A Scoping Study on the Levels of Antimicrobials and Presence of Antibiotic Resistant Bacteria in Drinking Water

Report to the

WATER RESEARCH COMMISSION

by

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The publication of this report emanates from a WRC project entitled *A scoping study on the levels of antimicrobials and presence of antibiotic resistant bacteria on drinking water* (WRC Project No. K8/1103//3).

EXECUTIVE SUMMARY

BACKGROUND

International and local studies have demonstrated that trace levels of antibiotics as well as the presence of antibiotic resistant bacteria (ARB) in source water and finished drinking water is an emerging health and water quality issue. Treated sewage (sometimes partially treated or even untreated); animal production and healthcare facilities are usually implicated as contributors to the presence of antibiotics in the environment. These antimicrobial substances could individually or synergistically select for antibiotic resistant bacteria. In the present study, the levels of antimicrobials and presence of antibiotic resistant bacteria in drinking water produced from water sources from the Gauteng and North West Provinces was studied. These water sources receive wastewater from a variety of activities and in addition also have mining and industrial activities from which large quantities of metals and other organic chemicals with potential antimicrobial effects may leach into these sources.

AIM

The aim of this study was to provide an overview of the levels of antimicrobials and presence of antibiotic resistant bacteria in selected drinking water treatment systems (drinking water production facilities).

OBJECTIVES

- To determine the levels of selected antimicrobials (antibiotics) and prevalence of antibiotic resistant bacteria (ARB) at selected drinking water production facilities (DWPF).
- To determine the presence of antibiotic resistant bacteria in the drinking water produced from the impacted source water.
- To determine if there is a correlation between the antibiotic resistance patterns as well as levels of antibiotics in the source and drinking water.

METHOD

Raw and final water samples were collected at three drinking water production facilities (DWPF A, B and C). Selected physico-chemical and microbiological parameter data were provided by the three agencies. Sampling was done twice for each of the facilities. In order to isolate bacterial species, a dilution series was initially used to obtain sufficient heterotrophic plate count bacteria on R2A agar. Heterotrophic plate count bacteria were collected and purified using a successive streak plating approach. Antibiotic susceptibility tests were performed on all colonies to determine their antibiotic resistant profiles using the Kirby-Bauer disc diffusion method. Antimicrobial substances that were tested for in this study included antibiotics (ampicillin, chloramphenicol, erythromycin, neomycin, oxytetracycline, streptomycin and trimethoprim), fungicides (amphotericin B, tolnaftate), PPCP (triclosan, 3,4 methylbenzylidene, DEET) and agrochemicals (BenfuraCarb, carbofuran, 2, chlorophenol, 2,4 chlorophenol, chlorpyriphos). Quantification of the selected antibiotics and antiseptics were carried out following the analytical methodology of the LiquidTech a

commercial water analysis laboratory associated with University of the Free State. This was only done once for the DWPF A. The antibiotics and antiseptics selected are the most commonly used ones. Antibiotic resistance profiles from previous studies were also used in the selection of these. The data were analysed using Microsoft Excel (2011) and XL STAT (Addinsoft) software. Raw data were standardised for Principal Component Analysis (PCA).

RESULTS AND DISCUSSION

With regards to the presence of antimicrobials, agrochemicals and pharmaceutical personal care products (PPCPs) in source waters, results obtained in this study showed that a cocktail of these organic substances enter drinking water production facilities as part of the source water. The levels of these substances were very low. Various antibiotics and other pharmaceutical personal care products and agrochemicals were detected in source water of DWPF A, and the levels of these substances ranged from 0.0040 to 0.9700 µg/ℓ. Generally, the levels of these substances, when present in the source water, were reduced by the drinking water production processes. In some cases the levels were reduced to below limits of detection.

The diversity of substances indicate that both sewage and agricultural sources are potentially the origins of the pollution. This diversity raises questions about synergistic effects, with respect to antibiotic resistance selection, that these substance could have in the drinking water production systems. Antibiotic resistant bacteria (ARB) were detected in source waters of all three DWPFs. A higher number of bacterial isolates from all the raw water could be processed and tested for antibiotic resistance patterns. At DWPF A isolates resistant to both β-lactam antibiotics (ampicillin and cephalothin) were isolated from the source water. Resistance to erythromycin (macrolide) and streptomycin (aminoglycoside) were also observed. The obtained results showed that some ARBs present in the source water may survive the drinking water treatment process and manifest in the distribution system. However, the numbers of HPC that could be purified and survived the sub-culturing process were generally low. This was the case for all three (DWPF A, B, and C).

CONCLUSION AND RECOMMENDATIONS

The obtained results indicated that antimicrobial substances and ARBs originating from various pollution sources are present in source waters used for drinking water production. Some of these compounds were not completely removed during drinking water production process. Results further demonstrate that these ARBs are not completely removed during drinking water production processes and could also survive in distribution systems. It is thus recommended that:

- Further research be conducted so that statistical analyses could be done to investigate the relationship between ARB, antibiotics and physico-chemical parameters.
- The presence of antibiotic resistance genes/genetic materials in the ARBs be investigated. In addition, the presence of these antibiotic resistance genes/genetic materials in bulk water should also be investigated with a focus on the potential for transfer to susceptible bacteria.

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ACRONYMS & ABBREVIATIONS

ARB	Antibiotic resistant bacteria
ARG	Antimicrobial resistant gene
ARM	Antimicrobial resistant microbe
DWA	Department of Water Affairs
DWAF	Department of Water Affairs and Forestry
DWPF	Drinking water production facility
GC-MS	Gas chromatography-mass spectrometry
HPC	Heterotrophic plate count bacteria
HPLC	High performance liquid chromatography
ICP-ES	Inductively coupled plasma-atomic emission spectrometry
LOD	Level of detection
LLOD	Lower level of detection
MeOH	Methanol
MPN	Most probable number
MRM	Multiple reaction monitoring
NTU	Nephelometric turbidity units
PCA	Principal component analysis
SANS	South African National Standard
TDS	Total dissolved solids
Temp	Temperature
PPCP	Pharmaceutical personal care products
WWTP	Wastewater treatment plant

1 INTRODUCTION

1.1 RATIONALE

International and local studies have demonstrated that trace levels of antibiotics as well as the presence of antibiotic resistant bacteria (ARB) in source water and finished drinking water is an emerging health and water quality issue. Treated sewage (sometimes partially treated or even untreated); animal production and healthcare facilities are usually implicated as contributors to the presence of a variety of contaminants in the environment. Antimicrobial substances from various pollution settings (agriculture, mining, storm water, sewage, industrial, hospitals, abattoirs, etc.) land in streams and rivers which are the sources of water that are used to produce drinking water (Bergeron et al., 2015). These antimicrobial substances could individually or synergistically select for antibiotic resistant bacteria (ARB), and thus act as reservoirs for antibiotic resistant bacteria (ARB) and genes (ARGs) (Biyela et al., 2004).

Such bacteria and genes, if present in the source water have the potential of surviving the drinking water production process and manifest in the final drinking water and distribution system. Various studies have, to a limited degree, supported this hypothesis. The extent to which water sources act as reservoirs for antimicrobial resistant microorganisms (AMRMs) and antimicrobial resistant genes (AMRG's) is relatively undetermined, particularly in South Africa. It is also known that physical and chemical conditions in aquatic systems affect the survival of microorganisms. In particular, various naturally occurring cations may also be responsible for the electrochemical conditions in such habitats. Such conditions may also impact on the ability of microbes to exchange and take up genetic material.

In South Africa several studies have shown that such bacteria and genes are present in surface and groundwater sources (e.g. Biyela et al., 2004; Bezuidenhout et al., 2013; Mulamattathil et al., 2014b). However, limited research has been conducted on the impacts of this scenario on drinking water production (e.g. Mulamattathil et al., 2014ab). This study is thus a timely one and although exploratory, may provide some useful guidance into further research needs.

1.2 PROJECT AIM AND OBJECTIVES

The main aim of this study was to provide an overview of the levels of antimicrobials and presence of antibiotic resistant bacteria in selected drinking water treatment systems (drinking water production facilities). The aim was achieved via the following the following objectives:

- To determine the levels of selected antimicrobials (antibiotics) and prevalence of antibiotic resistant bacteria (ARB) at selected drinking water production facilities (DWPF)

- To determine the presence of antibiotic resistant bacteria in the drinking water produced from the impacted source water.
- To determine if there is a correlation between the antibiotic resistance patterns as well as levels of antibiotics in the source and drinking water.

