Pol. J. Environ. Stud. Vol. 21, No. 5 (2012), 1349-1358

Original Research

Groundwater Quality in a South African Rural Community: A Possible Threat to Public Health

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Received: 5 July 2011
Accepted: 12 January 2012

Abstract

Access to clean and potable water is a great challenge in most rural areas of South Africa's Mpumalanga Province. The aim of this study was to conduct a preliminary investigation to determine whether the quality of the groundwater supply poses a possible threat to the health of these communities. Groundwater samples collected from 100 boreholes in rural areas of Mpumalanga were subjected to culture-based methods and molecular analysis targeting 16S RNA gene. The physical and chemical contents were also determined. The results of the study revealed that all the physico-chemical parameters were within the limits set by the national guidelines for domestic purposes, with the exception of magnesium, calcium, fluorides, nitrate, and turbidity. Seventy percent of the boreholes complied with the fluoride limit (0-1 mg/l), 98% with the nitrate (0-6 mg/l as N), 85% with the magnesium (0-30 mg/l as Mg), 68% with the calcium (0-32 mg/l as Ca, and 52% with turbidity (<1 NTU). The results also indicated that 78% and 81% of the boreholes did not comply with the limits set by the national guidelines in terms of fecal (0 cfu/100 ml) and total coliforms (0-5 cfu/100 ml), respectively. Of 100 boreholes, molecular study revealed the presence of *Citrobacter freundii* in 35%, *Serratia marcescens* in 19%, *Bacillus cereus* in 11%, *Enterobacter cloacae* in 9%, *Salmonella enterica* in 7%, and *Pseudomonas maltophilia* in 7%. *Escherichia coli* O157:H7, *Shigella flexineri*, *Klebsiella oxytoca*, and *Cronobacter sakazakii* were found in 1% of 100 boreholes. The results of this study were conclusive evidence that some groundwater supplies in rural areas of Mpumalanga pose a serious threat to the health of consumers.

Keywords: groundwater, microbiological quality, physicochemical quality, public health

Introduction

An adequate supply of fresh and clean drinking water is a basic need for the entire human race, yet it has been observed that millions of people worldwide are deprived of this service. It is estimated that 1.2 billion people across the world do not have access to safe drinking water [1]. A report by the World Health Organization [2] shows that approximately 1.6 million deaths can be attributed to unsafe water and sanitation, including a lack of hygiene.

South Africa has a population of 49.4 million people, 52% of whom are estimated to be living in rural areas [3]. Of this part of the population, 6.0 million still do not have access to a reliable source of drinking water [4], although the South African constitution states that every citizen has the right to be supplied with clean, safe drinking water [5]. This implies that a large number of communities in rural areas depend on untreated surface and groundwater sources

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for their daily water needs. Water from these sources is faecally contaminated and devoid of treatment [6, 7].

Groundwater is a vital natural resource for the provision of water to South African communities for domestic, industrial and agricultural purposes. Currently, groundwater contributes between 13% and 15% of the total available water in South Africa [8]. It is estimated that approximately more than 400 rural communities in South Africa depend on groundwater sources for domestic purposes [8]. In Mpumalanga Province the rural communities, which include villages, farmers, and formalized towns, rely mostly on groundwater for their daily needs, and this water source is used directly by these communities without any prior treatment. However, the groundwater sources can be contaminated by chemical and microbiological pollutants originating from natural sources, human activities, and on-site sanitation systems used in rural areas.

Chemical pollution of groundwater sources can include nitrate, fluoride, and trace metals, especially arsenic, sulphate, or chloride, which could have detrimental effects on the health of users [9]. Microbiological pollution is caused by bacteria, viruses, and protozoa. The major health risk associated with groundwater is from the microbial pathogens derived from human and animal faeces [10]. Pathogenic organisms found in groundwater with high counts of faecal coliforms include especially *Escherichia coli* and/or other pathogenic microorganisms such as *Vibrio cholerae*, *Aeromonas hydrophila*, *Shigella dysenteria*, *Salmonella tyhimurium*, *Pseudomonas* spp, and *Klebsiella* spp [7, 11, 12], and these organisms contribute to waterborne diseases.

The transmission of diseases by polluted water has a long history and remains a worldwide problem to this day. Diarrhoea is a symptom of these waterborne diseases, although not all cases of diarrhoea are related to water [13, 14]. Diarrhoeal diseases due to contaminated drinking water result in 2.5 million childhood deaths yearly [15]. Sporadic outbreaks of waterborne diseases such as cholera, typhoid fever and dysentery have been recorded due to polluted groundwater, with serious public health implications and risks for users [16-18].

