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Full Length Research Paper

Microbial water quality in the upper Olifants River catchment: Implications for health

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The Olifants River has been described as one of the most environmentally polluted rivers in Southern Africa, particularly in the upper catchment. Untreated water from the catchment may be used for many purposes including irrigation, recreation, consumption, and domestic purposes. As poor quality water remains a leading cause of morbidity and mortality, it is essential to understand the health risks that the water users in the Olifants catchment are exposed to. The aim of this research was to identify the extent of the microbiological pollution in the upper Olifants River catchment and determine the sources of the microbial contaminants by monitoring faecal indicator levels and selected water-borne pathogens. It was shown that sections of the upper Olifants River catchment are highly contaminated with faecal indicator bacteria and pathogenic micro-organisms. A quantitative microbial risk assessment was also performed and it showed that the polluted waters pose an unacceptably high risk to water users within this catchment. In all regions where extreme levels of faecal pollution were observed, the source could be traced back to inadequate wastewater treatment. The microbial water quality in the upper Olifants River catchment could therefore be remediated to a large extent by ensuring proper functioning of wastewater treatment works.

Key words: Faecal indicator organisms, Olifants river catchment, wastewater, pathogens, quantitative microbial risk assessment, microbial water quality.

INTRODUCTION

In South Africa where water is a scarce resource, the water quality of natural resources is of paramount importance. Apart from serving as a source of drinking water (treated or untreated), water needs to be fit for a variety of other domestic and economic activities. The Olifants River is presently one of the most threatened river systems in South Africa (Ballance et al., 2001; Van Vuuren, 2009). There is an intense demand for natural resources in the catchment, and rigorous mining activities occur in most of the nine secondary catchments. The South African Department of Water Affairs State of the Rivers Report on the Olifants River system, states that river ecosystems in this area are generally in a poor to fair condition. Mining-related disturbances were seen as the main cause of impairment of river health in the upper parts of the catchment, with the exception of the lower reaches of the Olifants River, which are protected by conservation activities (Ballance et al., 2001). However, most of the water quality parameters currently routinely measured in the Olifants River catchment by the National Chemical Monitoring Programme have little relevance to human health (de Villiers and Mkwelo, 2009).

Micro-organisms may adversely affect water quality, as microbial pathogens present in water can cause serious human disease. According to the World Health Organization diarrhoeal disease linked to unsafe water supplies remains a major contributor to mortalities in developing countries (WHO, 2004). The health risks are of particular concern for immunocompromised individuals, who are more susceptible to infection and disease than healthy individuals. Water resources that are contaminated by pathogenic micro-organisms would in most instances be unsuitable for use in untreated form,

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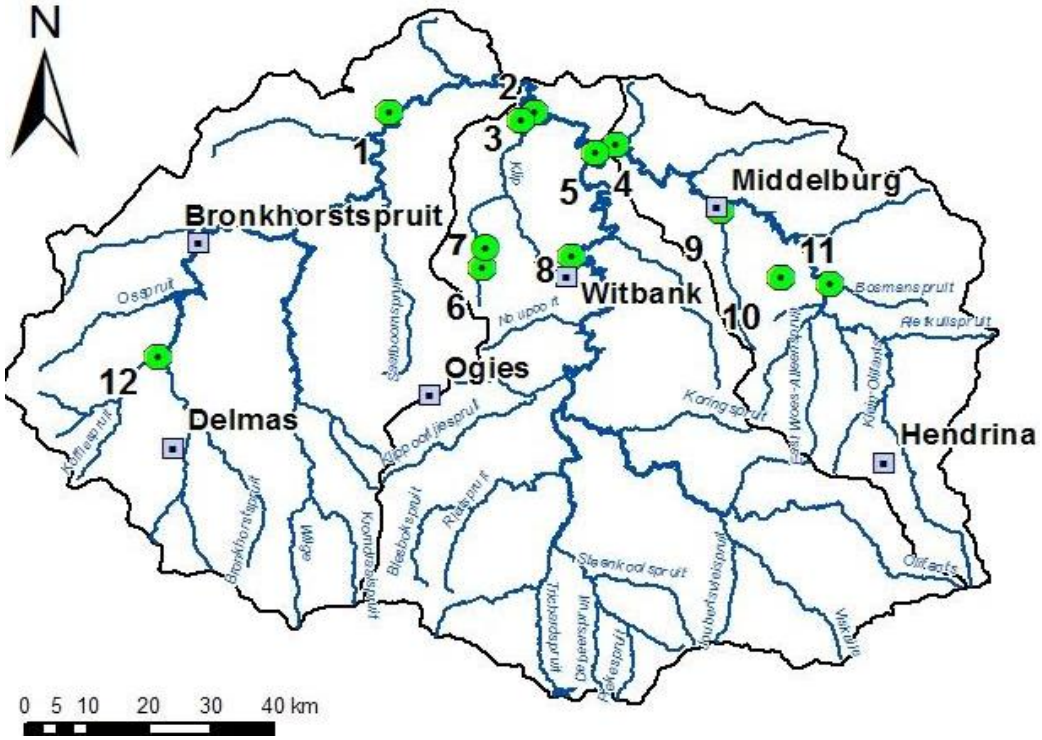