1.3 SCOPE AND LIMITATIONS

This was a scoping study and concerned three drinking water production facilities. The results presented are exploratory but would form the basis for further work and could be incorporated into future research. Analysis of antibiotic resistance patterns was limited to two sampling periods for all three plants. These sampling periods were however, were not in exactly the same time frames. This is an aspect that will have to be addressed in the future. Synchronizing the sampling time frames will ensure that similar climatic conditions prevail during sampling and will allow for direct comparison of the data.

2 BACKGROUND AND OVERVIEW

2.1 DRINKING WATER QUALITY

2.1.1 Physical and chemical parameters

Physical and chemical parameters affect the survival capacity of microbes as well as various other biological processes. These parameters include the temperature, pH, turbidity, dissolved solids (including various ions such as magnesium, calcium and chloride, amongst others), various organic molecular species (Lopes et al., 2013). The quality of raw water has an impact on the quality of the produced water (Bai et al., 2015; Watkinson et al., 2009). Physico-chemical water quality aspects also impact on biological processes within aquatic systems (DWAF, 1996b). These processes determine the fitness and survival capability of organisms such as bacteria.

Temperature for example affects the ability of non-sporulating bacteria and certain viruses to survive. At high temperatures, above 65°C, these organisms are inactivated. This is, however, a temperature level that is not achieved in natural water systems. In the temperate climatic conditions water temperatures in the Northern provinces of South Africa would fluctuate between approximately 10 and high 20°C between winter and summer. This would at most result in inhibition of bacterial levels, which could potentially be seen as seasonal fluctuations (Bezuidenhout et al., 2013). Such reductions are not sufficient to dramatically affect bacterial levels in source water. It is thus still important that normal drinking water treatment processes be followed to reduce bacterial levels and completely inactivate indicator bacteria and pathogens.

Water pH determines the chemical species of many elements. This may thus also have an impact on the potential toxicity thereof (Dallas & Day, 2004). Acidification (low pH; high concentration H^+-ions) of water bodies can be caused by low-pH from industries, mine drainage and acid precipitation resulting largely from atmospheric pollution (Dallas & Day, 2004). Such low pH may also leach base cations, particularly calcium and magnesium (Cresser & Edwards, 1988). At high pH deprotonated species may and this may pose a health risk to consumers (DWAF, 1996b). According to SANS 241 (2015) pH for drinking water is 5.0-9.7.

Inactivation and inhibition of microbial activity is most rapid in pure water. When salts, especially calcium and magnesium are present in water this inactivation and inhibition capacity of extreme temperatures are reduced (Douglas, 1975). Thus in, water systems with high TDS, particularly high concentrations of salts such as calcium and magnesium, some protection is provided for bacteria and other microbes to survive unfavourable conditions. The SANS 241 (2015) limit for TDS in drinking water is 1200 mg/ℓ. At this level

high the water could be rich in calcium and magnesium. Both these substances are macronutrients of organisms, normally required for cellular activity. However, these chemicals are also needed for uptake of free DNA from aquatic systems, particularly in the case of bacteria. Tsen et al. (2002) demonstrated that these cations are critical for rendering bacteria naturally competent to take up foreign DNA. The calcium-chloride-heat-shock process is an essential method used in molecular biology to facilitate the uptake of DNA during transformation in the laboratory (Aich et al., 2012). Free DNA originates from organisms in the aquatic system and may contain genes that provide bacteria with the ability to resist the impacts of antimicrobial substances. In view of the preceding discussion the authors would thus argue that it may be important that these levels of chemicals (calcium and magnesium) are considered when antibiotic resistance processes in aquatic systems are considered.

2.1.2 Microbiological parameters

Microbe levels are measured and monitored by specifically testing for the levels of indicator bacterial and viruses. The microbes include heterotrophic plate count bacteria, total coliforms, *E. coli* and bacteriophages (DWAF 1996b; Lopes et al., 2013). The heterotrophic plate count is used to assess the general microbial quality of drinking water (Reasonar 1990; WHO 2003). When the levels are high it may indicate inadequate water treatment, post treatment contamination or bacterial re-growth in the distribution system (Reasonar 1990; Allen et al., 2004). This, however, do not necessarily poses a risk to human health (Edberg et al., 1996; Allen et al., 2004). Coliform bacterial levels are also used to assess the quality of water. Several members of this group are not of faecal origin (Edberg et al., 2000). However, if the levels of these are high in drinking water it may suggest that pathogenic enteric microorganisms may also be present (da Silva *et al.*, 2008).

The most commonly used indicator is *E. coli*. This species is commensal of the human colon micro-flora and it presence in drinking water is a direct indication on faecal pollution. Several strains of this species are also pathogenic (Nataro and Kaper, 1998). These standards are all important, but as with any indicator criteria they do not provide any evidence of all the risks and in this case spread of antibiotic resistance. The latter phenomenon, antibiotic resistance, is normally only a requirement in the clinical scenario, particularly when a recommended antibiotic therapy procedure fails (Willey et al. 2015). Knowledge on what the antibiotic susceptibility patterns are in source and drinking water in specific areas may thus useful to health practitioners, guiding them on what to prescribe to avoid treatment failures.

2.2 DRINKING WATER PRODUCTION

The section above demonstrates why it is important that drinking water production facilities are thus compelled to regularly determine the physico-chemical and microbiological quality of source and drinking water and to keep records of this (DWA, 2005, 2012; Lopes et al., 2013). In terms of Blue Drop certification requirements, monitoring of certain parameters during drinking water production is required in

order to manage the risks associated with drinking water production (DWA, 2012). These are, however, usually critical physico-chemical and microbial parameters, since it is impractical and costly to constantly monitor all aspects. During drinking water production it is essential to reduce (and in some cases completely remove) microbes and dissolved substances (such as salts and organic compounds; Lopes et al., 2013). Most commonly used drinking water production processes involve addition of chemicals to create flocs, allowing these to settle as sludge, to filter the produced water and then to disinfect before distribution. There are, however, a number of variations and these may include additional steps or some advanced technologies. The general process has been around for more than a century and do not take into account dissolved organic substances such as the medicinal compounds (including antimicrobials), pharmaceutical care products, recalcitrant industrial compounds,etc. that could be present in in source water very low concentrations, in a dissolved format (Lopes et al. 2013). Removal of these substances from drinking water has recently become a cause for concern since impacts of these are generally unknown.

2.3 ANTIBIOTICS, ANTIBIOTIC RESISTANT BACTERIA (ARBs) AND ANTIMICROBIAL RESISTANT GENES (AMRGs) IN DRINKING WATER

2.3.1 Antibiotics in water environments

Several drugs/substances act as antimetabolites because they antagonize or block the functioning or metabolic pathways. Antimetabolites are structurally similar to the substrates of key enzymes and compete with the metabolites for the binding site of these enzymes. These antimetabolites bind to specific target enzyme but they are dissimilar enough to block enzyme activity and further progression of the pathway (Willey et al., 2015). Antibiotics are used in human medicine and animal husbandry practices to treat infections. The mechanisms of antibiotics differ with respect to the site of action. What is important is that the actions of antibiotics are affecting important metabolic processes as well as critical structures or enzymes (Willey et al., 2015). Effects of these antibiotics include bacteriocidal (killing) and bacteriostatic (inhibition).

Table 1 shows examples of antibiotics considered in the present study. The antibiotics listed in Table 1 include cell wall, protein and nucleic acid synthesis inhibitors. They belong to various classes. Although the effect may be similar the mode of action are in many cases different. For example, aminoglycosides and macrolides have the same effect, protein synthesis. However, the target sites are different. Aminoglycosides bind to the 30S subunit of the ribosome causing misreading and altering the amino acid sequence of the proteins, making them obsolete. Such cells will thus not be able to provide enzymes and proteins needed for metabolism and will thus die. Macrolides on the other hand binds directly to the 23S rRNA of the large ribosomal subunit. This results in protein elongation being terminated. Enzymes and proteins needed for metabolism will thus not be produced.