Outbreaks of cholera and typhoid infections in the South African provinces of Mpumalanga, KwaZulu-Natal, and the Eastern Cape have been reported since 2000 [19]. By the end of 2003, the cholera outbreak had spread to eight of South Africa's nine provinces, with 106,389 reported cases of cholera and 229 reported deaths [20]. The majority of the reported cases and deaths occurred in rural communities of KwaZulu-Natal and the Eastern Cape. In September 2005 a typhoid and diarrhoea outbreak due to contaminated groundwater in Delmas in Mpumalanga Province caused a month-long health crisis. A total of 3,000 people were diagnosed with diarrhoea and 561 with typhoid infections, and five deaths occurred, according to official figures. However, the community claimed that more than 49 deaths were caused due to typhoid and diarrhoea [21, 22].

The risk posed by microbial pathogens in water necessitates monitoring water for various types of microbial pathogens. Faecal coliform bacteria have been widely used as indicators of water contamination by humans and other warm-blooded animals [23], and have been included in water quality standards in different parts of the world [24-27]. Particular attention has been given to the survival of faecal indicator bacteria for sanitary reasons [25]. The WHO and the SANS 241 recommend the measurement of *E. coli* in drinking water samples as the best indicator of water quality. The WHO guidelines and the SANS 241 state that potable water must contain less than one *E. coli* per 100 ml of drinking water [26, 27].

In most developing countries, the detection and identification of a microbial species in water are conventionally based on the isolation of the microorganisms in pure culture and the examination of their morphological and physiological properties. The limits of conventional culture-dependent and phenotypic characterization are nowadays seen as a severe constraint for precise and reliable diagnostics of microbial pathogens. With the scientific progress in the field of genetic and molecular biology, a variety of DNA, RNA, and protein-based methods have been developed. The use of molecule-based technologies in microbial diagnostics has greatly enhanced the ability of researchers to detect and quantify pathogenic bacteria in water.

In this study, conventional and molecule-based techniques were used to determine whether the microbiological quality of the groundwater supply to the rural communities of Mpumalanga Province poses a possible threat to the health of consumers. The physical and chemical quality of this water supply also was determined. The intention of this study was to provide information that could assist water authorities in addressing problems with the management of groundwater systems in South Africa.

Materials and Methods

Study Area and Sampling Points

Mpumalanga Province is located in South Africa, bordered by Mozambique and Swaziland in the east and Gauteng in the west. It is situated mainly on the high plateau grasslands of the Middleveld, which roll eastwards for hundreds of kilometers. In the northeast, it rises toward mountain peaks and terminates in an immense escarpment. In some places, this escarpment plunges hundreds of meters down to the low-lying area known as the Lowveld. The best-performing sectors in the province are mining, manufacturing, and agriculture. The province has a total population of more than 3.5 million, 42% of whom live in rural areas and depend on groundwater as their main source of drinking water [28]. The bulk (65%) of the water available in Mpumalanga comes from surface water resources. Water transfers into the province constitute 19% of the total water available. Groundwater contributes 6% of the available water and the return flows from mining, industrial, irrigation, and urban sectors contribute 10% [28]. Groundwater sources are surrounded by weathered rock, dolomitic or karst, and alluvial aquifers. During the study period, none

Table 1. List and locations of the boreholes surveyed in Mpumalanga.

Municipalities surveyed	Number of boreholes	Locations of boreholes
Delmas	10	C1, C2, C3, C4, A4, A7, A3, BOT6, BOT 4, and D10
eMalahleni	6	Leeufontein, Kendal, Emakhosi, Borax 1 & 2, Legdaar Farm 78
Emakhazeni	6	Mooifontein, Nhlupheko P.S., Sanbery and Belfast 1, 2 & 3
Mbombela	18	Katoen, Mphatheni Mahushu, Mphatheni Guesthouse, White River 73B, White River 73A, White River 53, White River 60, Heide Eggs, Mtimba B, Malapa Farm, Heidelberg Farm 7, Heidelberg Farm 25, Heidelberg Tevrede, Moegeploeg Plot 36, Moegeploeg Plot 37, Weltevreden Plot 455A, Weltevreden Plot 455B, and Weltevreden Plot 43
Nkomazi	15	River View P.S., Beyers Farm 1 & 2, Baundi Farm, Mpapani Farm, Bambinyati Farm Plot 19, Bambinyati Farmhouse, Boland Farm, Malelani Farm, Eskom Distribution, Lilly Pond, Voorspoed Farm, Voorspoed Commonage 1 & 2, and Elenberg Komatipoort Farm
Albert Luthuli	11	Travelpoort Garage Badplaas, Rooihoogte Farm B16, Elstone Farm, Mosley Farm 13, Mosley Farm 17, Mosley Farm 27, Natal Drift Plot 13, Pullenshope, Drankenstein, and Bosmansfontein 1 & 2
Mkhondo	19	Groenvlei-Buhleni Farm, Vezinyaho 1, 2 & 3, Weeber Farm Piet Retief, Goedetrouw 1 & 2, Driehoek, Anyspruit, Ematafeleni, Kwa-Ngema 1 & 2, Zeelie Farm, Dirkiesdorp, Dirkiesdorp Farm, St Helena Farm, Themba Trust School, Injabulo Combined School, and Dirkiesdorp Police Station
Pixle Ka Seme	15	Goededorp Amersfoort, Piet Zyn Drift Farm, Langspoort Volksrust, Wenber Wakkerstrroom, Pietskop Wakkersttroom 1 & 2, Wakkerstroom plot 40, Uithenden Wakkerstroom, Kranspoort, Heins Farm Resort, Drinkwater 1 & 2, Tafelkop, Rietvlei, and Winkelhaak