Figure 1. The upper Olifants River catchment study area. Routine sampling sites, numbered from 1 to 12, are indicated.

and in addition pose a health risk to water users in applications that do not necessarily involve consumption, such as bathing, washing of clothes, and gardening. Catchments can also be regarded as the first potential barrier to pathogen hazards in the water supply system (Ferguson et al., 2007). Reducing pathogen loads exported from environmental sources to drinking water reservoirs is thus an important priority in applying a risk-based approach to managing water supplies.

Two approaches can be used to determine the microbial quality of a water resource; monitoring for the presence of pathogenic micro-organisms or alternatively assessing the levels of faecal indicator organisms. Indicator organisms (like *Escherichia coli*) are valuable tools to measure the occurrence and extent of faecal contamination in water sources.

These organisms act as indicators of faecal pollution, and have similar fate, transport, and survival characteristics to the related bacterial pathogens that they represent (Wu et al., 2011). Methodologies to ascertain the presence of pathogens, and their concentrations, are generally expensive and time-consuming, but provide valuable information that can be used to perform quantitative microbial risk assessments. In contrast, the monitoring of faecal indicator organism levels is relatively rapid and inexpensive, but relies on the assumption that the chosen indicator is an accurate surrogate for pathogen occurrence. Various South African and international guidelines exist that state the acceptable limits of faecal indicator organisms in water, the most applicable being the South African Water Quality Guidelines (DWAF, 1996). Other standards such as the Health Guidelines for the Use of Wastewater in Agriculture and Aquaculture (WHO, 1989), also provide good guidance.

In order to manage and mitigate health risks, monitoring of environmental waters for the presence of water-borne pathogens and high levels of faecal indicators is required. In this study faecal indicator levels and selected water-borne pathogens were monitored in the upper Olifants River catchment.

The data was used to perform a quantitative microbial risk assessment, and was also used to identify possible sources of microbial contaminants. Insights gained can be used to shape and guide mitigation strategies for the remediation of microbial water quality problems within this catchment.

METHODOLOGY

Study area

The Olifants River catchment is located in the Mpumalanga province of South Africa and covers 54, 570 km^2. The Wilge, Bronkhorstspruit, and Klein Olifants Rivers are tributaries of the Olifants River that, together with the Olifants River, originate in the Highveld grasslands (Ballance et al., 2001). The study was conducted in the upper section of the Olifants River catchment (Figure 1).

Table 1. Formulae used to calculate daily risk of infection.

Description	Formulae	Reference
Equation 1	$P_i = 1 - \left[1 + \frac{d}{N_{50}}(2^{\frac{1}{\alpha}} - 1)\right]^{-\alpha}$	WHO (2001)
Equation 2	$Pi = 1 - (1 + d/\beta)\text{^}{-\alpha}$	Haas et al. (1999)
Equation 3	$Pi = 1 - \exp(-rN)$	Haas et al. (1996)

P_i: Probability (risk) of infection, d: dose or exposure (number of organism ingested based on consumption of 100 ml per day resulting from accidental or unintentional ingestion), β: parameter characterised by dose-response relationship, α: parameter characterised by dose-response relationship, N_{50}: median infectious dose, r: parameter characterised by dose-response relationship.

