

Microbiological evaluation of the Mhlathuze River, KwaZulu-Natal (RSA)

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Abstract

Continuous faecal pollution in source water is a global problem that is particularly debilitating to rural communities that are directly dependent on untreated source water for all their domestic and other purposes. The elevation of indicator bacteria levels (such as the faecal coliforms) in the water may pose a public health risk. This study reports the results of microbial monitoring of the Mhlathuze River over a 21-month period. Elevated levels of indicator micro-organisms (both faecal and total coliforms) and heterotrophic plate count bacteria were observed from March 1998 to November 1999. Surface water temperature and rainfall during this period appeared to be some of the factors affecting the increased bacterial counts. Bacteria isolated from the river included *E. coli*, *Pseudomonas* spp., *Enterobacter* spp. (detected frequently), *Serratia* spp., *Klebsiella* spp., and *Aeromonas hydrophila* (detected less frequently). This study generated some essential baseline information of the microbial population for a section of the river utilised for domestic, agricultural and industrial purposes.

Introduction

The quality of water is typically determined by monitoring microbial presence, especially faecal coliform bacteria, and physico-chemical properties (Gray, 1994; DWAF, 1996; USA-EPA, 1999). These parameters could be affected by external and internal factors. There is an intricate relationship between the external and internal factors in aquatic environments. Meteorological events and pollution are a few of the external factors which affect physico-chemical parameters such as temperature, pH, salinity, hardness, dissolved oxygen and phosphates of the water. These parameters have major influences on biochemical reactions that occur within the water. Sudden changes of these parameters may be indicative of changing conditions in the water. Internal factors, on the other hand, include events, which occur between and within bacterial and plankton populations in the water body (Nübel et al., 1999; Byamukama et al., 2000; Goni-Urriza et al., 2000; Lobitz et al., 2000; Nishiguchi, 2000).

The population densities of heterotrophic and indicator bacteria (coliform) belonging to the Enterobacteriaceae are commonly used as indices for microbial water quality (Gray, 1994; DWAF, 1996; Sartory and Watkins, 1999). Faecal population assessment in waters is generally achieved by determining the number of faecal coliforms that are present in the sample. The faecal coliform count is a more reliable indicator of the sanitary quality of water because some coliform genera, such as *Citrobacter* spp., *Klebsiella* spp. and *Enterobacter* spp., include some species, which are of faecal origin, and others which are of non-faecal origin (Alonso et al., 1999). Standards clearly defined by water supply- and -monitoring agencies are used to determine whether water is suitable for human

consumption, recreation and other purposes such as agricultural use (Gray, 1994; DWAF, 1996; Kempster et al., 1997; USA-EPA, 1999).

Rural communities in the Mhlathuze River catchment area are directly dependent on this river for all their water needs including that of drinking, washing, recreation and agriculture. Treated water is, in many cases, unavailable to these communities. Communities such as these have been more prone to water-borne diseases (Pegram et al., 1998). There are also controversies about supplementing the water supply of the Mhlathuze River with water from an adjacent catchment area because the water from the Mhlathuze River is regarded as "over allocated" (*Zululand Observer*, RSA, May 28, 2001). It is therefore imperative that microbial and physico-chemical data of the river are collected to provide comprehensive baseline information that can be compared to other existing biological databases for the river as well as utilised for future monitoring purposes.

The primary aim of this study was to determine the microbial quality (heterotrophic bacterial, total coliforms and faecal coliform counts) and some physico-chemical parameters of the water in the Mhlathuze River. The secondary aim of the study was to determine seasonal changes in the bacterial population. These findings will reveal the effects of the rural population and industries situated along the river on the microbial population changes.

Materials and methods

Sampling

Water samples from five different locations along the Mhlathuze River (KwaDlangezwa: Site 1, KwaDlangubo: Site 2, Mhlathuze Estuary: Site 3, Mhlathuze pump station: Site 4 and Felixton bridge: Site 5) (Fig. 1) were collected bi-weekly in sterile Schott bottles for the period of March 1998 to November 1999. Once collected, the samples were immediately stored at 4°C in a dark cooler box and analysed within 6 h of collection.