of the boreholes was measured for depth. Water from groundwater sources is distributed directly to communities without any purification.

Groundwater samples were collected from 100 boreholes located in the villages of the Nkangala district municipality (which includes the Delmas, eMalahleni, and Emakhazeni municipalities); the Gert Sibande district municipality (Mkhondo, Pixle Ka Seme, and Albert Luthuli), and the Enhlanzeni district municipality (Mbombela and Nkomazi) in Mpumalanga (Table 1). The eMalahleni communities obtain underground water from boreholes using a rotary hand pump connected to a standpipe, while the Delmas, Emakhazeni, Mkhondo, Pixle Ka Seme, Albert Luthuli, Mbombela, and Nkomazi communities obtain their drinking water directly from standpipes connected to boreholes. Although the boreholes are covered, some are surrounded by animal excreta and others are located close to pit latrines. Seventy-six of the boreholes are privately owned (n=76), and the remaining 24 are communal boreholes (n=24).

Collection of Water Samples

Water samples from the abovementioned sources were collected between September and November 2008 in a once-off sampling exercise. The standpipes or the taps in the operation room were flushed for approximately 5 min. before collecting samples using 2l sterile glass bottles. All the bottles were sealed and properly labeled. A mobile laboratory containing all necessary equipment (a membrane filtration unit, a vacuum pump, kettles, portable incubators, sterile Petri dishes containing selective cultural media, sterile membrane filters, a pH meter, thermometer, turbidity meter, conductivity meter, etc.) was used for the on-site analysis of water quality. The plates containing coliform isolates and samples for chemical analysis were then placed in ice bags and transported to the Tshwane University of Technology Water Research Group laboratory for analysis.

Water Quality Variables

The water quality tools used to measure the environmental health risk in this study were the South African National Standard [27] and the South African Water Quality Guidelines for domestic use [25]. Molecular identification of the coliform isolates was also used as a proxy measure that confirmed the possible threat that the microbial quality of groundwater poses to public health in rural Mpumalanga communities.

pH and turbidity were measured on site using a pH meter (Metrohm Co. Model 713) and a microprocessor turbidity meter (Eutech Instrument Turbidimeter TN-100), while temperature and total dissolved solids (TDS) of the water samples were determined using a conductivity meter (Hach Co. Sension7). The concentrations of nitrates, fluoride, sulphate, and chloride were determined in the laboratory using a Spectroquant Nova 400 manual water analyzer (Merck) and photometric test kits (Merck), while the concentrations of magnesium, sodium, calcium and potassium in water samples were determined by atomic absorption spectrophotometry (SpectrAA 220FS), according to the standard method [23].

The initial microbiological analysis of water samples performed on site was limited to total and faecal coliforms. The membrane filtration technique, the Chromocult coliform agar (Merck), and the M-FC agar (BioLab) were used for the enumeration of coliforms. Water samples were analyzed for this group of indicator bacteria using internationally accepted techniques and principles. The physicochemical and microbiological water quality parameters were then compared to the standards set by [27] and DWAF Water Quality Guidelines for Domestic Use [25].

Molecular Identification of Coliform Isolates

For the identification of bacterial isolates, individual coliform colonies from water samples were randomly selected from different plates based on their size, shape, and color. They were transferred onto chromocult coliform agar (Merck) by the streak plate technique and incubated at 35±2ºC for 24 h. The colonies were further purified by the same methods at least three times using the same medium (Biolab) before Gram staining. Oxidase tests were then done on those colonies that were Gram negative. The oxidase-negative colonies were transferred onto nutrient agar slants, incubated at 35±2ºC for 24 h, and kept at 4ºC until further use.

Extraction of the Total Genomic DNA

A total of 60 oxidase-negative isolates were used for molecular study. Individual isolates were grown in nutrient broths, followed by incubation at 35 ± 2ºC for 24 h. The inoculated broths (1 ml) were centrifuged at 13,300 g for 5 min. The pellets were washed twice with sterile molecular-grade water. The total genomic DNA from the bacterial pellet was extracted using the DNeasy DNA purification kit (QIAGEN) according to the manufacturer's instructions. The quality and quantity of the isolated nucleic acids were determined using the NanoDrop™ 2000 spectrophotometer (Thermo scientific) and agarose electrophoresis (BioRad).

