A scoping study on the environmental water (groundwater and surface water) quality and management in the North-West Province, South Africa

Report to the
WATER RESEARCH COMMISSION

by

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EXECUTIVE SUMMARY

BACKGROUND & RATIONALE

Water in the North West Province is obtained from ground and surface water sources. The latter are mostly non-perennial and include rivers and inland lakes and pans. Groundwater is thus a major source and is used for domestic, agriculture and mining purposes mostly without prior treatment. Furthermore, there are several pollution impacts (nitrates, organics, microbiological) that are recognised but are not always addressed. Elevated levels of inorganic substances could be due to natural geology of areas but may also be due to pollution. On the other hand, elevated organic substances are generally due to pollution from sanitation practices, mining activities and agriculture. Water quality data are, however, fragmented. A large section of the population of the North West Province is found in rural settings and most of them are affected by poverty. It is also generally recognised that because poorer communities may not have the financial resources or may not be able to contribute financially to local management agencies, such communities may have to be satisfied with water that is of poorer quality. The present scoping study was thus formulated against this background.

OBJECTIVES AND AIMS

AIM 1 To establish a forum with role players in the water sector in the NWP to facilitate data sharing on aspects of water quality, (particularly microbiological aspects) and obtain information regarding the general management (modern as well as indigenous knowledge systems) of water in the communities of the North-West Province.

AIM 2

To identify geographical areas where no or little water quality data is available and to develop a water quality monitoring framework for those areas focusing on issues that would constitute the greatest health risk.

AIM 3

To determine microbiological (bacteriological and mycological) and physico-chemical quality of environmental water (ground and surface) as well as water in storage facilities in the selected areas to be used as a pilot implementation study of the framework.

METHODOLOGY

A. Microbiological and physico-chemical analysis

Water samples were collected from surface and ground water sources. Physico-chemical parameters (pH, temperature, conductivity, nitrates) were determined on site using portable instruments. Levels of indicator bacteria as well as levels and diversity of moulds were determined in the laboratory using standard methods.

B. Setting up a team of role players, find available water quality data, determine management needs as well as development of a framework for water quality monitoring in the North-West Province.

RESULTS AND DISCUSSION

A general overview of water quality based on microbiological and physico-chemical results for selected ground- and surface water sources are provided. The results coincide with concerns that had been raised by various researchers and management groups in the recent past. Firstly, some physico-chemical results are presented and indicate that pH was within target water quality ranges for domestic and agricultural use. However, electrical conductivity and nitrate levels for several of the samples were very high and made the water unfit for human or animal consumption. Various water samples were also microbiologically of poor quality. Some of the samples were also positive for the opportunistic bacterial pathogen *Pseudomonas aeruginosa*. In addition to the bacterial species several filamentous fungi and yeasts were also isolated from the various water sources. Both these mycological groups included some opportunistic pathogenic species. This is cause for concern, considering that the groundwater sources are used for domestic purposes without prior treatment.

The bacteriological results reported were mainly concerned with detection of faecal coliforms and in particular *E. coli*. However, the membrane filtration approach and media used was not always effective for *E. coli* detection. A different medium was also tested. Preliminary results indicated that it could better distinguish *E. coli* from other faecal indicator species. Furthermore, to quickly determine identity of the *E. coli*, a polymerase chain based method is presently being investigated. Some preliminary results are also presented.

RECOMMENDATIONS FOR FUTURE RESEARCH

The study should be repeated and expanded. In addition to the groundwater a more detailed surface water study should also be conducted. Furthermore, the future groundwater study as part of K5/1966 must also include DEWA monitoring boreholes. This approach should provide data that could indicate a clearer scenario of which areas are the most vulnerable to pollution. Such areas will have to be scrutinized in more detail for the presence of pathogenic enteric bacteria and viruses.

As part of K8/853 a role-player meeting was convened. Most of the researchers attended this meeting that was held on 29 April 2010. At the meeting, a context for the interdependence of K8/853 and K5/1966 was provided. Various students and researchers presented either details of how they will contribute towards K5/1966 or preliminary results obtained. These were discussed and certain recommendations made. It was also recommended that K8/853 be concluded at the inaugural meeting for K5/1966.

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LIST OF ABBREVIATIONS

ATCC	American Type Culture Collection
Cfu	Colony Forming Units
COD	Carbon Oxygen Demand
DWA	Department of Water Affairs
DWAF	Department of Water Affairs and Forestry
FC	Faecal Coliforms
MLG	Membrane Lactose Glucuronide
NWA	National Water Act
NWP	North West Province
NWP-SoER	North West Province State of the Environment Report
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
Spp.	Species (plural)
TC	Total Coliforms
TDS	Total Dissolved Solids
TMV	Thymine Mineral Vitamin
TSI	Triple Sugar Iron
TWQR	Target Water Quality Range
YM	Yeast Malt

1 INTRODUCTION AND OBJECTIVES

1.1 General introduction and rational

The North West Province (NWP) is known as the platinum province for the wealth of the metal it contains underground. The region is also known for cultivation of crops such as mealies, sunflour, sorghum as well as livestock such as cattle and sheep. It is the country's fourth-smallest province, taking up 8.7% of South Africa's land area and with a mid-2006 population of 3.4-million people. The NWP is predominantly populated by a rural population, but towns and big cities such as Potchefstroom, Klerksdorp, Rustenburg, Brits, Lichtenburg, Vryburg and the capital, Mafikeng, also contributes to an urban population. To the west, the landscape is largely flat regions of scattered trees and grassland. Rainfall on average for the western region is less than 300 mm per annum, the central region receiving around 550 mm per annum, while the eastern and south-eastern region receives over 600 mm per annum (NWP-SOER, 2002). According to the state of the environment report of 2008, the rural community of the North West Province require about 70 million m^3 water per annum, from which 25 million m^3 per annum is used for domestic consumption and the remainder is used for live stock watering and other agricultural purposes (NWP-SOER, 2008; NWRS, 2004).

Water from the North West Province catchment areas and aquifers also support prosperous gold, platinum and chrome mining and related support manufacturing industries as well as its growing urban and rural populations. Cities and major towns in this province provide treated drinking water to their consumers. However, the source water within catchment area as well as the aquifers is exposed to pollution from the mentioned mining, agricultural and other anthropogenic activities (Kalule-Sabiti & Heath, 2008; Van der Walt *et al*., 2002). Heavy metals including uranium, mercury, arsenic, copper, chrome, vanadium, cadmium, lead, zinc show elevated levels in sediment, water and in tissues of aquatic animals of the Mooi River catchment area (Erdman, 1999; Venter, 2001).

In urban affluent communities in the North-west Province the health risk associated with water borne potential pathogens is virtually non-existent because these communities have access to a supply of disinfected water provided to them by reasonably well maintained distribution systems. In such communities the quality of the water supplied may be further enhanced by end-user reverse-osmosis and other water filtering systems (Bezuidenhout *et al*., 2010; Walter, 2010).

However, in South Africa and other developing countries, poverty and poor water quality are closely intertwined (Gerlach & Franceys, 2009; Schreiner & Van Koppen, 2002). The poor are typically deprived of the benefits of good quality water because they lack the technological assets and financial resources to assess the quality of the water and if necessary initiate remediation processes. They are thus forced to drink water, particularly surface water, of low quality (Gerlach & Franceys, 2009; Schreiner & Van Koppen, 2002).

The mining, industrial and agricultural activities of the land locked North-West Province contribute greatly to the economy of this province, and South Africa in general. This comes at a cost of environmental challenges (Cho *et al*., 2000; Crafford *et al*., 2004), including source water pollution, as recently summarised by Kalule-Sabiti and Heath (2008). Pollution from such economic activities could have had long term adverse effects on the health of the population of the North-West Province, particularly those that are less affluent (Crafford *et al*., 2004).

Although this area contributes greatly to the economics of the country, statistical estimates indicate that 62 % of its population is affected by poverty. This sector of the population is scattered in rural, peri-urban and urban communities. It is further estimated that more than 80% of these communities depend on groundwater and (in some cases) untreated surface water sources for their domestic water needs (NWP-SOER, 2002). These communities have on-site sanitation facilities and livestock may also be kept close to households. Such practices may negatively impact on the water quality (Cho *et al*., 2000; Crafford *et al*., 2004; Fourie & Rynefeld, 1995)

Health statistics for the province indicates health burden increases in the economically marginalised group. For instance, in 1998 the incidence of diarrhoea among children younger than 5 years was 5th highest among the nine provinces of South Africa. In 2000 these statistics (Figure 1) showed that diarrhoea among this age group has increased and was the highest in the country. The situation has improved since then but for the years 2001, 2003 and 2005 it was still the 3rd highest in South Africa. Direct links with consumption of poor water quality and diarrhoea has long been established (Barrel *et al*., 2000; Pedley & Howard, 1997) and demonstrated in the South Africa (Gaoganediwe, 2006; GCIS, 2005; Hemson & Dube, 2004). Children, the elderly and immunocompromised individuals are the most vulnerable within society and all effort should be made to ensure that they obtain safe drinking water (Nwachuku & Gerba, 2004).