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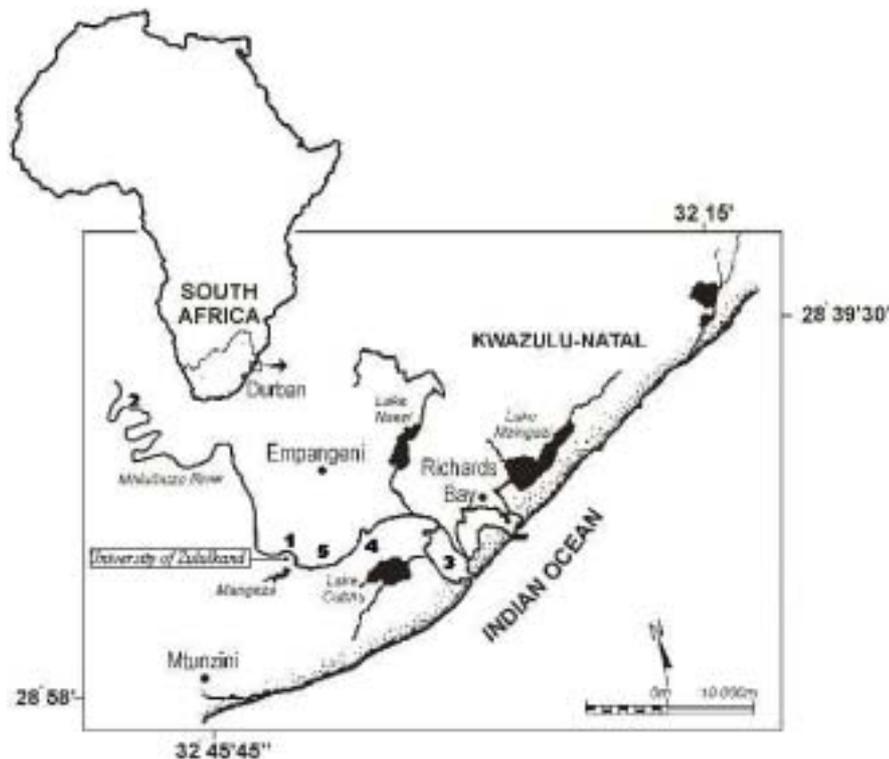


Figure 1

Map of the eastern region of the Mhlathuze catchment area indicating the sampling sites (Site 1: Kwadlangezwa, Site 2: KwaDlangubo, Site 3: Mhlathuze Estuary, Site 4: Mhlathuze Pump Station and Site 5: Felixton Bridge)

Microbiological analysis

A series of tenfold dilutions of the water samples were used for the enumeration of bacterial contents using plate count and membrane filtration (Millipore, HANG 47 mm) methods. Nutrient agar (NA), mEndo Les, mFc and S-S agars (Merck, SA) were used to determine heterotrophic bacterial, faecal and total coliform, and *Salmonella-Shigella* respectively. All plates, with the exception of mFc plates (incubated at 44.5°C for 24 h), were incubated at 35°C for 24 h. Analyses were performed in duplicate.

Identification of bacteria

Single colonies from each plate were randomly isolated, based on the morphology. The isolates were then identified by Gram stain and biochemical reactions, then confirmed by API 20E strips (BioMerieux, France) according to the manufacturer's instructions.

Temperature and pH analysis

Temperature was measured *in situ* using a mercury bulb thermometer (Brannan, England) and pH measurements were taken in the laboratory at room temperature, using a PHI 34 pH meter (Beckman, USA).

Rainfall data

Rainfall data (South African Weather Bureau number 0304823) for the sampling region were obtained from the Computing Centre for Water Research (CCWR) at the University of Natal-Pietermaritzburg, Republic of South Africa.