Amplification of the 16S rRNA Gene

Eubacterial universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') [29] and 1507R (5'-TACCTTGTTACGACTTCACCCCA-3') [30] were used in PCR reactions for the amplification of the 16S rRNA gene of each of the isolates. The PCR reaction mixtures contained 12.5 µl DreamTaq Master mix (2x) (Fermentas, 140 St. Leon-Rot, Germany), 0.5 µl of each primer (10 pmol), 8.5 µl of nuclease-free water (Fermentas, 140 St. Leon-Rot, Germany), and 5 µl of DNA template. The PCR reaction mixtures were placed in an MJ MINI thermal cycler (BIORAD), and the following thermal cycling conditions were used: pre-denaturation for 10 min, followed by 35 amplification cycles of denaturation at 94ºC for 30 s, annealing of primers with template DNA at 55ºC for 30 s, and primer extension at 72ºC for 30 s. This was followed by a final extension at 72ºC for 7 min. The PCR amplicons were resolved through electrophoresis of 1% (w/v) agarose gel stained with ethidium bromide, followed by visualization under ultraviolet light. The low-range Fast Ruler (Fermentas) was included in all gels as a size marker. All results were captured using a gel documentation system (Syngene, Cambridge, U.K).

Restriction Analysis of PCR Amplicons

In order to select representative isolates for sequencing, all PCR amplicons were subjected to restriction fragment length polymorphism (RFLP) analysis. For this purpose, 10 µl of the 16S rRNA amplicons was digested with *Taq1* and *Cs6pI* (Fermentas) according to the manufacturer's instructions. The restriction digests were resolved through electrophoresis of conventional 1.5% (w/v) agarose gel stained with ethidium bromide, followed by visualization under ultraviolet light. The hyperladder 1 (marker) 100 lines (Bioline) was included in all gels as a size marker. All results were captured using a gel documentation system (Syngene, Cambridge, U.K.). The restriction patterns were determined manually and for every five similar profiles, the selected isolate was then ready for sequencing.

Sanger Sequencing of the 16S rRNA Gene

After grouping the isolates using the PCR-RFLP, the genomic DNA from 32 representative water samples was amplified using the existing 27 F and 1507 R primers as described above. The 1500 PCR amplicons were further studied by conventional Sanger (dideoxy) sequencing in both directions using 27 F and 1507 R primers. For this purpose, BigDye for ABI 3130XL was used according to the manufacturer's instructions and the gel was run on a 3130XL sequencer. All the sequences were inspected and manually corrected using Bioedit v.5.0.9 (33) software. For preliminary identification of the bacterial isolates, the corrected sequences were then compared to those in the National Centre for Biotechnology Information [31] using *blastn*.

Results and Discussion

Physical and Chemical Characteristics of the Groundwater Samples

The physical characteristics of the groundwater used by rural Mpumalanga communities between September and October 2008 are summarized in Table 2. Generally, the results of the analyses revealed that water samples collected from boreholes in all local municipalities were within the recommended limit for no risk in terms of pH (5-9.5), temperature (1-25ºC), and TDS (500 mg/l) [25, 27]. The average values ranged between 7.4 and 8.0 for pH, between 19.0 and 24.9ºC for temperature, and between 107.1 and 499.5 mg/l for TDS. The results suggest that the physical quality of boreholes did not have any negative effect on the health of consumers [25]. The turbidity levels of water samples ranged between 0.1 and 20.3 NTU and 52% of the

Table 2. Physical quality of borehole samples (mean value) analyzed in Mpumalanga Province during the study period.

Local municipalities	pH value	Temperature ºC	Turbidity, NTU	Total dissolved solids, mg/ℓ
Delmas	7.4 (±0.40)	19.4 (±0.44)	1.2 (±1.42)	224.5 (±73.26)
eMalahleni	7.1 (±0.40	20.4 (±0.48)	3.8 (±3.58)	439.0 (±73.20)
Emakhazeni	7.8 (±0.16	22.9 (±0.77)	6.6 (±4.00)	158.0 (±52.26)
Mbombela	7.6 (±0.27)	23.8 (±2.00)	5.1 (±0.48)	116.9 (±75.08)
Nkomazi	8.0 (±0.28)	24.6 (±1.25)	0.7 (±0.31)	393.6 (±95.34)
Albert Luthuli	7.6 (±0.14)	24.9 (±2.66)	1.9 (±0.24)	171.1 (±79.49)
Mkhondo	7.5 (±0.46)	24.1 (±1.18)	2.2 (±0.83)	107.1 (±56.46)
Pixle Ka Seme	7.6 (±0.25)	24.9 (±0.95)	1.7 (±0.13)	284.4 (±96.67)

borehole samples were within the recommended limits (0-1 NTU) for potable water [25, 27]. These water samples were collected from Emakhazeni (2%), eMalahleni (4%), Delmas (6%), Mbombela (6%), Albert Luthuli (7%), Mkhondo (8%), Pixle Ka Seme (8%), and Nkomazi (11%). The highest concentration of 20.3 NTU was found in the eMalahleni (Kendal) local municipality. Turbidity is caused by the presence of suspended clay, silt, organic matter, inorganic matter, plankton, and other microscopic organisms [25]. The measurement of turbidity gives only an indication of the extent of pollution [12]. High turbidity levels are associated with poor water quality and promote the survival of microorganisms [25].