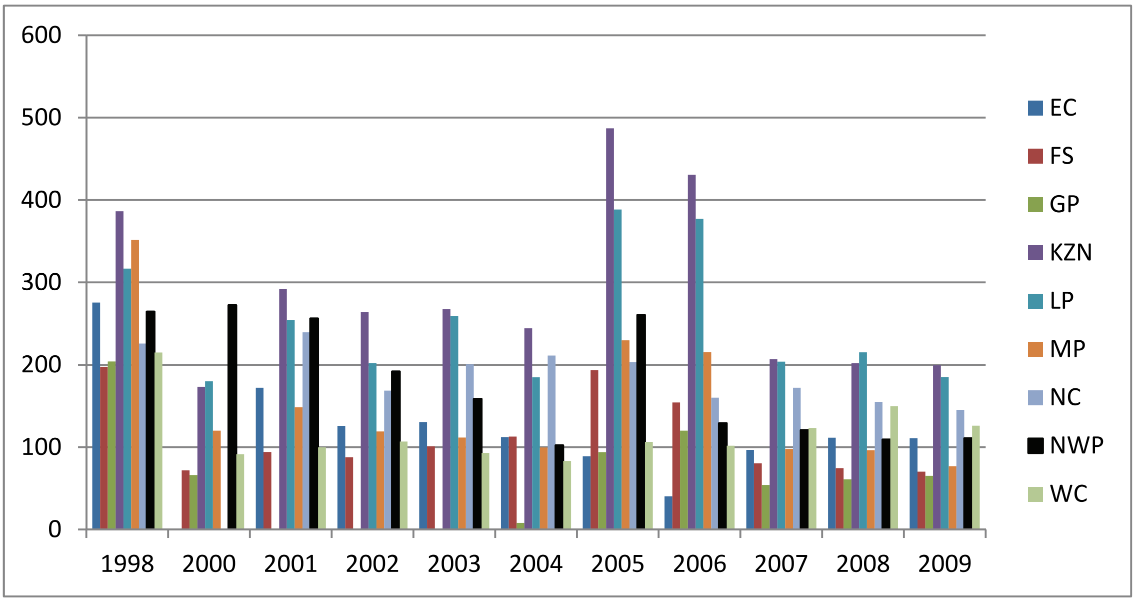


Figure 1: Incidence of diarrhoea among children under 5 years (per 1000) ; http://www.healthlink.org.za/healthstats/132/data). EC: Eastern Cape FS: Free State GP: Gauteng KZN: KwaZulu-Natal LP: Limpopo MP: Mpumalanga NC: Northern Cape NW: North West WC: Western Cape.

The province has also seen a general increase in the prevalence of HIV (Figure 2). It increased from less that 2 % of the population in 1994 it increased to over 10 % by 2009. This figure is expected to stabilize. Provision of good quality domestic water will be critical in establishing such a maturation rate for HIV prevalence.

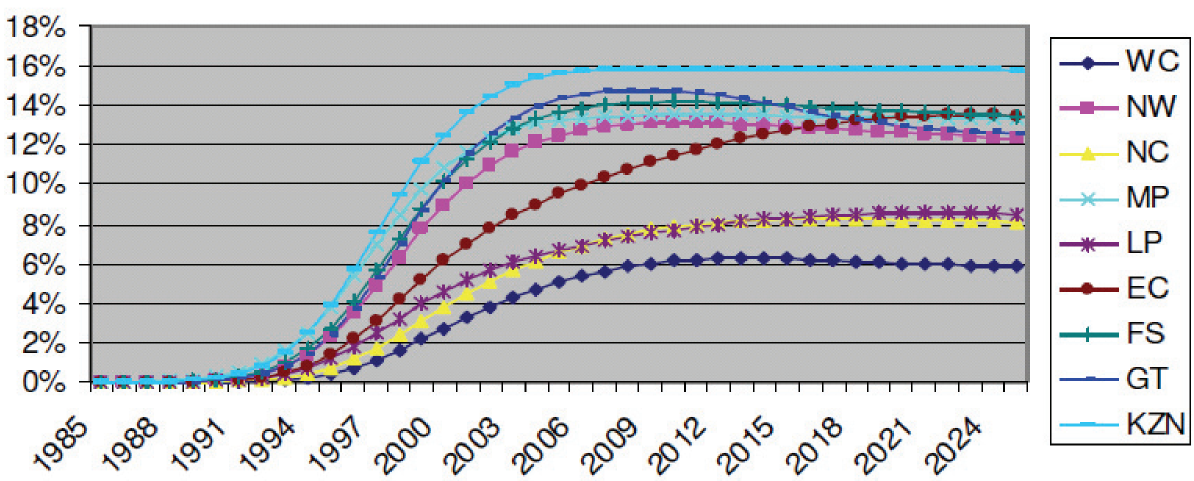


Figure 2: HIV prevalence and projected prevalence rate in South Africa (http://www.metam.co.za/documents_v2/File/RedRibbon_2009/Provincial%20HIV%20and%20AIDS%20statistics%20for%202008.pdf) EC: Eastern Cape FS: Free State GP: Gauteng KZN: KwaZulu-Natal LP: Limpopo MP: Mpumalanga NC: Northern Cape NW: North West WC: Western Cape.

Available (scattered) data on water quality in the North-West Province demonstrated that a large number of these water sources are generally of a poor quality and that it may be deteriorating (Kalule-Sabiti and Heath, 2008; Kwenamore and Bezuidenhout, 2008;

Mulamathathil *et al*., 2000; NWP-SOER, 2002; Van der Walt *et al*., 2002). Furthermore, available data includes mostly physical and chemical parameters that were measured. Even though water of a poor microbiologically quality can have detrimental effects on individuals almost immediately, this aspect has until recently generally been neglected in water quality monitoring regimes.

This is cause for concern. There is a dire need to establish a water quality monitoring regime in a coordinated manner and to use such data to influence policy towards protection of the water sources (Kalule-Sabiti and Heath, 2008). This resource, water, is the fibre of the socio-economics of the province and cannot be managed in a nonchalant manner. However, coordinated research efforts are necessary to demonstrate the impact of agriculture and the mining (as well as related) industries on the water quality in the province. Based on such data, these industries could be held accountable and required to assist in purifying and protection efforts. For this to be valid it would essential that appropriate source tracking of the pollution is demonstrated (Nicols & Leeming, 1991; Samadpour, 2009). It is also critically important to generate data about water related social and water management issues in communities where sampling is conducted (Templehoff, 2009).

1.2 Groundwater of the North West Province

Apart from the few surface water resources, the NWP has a large reservoir of subterranean water in the form of fractured aquifers and dolomitic compartments (DWAF, 2002; Woodford *et al*., 2005) of which more than 80% of the rural community solely depend on as a source of water, especially the more arid western region Sixty percent of the Province's population resides in rural settlements (NWP-SOER, 2008). Anthropogenic activities, which increase with population growth and urbanization, exert strong pressures on groundwater resources (Murray *et al.,* 2004; NWP-SOER, 2002;2008; Usher *et al.*, 2004) and due to the limited surface water resources in the Province (especially the arid western region of the Province), it is imperative to protect the groundwater resources of the North-West Province from further degradation.

A study by Kwenamore (2006) showed relatively high bacteria counts of total and faecal coliforms in groundwater from the central Disobotla and Molopo districts of the Province. This was the case throughout a two year seasonal sampling period, indicating the possibility of constant faecal contamination of the groundwater. Two opportunistic pathogenic coliform bacteria, namely *Klebsiella* spp. and *Citrobacter* spp. were isolated and characterized. The majority of isolates from these groundwater sources exhibited multiple antibiotic resistances (MAR) phenotype patterns to various antibiotics tested. Multiple antibiotic resistant

Enterobacteriaceae and other faecal indicator bacteria were frequently isolated from water sources in the North West Province (Ateba *et al*., 2007; 2010; Ateba & Bezuidenhout, 2009; Bezuidenhout *et al*., 2010; Kwenamore & Bezuidenhout, 2008; Monoeang & Bezuidenhout, 2009; Mulamattathil *et al*., 2000; Venter 2010; Walter 2010).