Table 1: Classes and examples of antibiotics as well as the mode of action and primary effect (Willey et al., 2015).

Cell wall synthesis inhibition:			
Classes	Members and Spectrum	Mode of action	Primary effect
Penicillins Cephalosporins	Penicillin G, Narrow (G+); Ampicillin, carbenicillin: Broad (G+, some G-) Cephalothin, Broad spectrum	• Inhibit trans-peptidation – Structural similarity between terminal D-alanyl-D-alanine and the B-lactam is thought to block the enzyme responsible for trans-peptidation reaction that forms the peptidoglycan cross-links. • Cause changes to cell shape and size. • Induce of cell stress responses. • Activate cell wall lytic enzymes.	Bactericidal
Vancomycin	Vancomycin: Narrow (G+)	• Is composed of a peptide portion that blocks the trans-peptidation reaction. Binds to the D-alanyl-D-alanine terminal sequence on the pentapeptide portion of peptidoglycan. • Has a different binding site than that of the penicillins.	Bactericidal
Protein synthesis inhibitors			
Aminoglycosides	Neomycin, Kanamycin, Gentamycin, Streptomycin: Broad (G+) Narrow (aerobic G-)	• Binds to 30S ribosomal subunit and causes misreading of mRNA • Alters amino acid sequence of protein • Increase of radical oxygen production	Bactericidal
Tetracyclines	Oxytetracycline, Tetracycline Broad spectrum	• Combine with 30s subunits of ribosomes causing misreading of mRNA.	Bacteriostatic
Macrolides	Erythromycin, clindamycin: Broad (aerobic and anaerobic G+, some G-)	• Contains a lactone ring and binds to the 23S rRNA of large ribosomal subunit to inhibit peptide chain elongation.	Bacteriostatic
Chloramphenicol	Chloramphenicol: Broad spectrum	• The mechanism same as macrolides, binds to 23S rRNA of large ribosomal subunit and inhibit peptide chain elongation.	Bacteriostatic
Nucleic acid synthesis inhibition			
Fluoroquinolones	Ciprofloxacin: Narrow (G- better than G+) Levofloxacin: Broad spectrum	• Blocks DNA replication and transcription • Inhibit bacterial topoisomerase II and DNA gyrase activity. The inhibition disrupts DNA replication and repair.	Bactericidal
Metabolic antagonist			
Trimethoprim	Trimethoprim Broad spectrum	• Block folic acid synthesis by inhibiting the enzyme tetrahydrofolate reductase • Also binds to dihydrololate reductase. This enzyme is responsible for converting dihydrofolic acid to tetrahydrofolic acid. Trimethoprim is often used in combination with sulfa drugs to increase the efficacy of antibiotic treatment by blocking two key steps in the folic acid pathway	Bacteriostatic

Antibiotics in the aquatic environment may be due to several point sources of pollution. It has been demonstrated that aquatic environments down-stream from sewage treatment plants may be hotspots for low concentrations of pharmaceutical personal care products (PPCPs), biocides and various medicines and agro-chemicals (Bengtsson-Palme and Larsson, 2015). Furthermore, agricultural practices such as animal husbandry have also been implicated as sources of antimicrobial substances that land in environmental water. This is due the varied uses of antibiotics. In addition to treatment of infectious disease, some antibiotics are also used in sub-therapeutic concentrations to stimulate growth. This is particularly the case in battery rearing of chickens and feed-lot practices. Watkinson et al. (2009) conducted a study on the occurrence of antibiotics in Australian water system. These authors found antibiotics from various classes in environmental surface water, waste water treatment plants but could not detect any antibiotics in drinking water. They also found that the antibiotics were in the ng/ℓ range. Low concentrations of antibiotics in aquatic systems are ideal pressures for the selection and maintenance of antibiotic resistant bacteria (Bergeron et al., 2015) These antibiotics (including other antimicrobial agents) and antibiotic resistant bacteria may thus find their way into water sources that are abstracted for drinking water production (Bergeron et al. 2015).

2.3.2 Bacterial resistance to antibiotics

Antimicrobial agents (PPCP's, biocides, etc.) in the source water can synergistically act as selection pressure for the maintenance of resistant microbes (Pal et al., 2015). Mechanisms causing resistance to antibiotics and several other antimicrobial substances are overlapping thus resulting in what is known as co-selection. These may be due to genetic elements that are, in many instances, found on mobile elements such as plasmids (Bergeron et al., 2015; Pal et al., 2015). Sub-therapeutic levels of one or both of these chemicals would thus result in such mobile elements being retained in these habitats (Bergeron et al., 2015; Pal et al., 2015). Disinfection at drinking water production facilities (DWPFs) may also select for antibiotic resistant bacteria (Bai et al., 2015). Chlorination is a commonly used disinfection processes but recent studies have demonstrated the this process could be directly linked to selection of antibiotic resistant bacteria (ARB) and antibiotic resistant genes (ARGs) (Shrivastava et al. 2004; Bai et al., 2015; Bergeron et al. 2015).

Bacterial resistance to antibiotics may also be due to intrinsic factors, e.g. Gram negative bacteria are resistant to vacomycin and penicillin-G. These are cell wall inhibitors (Willey et al., 2015). Such antibiotics are thus not included when testing antibiotic susceptibility of Gram negative bacteria.

3 SAMPLING AND ANALYSIS OF SAMPLES

3.1 INTRODUCTION

This section provides the details of the methods that were used. The methodology is standard but is supported by literature that is cited at the various sections. Sufficient details are provided for this study to be repeated elsewhere.

3.2 SAMPLING

3.2.1 Description of study sites

Water samples were collected at three drinking water production facilities (DWPFs), labeled A, B and C.

- DWPF A – is a conventional system but has granular activated carbon as part of the filtration step.
- DWPF B – source water is abstracted from a dam. The raw water is pre-chlorinated after which chemical dosing takes place. Clarification-flocculation, filtration and post-chlorination then follow.
- DWPF C – source water is abstracted directly from the river. Following sedimentation and filtration the resultant water is chlorinated and distributed.

3.2.2 Sample collection

Sampling was done at all DWPFs and included raw water as it enters the production facility as well as final water. For the heterotrophic plate count bacteria, grab samples were collected in sterilized 1 ℓ Schott bottles. This was done accordance with the sampling guide for domestic water (DWAF, 2000). Samples were stored on ice and transported to the laboratory. Analysis was conducted within 6 hours of collection. There were two sampling periods for each of the DWPFs. Sampling periods were, however, not synchronized.

3.3 ANALYSIS OF PHYSICO-CHEMICAL AND MICROBIOLOGICAL WATER QUALITY PARAMETERS

Selected physico-chemical water quality data were provided by the participating agencies mostly for the period of January to December 2014. Some 2015 data were also available. Water quality data for parameters supplied was as follows:

3.3.1 Total Dissolved Solids

The bottled water samples were filtered through a glass-fiber filter, evaporated and dried in a weighed dish. It was then dried to constant weight at 180 ± 2°C. The increase in dish weight represented the total dissolved solids.

3.3.2 Turbidity

Turbidity was measured using a 2100 turbidity meter (HACH) and it was expressed nephelometric turbidity units (NTU).

3.3.3 Conductivity, alkalinity and pH

The conductivity, alkalinity and pH of the samples were analysed using Tiamo autotitrator (Metrohm) where conductivity was measured in units of millisiemens per metre (mS/m), pH in pH units and alkalinity (in mg/ℓ as CaCO3). Potassium Chloride (KCℓ) was used as a verification standard when measuring conductivity.

3.3.4 Calcium and Magnesium

Inductively coupled plasma-Atomic Emission Spectroscopy (ICP-ES) was used to determine mineral components. The unit of measurement is mg/ℓ.

3.3.5 Silica and Chloride

Ion chromatography (Metrohm 761 Compact IC-system A). Verification standards were run after every 10th sample during the analysis.

3.3.6 Indicator microorganisms

Escherichia coli and coliform bacteria were analysed using the Colilert®-18/Quanti-Tray® and Colilert®-18/Quanti-Tray® 2000 in most probable number (MPN). The pour plate method was used to determine plate count bacteria. Yeast Extract Agar was used as the media. Somatic coliphages were determined using the ISO 10705-1&2 methods and the results expressed in plaque forming units per 10 mℓ.