Although variations were found in the chemical characteristics of the groundwater samples, the concentrations of chloride and sulphate were within the limits set by the national guidelines for domestic purposes [25, 27] for all water samples, while the magnesium, calcium, fluoride, and nitrate concentrations in some boreholes exceeded the recommended limits (Fig. 1). In general, the concentrations of chloride and sulphate in water samples ranged from 22.84 to 55.94 mg/l and from 21.06 to 84.31 mg/l, respectively. The nitrates and fluoride concentrations in the borehole samples ranged from 0.45 to 7.27 mg/l as N and from 0.46 to 1.55 mg/l, respectively (Fig. 1). The nitrate concentrations for most of borehole samples compiled with the limits for no risk (0-6 mg/l as N), except for two boreholes at eMalahleni (Leeufontein and Emakhosini) where nitrate concentrations were higher (13.4 and 46.1 mg/l as N) than the recommended limits (Fig. 1). High nitrate-nitrogen concentrations in groundwater might occur due to the leaching of on-site sanitation, animal and agricultural manures. High nitrate levels in groundwater sources have been noted in urban and rural areas of other provinces of South Africa, namely the Northern Cape, the North West, and Limpopo provinces [32]. Nitrate concentrations above the recommended value of 6.0 mg/l [32] have been reported to be dangerous to pregnant women and pose a serious health threat to infants younger than three to six months of age, because of its ability to cause methamoglobin, or "blue baby syndrome," in which blood loses its ability to carry sufficient oxygen and it is also reported to be carcinogenic [32, 33]. Furthermore, high nitrate levels can be used as a crude indicator of faecal pollution where microbiological data are unavailable, due to the fact that nitrate is used by microorganisms as a food resource [34]. Seventy percent of borehole samples complied with the fluoride limit of 0-1 mg/l for no risk. Of the remaining 30% of boreholes that had higher fluoride concentrations than the recommended limits of 0-1 mg/l; 11 boreholes were located at Mbombela; six at Nkomazi; five at Delmas; three at Albert Luthuli, eMalahleni, and Emakhazeni; and two at Mkhondo (Fig. 1). Chronic endemic fluorosis caused by an excess of fluorides in drinking water affects the calcification of the teeth, resulting in what is commonly known as dental fluorosis [35]. Fluoride also has been implicated in higher rates of bone cancer (Osteosarcoma) [36].

As shown in Fig. 2, sodium and potassium concentrations for borehole samples from different local municipalities ranged from 5.59 to 50.6 mg/l and from 0.72 to 3.24 mg/l, respectively. The borehole samples were within the recommended limits for no risk of sodium (0-100 mg/l) and potassium (0-50 mg/l) [25]. The concentration of magnesium in water samples ranged between 17.31 and 76.87 mg/l. Eighty-five percent (85%) of borehole samples complied with the recommended limits of 0-30 mg/l for magnesium [25] and 15% of the boreholes had more than 30 mg/l magnesium in the water samples. Among the latter

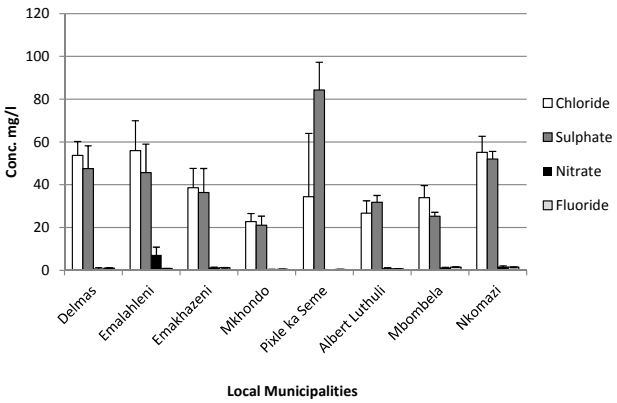

Fig. 1. Concentrations of chloride, sulphate, nitrate, and fluoride in eight local municipalities.