The farmlands in the NWP are mainly cultivated land where livestock are watered and crops are irrigated with groundwater (NWP-SOER, 2002). Return flows from the irrigation water may contain many fertilizer and pesticide residues which exert further pressures on the receiving ground waters (NWP-SOER, 2008). Mining also cause stress on water resources. It impacts on quantity and quality. Many local aquifers are depleted of its water in order to promote safe deep mining practises, due to the dangers involved in mining below dolomitic compartments (NWP-SOER, 2002).

Farmers are usually financially more secured than informal settlement dwellers, and therefore may have boreholes that are drilled much deeper into the aquifers. Hence the greater horizontal distance, water from these deeper boreholes and the absence of open pit latrines may lead to the assumption of less-contaminated water. The following should also be considered in the risk prediction of farmland: the leaching of livestock manure, as well as pesticides, herbicides, remnant fertilizer and other agricultural waste into the groundwater (Cho *et al*., 2000; Crafford *et al*., 2004; Koplin, 1997; Owens *et al.,* 1994; Silva *et al*., 2006). Nitrate loading in groundwater is promoted by the latter pollutants. Several other non-agricultural sources may also be responsible for nitrate pollution (Wakida & Lerner, 2005).

A study monitoring the groundwater of the North West Province throughout the period of 2002 to 2005 concluded that the average measured nitrate concentration of two out of the five monitored secondary drainage basins exceeded the DWAF TWQR of 6 mg/l for domestic use (Figure 2). The study further established that groundwater nitrate concentration in the North West Province was higher than that of surface water. This higher nitrate concentration was deemed as a gradually prevalent trend. An increase in pit latrines in rural areas together with the rapid population growth of informal settlements contributed to the latter nitrate loading of ground waters (NWP-SOER, 2008).

In many of the rural populations, households have to share water being pumped from the ground to a communal tap and tanks by collecting the water in buckets (Zamxaka *et al*., 2004). In some of these settlements water is only pumped two to three times a week, resulting in water to stand in tanks for extended periods before use. Microbial biofilms can form in these containers (Ateba *et al*., 2010; Foit, 2010; Momba & Kaleni, 2002). More affluent households may have a private shallow-well borehole that is pumped by hand. Shallow boreholes may be more readily contaminated than deeper ones due to the shorter horizontal distance that contaminants (microbes and chemicals) need to travel to reach the water table. In rural settlements, open pit latrines as well as poorly serviced septic tanks are also frequently located in close proximity to these shallow boreholes (Kalule-Sabiti & Heath, 2008; Kwenamore, 2006; Fourie & Rynefled, 1995).

From the above it is evident that microbial and chemical pollution of the groundwater in the North West Province of South Africa is a reality. However, data of the water quality for the province are fragmented when these exist (Kalule-Sabiti & Heath, 2008). It is thus important that the ecology of this water source be properly managed (Grieber *et al*., 2010).

1.3 Surface water of the North West Province

Several rivers, pans and inland lakes are indicated in Figure 3. This creates the impression that sufficient surface water sources should be available for the North West Province. It is, however, not the case. The water sources from this province are non-perennial and decreases from east to west (NWP-SOER, 2008). The three major catchments are the Limpopo, Vaal and Crocodile (Figure 3). The Vaal River forms the southern border of the NWP and the Molopo the part of the northern border. On the, other hand, the Harts, Schoonspruit and Mooi rivers run through a large part of the province and are tributaries of the Vaal river. Water quality for these surface water sources are variable and impacts include mining, agriculture, surface run-off, non-compliant waste water treatment facilities and sanitation back-logs (Figure 3; DWA, 2010; NWP-SOER, 2008). As examples the faecal coliforms levels in some of the dams in the province as well as nitrate levels in some of the secondary drainage areas are provided (NWP-SOER, 2008). The values could be well within the DWAF (1996) guidelines for some uses or several times over the limit for others. Extreme high levels of nitrates were previously reported for areas in the North West Province (Marais, 1999).

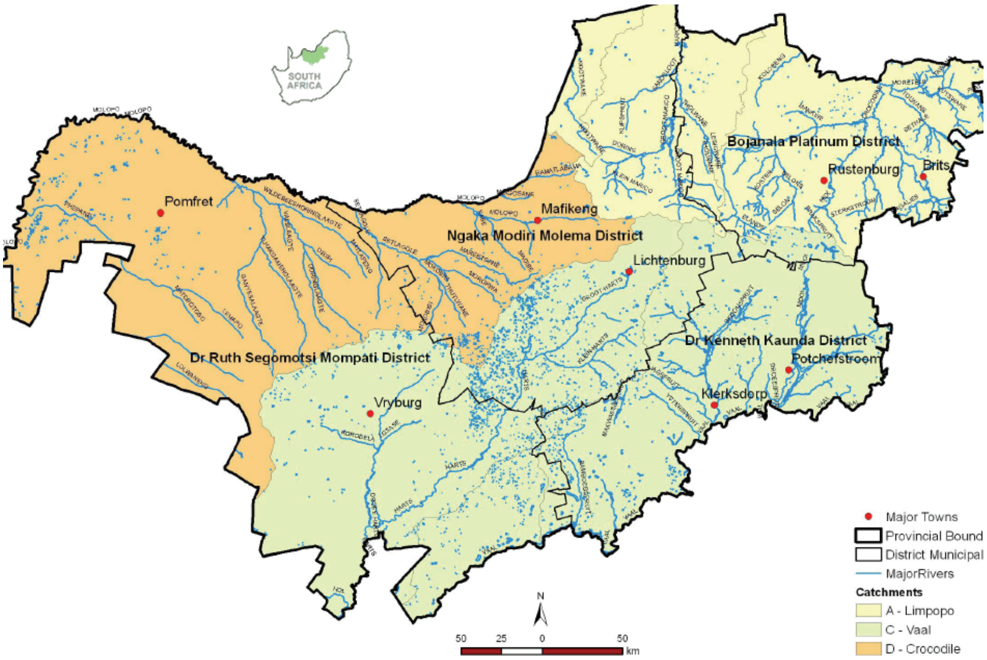
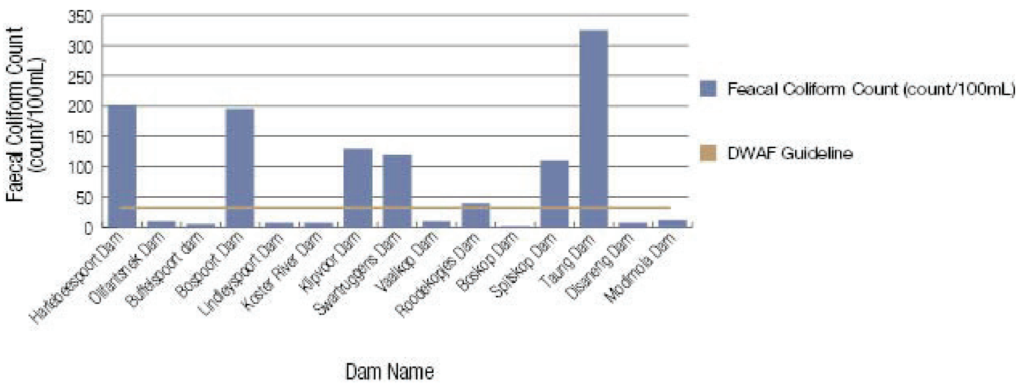


Figure 3: Map of the North West Province indicating some of the major towns and rivers (NWP-SOER, 2008).



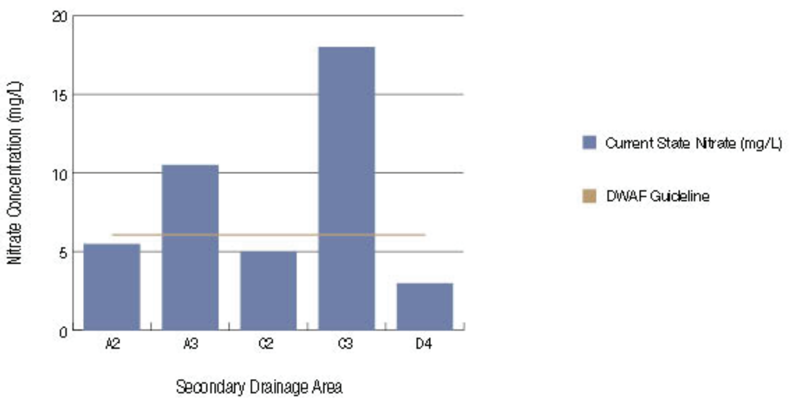


Figure 4: Faecal coliform and nitrate levels in some of the water sources (NWP-SOER, 2008).

