	.151
	p	***.000***	***.001***	.074
C. perfringens	r_s	−.085	−.085	−.025
	p	.356	.355	.786

Positive correlations are in bold

Table 4 Correlation between indicator and pathogenic organisms in water and sediments during the wet season

		V. cholerae	*Salmonella* spp	*Shigella* spp
Correlation between indicator and pathogenic organisms in the water column during the wet season				
E. coli	r_s	.401	.347	.456
	p	***.000***	***.000***	***.000***
C. perfringens	r_s	.305	.043	.125
	p	***.001***	.640	.174
Correlation between indicator and pathogenic organisms in the sediments during the wet season				
E. coli	r_s	.355	.119	.291
	p	***.000***	.162	***.001***
C. perfringens	r_s	−.017	−.015	.042
	p	.855	.867	.649

Positive correlations are in bold

of pathogens in the sediments. The strong correlation between *E. coli* and the pathogens supports the classification of *E. coli* as a good index organism for pathogens like *Salmonella*. However, there exist no data on suitable index organisms of *V. cholerae* and *Shigella* spp in the environment. Also, the notion of index organisms should be studied extensively within a given environment before it is fully used.

Conclusion

The water from the Apies river is unsafe for use for recreation and if untreated, for any household use. The sediments of the Apies River are polluted with indicator and pathogenic bacteria. There is a strong correlation between *E. coli* and pathogenic bacteria (*V. cholerae*, *Salmonella* and *Shigella*) in the water column suggesting that *E. Cole* is not only a good indicator of faecal pollution, but could also be a good indicator for the presence of other pathogenic bacteria. However, the lack of correlation between *E. coli* and some pathogens in the sediments depending on the season as observed in the present study highlights the need to investigate for more indicators that could better indicate the presence of pathogens in this complex matrix. The lack of correlation between *C. perfringens* and the pathogens does not eliminate its suitability as an indicator of long-term faecal pollution indicator. Although no toxigenic *V. cholerae* strain was detected in this study, due to the high percentage of *V. cholerae* detected we recommend that further research be carried out to investigate if the environmental strains of *V. cholerae* in sediments and water of the Apies River carry other virulence genes that could allow them to initiate infection under appropriate conditions. It would also be necessary to investigate how long these organisms can survive in the sediments of the Apies River and if human-induced or increased flow conditions may cause their resuspension.

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