were six borehole samples located at Nkomazi, three at Pixle Ka Seme, two at Delmas and eMalahleni, and one each at Emakhazeni and Mbombela. Calcium concentrations in borehole water samples ranged from 12.52 to 42.56 mg/l, where the highest concentration was observed in the Delmas local municipality. Sixty-eight percent (68%) of borehole samples complied with the recommended limits of 0-32 mg/l, except for nine boreholes at Nkomazi, eight at Delmas, six at Pixle Ka Seme, four at Mbombela, three at eMalahleni, and two at Emakhazeni. A high magnesium concentration in drinking water results in a bitter taste, and excessive magnesium and sulphate intake results in diarrhoea [25]. Magnesium, together with calcium, is responsible for scaling problems caused by deposits of carbonates in appliances using heating elements and plumbing that transports hot water, and also for inhibiting the lathering of soap, which results in scum formation. This study calls for urgent intervention in the management strategies of groundwater sources for the removal of turbidity, fluorides, nitrate, magnesium, and calcium.

Microbiological Characteristics of Groundwater Samples

The Department of Water Affairs and Forestry guidelines for domestic use of water [25, 27] recommend that potable water must contain zero to five total coliforms and less than one *E. coli* per 100 ml drinking water, which translates a negligible risk of microbial infection. Of the 100 boreholes in this study, 78% had more than 5 cfu/100 ml drinking water sample. The highest coliform counts, 425 cfu/100 ml, were recorded in the Delmas and Mkhondo local municipalities (Fig. 3). Total coliforms comprise a heterogeneous group that includes bacteria from the genera *Escherichia*, *Citrobacter*, *Enterobacter*, *Klebsiella*, *Serratia*, and *Rahnella*. Although most of these bacteria belong to the family of *Enterobacteriaceae*, there is an indication of the possible presence of bacterial pathogens such as *Salmonella* spp. and *Shigella* spp. [25, 37].

The results indicate that 81% of the boreholes had less than one faecal coliform per 100 ml drinking water, while 19% of the boreholes were faecally polluted and did not comply with the recommended limits (Fig. 3). This implies that these water sources pose a serious health risk to consumers. The highest faecal coliform counts of 150 cfu/100 ml were observed in the Delmas local municipality. Faecal pollution of groundwater sources in certain areas of Mpumalanga Province, especially in Delmas, is a matter of concern, as this area has a history of waterborne outbreaks that so far have resulted in 3,000 cases of diarrhoea, 561 cases of typhoid infection, and five deaths, according to the official figures, while the community claims that 49 deaths were caused by typhoid and diarrhoea [21, 22].

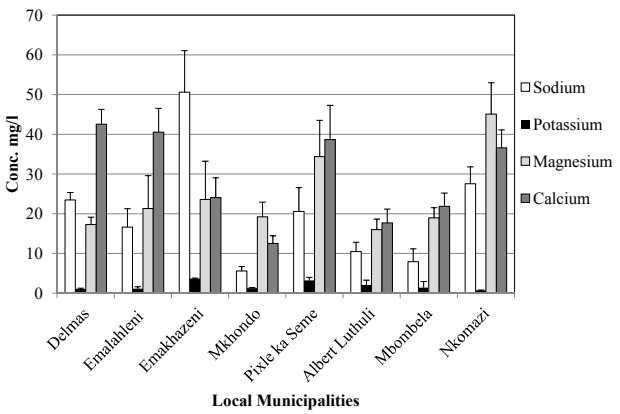

Fig. 2. Concentrations of sodium, potassium, magnesium, and calcium concentrations in eight local municipalities.

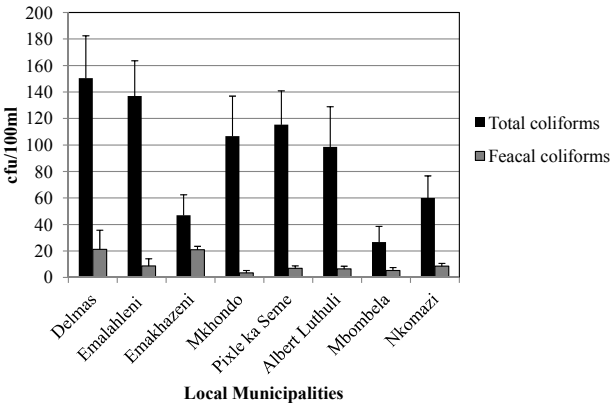

Fig. 3. Coliform counts of borehole water samples collected from eight local municipalities.

The conventional techniques used in this study gave only a simple indication of the faecal pollution of groundwater sources based on total coliform colonies that were detected in conjunction with faecal coliforms. Although these isolates might indicate the possible presence of bacterial pathogens such as *Salmonella* spp., *Shigella* spp., *Vibrio cholerae*, *Campylobacter jejuni*, *Campylobacter coli*, *Yersinia enterocolitica*, and pathogenic *E. coli* [25], the culture-based study could not result in the accurate identification of pathogenic bacteria that were present in water samples. In order to obtain an accurate detection of the range of bacterial pathogens from the groundwater samples, coliform isolates were subjected to a subsequent molecular analysis, since molecular tools for microbial diagnosis rely on the *in vitro* amplification of a DNA fragment and offer a higher level of the specificity of strain detection [38, 39].