Combined faecal, nitrate and organic pollution could have adverse health implications for the consumers of the water. This could lead to diarrhoea outbreaks such as those observed in the North West Province and elsewhere is South Africa (Gaoganediwe, 2006; GCIS, 2005; Hemson & Dube, 2004). Extremely high nitrate levels in drinking could lead to various types cancers (Gullis *et al*., 2002; Jensen, 1982), methaemoglobinemia in infants and young children (Craun *et al*., 1981; Sadeq *et al*., 2008) and other types of risks (Bauchard *et al*., 1992; Camp, 2007; Ghiglieri *et al*., 2009)

The present limited review indicates the need for a more elaborate study on water quality issues. The North West Provincial Government is concerned about the water quality issues and has realised that should the scenario not be turned around, future developments could be jeopardised. It is thus important that appropriate parameters are measured (Tredoux *et al*., 2004) and where appropriate, corrective measures be put in place.

2 EXPERIMENTAL PROCEDURES

Rivers and boreholes were sampled according to the guidelines provided in the sampling guide of WRC (2002). Boreholes with pumps (electrical and hand) were selected for sampling. Groundwater was obtained directly from the aquifer by purging for 3-5 minutes before sampling. Triplicate samples were collected per borehole. Samples were transported to the laboratory in ice boxes and analyzed within six to ten hours of collection.

2.1 Physico-chemical and bacteriological quality

For all samples (surface and groundwater) a culture based membrane filtration technique was used for the enumeration of indicator organisms. One hundred millilitres (100 ml) of sample water was filtered through a 0.45 µm filter from Whatman®. These were then placed on selective agar (m-Endo for total coliforms, m-Fc agar for heat tolerant faecal coliforms, mannitol salt agar for staphylococci, and KF-streptococcus agar for steptococci). Plates were incubated for the recommended period and at appropriate temperatures (m-Endo, mannitol and KF-streptococcus at 37^oC and m-Fc at 44.5^oC). All media used were Biolab products obtained from Merck (Germany). The results were expressed as number of colony forming units (cfu)/100 ml of water tested.

Following incubation, presumptive colonies observed on the surface of membranes were counted and recorded. Dilutions up to 10^{-6} were made for the enumeration of heterotrophic bacteria. R2A agar was used as selective agar for oligotrophic plate count bacteria. The incubation period was 5 days at room temperature. Results were expressed as colony forming units per ml.

Presumptive *E. coli* colonies from faecal coliform selective plates (mFc) were selected, isolated and purified by the streak plate method. Gram staining was performed and biochemical tests including the Triple Sugar Iron (TSI) test and Analytical Profiling Index for *Enterobacteriaceae* spp. (API 20E) were used to classify and identify the isolates.

Temperature, TDS, EC, pH and dissolved oxygen were determined on-site using a field 350i multi-probe from WTW (Germany). Nitrates were measured using a calibrated nitrate probe and appropriate standards. The latter was test was laboratory based.

2.2 Mycological quality

2.2.1 Filamentous fungi

To isolate fungi from the water samples, 100µl of each sample was accurately transferred onto potato dextrose agar (PDA) plates. The aliquots were then spread on the agar using the spread plate technique. The plates were then incubated at room temperature (± 25°C) for seven to ten days. Each sample was prepared in duplicate. PDA is a media generally used for the isolation of saprophytic fungi and bacteria. The use of this media will basically support the growth of a variety of microorganisms. Developed fungal colonies were counted, converted and expressed as colony forming units per millilitre.

After a period of ten days, fungal colonies were isolated from the original PDA plates, and then plated individually again onto fresh PDA plates for isolation into pure cultures. The plates were incubated at room temperature for about seven to ten days. Following incubation on PDA, pure culture isolates were subjected to microscopic investigation for morphological identification using two manuals, one compiled by Funder (1968) and the other by Onions *et al*. (1981).

2.2.2 Yeasts

In order to isolate for yeast colonies from soil and water sources two different types of selection media were evaluated. Soil dilution plates were prepared, using thymine-mineral-vitamin (TMV) agar and yeast-malt- extract (YM) agar. The pH was adjusted to 4.5. TMV agar was used for it selects for physiologically similar soil yeasts and simultaneously prevent over growth of media by filamentous fungi. YM agar supplemented with 100 ppm chloramphenicol was also used. The plates were incubated for 5-7 days at room temperature (± 25°C). Well isolated yeast colonies were purified and maintained on YM-agar.

The isolates were subjected to biochemical tests using the ID 32C system (bioMerieux, France). This system consists of a single-use disposable plastic strip with 32 wells containing substrates for 29 assimilation tests (carbohydrates, organic acids, and amino acids), one susceptibility test (cycloheximide), one colorimetric test (esculin), and a negative control. The yeast identification procedures were conducted in accordance with the manufacturer's instructions. Single colonies were aseptically transferred from a freshly inoculated stock culture to sterile distilled water to prepare a suspension with a final turbidity equivalent to McFarland standard #2. Five drops of this suspension was then dispensed to an ampule of C medium provided by the manufacturer and homogenized to prepare an even dispersion of inoculum. After homogenizing, the inoculum suspension was used to inoculate the wells in the strip, the lid of the strip was replaced, and the system was incubated at 30°C for 48 h. The strips were then visually examined, and growth was determined to be positive or negative based upon the presence or absence of turbidity in the wells. The results were transformed into numerical biocodes, and the isolates were identified through the use of the ID 32C Analytical Profile Index (bioMerieux, France).

2.3 Analysis

Where applicable, averages and standard deviations were used to interpret the data.

3 RESULTS, TREATMENT OF RESULTS AND DISCUSSION

The results for groundwater are presented in the following figures and tables. Eleven areas were identified and results for these grouped (Tables 1 and 3). The positions of the various sites are indicated in Figure 3.

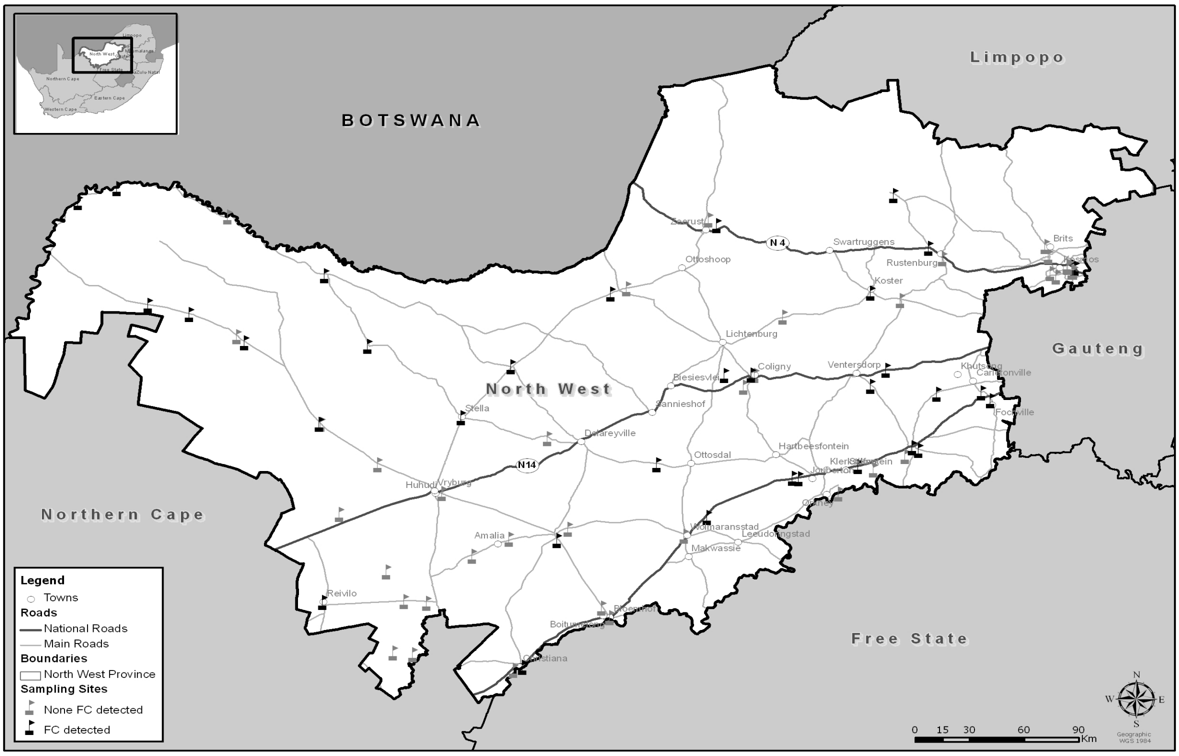


Figure 5: Borehole sample sites in the North West Province.