Microbial monitoring

Microbial water quality was monitored over a two year period. During the first year, faecal indicator counts (*E. coli)* levels were monitored bi-monthly at eleven sampling sites (Figure 1). Sampling at site 7 (included initially) was discontinued as the stream was contaminated with industrial pollution to a point where no viable bacteria could be detected. Pathogens (*Salmonella* species, *Shigella* species, *Vibrio cholerae*, *Giardia* species, *Cryptosporidium* species, Norovirus and Enterovirus) were tested for (bi-monthly) at the sites that exhibited high *E. coli* counts (Table 2). Faecal indicator counts were also measured at 12 additional sites during the year to aid in identifying sources of microbial pollution, and on average, these sites were monitored on three different occasions. During the second year, 86 sample sites (Global Positioning System (GPS) coordinates available on request) were monitored for *E. coli* during low flow conditions using a once-off sampling approach.

Quantification of *E. coli* was determined using the Colilert™ Most Probable Number (MPN) method (IDEXX, USA). This was carried out according to the manufacturer's protocol. For detection of *Salmonella* spp., one litre water samples were filtered and the filtrate incubated overnight in 30 ml of Selenite Broth (Merck, Germany). Cells were harvested from the broth and the DNA was extracted using InstaGene Matrix (Biorad Technologies, USA). *Salmonella* was detected by real-time polymerase chain reaction (PCR) targeting the *invA* gene (Malorny et al., 2003). Bacteria belonging to the genus *Shigella* were detected by concentrating one litre water samples (by filtration), followed by overnight incubation of the filtrate in 30 ml of Peptone Broth (Biolab, South Africa). Cells were harvested from the broth and DNA was extracted using InstaGene Matrix (Biorad Technologies, USA). *Shigella* (virulent types) and/or enteroinvasive *E. coli* (EIEC) were detected by real-time PCR targeting the *ipaH* (invasion plasmid antigen) gene (Theron et al., 2001).

For the detection of *V. cholerae*, one litre water samples were filtered and the filtrate was incubated overnight in 30 ml Alkaline Peptone Water (APW) (Merck, Germany). Cells were harvested from the broth and DNA was extracted using InstaGene Matrix (Biorad Technologies, USA). For the detection of *V. Cholerae* (all strains) the gene coding for the Outer Membrane Protein (*ompW*) was targeted using real-time PCR (Nandi et al., 2000; le Roux and van Blerk, 2011). For the detection of enterotoxigenic strains, the *ctxAB* genes were targeted in a real-time PCR amplifying a section of the cholera toxin A and B sub-unit (Goel et al., 2005; le Roux and van Blerk, 2011).

The water was analysed for the presence of Enterovirus by concentrating 10 L (using ultra-filtration) of water per sampling site. Genetic material was extracted from the concentrate, and quantitative real-time PCR was performed using TaqMan technology and primers and probes as described by Fuhrman et al. (2005). The same process was used to test for the presence of Norovirus, but with the real-time PCR assay making use of primers and probes as described by Loisy et al. (2005) and da Silva et al. (2007).

Protozoan parasite (*Giardia* and *Cryptosporidium*) analysis was carried out according to the EPA 1623 method (EPA, 2010). Briefly, parasites were concentrated (from 10 L of water) using 1.2 µm membranes filters, separated from the filtrate by immuno-magnetic separation (Invitrogen, Norway), labelled with fluorescent species-specific antibodies (Cellabs, Netherlands), and counts were obtained by fluorescence microscopy.

Quantitative microbial risk assessment (QMRA)

The average counts and/or detection data for indicator organisms, pathogenic bacteria, viruses and parasites were used to perform a quantitative microbial risk assessment. Doses were calculated based on the volume used for assessment (assuming, based on observations, that a conservative volume of 100 ml of untreated river water was consumed). The formulae used to calculate the daily risk of infection in the quantitative microbial risk assessment is given in Table 1.

Pathogen specific parameters used in the probability of infection formulae are given as follows. Equation 1: *Salmonella* spp. N_{50} = 23600 α = 0.21 (Haas et al., 1999), *Shigella* spp. N_{50} = 1120 α = 0.16 (Haas et al., 1999), and *Vibrio* spp. N_{50} = 243 α = 0.25 (Haas et al., 1999). Equation 2: *Norovirus* N_{50} = 50 α = 0.022 (Fankem, 2008), Enterovirus (Poliovirus 1) α = 0.097 β = 13020 (Haas et al., 1993), and *E. coli* α = 0.395 β = 2.473 (Strachan et al., 2005). Equation 3: *Giardia* spp. r = 0.0198 (Teunis et al., 1996) and *Cryptosporidium* spp. r = 0.00419 (Teunis et al., 1996).