The total genomic DNA of the isolates yielded a high amount of genomic bacterial DNA of good quality. The average concentrations of DNA were between 2.0 and 120.0 ng/µL. The feasibility of the selected primers and the size of amplicons were carried out with 60 DNA isolates extracted from pure cultures. The 16S rRNA eubacterial universal primers pair 27F and 1507R successfully amplified the targeted part of 16S rRNA gene from all the isolates, providing a single strain yielding a PCR product of the expected size (approximately 1,500 bp) (Fig. 4). A band of approximately 1,500 bp was visualized in all samples, confirming the amplification of the expected fragment. The gel showed the example of the amplified PCR product of

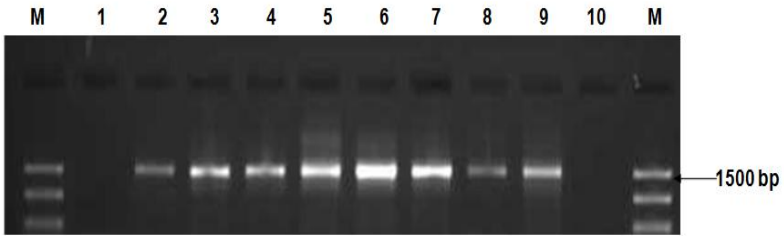

Fig. 4. An example of agarose gel electrophoresis for the amplified PCR product.

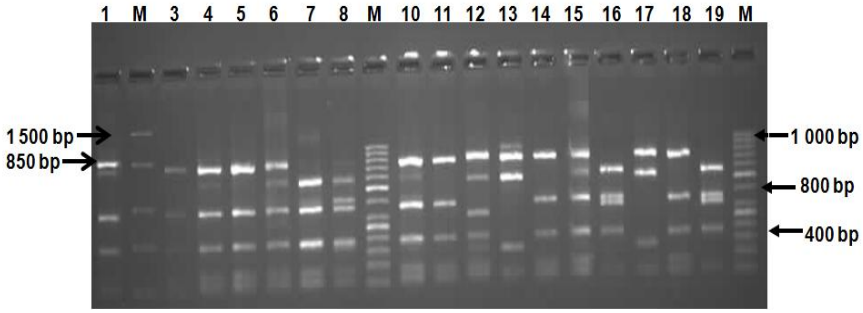

Fig. 5. Example of agarose gel electrophoresis for the restriction fragment profiles of the group-specific PCR products after digestion with *Taq1* enzyme. Lane M represents molecular weight marker.

the 16 isolates where Lane M represents the molecular weight marker (1,500 bp ladder). The products of restriction digestion are shown in Fig. 5. Using *Cs6pI* enzyme, four different species could be distinguished, while when using the *Taq1* enzyme, 10 different species could be distinguished. These PCR-RFLP types were collapsed into groups based on similarity. The 32 representative PCR amplicons were subjected to DNA sequencing with the original primers 27F and 1507R in both directions. The microorganisms found in NCBI Blast (Blastn) were *Serratia marcescens*, *Citrobacter freundii*, *Salmonella enterica*, *Bacillus cereus*, *Escherichia coli* O157:H7, *Shigella flexineri*, *Pseudomonas maltophilia*, *Enterobacter cloacae*, *Klebsiella oxytoca*, and *Cronobacter sakazakii*. Of the 100 boreholes, the molecular study revealed the presence of *Citrobacter freundii* in 35%, *Serratia marcescens* in 19%, *Bacillus cereus* in 11%, *Enterobacter cloacae* in 9%, *Salmonella enterica* in 7%, and *Pseudomonas maltophilia* in 7%. Each of the following microorganisms was found at a rate of 1% out of 100 boreholes: *Escherichia coli* O157:H7, *Shigella flexineri*, *Klebsiella oxytoca*, and *Cronobacter sakazakii* (Fig. 6).