3.4 DETERMINATION OF ANTIMICROBIALS IN SOURCE AND FINAL WATER

3.4.1 Selection of antimicrobial agents

In order to understand the patterns of antibiotic resistance in bacteria from source to final water, it is important to generate data on levels of substances (antibiotics, biocides, agrochemicals, PPCPs, etc.) that could potentially provide selection pressures for such patterns. Several studies on aquatic systems in the North West Province of South Africa have shown that bacteria resistant to these antibiotics were isolated (Mulamattathil et al., 2014a,b; 2015; Bezuidenhout et al., 2013; Cartens et al., 2014; Molale and Bezuidenhout, 2016; Ferreira et al. submitted). Thus in the present study, the presence of antibiotics listed in Table 1 as well as some other representatives from the same group in source and final waters, as well the presence of associated bacterial resistance was studied. The agrochemicals were selected on the basis that the sample area is impacted by agricultural activities that uses the listed agrochemicals. Three most commonly used pharmaceuticals and personal care products (PPCPs) were also included.

The list of analytes that were tested for included antibiotics (ampicillin, chloramphenicol, erythromycin, neomycin, oxytetracycline, streptomycin and trimethoprim), fungicides (amphotericin B, tolnaftate), pharmaceuticals and personal care products (triclosan, 3,4 methylbenzylidene, DEET) and agrochemicals (BenfuraCarb, carbofuran, 2, chlorophenol, 2,4 chlorophenol, chlorpyriphos). Previous studies on aquatic systems in the North West Province of South Africa have shown that bacteria is resistant to certain antibiotics (Mulamattathil et al., 2014a,b; 2015; Bezuidenhout et al., 2013; Cartens et al., 2014; Molale and Bezuidenhout, 2016; Ferreira et al. submitted).

3.4.2 Extraction of the selected antimicrobials, agrochemicals and PPCPs

Water samples collected (1 ℓ) were filtered through glass fiber filters to remove particulate matter before being concentrated onto methanol conditioned C18, 6 mℓ solid phase extraction cartridges (Strata, Phenomenex) at a flow rate of 5 mℓ/min. Bound sample was slowly eluted off the dried cartridges using 2 mℓ methanol followed by 2 mℓ ethyl acetate. The eluent was vacuum dried (Thermo Scientific Savant Speedvac) until almost dry, and reconstituted in a suitable reconstitution buffer depending on the ionization mode.

3.4.3 Quantification of the selected antimicrobials, agrochemicals and PPCPs

Quantification of the selected antibiotics, antiseptics, agrochemicals and PPCPs was carried out in all samples collected following the analytical methodology of the LiquidTech a commercial water analysis laboratory associated with University of the Free State. These prepared water samples were analysed using an ABSCIEX 4000 QTRAP hybrid triple quadrupole ion trap mass spectrometer with a Shimadzu HPLC stack as a front end. Two types of analyses were performed depending on whether screening for

unknown analytes or performing a targeted analyte quantitation. All data acquisition and processing was performed using Analyst 1.5 (AB SCIEX) software.

Twenty microliter of each extracted sample was analysed in both positive and negative ionisation mode. For negative ionisation mode the analyte separation was performed using a C18 (150 mm x 4.6 mm, Gemini NX, Phenomenex) column at a flow rate of 0.3 mℓ/min using a 5 min isocractic run at 90% solvent B (MeOH/0.1% NH_4OH). In positive ionization mode the separation was performed using a C18 (150 mm x 3.0mm, Clipeus, Higgins Analytical) column. The flow rate was 0.300 mℓ/min using a step wise gradient starting at 50% solvent B (MeOH/0.1% formic acid) for 2 min. The gradient was gradually increased to 70% for 2 min followed by 90% for 2 min before re-equilibration at 50% solvent B for a total run time of 20 min. Eluting analytes were electrospray ionised in the TurboV ion source using 500°C heater temperature to evaporate excess solvent, 50 psi nebuliser gas, 50 psi heater gas and 25 psi curtain gas. Ion spray voltage was set at 5500 V (positive mode) and 4500 V (negative mode).

Targeted analyses of pesticides were performed using 2 MRM transitions per analyte. The peak area on the chromatogram generated from the first and most sensitive transition was used as the quantifier while the second transition is used as a qualifier. The qualifier serves as an additional level of confirmation for the presence of the analyte, the retention time for these two transitions must be the same.

3.4.4 Quality control

Samples were submitted into batches that include solvent blank runs between each sample analysed. These were interspersed with quality control samples of known concentration to verify instrument performance. An 8 point calibration curve was generated for each analyte ranging in concentration from 2 ppm (part per million; mg/ℓ) down to below the lower limit of detection (LLOD) for each analyte with a linear fit through the origin producing a correlation coefficient (r value) in excess of 0.98. The limit of detection of each analyte was determined to be the lowest concentration of the analyte that gives an instrument response with a signal to noise (S/N) ratio of ~3.

3.5 DETERMINATION OF ANTIOBITIC RESISTANCE IN SOURCE AND FINAL WATER

3.5.1 Isolation of bacterial species

A dilution series was used to obtain sufficient heterotrophic plate count bacteria on R2A agar (Merck, Germany). Heterotrophic plate count bacteria were collected and purified using a successive streak plating approach [see Carstens et al., 2014 for details]. Various representative morphotypes were selected, and Gram staining was performed (Carstens et al., 2014).

3.5.2 Determination of bacterial antibiotic resistant profiles

Antibiotic susceptibility tests were performed on all colonies to determine their antibiotic resistant profiles using the disc diffusion method (Bauer et al., 1966; Carstens et al., 2014). Briefly, the pure isolated colonies were individually placed in nutrient broth (Merck, Germany) and incubated at 35°C for 24 hours. After 24 hours, individual bacterial lawns were prepared on Mueller-Hinton agar (Merck, Germany) by spreading 0.1 mℓ of the cultures using sterile technique. Ten different antibiotics were used (Oxoid, United Kingdom). The following antibiotics were tested: ampicillin (10 µg); cephalothin (30 µg); erythromycin (15 µg); chloramphenicol (30 µg); ciprofloxacin (5 µg); kanamycin (30 µg); neomycin (30 µg); streptomycin (300 µg); vancomycin (30 µg); penicillin-G (10 units); oxy-tetracycline (30 µg); trimethoprim (2.5 µg). All of these discs were from Mast Diagnostics (UK). The discs were placed onto Mueller Hinton agar inoculated with of a pure bacterial culture. These petri dishes where incubated at 35°C for 24 hours. The inhibition was determined by measuring (in mm) the clear zones around the antibiotic containing disc.

3.6 STATISTICAL ANALYSES

The data were analysed using Microsoft Excel (2011) and XL STAT (Addinsoft) software. Raw data were standarised for Principal Component Analysis (PCA).

4 RESULTS AND DISCUSSIONS

4.1 INTRODUCTION

The results in this section are provided in the following order. First the historical data, over a one year period is provided. This is done for each of the drinking water production facilities (A, B and C). For each of the facilities the data and analyses are provided separately for source and final water.

4.2 DRINKING WATER PRODUCTION FACILITY (DWPF) A

4.2.1 Source water quality

In the case of DWPF A the source water and drinking water changes in terms of the listed physico-chemical and microbiological parameters observed reflect typical seasonal changes. The turbidity in the source water was generally very high during the rainy season, pH was throughout slightly alkaline, TDS ranged between 237 and 454 mg/ℓ and levels were in the high range during the drier periods. Furthermore, microbial pollution indicators were detected at all the sites, also following seasonal variations linked to temperature fluctuations (Table 2).

Principal component analysis showed that there were significant correlations between TDS and all the other physico-chemical parameters (Table 3). Turbidity, however, also demonstrated a significant correlation with *E. coli*. In Figure 1 associations of the various monthly measurements and levels of the measurements are demonstrated. For example January was associated with turbidity and June, July, August, September and October were all associated with pH. Calcium and magnesium also had a significant negative correlation with turbidity and *E. coli*. There were also other associations.