For the QMRA making use of quantitative pathogen data, the average concentrations per 100 ml were calculated from the results of five sampling rounds and used in the risk calculations as described earlier. Where only qualitative data was available (presence/absence), namely for *Salmonella*, *Vibrio*, and *Shigella*, the number of times the organisms were detected was used as a conservative input for the dose calculation. *E. coli* concentrations were adjusted to represent pathogenic bacteria numbers. To achieve this, a ratio was calculated based on the lowest concentration of *E. coli* detected when pathogens were detected. The ratio used for the *E. coli* pathogenic to non-pathogenic in this study was 200.

RESULTS

Eleven sites (Figure 1) within the upper Olifants River catchment were monitored on five occasions (bi-monthly) over one year for faecal indicator levels. Six of these sites (located in the Olifants-, Wilge- and Klip River, and tributaries of the Klein Olifants River) were also monitored for the presence and/or levels of seven water-borne pathogens. A summary of the monitoring data is provided in Table 2.

High levels of *E. coli* were recorded at three sites (Sites 6, 8, and 9), and these sampling points were all located in urban areas and were directly downstream of wastewater treatment works (WWTWs). A fourth sampling point (Site

Table 2. Summary of pathogen and indicator organism detection results for five sampling rounds.

Site	Mean counts					Percentage detection		
	***E. coli* (average) most probable number/100 ml**	**Norovirus (average) virions/10 L**	**Enterovirus (average) virions/10 L**	***Giardia* (average) cysts/10 L**	***Cryptosporidium* (average) cysts/10 L**	***Salmonella* (% detection out of 5 samples)**	***Vibrio cholerae* (% detection out of 5 samples)**	***Shigella* (% detection out of 5 samples)**
Site 1	34	ND	ND	0.5	ND	40	ND	ND
Site 2	264	ns	ns	ns	ns	ns	ns	ns
Site 3	441	ns	ns	ns	ns	ns	ns	ns
Site 4	364	ns	ns	ns	ns	ns	ns	ns
Site 5	611	ns	ns	ns	ns	ns	ns	ns
Site 6	44,110	162	1620	ND	ND	80	20	60
Site 8	15,999	48	ND	ND	ND	80	40	60
Site 9	13,632	828	2760	0.25	0.25	80	100	80
Site 10	2,319	ND	ND	7.5	1.2	40	60	ND
Site 11	723	ND	ND	1	0.25	40	60	ND
Site 12	163	ns	ns	ns	ns	ns	ns	ns

ns: Not scheduled, ND: not detected. Site 7 is not included in the table as no viable micro-organisms were detected in early sampling efforts, and sampling at this site was discontinued.

10, situated downstream of a cattle feedlot) also exhibited elevated *E. coli* levels. The sites with the highest levels of *E. coli* also harboured the most water-borne pathogens. A site in the Wilge River (Site 1) had low indicator bacteria levels and similarly few water-borne pathogens were detected. Bacterial pathogens were present at many of the tested sites. *Salmonella* spp. and *V. cholerae* (non-enterotoxigenic) were highly prevalent, with *Shigella* spp. detected at fewer sites. It is noteworthy that the two protozoan parasites (*Giardia* and *Cryptosporidium*) were more prevalent at sites 10 and 11 as these sites were directly down-stream of cattle feed-lots. *Giardia* was detected more often than *Cryptosporidium.*

The data presented in Table 2 was used to perform a quantitative microbial risk assessment. Figure 2 shows the combined probability of infection (risk of infection of all pathogens summed) for the seven pathogens that were monitored for during the study, the pathogens were: *Salmonella* spp., *Shigella*, *V. cholerae* (non-enterotoxigenic), *Cryptosporidium* spp., *Giardia* spp., Norovirus and Enterovirus. The risk was based on a conservative estimate of a once-off consumption of 100 ml of untreated water.

Water from site 9 (a tributary of the Klein-Olifants River) resulted in the highest risk of infection (Pi = 26%), followed by site 10 (a tributary of the Klein-Olifants), site 8 (Olifants River), and site 6 (Brugspruit/Klip River). The risks ranged from 26% probability to 12% probability based on very conservative single event exposure consumptions. In all sites, with the exception of site 10, the high risk was due to the presence of Norovirus followed by the bacterial pathogens, *V. cholerae* and *Shigella*. The high risk of infection calculated for site 10 was due to the presence of *Giardia* spp.