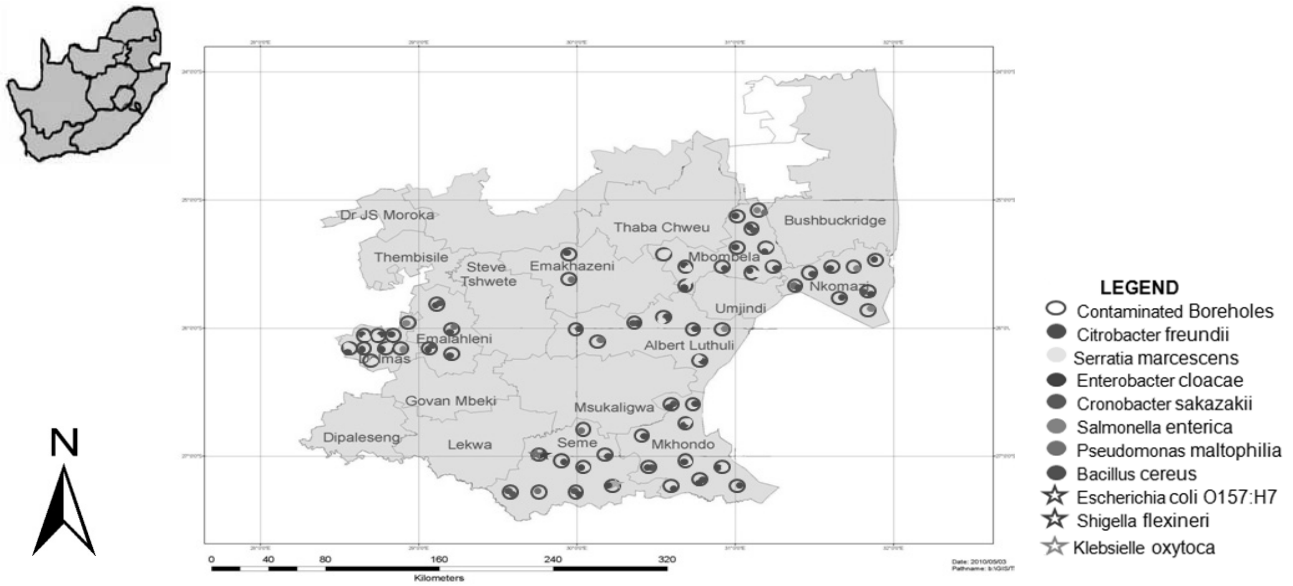

Fig. 6. Map of Mpumalanga Province indicating contaminated boreholes with microorganisms identified in the local municipalities of Delmas, eMalahleni, Emakhazeni, Mbombela, Nkomazi, Albert Luthuli, Mkhondo, and Pixle Ka Seme.

The above enteric bacteria are reported to be disease-causing microbes (pathogens) that can cause diarrhoea, cramps, nausea, headaches, or other symptoms. These pathogens may pose a special health risk for infants, young children, and people with severely compromised immune systems. These opportunistic pathogens are commonly found in the environment, mainly in soil, water, and sewage. The opportunistic pathogens like *E. coli* O157:H7, *Shigella flexineri*, *Salmonella enterica*, and *Bacillus cereus* are important causes of diarrhoea in the world [37, 40, 41]. A waterborne outbreak of *E. coli* O157:H7 took place in Scotland with 496 cases (272 laboratory-confirmed cases) and 19 deaths [42]. In South Africa, diarrhoea is responsible for about 20% of all deaths of one- to five-year-olds [43]. *Citrobacter freundii*, *Serratia marcescens*, *Cronobacter sakazakii*, and *Enterobacter cloacae* are known to cause a wide variety of nosocomial infections of the respiratory tract and urinary tract [44-47]. *Pseudomonas maltophilia* is a species of *Stenotrophomonas*, formerly called *Xanthomonas maltophilia*, which reduces nitrate. It is a cause of hospital-acquired ocular and lung infections, especially in patients with cystic fibrosis and those who are immunosuppressed [48]. *Klebsiella oxytoca* can cause systemic infections such as meningitis, adrenal haemorrhage, haemorrhagic colitis and septic shock [49, 50]. Outbreaks of *K. oxytoca* infections have been reported in newborn babies following colonization of their digestive tracts, in oncology patients following contamination of intravenous fluids, and in cardiac patients following contamination of invasive blood pressure monitoring devices [51-53]. The findings of this study, therefore, predict a possible threat for public health because of the quality of the groundwater supply in some rural communities in Mpumalanga Province.

Conclusions

The findings of this study have revealed that groundwater, the main source of drinking water in Mpumalanga Province, is not fit for human consumption in some rural areas. High concentrations of magnesium, calcium, fluoride, and nitrate, and the turbidity levels and especially poor bacteriological quality of this water source pose a serious threat to consumers. The detection of various opportunistic pathogens and pathogenic strains such as *Serratia marcescens*, *Citrobacter freundii*, *Salmonella enterica*, *Bacillus cereus*, *Escherichia coli* O157:H7, *Shigella flexineri*, *Pseudomonas maltophilia*, *Enterobacter cloacae*, *Klebsiella oxytoca*, and *Cronobacter sakazakii* indicates a potential risk of infections and waterborne diseases. This study therefore calls for urgent intervention for the protection of groundwater sources and implementation of drinking water treatment barriers ensuring the production of safe potable water in the province.

Acknowledgements

We thank the National Research Foundation of South Africa and Tshwane University of Technology for funding this study.

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