Statistical analysis

Geometric mean of microbiological organisms, rainfall, temperature and pH analysis data were used to present monthly values for these parameters. Pearson's correlation coefficient (r) was used to show correlation between microbiological data on the one hand and rainfall and surface water temperature on the other. The Student's t-test was used to determine the statistical significance. Probability was set at $p < 0.05$.

Results

Table 1 is a summary of geometric mean monthly bacterial populations, surface temperature and rainfall data from five sampling sites along the Mhlathuze River for the period March 1998 to November 1999. During this period the mean total coliform counts were generally two-fold greater than the mean faecal coliform counts. However, the period between November 1998 and February 1999 shows an enormous (fivefold) increase in the mean total coliform counts, compared to the mean number of faecal coliform counts. The trends in Fig. 2 depict typical seasonal changes. Lowest numbers of colony forming units (CFU's) were detected during the winter season (May

to August) and large numbers of CFUs were detected in the summer season (November to February). Heterotrophic plate count bacteria detected during the summer season also shows a peak which had four Log_{10} increases compared to the winter. The changes in heterotrophic bacterial counts were more gradual than those of the indicator micro-organisms. The two environmental factors (surface water temperature and rainfall) also showed similar cyclic changes to the microbial data and may have influenced the bacteriological observations. The mean monthly pH values ranged between 6.5 and 8.5. These values are well within the SA standards for potable water (DWAf, 1996) and sudden fluctuations, which are indicative of adverse conditions, were not observed.

The faecal coliform counts detected at each sample site for the study period are shown in Fig. 3. Similar results were observed for total coliform and heterotrophic plate counts. It is evident that the sampling site at Felixton (Site 5) was the major contributor to the observed peak in CFUs especially during the summer season.

Monthly comparisons of the mean faecal coliform counts in 1998 to those observed in 1999 show that, except for May and June, greater numbers of these indicator micro-organisms were detected in the Mhlathuze River in 1999 than in 1998 (Fig. 4). The heterotrophic plate count and total coliform bacteria detected during this period showed a similar trend. The relationship between surface water temperature, rainfall figures and faecal coliform counts is also illustrated in Fig. 4. The mean surface water temperatures and the mean rainfall figure of the corresponding periods were ± 5 to 10°C and ± 5 mm higher, respectively, in 1999 than those in 1998 (Table 1; also Fig. 4). Pearson's correlation coefficient analysis showed that there was a positive correlation between increase in surface water temperature and increase in bacterial counts ($r = 0.805$ for total coliform and $r = 0.678$ for faecal

TABLE 1
A summary of the microbiological, surface water temperature and rainfall data obtained for the Mhlathuze River (March 1998 – November 1999). The mean values of the microbial data are depicted in Fig. 2. Mean monthly heterotrophic plate count bacteria detected are expressed as Log₁₀ CFU/ml and the mean coliforms (TC – total coliform, FC – faecal coliform) as CFU (x 10² colony forming units) per 100 ml.

	HPC (Log ₁₀ CFU/ml)	Total coliforms (10 ² CFU/ 100ml)	Faecal coliforms (10 ² CFU/ 100ml)	Surface water temperature	Rainfall in mm	
Month	Mean	Mean	Mean	Mean	Mean	Total
March	5.14	25.4	8.6	26.5	11.7	367
April	4.86	14.0	6.2	24.4	18.7	560
May	5.10	23.4	7.8	20.0	3.4	105
June	4.95	16.0	5.8	20.0	0	0
July	2.38	13.8	4.0	18.0	0.1	623
August	1.70	9.8	4.2	16.0	4.3	133
September	2.13	10.8	2.8	19.5	25.6	803
October	2.74	14.2	7.2	20.5	38.0	1177
November	2.69	22.8	9.8	27.5	47.1	1413
December	3.52	70.0	15.7	30.7	29.9	928
January	6.36	125.2	25.6	34.0	53.7	1666
February	6.51	96.8	32.25	27.5	126.0	3529
March	6.42	22.8	10.6	26.5	18.1	560
April	5.69	19.4	10.2	21.5	13.5	405
May	5.13	14.6	5.8	21.0	9.8	305
June	4.51	8.2	3.8	17.5	4.2	126
July	2.69	19.0	8.2	21.0	24.6	762
August	2.75	37.0	13.0	26.0	12.4	384
September	3.39	38.4	16.6	24.0	26.8	803
October	4.76	41.0	27.8	25.5	68.6	2128
November	4.85	49.4	36.2	26.5	12.0	360