From Tables 1 and 2 it is evident that that pH was constantly within the TWQR (6-9) for drinking water, recreation, livestock watering as well as irrigation. Based on TDS and electrical conductivity, the water could generally be classified as hard. This is potentially due to the Mg and Ca salts occurring in the dolomitic waters of the province. What is of concern is the very high levels of nitrates in the water (Table 2; Figure 6). There could be health implications for the consumers of the water (Craun *et al*., 1981; Gullis *et al*., 2002; Silva *et al*., 2006; Suthar *et al*., 2009). The COD levels of some of the samples were also very high. If the combined nitrate and COD levels are considered then it is evident that areas D, K, J and I are the most affected areas. Table 4 illustrates this trend further.

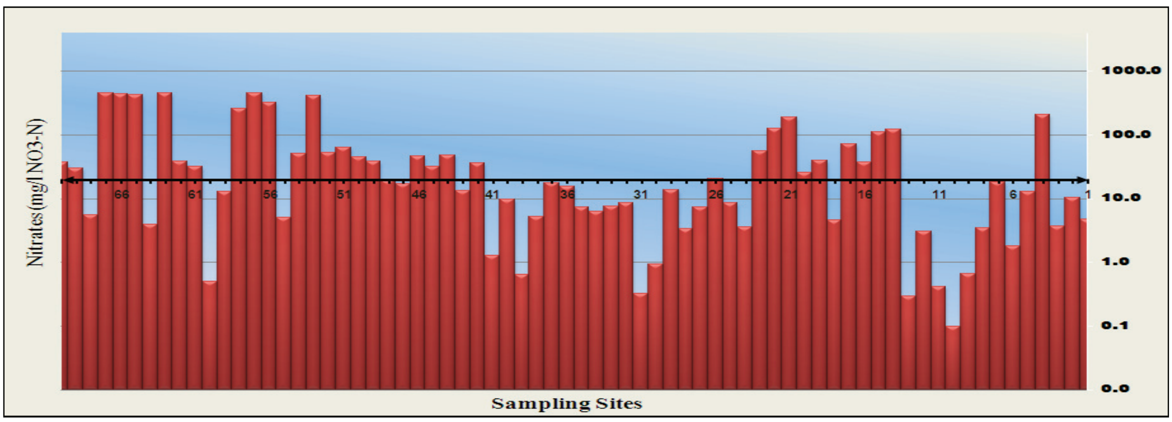


Figure 6: Nitrate levels of the boreholes sampled in 2009.

Table 1: Target water quality ranges for domestic, recreation, livestock watering and irrigational purposes (DWAF, 1996).

Variable	Target water quality range (TWQR)			
	Domestic	**Recreation**	**Livestock watering**	**Irrigation**
pH	6.0-9.0	6.5-8.5	NA	6.5-8.4
TDS (ppm) ;	0-450	NA	<1000[a] <2000[b] <3000[c]	260
EC (µs/cm)	0-700	NA	NA	400
Nitrate (mg/L NO3-N)	0-6	NA	0-100	NA[d]
HPC (cfu/ml)	0-100			
Coliforms (cfu/100 ml)	0-5 (TC) 0 (FC)	0-150 (FC) 0-130 (*E. coli*)	0-200 (FC)	<1 (FC)
Faecal Streptococci	NA	0-30 (Full contact)	NA	NA

NA – Not Available; HPC – Heterotrophic Plate Counts; TC – Total Coliforms; FC – Faecal Coliforms; cfu – colony forming unit ; [a]Dairy, pigs and poultry; [b]Cattle and horses; [c]Sheep; [d]Nitrogen levels >30 mg/L will cause groundwater contamination

Table 2: Physico-chemical parameter data of groundwater samples measured in 2009.

Sample date & Season	Sample area n – quantity boreholes sampled	Ass. Lett. *A-K*		pH	Temp *(°C)*	EC *(µs/ cm)*	TDS *(ppm)*	Nitrate - nitrogen *(mg/L NO_3-N)*	COD *(mg/ L)*
16/2/2009	Klerksdorp-Stilfontein Orkney		Min	6.8	20.4	483	407	1.8	16
			Max	7.6	24.5	1400	970	209.8	114
Summer	n = 6	**A**	Ave	7.1	22.6	968	653	40.5	57
4/3/2009	Potchefstroom		Min	6.9	22.4	347	322	3.5	142
			Max	7.1	24.4	920	630	18.3	325
Autumn	n =2	**B**	Ave	7	23.4	634	476	10.9	234
10/3/2009	Carletonville-Ventersdorp Fochville-Welverdiend		Min	6.8	21.1	67	41	0.1	61
			Max	7.9	22.3	700	470	3.0	376
Autumn	n = 5	**C**	Ave	7.4	21.6	447	301	0.9	158
26/3/2009	Lichtenburg-Koster Doornkop-Makokskraal		Min	6.9	20.6	127	85	4.5	47
			Max	7.5	24.4	1350	906	120.9	1100
Autumn	n = 7	**D**	Ave	7.1	23.0	662	444	58.7	429
4/4/2009	Hartbeespoortdam-Brits Matrooster-Rustenburg		Min	7.1	20.2	489	348	0.9	11
			Max	8.1	24.1	1225	860	55.2	80
Autumn	n = 8	**E**	Ave	7.3	23.0	900	611	14.1	50
17/4/2009	Hartbeespoortdam		Min	6.4	19.2	312	223	0.3	20
			Max	7.7	24.3	1146	812	8.4	68
Autumn	n = 5	**F**	Ave	7	22.4	819.4	582.4	5.9	41
9/5/2009	Delareyville-Zeerust Mafikeng-Stella		Min	6.8	21.0	214	152	0.6	34
			Max	7.6	22.4	1218	865	160	108
Winter	n = 8	**G**	Ave	7.2	21.6	902	635	12.4	87
26/5/2009	Schweizer-Renecke Bloemhof-Wolmaransstad		Min	6.9	18.6	574	404	16.9	0.5
			Max	7.5	19.5	1758	1250	47	96
Winter	n = 5	**H**	Ave	7.1	18.7	976	691	32.2	31.9
10/6/2009	Vryburg-Ganyesa		Min	6.7	16.6	960	675	38.6	56
			Max	7	18.5	1731	1310	63.6	142
Winter	n = 4	**I**	Ave	6.9	17.7	1247	904	49.9	97
23/6/2009	Bray-Vostershoop Piet-Plessis-Makopeng		Min	7.1	16.2	637	624	0.5	19
			Max	8.2	24.0	1134	926	454.5	540
Winter	n = 9	**J**	Ave	7.4	20.6	912	701	171.6	153
26/7/2009	Taung-Salpeterspan-Sekhing-Geluk-Amalia		Min	7	15.5	758	548	3.9	4
			Max	8.8	21.5	1445	1030	454.5	565
Winter	n = 9	**K**	Ave	7.4	19.7	1062	754	210.7	182

Levels of some of the parameters were such that it was not suitable for domestic, recreation or any of the agricultural purposes (Table 1). In the large-scale study (K5/1966), these areas are being retested to verify the results. The high levels of nitrates observed in this study is not uncommon and had been reported previously (Marais, 1999).

Faecal indicator bacteria were detected in a large number of samples. This result is summarized in Table 3. It demonstrates that water from few of the boreholes tested, complied with drinking water standards.

Table 3: Indicator bacterial counts and % of sites that had *P. auruginosa* present in the areas A-K sampled during 2009.

Sample area		HPC *cfu/ml*	TC *cfu/100ml*	FC *cfu/100ml*	*E. coli* *cfu/100ml*	FS *cfu/100ml*	*P. aeruginosa* *% Present*
A	Min	34	76	ND	ND	ND	0%
	Max	3076	>300	200	2	68	
B	Min	287	43	24	ND	>300	0%
	Max	487	57	44	ND	16	
C	Min	60	17	8	0	2	0%
	Max	3 100	>300	260	0	>300	
D	Min	87	103	ND	ND	ND	0%
	Max	4 666	>300	163	ND	50	
E	Min	180	23	ND	ND	ND	75%
	Max	3 360	>300	106	ND	>300	
F	Min	47	130	ND	ND	ND	100%
	Max	1 333	>300	92	ND	ND	
G	Min	43	37	ND	ND	ND	75%
	Max	7 500	>300	104	1	5	
H	Min	10	2	ND	ND	ND	100%
	Max	1 900	>300	2	1	54	
I	Min	133	260	ND	ND	5	0%
	Max	3 000	>300	42	ND	7	
J	Min	166	53	ND	ND	1	75%
	Max	1 263	>300	280	ND	159	
K	Min	50	19	ND	ND	ND	44%
	Max	400	>300	3	ND	94	