Table 2: Physical, chemical and microbiological parameters of source water supplied to the drinking water production facility (DWPF) labelled A.

	pH	Temp °C	Cl mg/ℓ	Ca mg/ℓ	Mg mg/ℓ	TDS mg/ℓ	Turbidity NTU	*E. coli* cfu/100 mℓ	Total coliforms cfu/100 mℓ	HPC cfu/mℓ	Phage pfu/10 mℓ
Jan-2014	7.84	24.55	15.75	24.75	11.25	237.50	49.75	57.75	6668.75	6103.88	7.00
Feb	7.74	23.43	18.75	29.75	11.50	253.75	51.25	107.00	26227.25	31311.25	2.50
March	7.51	22.36	18.16	30.40	12.40	259.00	65.20	441.80	25754.00	1655.67	42.80
April	7.71	19.85	27.50	40.50	14.50	280.00	37.50	1.50	59815.00	3861.00	3.00
May	7.91	16.25	43.25	44.25	16.25	342.50	11.25	11.50	7709.00	7993.38	3.25
June	8.14	12.95	55.75	45.25	15.50	413.75	9.08	9.25	251.50	11572.63	5.50
July	8.42	9.63	55.75	44.75	17.75	391.25	9.85	5.00	211.25	18049.78	4.00
August	8.10	12.39	42.00	46.25	18.00	421.25	13.58	24.00	842.25	17294.13	7.50
Sept	8.31	16.20	44.40	51.60	19.00	454.00	9.14	14.80	1513.60	12219.78	1.20
Oct	8.52	19.93	50.75	44.75	16.50	420.00	7.88	18.25	12142.50	13562.33	3.00
Nov	7.83	20.73	47.75	49.50	17.25	382.50	7.90	33.25	225365.00	6329.38	24.00
Dec	7.85	23.70	35.60	46.40	15.60	369.00	10.36	10.20	81277.40	12550.38	10.40
Jan-2015	8.19	25.93	37.50	42.25	15.00	370.00	14.23	25.50	117576.50	33594.00	3.50
Feb	8.37	24.35	43.00	46.25	15.50	377.50	7.95	39.25	71034.00	78007.50	1.50
March	8.15	23.02	46.00	47.60	15.20	406.00	14.40	64.20	28995.60	106320.67	5.80
April	7.88	21.65	45.50	57.50	17.00	462.50	6.40	19.50	6960.00	18031.86	3.00
May	7.92	17.55	57.75	68.25	18.50	458.75	9.33	11.50	7980.75	7492.13	3.25
June	7.87	13.04	46.00	56.20	17.80	370.00	7.10	12.20	617.00	2685.56	11.00
July	7.84	12.18	49.00	62.50	22.00	338.75	7.60	13.75	676.00	5282.78	15.50

Table 3: Correlation matrix (Pearson (n)) of physico-chemical and microbiological data of the source water entering DWPF A

Variables	pH	Temp	Cl	Ca	Mg	TDS	Turbidity	E. coli	Total coliforms	HPC
pH	**1**	**-0.630**	**0.776**	0.564	**0.680**	**0.787**	**-0.718**	-0.561	-0.354	0.313
Temp	**-0.630**	**1**	**-0.755**	-0.569	**-0.699**	**-0.657**	**0.586**	0.363	0.360	-0.171
Cl	**0.776**	**-0.755**	**1**	**0.847**	**0.845**	**0.890**	**-0.916**	-0.557	0.043	0.090
Ca	0.564	-0.569	**0.847**	**1**	**0.945**	**0.904**	**-0.913**	-0.555	0.250	-0.011
Mg	**0.680**	**-0.699**	**0.845**	**0.945**	**1**	**0.921**	**-0.878**	-0.516	0.095	0.019
TDS	**0.787**	**-0.657**	**0.890**	**0.904**	**0.921**	**1**	**-0.901**	-0.507	-0.015	0.137
Turbidity	**-0.718**	**0.586**	**-0.916**	**-0.913**	**-0.878**	**-0.901**	**1**	**0.738**	-0.141	-0.103
E. coli	-0.561	0.363	-0.557	-0.555	-0.516	-0.507	**0.738**	**1**	-0.046	-0.260
Total coliforms	-0.354	0.360	0.043	0.250	0.095	-0.015	-0.141	-0.046	**1**	-0.260
HPC	0.313	-0.171	0.090	-0.011	0.019	0.137	-0.103	-0.260	-0.260	**1**

Values in bold are different from 0 with a significance level alpha=0.05

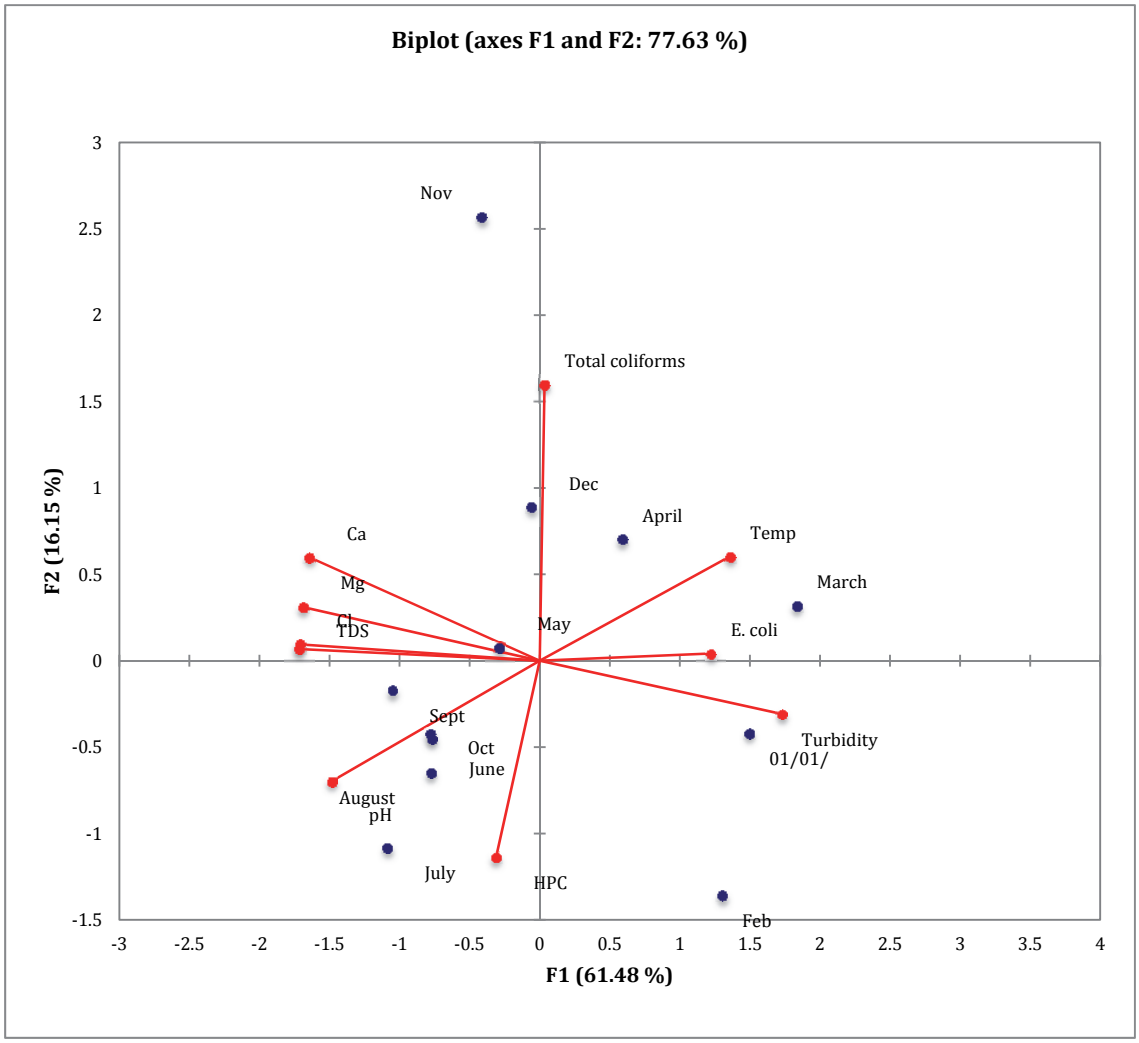

Figure 1: PCA biplot indicating the relationship of physico-chemical and microbiological data of the source water at DWPF A measured over a one year period.