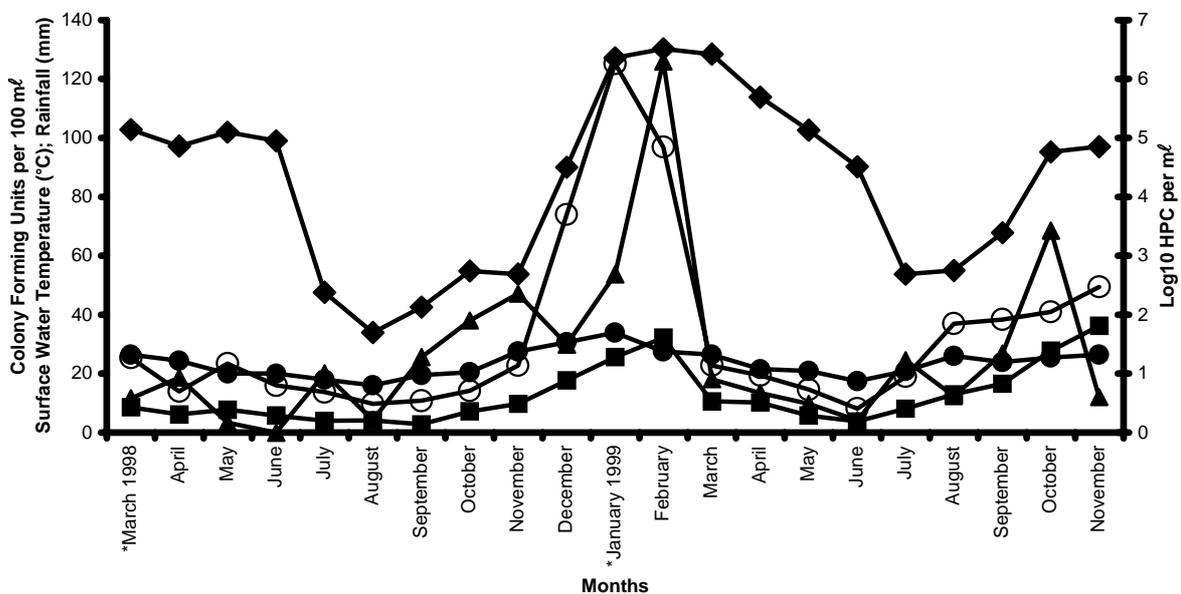


Figure 2
Seasonal variation in heterotrophic plate count (♦), total coliform (○) and faecal coliform bacteria (□) in the Mhlathuze River as well as rainfall (▲) and surface water temperature (●) data collected from March 1998 to November 1999. The monthly values for all bacteria are the mean values of five sampling sites per month that were sampled and analysed, in duplicate, twice per month. Temperatures were measured in situ and rainfall figures were supplied by the CCWR of the University of Natal, Pietermaritzburg.