The seven pathogens represented in Figure 2 are only a small fraction of the total pool of water-borne pathogens that may be present in contaminated waters. In an effort to provide a representative risk, *E. coli* was used as a surrogate for pathogens in calculating the probability of infection as described earlier (Figure 3).

Understandably the sites with the highest levels of *E. coli* (sites 6, 8, 9, and 10) had the highest probability of

infection. Once again the risk assessment was based on the consumption of 100 ml of untreated water. The probability of infection at these sites ranged between 70 and 82%.

In order to gain insight on the sources of microbial pathogens, 86 additional sample sites were sampled in the upper Olifants River catchment for faecal indicator levels. All major tributaries of the Wilge, Klein Olifants, and Olifants Rivers were sampled during low flow conditions using a once-off sampling approach. A summary of the results is given subsequently.

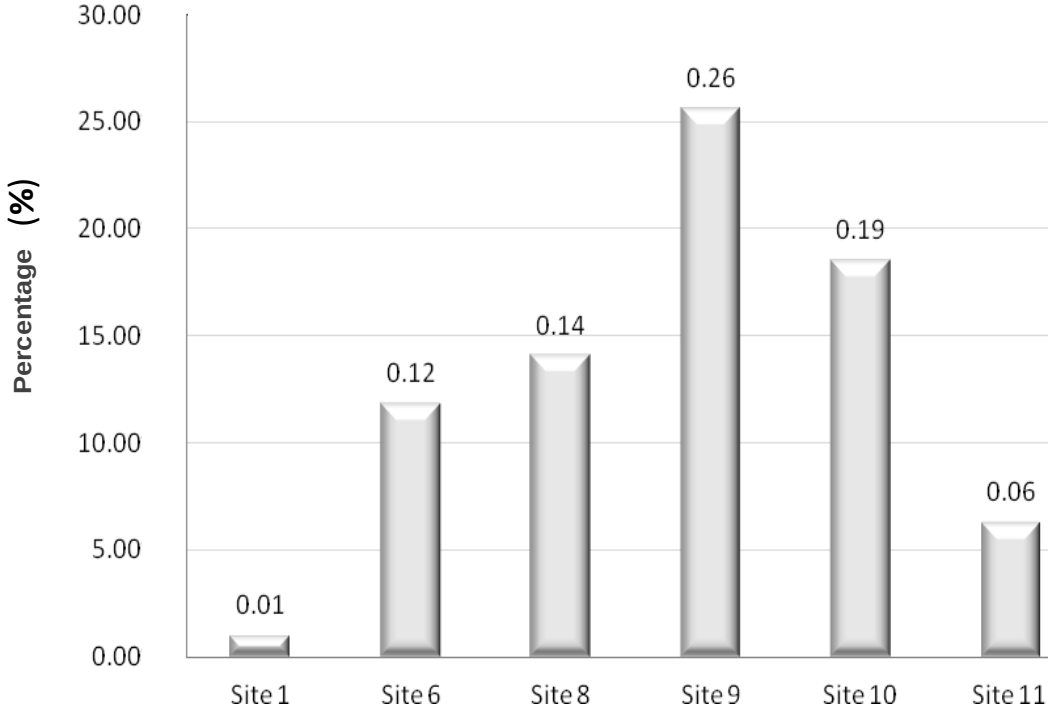

Figure 2. Combined probability of infection (Pi) for the seven water-borne pathogens monitored in this study.