In 2009, faecal coliform bacteria were selected and subjected to biochemical tests only. This resulted in misidentification of *E. coli* and may explain the results in Table 3. In 2010, the selective medium was changed from mFC to MLG agar. Furthermore, a PCR based method was optimized to rapidly determine whether isolated faecal coliform bacteria (putative *E. coli)* are *E. coli* or not. The gel in Figure 7 is an example of the results generated with this method. Details of the approach is provided in the literature review – K5/1966, Deliverable 1, also see Beij *et al.,* 1991 for an overview). This test is based on detection of 3 bands in an agarose gel, indicating the presence of 3 markers, 2 of which are specific for *E. coli.*

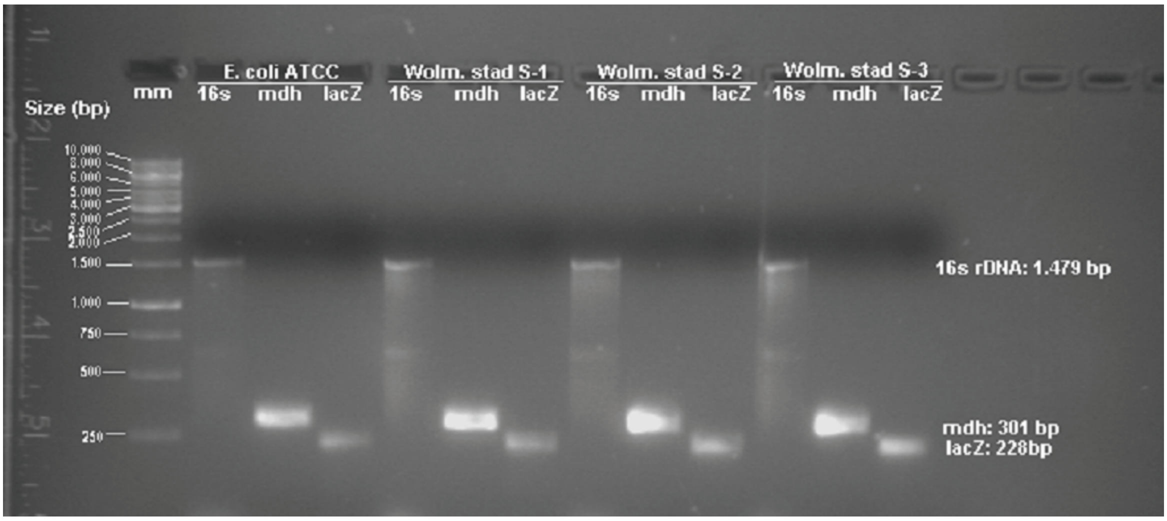


Figure 7: A 1.5% w/v ethidium bromide stained agarose gel depicting PCR results of ATCC *E. coli* (positive control), and three presumptive and now confirmed *E. coli* strains. The 16S, mdh and lacZ gene was amplified in all four strains.

Table 4: Percentage boreholes in each sampling area in 2009 exceeding the DWAF TWQR for domestically used water.

	% boreholes exceeding DWAF TWQR						
Sample Area	**pH**	**TDS**	**EC**	**Nitrate**	**HPC**	**TC**	**FC**
A	0	67	67	50	83	100	50
B	0	50	50	50	100	100	100
C	0	20	20	0	60	100	100
D	0	44	44	89	89	100	63
E	0	86	71	57	100	100	57
F	0	75	63	63	75	100	14
G	0	88	88	63	88	100	50
H	0	88	75	88	75	88	38
I	0	100	100	100	100	100	25
J	0	100	89	78	100	100	78
K	0	100	100	78	78	100	11

1.2 Fungal diversity and distribution

Filamentous fungi were isolated from all of the surface water sites throughout the sample period and from 22.2% of groundwater sites (Tables 5 and 6). Six potentially opportunistic pathogenic species were isolated and identified namely; *Acremonium* spp., *Altenaria* spp., *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp., and *Verticillium* spp. Four more genera were isolated from both surface and ground water but were unidentified. All of the isolates are common plant pathogens. The occurrence of fungi in surface and groundwater means that during irrigation the microbes could be distributed to crops.

Table 5: The number of sites tested for fungal presence, the percentage of sites that tested positive for fungal presence and the number of sites from which the fungal species were isolated from in surface- and groundwater.

	Surface water	Groundwater
Number of sites tested	6	45
Percentage positive	100 %	22.2 %
Isolated species	**Number of sites tested positive**	**Number of sites tested positive**
Acremonium spp.	2	2
Altenaria spp.	2	1
Aspergillus spp.	2	2
Fusarium spp.	1	1
Penicillium spp.	2	2
Verticillium spp.	2	2
Unidentified	1	4

Acremonium spp., *Aspergillus* spp., and *Verticillium* spp. were β-haemolytic when grown on blood agar at 37°C (Table 4). A concern was that all of the identified fungi were also opportunistic human pathogens. Literature reveals that infections by these fungi may be superficial, subcutaneous or systemic.

The fungi recovered from water in most studies performed include some yeast, but mostly are filamentous fungi (Hageskal *et al*., 2006; 2009). Among the isolated filamentous fungi were some that are known to be potentially pathogenic, allergic and toxigenic species.

Human pathogenic fungi can be transported in water (Mara & Horan, 2003). In most cases, fungi do not pose serious threats to healthy individuals, although there may be potential risks for immuno-compromised individuals (Carlile *et al*., 2001). There seem to be a possibility for filamentous fungi to produce mycotoxins and other metabolites in water. According to Hageskal *et al*. (2009) mycotoxins produced in water will often be diluted and may be of minor concern, but upon storage in reservoirs or bottles for prolonged periods the concentration of mycotoxins may increase and intake of such water may be hazardous to human health. Before the onset of chemotherapy causing severe immune-suppressions, and before the AIDS epidemic, invasive infections from saprophytic fungi were extremely rare.

Table 6: Isolated fungal spp., sites tested positive for the presence of isolates, haemolytic response, and a brief description of pure colonial growth.

Species Isolated	Sites tested positive	Haemolytic
Acremonium spp.	Sekhing;Molopo; Christiana; Barkly West	β
Altenaria spp.	Parys; Bloemhof 1; Bloemhof	None
Aspergillus spp.	Hartebeesfontein B3/3; Barkly West; Windsorton	β
Fusarium spp.	Parys;Ganyesa-Vryburg	None
Penicillium spp.	Hartebeesfontein A1; Barkly west; Hartebeesfontein B3/3; Bloemhof	None
Verticillium	Barkly west; Midvaal Hartebeesfontein C; Ganyesa; Vryburg	β
Not Identified	Christiana; Piet Plessis	None
Not Identified	Hartebeesfontein C	None
Not Identified	HartebeesfonteinB3/1	None
Not Identified	Mokgareng	None

The *Acremonium* species are filamentous soil opportunistic molds. They produce mycotoxins like ergopeptine and lolitrem alkaloids (Jivan, 2006). Infections with species of these genera have been reported, whereby human diseases have occurred in healthy persons who wear contact lenses and develop keratitis (Vasiloudes *et al*., 1997).

Alternaria species are capable of growing at low temperatures and are often involved in the spoilage of refrigerated products (Jivan, 2006). Studies indicate that *Alternaria* species have also been isolated from drinking water utilities. The work of Gonçalves *et al*. (2006) points out that *Alternaria* sp. may be involved in the production of bad taste and odour of drinking water.

Aspergillus species are tolerant of low water activity, being able to grow on substrates of high osmotic potential and sporulate in an atmosphere of low relative humidity (Carlile *et al*., 2001). *Aspergillus* species are a serious problem in immuno-compromised cancer patients. Environmental exposure is a major risk factor for invasive aspergillosis in these patients. Studies show that, fewer patients would have died from transplantation or its complications if they had not developed *Aspergillus* infections (Ribaud, 1997). Aflatoxin is a mycotoxin produced by certain *Aspergillus* species. This mycotoxin is known to cause liver damage and cancer.

The fusaria have slime spores that are readily wettable (Carlile *et al*., 2001). The fusarial spores can be dispersed by dew, rain drops, rain or flowing water. Fusarium species are commonly known to cause major plant diseases such as the maize ear infection (Leslie and Summeril, 2006). However, associations with other plants including indigenous ones have also been demonstrated (van der Walt *et al*., 2005; 2006). Research has recognized *Fusarium* spp. as a cause of opportunistic infections in immuno-suppressed patients. Fusarium species produce a toxin called fumonisins amongst others, and this toxin is known to cause oesophageal cancer (Carlile *et al*., 2001).

Penicillium species may be pathogenic to humans and are often found in immuno-compromised hosts. Studies conducted by Jan *et al*. (2008) show that *Penicillium* species can be isolated lungs, spleen, liver and lymph nodes of infected hosts. He concluded that, *Penicillium* species cause disseminated infections and can be the initial manifestation in HIV-infected patients. In addition to their infectious potential, some penicilliums also produce harmful mycotoxins (Jivan, 2006). *Penicillium* species produce patulin, a mycotoxins associated with kidney damage. *Acremonium* and *Penicillium* have been isolated in studies conducted by Hageskal *et al*. (2006). These isolates were associated with water-related problems like off flavour and odour.