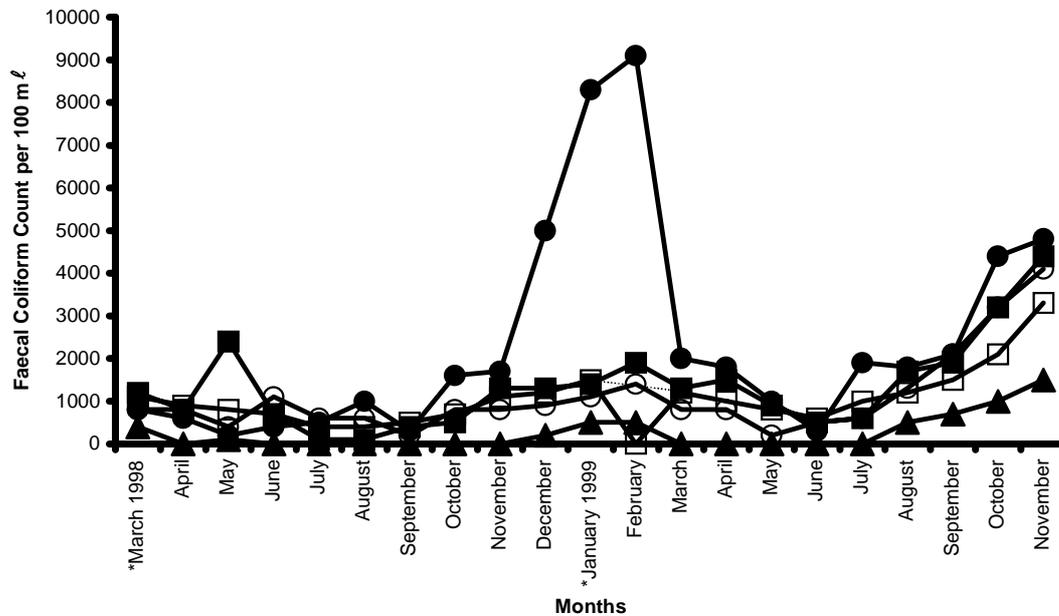


Figure 3

The mean faecal coliform count in the Mhlathuze River at each site (Site 1-□, Kwadlangezwa; Site 2-■, KwaDlangubo; Site 3-▲, Mhlathuze Estuary; Site 4-o, Mhlathuze Pump Station and Site 5-◆, Felixton Bridge) during the study period (March 1998 to November 1999). The dashed line (KwaDlangezwa, January to March 1999) indicates missing data. During February 1999 samples could not be collected at Site 1, due to inaccessibility of the sample area due to rain.

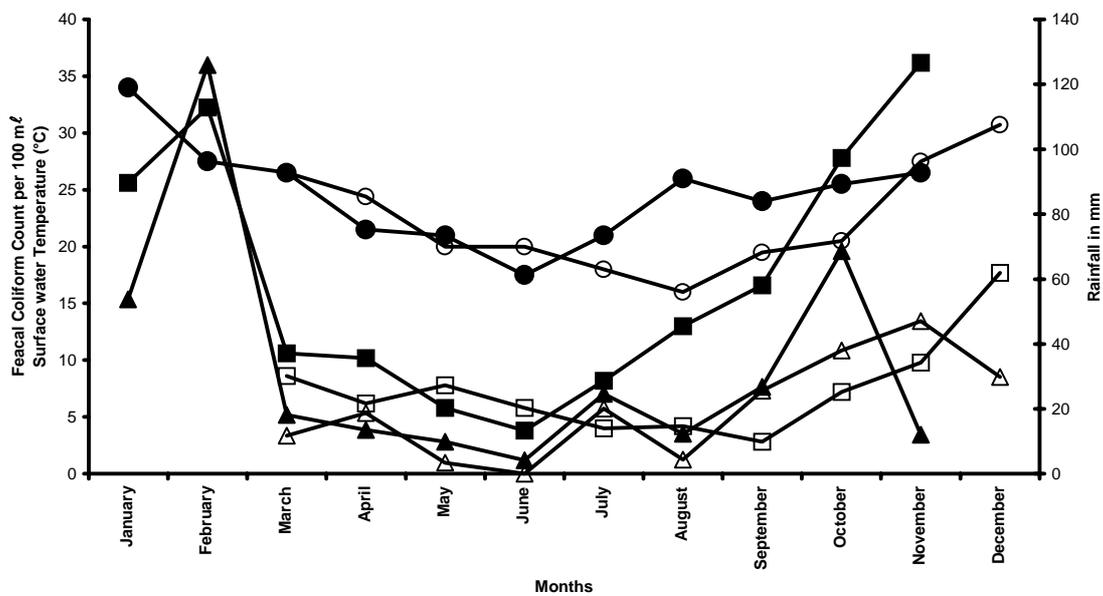


Figure 4

Comparison of the mean faecal coliform count (98, □; 99, ■), surface water temperature (98, O; 99, ●) and mean rainfall (98, △; 99, ▲) in the Mhlathuze River between 1998 and 1999 during the period of study. The monthly values are the mean values of biweekly sampling of the five sampling sites, of the study.