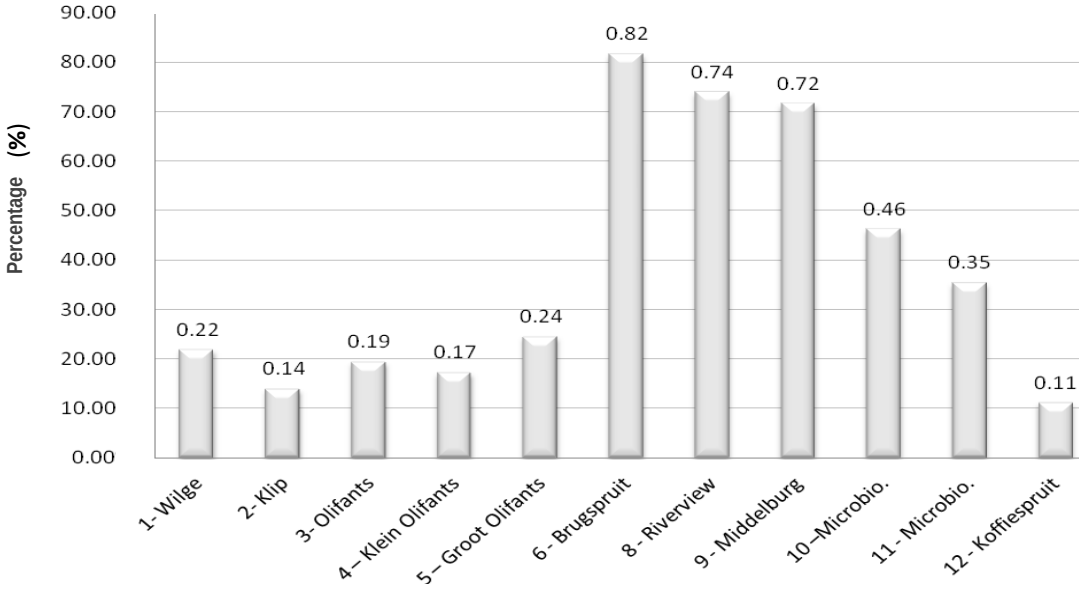

Figure 3. Risk of infection calculated using *E. coli* as a surrogate for water-borne pathogens.

The Wilge River had many sites where moderate *E. Coli* counts were measured (100 to 1000 MPN/100 ml), with only one site exceeding 1000 MPN/100 ml. In the Olifants River, eight sites had *E. coli* numbers above 1000 MPN/100 ml. Six of these eight sites were located directly downstream of WWTWs, one was directly down-

stream of an urban area and another was situated close to an intensive mining area. From a microbiology perspective the Klein Olifants River had fairly good quality water. However, the water quality in the vicinity of urban and intensive mining areas was found to be poor at certain sites. Elevated faecal indicator numbers were recorded in the Klein Olifants as it flowed through Middelburg. The microbial quality improved downstream of the WWTW indicating that this facility may not be the source of contaminants. Further upstream in the Klein Olifants River three sites had intermediate faecal indicator counts (100 to 1000 MPN/100 ml), and this area is characterized by intensive mining. The only other sampling site to show very high numbers of faecal indicator bacteria was located directly downstream of a WTWW on the outskirts of the town of Hendrina.

Of the 86 sites sampled, 12 sites had *E. coli* levels exceeding 1000 MPN/100 ml. Of the 12 sites, 7 were located directly downstream of WWTWs, two sites were directly downstream of urban areas, one was located in an area characterized by intensive mining activities, and another two sites were located in agriculture regions. On average, the *E. coli* levels were an order of a magnitude higher for sites linked to WWTWs as compared to non-point sources like agriculture and other intensive land uses (average *E. coli* counts were 40,000 MPN/100 ml and 2700 MPN/100 ml, respectively). Where possible water samples were taken from the stream or river directly upstream and downstream of the effluent discharge point to confirm that a specific treatment works was the cause of the contamination.

In other cases streams only existed as a result of effluent discharge and had no other source of flow. In this regard, in four of the seven sites linked to WWTWs, the faecal contamination could only be accounted for by below par WWTW functioning. Indications are that the three remaining sites located close to WWTWs also receive their contaminants from inadequate wastewater treatment facilities.

DISCUSSION

Faecal indicator levels

Many sampling sites had high counts of *E. coli*, indicating that sections of the upper Olifants River system were heavily contaminated by faecally derived microbes (Table 2). Sites that exhibited lower numbers of *E. coli* were generally located in regions of lower population density, whereas sampling sites located closer to settlements and towns generally had higher counts. The exceptions to this rule were two sites located in tributaries of the Klein Olifants River. The periodically high counts observed here could be explained by runoff from nearby cattle feedlots. Indicator counts for sites that were not directly downstream of WWTWs were higher during the high rainfall months of January and March than the low rainfall months of July and September. Surface water runoff from polluted areas is known to carry high loads of microbial contaminants (Doran and Linn, 1979; Curriero et al., 2001; Tate et al., 2006). Therefore, the most likely explanation for the increase in *E. coli* counts is the high rainfall that preceded the sampling days. The sites that receive inflow from WWTWs did not show a decrease in faecal indicator counts during the dry months, and instead showed a general increase. This is most likely due to the effluent being more concentrated in the absence of rain water runoff.