4.2.2 Final drinking water quality

Once again seasonal variations were observed that could be linked to changes in temperatures. The turbidity was very low (<0.5 NTU). However, TDS as well as chloride, calcium and magnesium levels were higher in the drinking water (Table 4) compared to the source water (Table 2). The faecal indicator microbial parameters were zero and the heterotrophic plate count bacteria were generally very low (<10^2 cfu/mℓ) except for two months (January and February 2015).

Principal component analysis showed certain trends: Total dissolved solids (TDS) significantly correlated with temperature, chlorine, magnesium and phage levels. The levels of the later parameter also correlated with calcium. Heterotrophic plate count bacteria levels correlated with magnesium levels (Table 5).

Table 4: Physical, chemical and microbiological parameters of drinking water from the DWPF A.

	pH	Temp °C	Cl mg/ℓ	Ca mg/ℓ	Mg mg/ℓ	TDS mg/ℓ	Turbidity NTU	*E. coli* cfu/100 mℓ	Total coliforms cfu/100 mℓ	HPC cfu/mℓ	Phage pfu/10 mℓ
Jan-2014	8.00	25.13	66.25	51.25	11.25	318.75	0.26	0.00	0.00	2.67	0.00
Feb	7.95	23.46	55.25	48.50	11.75	336.25	0.28	0.00	0.00	129.50	0.00
March	7.98	21.77	37.75	38.75	10.93	264.00	0.35	0.00	0.11	21.00	0.11
April	7.96	18.73	57.00	52.00	14.00	337.50	0.36	0.00	1.00	4.83	0.00
May	8.22	15.63	69.00	55.75	16.50	408.75	0.33	0.00	0.00	3.38	0.00
June	8.03	12.61	76.33	57.00	17.00	447.50	0.34	0.00	0.00	16.00	0.00
July	7.87	10.29	85.25	55.50	16.75	476.25	0.36	0.00	0.00	67.25	0.00
August	7.85	14.11	74.50	46.25	22.00	496.25	0.33	0.00	0.00	437.78	0.00
Sept	8.23	17.40	75.75	58.25	19.00	427.00	0.37	0.00	0.00	91.38	0.00
Oct	8.23	20.74	68.25	54.75	17.75	463.75	0.36	0.00	0.00	101.67	0.00
Nov	8.12	20.55	66.25	56.75	17.75	400.00	0.32	0.00	0.00	34.88	0.00
Dec	8.04	23.73	51.00	53.67	15.00	429.00	0.36	0.00	0.00	61.25	0.00
Jan-2015	7.96	25.35	55.75	46.75	15.00	417.50	0.30	0.11	0.56	387.88	0.00
Feb	8.13	25.24	64.50	52.50	16.00	433.75	0.40	0.00	0.00	1918.88	0.00
March	8.12	22.13	62.25	54.50	16.50	453.00	0.33	0.00	0.00	1427.50	0.00
April	8.28	18.79	56.50	63.00	16.50	508.33	0.26	0.00	0.00	151.25	0.00
May	8.19	16.56	87.00	74.75	20.75	520.00	0.29	0.00	2.00	19.13	0.00
June	8.04	11.27	73.25	70.75	19.75	527.50	0.30	0.00	0.00	7.38	0.00
July	8.10	12.87	66.33	78.00	21.25	506.67	0.33	0.00	0.00	21.00	0.00

Table 5: Correlation matrix (Pearson (n)) of physico-chemical and microbiological data of the drinking water from DWPF A

Variables	*pH*	*Temp*	*Cl*	*Ca*	*Mg*	*TDS*	*Turbidity*	*TC*	*HPC*	*Phage*
pH	**1**	0.177	0.064	0.531	0.181	0.088	0.219	-0.205	-0.386	-0.142
Temp	0.177	**1**	**-0.726**	-0.318	**-0.630**	**-0.647**	-0.447	0.026	-0.240	0.206
Cl	0.064	**-0.726**	**1**	**0.674**	**0.685**	**0.766**	0.085	-0.272	0.240	**-0.663**
Ca	0.531	-0.318	**0.674**	**1**	0.445	0.561	0.205	-0.106	-0.293	**-0.768**
Mg	0.181	**-0.630**	**0.685**	0.445	**1**	**0.881**	0.437	-0.221	**0.599**	-0.458
TDS	0.088	**-0.647**	**0.766**	0.561	**0.881**	**1**	0.404	-0.345	0.495	**-0.602**
Turbidity	0.219	-0.447	0.085	0.205	0.437	0.404	**1**	0.246	-0.013	0.138
TC	-0.205	0.026	-0.272	-0.106	-0.221	-0.345	0.246	**1**	-0.218	0.019
HPC	-0.386	-0.240	0.240	-0.293	**0.599**	0.495	-0.013	-0.218	**1**	-0.157
Phage	-0.142	0.206	**-0.663**	**-0.768**	-0.458	**-0.602**	0.138	0.019	-0.157	**1**

Values in bold are different from 0 with a significance level alpha=0.05

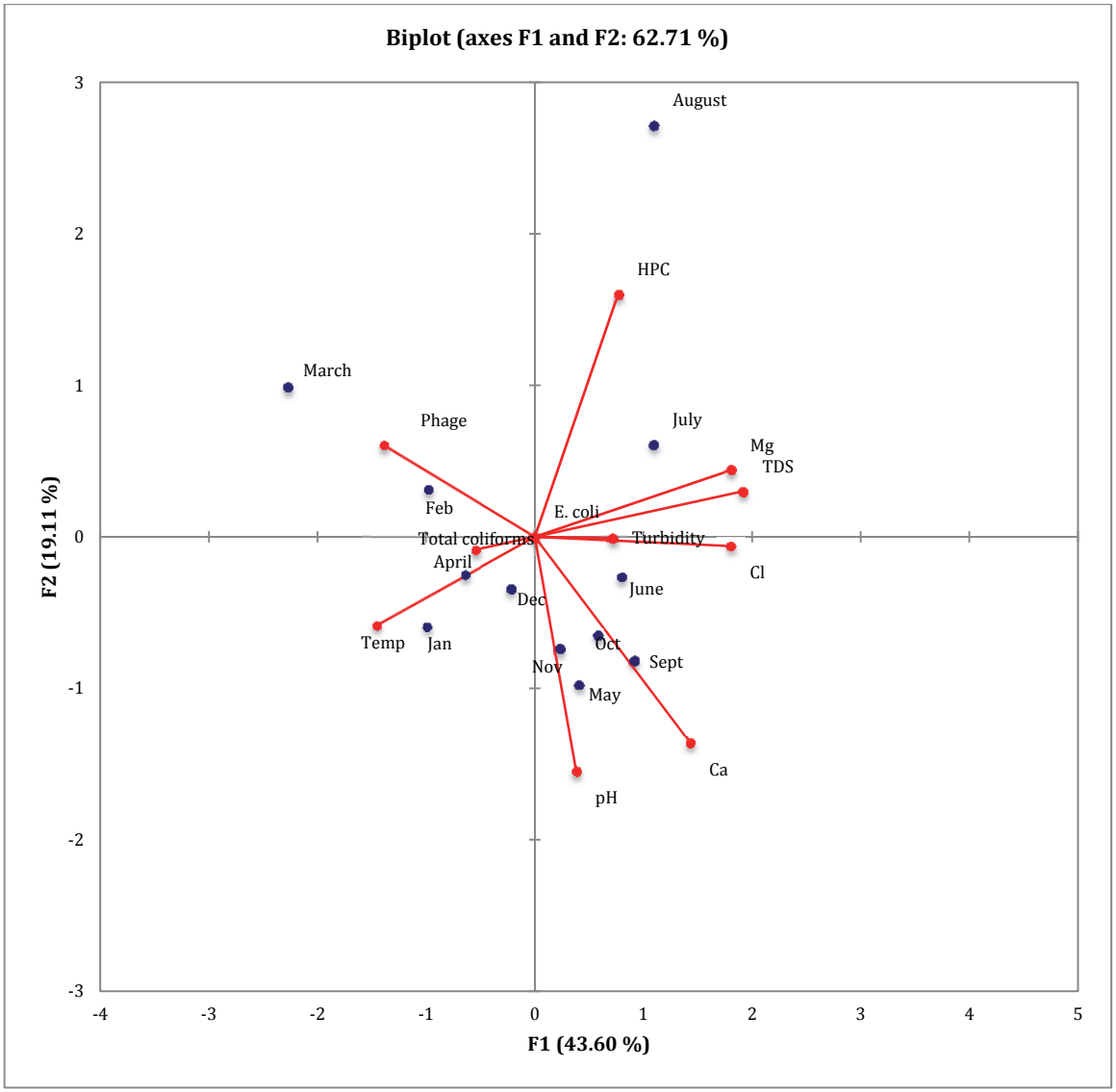

Figure 2: PCA biplot indicating the relationship of physico-chemical and microbiological data of the drinking water at DWPF labeled A measured over a one year period.