coliform). Similar analysis for rainfall figure changes and bacterial count changes also showed a positive relationship ($r = 0.646$ for total coliform and $r = 0.622$ for faecal coliform). These values were significant ($p < 0.05$).

E. coli, *Enterobacter* spp. and *Pseudomonas* spp. were constantly detected at all five sampling sites. *Klebsiella* spp., *Proteus* spp., *Enterobacter* spp., *Serratia* spp., *Aeromonas hydrophila* and *Citrobacter freundii* were also occasionally isolated from the various sites. These species were observed at the Felixton Bridge (Site 5). There were no *Salmonella* spp. or *Shigella* spp. detected in the Mhlathuze River during the study period.

Discussion

The Mhlathuze catchment supports a rapidly growing agricultural and industrial community in Northern KwaZulu-Natal, South Africa (Steyl et al., 2000). With increasing demands on water resources and contamination from industrial waste and human activities, the potential outbreaks of water-borne diseases continue to grow. Insufficient data on the population of human pathogens and indicator micro-organisms for the Mhlathuze catchment and surrounding areas motivated this study. The intention of this study was to generate more extensive microbial data for this important

environmental reserve.

All results include heterotrophic bacteria counts, total- and faecal-coliform counts, temperature, pH and rainfall figures at five different sites of the Mhlathuze River and demonstrate seasonal patterns for the period March 1998 to November 1999 (Fig. 1). There was a two- to fourfold increase in bacterial numbers for corresponding periods in 1998 to 1999 (Table 1, Figs. 2, 3 and 4). This important observation coincided with the higher mean surface water temperatures and increased rainfall during 1999. Strong correlation was demonstrated between bacterial counts and rainfall figures (total coliform $r = 0.646$ and faecal coliform $r = 0.622$). A stronger correlation existed between bacterial counts and surface water temperature (total coliforms $r = 0.805$ and faecal coliforms $r = 0.678$). The mean surface water temperature (constantly between 20-34 °C) of the Mhlathuze River during the summer period is ideal for the prolonged survival of coliform bacteria originating from the intestines of warm-blooded organisms (Nübel et al., 1999; Lobitz et al., 2000; Nishiguchi, 2000). In addition to the high surface water temperature, runoff from high rainfall may have influenced the increased number of bacteria detected during the summer period. The correlations between surface water temperature, rainfall and bacterial counts, specifically *E. coli*, and other indicator micro-organisms observed in this study are significant ($p < 0.05$) and are strongly supported by other studies (Byamukama et al., 2000; Solo-Gabriele, 2000). Studies on heterotrophic, autotrophic and other bacterial types showed that temperature, light, salinity, rainfall, predation, available nutrients and environmental pollutants are the key factors that influence bacterial growth and abundance in water bodies (Pernthaler et al., 1998; Lobitz et al., 2000; Solo-Gabriele et al., 2000).

Solo-Gabriele et al. (2000) demonstrated that the cyclic variations in *E. coli* counts in water could also be ascribed to runoff due to storms. The present study also shows a direct relationship between elevated faecal and other indicator micro-organisms and changing meteorological conditions in the Mhlathuze River. Although this study did not measure the effect of soil on the faecal and total coliforms of the water, it recognises that soil and runoff after rains may have had an influence on the bacterial numbers detected. The Mhlathuze River is in a subtropical area and has high rainfall (mean total rainfall = 1092.6 mm, CCWR). A high water content of the soil and moderate to high ambient temperatures would provide favourable conditions for the soil to act as a reservoir for coliform bacteria and to provide conditions which are favourable to allow contaminated soil runoff to influence seasonal bacterial count variations. High levels of these bacterial types pose a potential risk for contracting human intestinal pathogens.