Pathogen monitoring

Bacterial pathogens were readily detected at the sampled sites, with *Salmonella* being the most prevalent (Table 2). *Salmonella* causes diseases such as typhoid and paratyphoid. The widespread occurrence of this organism in waters from the upper Olifants River catchment is a concern. This bacteria remains an important pathogen, with an estimated 216,000 deaths per year (globally) attributed to typhoid (Crump et al., 2004). Another devastating disease, dysentery, causes an estimated 1 million deaths per year globally (Niyogi, 2005). Dysentery is caused by the bacteria of the genus *Shigella*, and was readily detected at three sampling sites in the upper Olifants River catchment (Table 2). The causative agent of cholera is the bacterium *V. cholerae*, and more specifically enterotoxigenic *V. cholerae* sub-types. Although, *V. cholerae* was widely detected in water from the upper Olifants River (Table 2), none of these strains were enterotoxigenic. The detected strains cannot cause epidemic cholera, but may cause sporadic disease as non-toxigenic strains may cause opportunistic infections (Bubshait et al., 2000). Predictably, the sites that had the highest faecal indicator (*E. coli*) counts also harboured microbial pathogens more often (Table 2), highlighting the value of indicator bacteria during monitoring (Wu et al., 2011).

The protozoan parasites (*Giardia* and *Cryptosporidium*) were detected at two sampling sites; sites 10 and 11 (Table 2), situated in close proximity and directly downstream of cattle feedlots. *Giardia* and *Cryptosporidium* are associated with livestock farming and domesticated animals (Olsona et al., 1997; Traub, 2008). It is highly likely that the parasites detected originate from the cattle feedlots. Rainfall events, and the associated run-off, can cause higher loads of the pathogens to be present in regional waters. *Giardia* and *Cryptosporidium* can survive for extended periods as (oo)cysts, and have low infectious doses (Adam, 2001; DuPont et al., 1995; Guillot and Loret, 2010). In addition, disease caused by *Cryptosporidium* is difficult to treat, and is a concern for immuno-compromised individuals

(Guillot and Loret, 2010).

Investigation of sources of microbial pollution

Initial monitoring efforts were directed at 12 sampling sites within the upper Olifants River catchment during the first phase of this project (Figure 1). Repeat sampling was performed over a period of one year and provided microbiological base-line data. However, due to the limited number of sites that were investigated, a complete picture was still lacking. To address the knowledge gaps, more sample sites were investigated during a second monitoring phase. These sample sites were chosen in such a way that they could provide information on how each tributary and contributing stream affected water quality, both locally and to the main stem of each river. The Wilge, Klein Olifants, and Olifants Rivers were sampled during separate sampling trips, all during low flow. Each river, and all sites linked to it, were sampled within the shortest possible time frame (usually within two days) in order to minimize time-related variances. For this reason the data may have limited value for comparing microbial quality between the three river systems, but are more suited to infer what the drivers and sources of microbial contaminants within a specific system were at a given time.

Sites were focussed on where indicator levels were found to exceed 1000 *E. coli* MPN/100 ml of water. This value was chosen because WWTWs should not release effluent that exceeds this value and South African irrigation guidelines also advise against using water that exceeds this value for agricultural purposes (assuming that no purification steps are employed) (Department of Water Affairs and Forestry, South Africa, 1996). In addition, the recommended water quality guidelines for wastewater use in agriculture for the irrigation of crops likely to be eaten uncooked, public parks and sports fields is ≤1000 faecal coliforms per 100 ml of water (WHO, 1989). It should be noted that water containing faecal indicators at this level is deemed to carry a significant health risk to water users. Twelve of the 86 sites sampled were found to contain *E. coli* at levels exceeding this value, with the Olifants River and its tributaries accounting for eight of the sites. Of the twelve sites, seven were directly downstream of WWTWs, two sites were directly downstream of urban areas, one was located in an area characterized by intensive mining activities, and another two sites were located in agricultural regions. Intensive land uses are known to increase faecal indicator loads to rivers and streams, but these non-point sources would in many cases only be sporadic contamination events (Loague and Corwin, 2005).

It should be noted that not all WWTWs in the upper Olifants River catchment are dysfunctional, because the data suggested that a treatment works on the northern side of Middelburg was operating within specification. Another treatment works on the south side of Middelburg was recorded to repeatedly contaminate a tributary of the Klein Olifants River with extremely high levels of faecal indicator organisms and human pathogens. However, a drastic improvement in this stream's microbial quality was observed during the latter part of the monitoring.