In Figure 2 the relationship between the monthly measurements was demonstrated. From this it is evident that in the drinking water, January, April and December associated with temperature and to a certain extend total coliforms. February and March associated with phage levels, November and May with pH and October and September with calcium levels. The levels of latter parameter (calcium) also had associations with December, November, May and June. Levels of magnesium, chloride TDS and turbidity were important impacts during June and July. There were also other associations.

4.3 DRINKING WATER PRODUCTION FACILITY (DWPF) B

4.3.1 Source water quality

The source water entering the DWPF B had a pH that was constantly above pH 8. Turbidity was very low and TDS generally high (+380 mg/ℓ). Total coliform and *E. coli* levels were high (Table 6). The levels of *E. coli* show some seasonal variations (log differences between the warmer rainy seasons (February to April) compared to the colder dry period (June to August). Table 7 shows the correlation matrix of physico-chemical and microbiological data of the source water entering DWPF B.

Principal component analysis showed that the only significant correlation was between the levels of *E. coli* and total coliforms (Figure 3). The PCA biplot demonstrated associations between the following levels of measured parameters and month in which it was measured: Turbidity, magnesium, *E. coli* and total coliforms levels impacted February, March April and September. Chloride and TDS impacted November and October. May, August and December were impacted by pH and June and July were impacted by calcium levels.

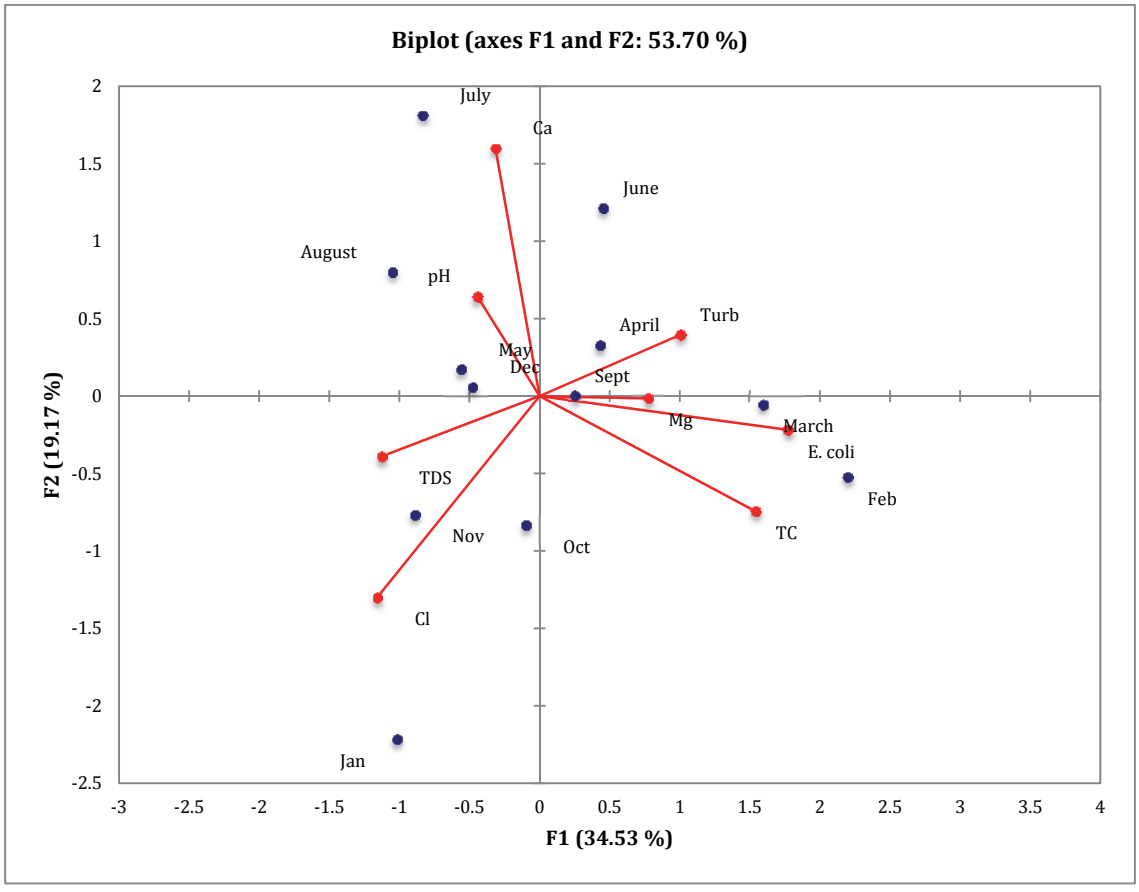

Figure 3: PCA biplot indicating the relationship of physico-chemical and microbiological data of the source water at DWPF B measured over a one year period

Table 6: Physical and microbiological parameters of source water entering the DWPF B

	pH	*Cl (mg/ℓ)*	*Ca (mg/ℓ)*	*Mg (mg/ℓ)*	*TDS (mg/ℓ)*	*Turbidity (NTU)*	E. coli *(cfu/100 mℓ)*	*Total coliforms (cfu/100 mℓ)*
Jan	8.32	35.75	46.00	51.95	487.50	1.67	817.00	22456.50
Feb	8.32	28.25	45.60	57.08	388.75	4.06	17416.50	36743.50
March	8.45	29.25	52.40	54.66	426.25	3.75	16072.50	40898.00
April	8.27	28.60	55.36	54.65	439.80	2.36	6840.40	19938.80
May	8.30	30.75	56.00	51.23	466.50	2.56	1229.00	12038.00
June	8.32	29.75	58.80	59.80	441.00	5.99	2172.50	10312.75
July	8.57	29.80	61.44	49.20	427.80	2.47	509.80	2173.60
August	8.39	29.75	58.00	51.95	486.75	1.82	540.75	2542.25
Sept	8.34	31.60	51.84	68.68	387.80	2.40	2343.40	8517.20
Oct	8.35	31.50	46.40	61.70	491.25	3.94	2896.25	13711.00
Nov	8.40	34.25	47.20	45.85	419.75	3.20	378.75	3543.00
Dec	8.63	30.80	45.12	57.96	450.00	3.40	353.20	6562.40

Table 7: Correlation matrix (Pearson (n)) of physico-chemical and microbiological data of the source water entering DWPF B

Variables	*Ca*	*Mg*	*Cl*	*pH*	*TDS*	*Turb*	*E. coli*	*TC*
Ca	**1**	-0.171	-0.454	-0.006	0.028	-0.041	-0.206	-0.332
Mg	-0.171	**1**	-0.206	-0.171	-0.242	0.317	0.135	0.110
Cl	-0.454	-0.206	**1**	-0.057	0.312	-0.325	-0.540	-0.267
pH	-0.006	-0.171	-0.057	**1**	-0.065	-0.018	-0.181	-0.293
TDS	0.028	-0.242	0.312	-0.065	**1**	-0.234	-0.480	-0.240
Turbidity	-0.041	0.317	-0.325	-0.018	-0.234	**1**	0.305	0.207
E. coli	-0.206	0.135	-0.540	-0.181	-0.480	0.305	**1**	**0.909**
TC	-0.332	0.110	-0.267	-0.293	-0.240	0.207	**0.909**	**1**

Values in bold are different from 0 with a significance level alpha=0.05

4.3.2 Final drinking water quality

The number of overlapping parameters for the drinking water was even less for drinking water at DWPF B. The pH was lower in the drinking water (Table 8) compared to the source water (Table 6). Microbial parameters were zero and turbidity was generally low (<1.0 NTU). February and March was impacted by turbidity (Figure 4). August and December was impacted by TDS. This parameter also impacted September, October and November.