A diarrhoeal outbreak was observed within parts of the Mhlathuze River reserve in July 2000. This episode was followed by a severe cholera epidemic. It may be argued that the observed surface water temperature increase between 1998 to 2000 and the cholera outbreak of 2000/2001 were coincidental and that the *Vibrio cholerae* was introduced into the Mhlathuze River due to human movement. The influences of higher surface water temperature and the increased rainfall observed in this study should not be underestimated. Lobitz et al. (2000) used sea surface temperatures in addition to the sea surface heights and phytoplankton blooms as one of the factors to predict cholera outbreaks. It may be important to monitor events/phenomena (such as prolonged increased surface water temperature) that provide favourable conditions for rapid *Vibrio cholerae* growth (Lobitz et al., 2000; Nishiguchi, 2000). Such information may be useful as early warning signals to predict possible future cholera outbreaks.

Felixton (Site 5) was considered to be the most contaminated

during the study period. Human activities around this site were also the highest throughout the study period. These activities included the cultivation of large informal gardens, the use of the bridge adjacent to the sample area as a thorough-fare and absence of sanitation facilities. The Felixton site is also adjacent to a paper pulping factory, a sugar mill and is downstream from a sewage treatment plant outlet. These factors could have contributed, independently or in combination, to the very high levels in bacterial counts that were observed at this sampling site.

Mean faecal and total coliform counts throughout the study period were generally very high, but lower than those observed in other studies (Pretorius, 2000; Byamukama et al., 2000; Goni-Urriza et al., 2000). According to the DWAF (1996) guidelines, the bacteriological quality of the water of the Mhlathuze River posed an increased risk of infectious disease transmission to the communities that were dependent on the river for household, recreation and other purposes. According to the report to the Institute For Water Quality Studies of DWAF (IWQS, 2000) this catchment area (W12 C-J) is high on the National Microbial Water Quality Monitoring Programme's priority list (14th in a total of 120).

No pathogenic *E. coli* strains and other water-borne pathogens, such as *Vibrio cholerae*, *Salmonella* sp. or *Shigella* sp., were detected in the samples from the Mhlathuze River during the study period. The micro-organisms isolated from the river were mainly *Enterobacteriaceae*, *Pseudomonas* spp. and *Aeromonas hydrophila*. These micro-organisms are commonly isolated from river water and soil (Goni-Urriza et al., 2000). *Aeromonas hydrophila*, is associated with gastroenteritis and *Pseudomonas aeruginosa*, (although not specifically identified in the present study) on the other hand, affects patients that are immuno-compromised and ones with metabolic and haematological disorders (Pearson et al., 2000). The presence of the opportunistic pathogens as well as *Klebsiella* spp. may have serious implications for the consumers of the water directly from the river, especially for young children, the elderly and those infected with HIV/AIDS. According to statistics of the Department of Health of South Africa, KwaZulu-Natal has an HIV prevalence rate of 32.5 %, the highest in South Africa. In 1999 the Jozini/Empangeni region had 30% of the state hospital bed space occupied by HIV patients (Health Department, Report, 2000).

A microbial population study along the eastern/coastal area of the Mhlathuze catchment, which includes a detailed microbiological and physico-chemical evaluation, is in progress. The aim of this study is to determine the impact of the surrounding communities (rural, urban, agricultural and industrial) on the microbial quality of this important water source in Northern KwaZulu-Natal, South Africa. It is anticipated that the present and future studies will provide important information on sources of bacterial contamination; effects of discharged chemicals (pesticides, antibiotics, fertilisers etc.) and the effects of mining (sand and gravel mining for construction purposes as well as mining for metals and heavy minerals) on the microbial population characteristics. Data available to date show a correlation between antibiotic resistance patterns of bacteria from the Mhlathuze River and those isolated from stools of diarrhoea patients (Bezuidenhout et al., 2001).

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