The data show that small microbial water quality gains can be made by improving agricultural and industrial practises. However, this would entail a paradigm shift in the way that many small, and some large enterprises function; all of this to achieve a slight improvement in microbial water quality. In contrast, large gains can be achieved by ensuring the proper functioning of a few WWTWs within the upper Olifants River catchment.

Quantitative microbial risk assessment

Untreated water may be used for many purposes in the upper Olifants River catchment. These include irrigation, recreation, consumption, and domestic purposes. Poor quality water remains a leading cause of morbidity and mortality on the global scene (WHO, 2004), and it is vital to understand the risks that the water users in the Olifants catchment are exposed to.

Various sites within the upper Olifants River catchment have been shown to be highly contaminated with faecal pollutants. These sites harbour diverse human pathogens that pose an unacceptably high health risk to water users. The data show that an individual consuming 100 ml of untreated water from such a water source could expect to have up to a 26% chance of falling ill (from any of the seven pathogens that were monitored for in this study) (Figure 2). Important water-borne pathogens as they may be, these organisms are but a fraction of the total potential pathogen pool expected to occur in poor quality waters, and the true risk water user's face would in fact be much higher. Using *E. coli* as an indicator for pathogens, the risk of infection at some sites was calculated to be in the region of 80% based on a single exposure event (Figure 3). It is important to note that the probability of infection, resulting from exposure to water, makes use of dose-response models. These dose-response models typically make use of studies involving healthy volunteers, thereby not taking into account the sensitive subgroups, such as the young, elderly, and immune-compromised. Not all infected individuals would develop clinical symptoms. The percentage of infected people that would develop clinical illness would depend on a number of issues, such as the immunity of the person and the virulence of the pathogen. For this reason more illness may be expected (as opposed to infection) among the relatively high proportion of HIV positive individuals in this region.

Ideally, these contaminated waters should not be used for human consumption, but research data indicate that

consumption of untreated water by humans is not uncommon in the study area (personal communication, C. Wright.). According to the WHO, the “acceptable” annual risk of infection (from drinking water) is considered to be 1 in 10,000 (WHO, 2001), however, a higher risk of 1 in 1,000 is now being considered as more appropriate. Regardless of which of these should be considered as acceptable, the risk of infection in this study resulting from possible exposure to pathogens is significantly higher.

Priority areas

From the results, it is clear that sections of the Wilge, Klein Olifants, and Olifants River systems are highly contaminated with pathogenic micro-organisms. It is also clear that these organisms, at the level that they are present, pose a serious health risk to water users in this catchment. However, some polluted areas may be of low importance due to the absence of significant exposure mechanisms. For instance, poor quality river water in a region of low population density, where other sources of drinking water are readily available, will have less of an impact than poor quality water in a densely populated region with no formal service delivery. Some of the sites that exhibited poor water quality during the second monitoring phase of this study are located in low population density areas, even though the most likely cause of their poor state lies in densely populated areas (that is, WWTWs).

Two priority areas were identified where poor quality water co-existed with high population densities, the most prominent being Emalahleni and the surrounding areas. The Klip River, Blesbokspruit and sections of the Olifants River posed unacceptably high risks to water users within this area. The Klein Olifants in the vicinity of Middelburg was also highly contaminated with faecally derived microbes.

Conclusion

It was shown that sections of the upper Olifants River catchment are highly contaminated with faecal indicator bacteria and pathogenic micro-organisms. Data from the quantitative microbial risk assessment also showed that the polluted waters pose an unacceptably high risk to water users within this catchment. Making use of *E. coli* data as a surrogate for the possibility of other pathogens in the quantitative microbial risk assessment resulted in very similar probabilities of infection, illustrating the value of *E. coli* in a quantitative microbial risk assessment. Diffuse sources may be responsible for a portion of the observed faecal pollution, but in most cases the contamination associated with diffuse sources (such as informal and formal housing areas) was a few orders of magnitude lower than that of the point sources identified. Extreme levels of faecal pollution could in most instances be traced back to inadequate wastewater treatment.

In order to mitigate water-borne risks in the upper Olifants River, wastewater treatment works need to be maintained and operated in such a way that sewage effluent meets effluent discharge criteria at all times. In addition service delivery in developed and developing areas should be maintained and expanded where necessary to provide hygienic living conditions, with safe drinking water provision and wastewater infrastructure being paramount. Until the current short comings are addressed, water users at certain locations within this catchment will continue to be at risk from water-borne infections.

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