Species of the genus *Verticillium* infections are very rarely cause infections in humans. However, a study conducted by Wu *et al*. (2008), showed that *Verticillium* spp. may cause human infections and can be considered in a list of differential diagnosis of hepatosplenic abscesses in immuno-compromised patients.

Water quality surveillance programmes use enteric pathogens as indicators of polluted water (Barrell *et al*., 2000; Grabow, 1996). Infections are generally contracted by drinking contaminated water, recreational exposure to contaminated water, inhaling contaminated aerosols or the consumption of raw food exposed to polluted water (Noble *et al*., 2003),

A correlation between filamentous fungi and the generally recommended indicators of the microbiological safety of domestic water has not been clearly established (Arvanitidou *et al*., 2002). Filamentous fungi receive little attention in most water quality systems as compared to bacteria and viruses, and in most cases fungi are detected accidentally. It is a fact that bacteria and viruses are the most common pathogens known to contaminate drinking water leading to acute waterborne diseases in humans, but this does not out rule the importance or the significance of filamentous fungi in water consumed by humans.

To date there have been no published studies reporting the exact significance of filamentous fungi in drinking surface water. It is also difficult to compare data between laboratories due to lack of standardized and adequate data (Gonçalves *et al*., 2006). Water as a possible source of fungal infection is a controversial matter. Researchers seem to agree that proper studies need to be conducted to clear out the controversy surrounding the significance and the contribution of filamentous fungi in water related infections or diseases.

The largest percentage of yeast isolated was from soil samples (Figure 8) and included *Cryptococcus* spp. *Cryptococcus laurentii* is a common soil yeast. This predominance can be explained by the occurrence of capsules. The latter structures protect the yeast against several physical and biological stresses so that they are capable to survive under unfavourable conditions.

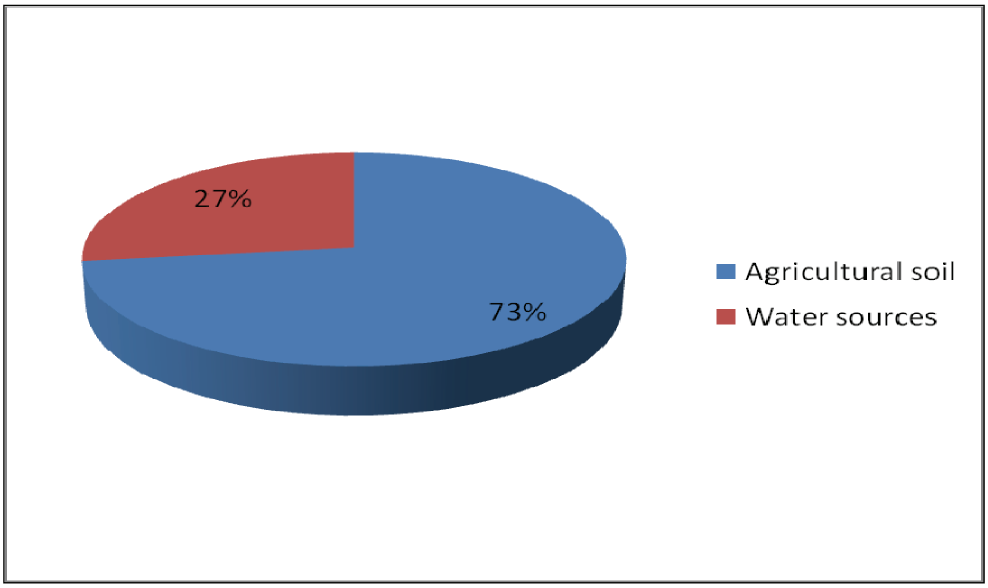


Figure 8: Yeast distribution according to habitat.

When these yeast isolates from this study were subjected to biochemical tests, the following yeasts were identified namely *C. laurettii* and *C. terreus* (from soils) as well as the ascomycete *Candida guilliermondii*. In the case of water, the red yeasts *Rhodotorula mucilaginosa* and *glutinis* were predominantly isolated. 26S rDNA sequences were used to confirm identities. Some of these species may be allergenic or cause infections in humans and can also include potentially pathogenic species. In recent years opportunistic infections associated with the genus *Cryptoccocus* have been reported and reports of cases of infection due to *C. laurentii* have been increasing.

4 CONCLUSIONS

The North West Province is land locked and a large section of the population is found in rural settings. Rainfall in the province is unevenly spread throughout the province. The western regions are drier than the eastern regions. Pollution of the water sources of this province is recognised local government and potential causes described in documentation. Groundwater is a major water source for domestic and agriculture purposes. In these cases the water is supplied without any treatment. Groundwater quality has received little attention because of the perception that this water has filtered through several layers of soil and rock and should be free from pollutants. Results presented in this preliminary report demonstrate that several quality issues, with respect to water, in particular groundwater, exist. These include elevated physical, chemical and microbiological parameters that could impact on disease burden of the population. However, the issues could not be resolved within one preliminary study. The present scoping study (K8/853) on water quality issues from a microbiological and physico-chemical perspective provided a basis for the large-scale study (K5/1966) will be launched in 2010. If water quality issue are not timeously resolved it could led to civil unrest (Thom, 2010; Templehoff, 2009) or could have disastrous implications (Van Riet & Templehoff, 2009; Van Vuuren, 2009ab).

5 RECOMMENDATIONS

It is recommended that:

- A comprehensive large scale study of water quality issues in the North West Province be conducted.
- Physico-chemical parameters include major ions, pH, Dissolved oxygen, COD, Nutrients in particular nitrates.
- Microbiological parameters include indicator bacteria (total and faecal coliforms), pathogenic and potential pathogenic bacteria, yeasts, phages and enteric viruses.
- Cytotoxic effects of polluted water on cell cultures be established.
- Pollution sources be tracked using chemical (faecal sterol ratios) and antibiotic resistance methods.
- Information on social impacts and indigenous management practices collated.
- All the available water quality data for the North West Province be collated into a comprehensive database.

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APPENDIX:

Role-player workshop held on 29 April 2010

Attendance register, agenda and proceeding of the meeting (minutes) are provided

Role-player Workshop 29 April 2010

	NAME	INSTITUTION	TEL	E-MAIL
1	CARLOS BEZUIDENHOUT	NWU-PUK	0824940221	CARLOS.BEZUIDENHOUT@NWU.AC.ZA
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24				

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26 April 2010

Dear Colleagues

The following two studies apply:

A SCOPING STUDY ON THE ENVIRONMENTAL WATER (GROUNDWATER AND SURFACE WATER) QUALITY AND MANAGEMENT IN THE NORTH-WEST PROVINCE, SOUTH AFRICA WRC K8/853 to be completed by end of April 2010.

As part of the abovementioned study that is currently underway a role-player meeting had to be held in early 2010. The report from this meeting and a summary of the results will be used to conclude K8/853. A final report will be presented to the WRC in this regard. You may or may not be part of the scoping study mentioned above.

However, the role-player meeting will bring together the various individuals that will be part of the next phase of the study: WRC K5/1966

A LARGE SCALE STUDY OF THE HUMAN-INDUCED IMPACTS ON THE MICROBIAL AND PHYSICO-CHEMICAL QUALITY OF GROUND- AND SURFACE WATER IN THE NORTH-WEST PROVINCE, SOUTH AFRICA

Funding for K5/1966 has been secured and the study will commence in 2010. You have indicated in 2009 or early 2010 that you will be contributing to this study (K5/1966). I have provided you with the final proposal as amended, and accepted by Mrs Annatjie Moolman of the WRC.

The meeting, in the form of a workshop, will be held on 29 April 2010 at Christa Galli Conference Centre in Potchefstroom (directions attached). As indicated in our various verbal discussions, it is important that we discuss our roles within the project at the onset and that we set realistic time frames within the broader time frames of the project. The meeting will also provide us with the opportunity to meet each other and to familiarise ourselves with the various expertise within the group.

PROGRAMME FOR THE DAY:

9:00-9:30	Arrival and tea
10:00-10:15	Welcome
10:15-10:30	Project overview = project leader
10:30-12:30	Expertise and Project foundation presentations
12:30-13:15	Lunch
13:15-14:15	Group discussion
14:15-15:00	Report back and way forward

In the time slot 10:30-12:00 a 4-5 slide presentation of 5 to 7 minutes will be provided by the individuals listed below. The slides should briefly provide an overview of the expertise the individual, or the group represented by the individual, will bring to the project. Where applicable some preliminary data sets (Max 5 slides and 7 minutes talk time).

All of the presentations must be loaded by 9:30 am. If you cannot attend meeting/workshop then please send the presentation. This will demonstrate commitment to the project.

The list of speakers:

1. Ms. Simone Ferreira – NWU PUK
2. Mr. Collins Ateba – NWU Mafikeng
3. Mr. Owen Rhode – ARC-GCI
4. Ms. Chani van Vuuren – NWU PUK
5. Dr. Thami Sithebe – NWU Mafikeng
6. Dr. Rialet Pieters – NWU PUK
7. Prof. Kayah Kaya – NWU Mafikeng
8. Prof. Gideon Steyl – UFS-IGS
9. Ms. Lesego Molale – NWU PUK
10. Mr. Wesley van Oeffelen – NWU PUK (Hoogenschool Avans, Netherlands)
11. Prof. Johann Templehoff – NWU Vaal Triangle
12. Ms. Karen Jordaan – NWU PUK
13. Prof. Mark Maboeta – NWU PUK

I hope that you will find the arrangements in order

If you have any queries then contact me on 018 299 2315 or 0824940221.

Kind regards

C.C. Bezuidenhout (PhD) Pr.Sci.Nat. MRSSA
Associate Professor and Chairperson of Microbiology

Proceedings of the meeting of 29 April 2010 (minutes)

The meeting was constituted at 10:30 and was chaired by Leandra Venter a Masters degree student. She welcomed all the participants especially Mrs Annatjie Moolman from the WRC and the participants from Mafikeng Campus. The programme was followed as set out above.

The list of speakers:

1. Prof C. Bezuidenhout – NWU PUK
Project leader:
An overview of the project K5/1966 was provided. This overview also demonstrated how K8/853 and K5/1966 was inter-related.

2. Ms. Simone Ferreira – NWU PUK
Ms Ferreira provided an overview of the information on microbiology and physico-chemical quality of water from domestic (drinking water) boreholes in the North-West Province.

3. Mr. Collins Ateba – NWU Mafikeng
Mr Ateba gave an overview of what expertise he could bring to the project particularly regarding the typing of bacterial pathogens. This is critical to achieve the overall success of the project.

4. Mr. Owen Rhode – ARC-GCI
Mr Rhode presented briefly the strength of his research group regarding yeast diversity and dynamics. These are expertise that will be crucial to the overall success of the project.

5. Ms. Chani van Vuuren – NWU PUK
Ms Van Vuuren presented some insights into what faecal sterols are and the challenges of determining faecal sterols ratios in environmental waters. She also provided some progress data on achieving deliverable 3.

6. Dr. Thami Sithebe – NWU Mafikeng
Dr Sithebe was represented by Mr Kawadsa and the role of the Biology Department from NWU Mafikeng in achieving deliverable 4.

7. Dr. Rialet Pieters – NWU PUK
Dr Pieters explained the role of her laboratory in achieving deliverable 5

8. Prof. Kayah Kaya – NWU Mafikeng
Could not attend due to international research visit.

9. Prof. Gideon Steyl – UFS-IGS
Could not attend but send a presentation in which he explained the potential role in generating some GIS data but also in assisting to assimilate the obtained data into a useful format.

10. Ms. Lesego Molale – NWU PUK
Presented some preliminary data on surface water microbiological and physico-chemical issues, focussing on isolated streptococci characteristic.

11. Mr. Wesley van Oeffelen – NWU PUK (Hoogenschool Avans, Netherlands)
Provided an overview of characterising *E. coli* and determining if these are pathogenic using multi-plex PCR.

12. Prof. Johann Templehoff – NWU Vaal Triangle

Could not attend due to research priorities.

13. Ms. Karen Jordaan – NWU PUK
Ms Jordaan provided an overview of the DNA profiling method to monitor environmental microbiological quality. She provided some insight into the progress to date on achieving the deliverable (deliverable 2) target date.

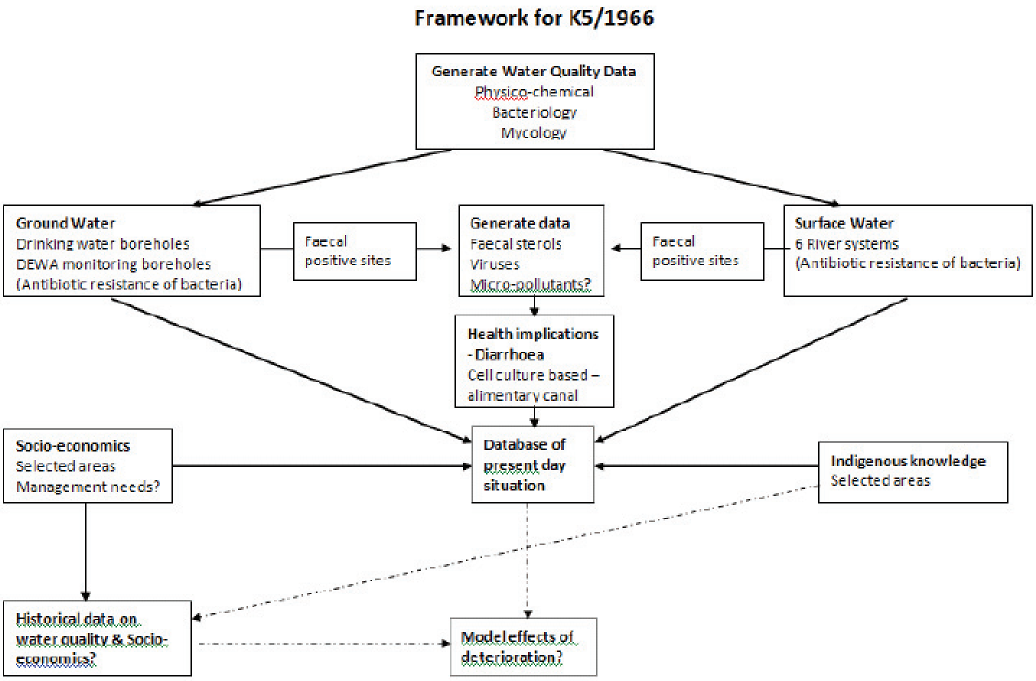
14. Prof. Mark Maboeta – NWU PUK
Prof Maboeta provided an overview of the statistics that will be necessary for successful presentation of the project data.

Some of the matters and questions listed in the post lunch session.

- Risk assessment should be understood by the students and be consistently brought into discussion of results.
- What is the percentage of death of blue babies specifically, and what other causes may also contribute to the total number of deaths of infants in a specific area?
- The natural occurrence of NO_3 + NO_2 should also be examined during study in comparison with nitrite and nitrate which is added to soil through anthropogenic activities.
- How are nitrate and nitrite linked specifically to fertilizers used during crop farming?
- How would/could/should antibiotic resistance of micro-organisms be communicated to the health authorities?
- What are the survival rates of micro-organism in bore-holes? (VBNC?)
- What are the specific sources of pollution? Human or animal?
- What identification methods would be more suitable for smaller water purification plants – cost implications?
- What are the survival rates of yeasts in water?
- Can yeasts be contained in in-house containers to prevent spores(?) from spreading, and what would the survival rate be of such said yeasts in the containers?
- Put CD with list of variable yeasts found in water and methods of how to identify the specific yeasts in with the final report.

The meeting ended at 3:00 pm.

APPENDIX B - FRAMEWORK



Appendix C

Outputs

A. BSc Hons projects completed:

D.A.B VAN WYK. 2009. Preliminary investigation into the isolation and characterisation of yeasts from local water sources and agricultural soils (Female, Coloured)

P. MOTHOBI. 2009. Isolation and identification of filamentous fungi from surface and groundwater in the North West Province (Female, Black)

B. MSc study in progress:

S. FEIRRERA. Microbial and physico-chemical quality of groundwater in the North-West Province, South Africa (Female, White)

C. Conference proceedings:

FERREIRA, S.L & BEZUIDENHOUT C.C. 2009. Microbial and physico-chemical groundwater quality in – selected areas in the North West Province, South Africa. GROUNDWATER CONFERENCE: PUSHING THE LIMITS, GEOLOGICAL SOCIETY OF SOUTH AFRICA, Cape Town, 15 to 18 November (Podium Presentation)

Permission requested to present the following as poster or podium:

? **FERREIRA, S.L & BEZUIDENHOUT C.C.** 2011. Microbiological and chemical quality of domestic groundwater sources in the North West Province, South-Africa. 4th CONGRESS OF EUROPEAN MICROBIOLOGISTS, FEMS 2011, Geneva, Switzerland 26 to 30June

D. Contribution to popular article:

Van Vuuren, L. 2010. Groundwater quality questioned in North West villages, *The Water Wheel*. March/April, 11.