Table 8: Physical, chemical and microbiological parameters for final water at DWPF B

	Chlorine mg/ℓ	pH	TDS mg/ℓ	Turbidity NTU	*E. coli* cfu/100 mℓ	Total coliforms cfu/100 mℓ
Jan	50.67	7.90	405.33	0.39	0.00	0.00
Feb	38.00	7.84	406.00	1.37	0.00	0.00
March	38.00	7.90	396.50	0.84	0.00	0.00
April	37.00	7.77	434.40	0.58	0.00	0.00
May	35.50	7.85	441.00	0.48	0.00	0.00
June	38.00	7.87	427.00	0.57	0.00	0.00
July	46.20	8.17	499.80	0.64	0.00	0.00
Aug	37.50	7.98	465.50	0.49	0.00	0.00
Sept	38.20	7.85	484.60	0.49	0.00	0.00
Oct	38.75	7.83	479.25	0.47	0.00	0.00
Nov	42.25	7.78	465.25	0.36	0.00	0.00
Dec	37.20	7.97	478.20	0.47	0.00	0.00

Figure 4 shows the PCA biplot indicating the relationship of physico-chemical and microbiological data of the drinking water at DWPF B measured over a one year period.

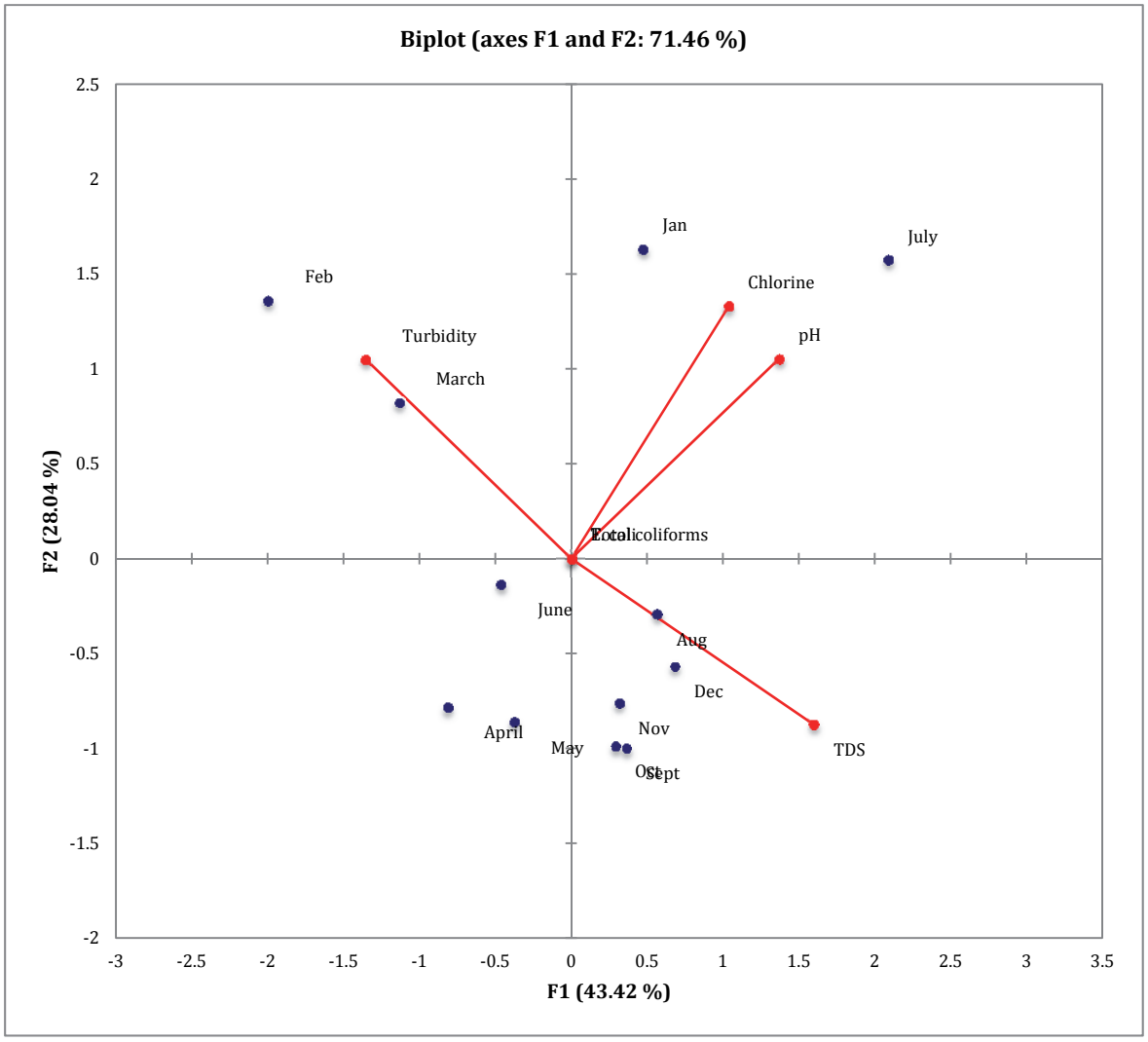

Figure 5: PCA biplot indicating the relationship of physico-chemical and microbiological data of the drinking water at DWPF B measured over a one year period.

4.4 DRINKING WATER PRODUCTION FACILITY (DWPF) C

4.4.1 Source water quality

No data were available for January and only some for July. TDS levels were generally lower than the previous two DWPFs' source water. The turbidity values provided were also very low. Microbiological parameters data were also not consistent (Table 9). However, statistical analysis was conducted on these measurements and showed the following (Table 10): Significant correlations were observed between TDS and chloride, total coliform and *E. coli* and HPC.

Table 9: Physical, chemical and microbiological parameters of source water entering the DWPF C

	pH	Cl mg/ℓ	Ca µg/ℓ	Mg mg/ℓ	TDS mg/ℓ	Turbidity NTU	*E. coli* cfu/100 mℓ	Total coliforms cfu/100 mℓ	HPC cfu/mℓ
Feb	7.62	6	57	32	256	0.2	38	114	200
March	7.69	8	44	25	492	0.2	2420	2420	1600
April	7.67	5	55	32	304	0.2	2	2	10
May	8.14	7	63	39	279	1	0	0	0
July	8.22						43		
Sept	8.34	5	42	25	284	0.9	172	1414	400
Oct	8.21	5	50	29	274	0.2	5	5	22
Nov	8.08	6	46	27	309	2.1	0	0	3
Dec	8.12	5	39	27	228	0.2	225	276	1200

Table 10: Correlation matrix (Pearson (n)) of physico-chemical and microbiological data of the source water entering DWPF C

Variables	pH	Cl	Ca_Raw	Mg	TDS	Turbidity	E coli	TC	HPC
pH	**1**	-0.379	-0.313	-0.128	-0.425	0.415	-0.385	-0.119	-0.192
Cl	-0.379	**1**	0.223	0.148	**0.764**	0.097	**0.726**	0.516	0.406
Ca_Raw	-0.313	0.223	**1**	**0.930**	-0.265	-0.028	-0.344	-0.497	-0.633
Mg	-0.128	0.148	**0.930**	**1**	-0.384	-0.013	-0.438	-0.592	-0.557
TDS	-0.425	**0.764**	-0.265	-0.384	**1**	-0.241	**0.993**	**0.846**	**0.750**
Turbidity	0.415	0.097	-0.028	-0.013	-0.241	**1**	-0.272	-0.186	-0.392
E coli	-0.385	**0.726**	-0.344	-0.438	**0.993**	-0.272	**1**	**0.878**	**0.817**
TC	-0.119	0.516	-0.497	-0.592	**0.846**	-0.186	**0.878**	**1**	**0.754**
HPC	-0.192	0.406	-0.633	-0.557	**0.750**	-0.392	**0.817**	**0.754**	**1**

Values in bold are different from 0 with a significance level alpha=0.05

From Figure 5 it is evident that February, April and May were impacted by calcium and magnesium; October and November by turbidity and pH and March by TDS and *E. coli*.

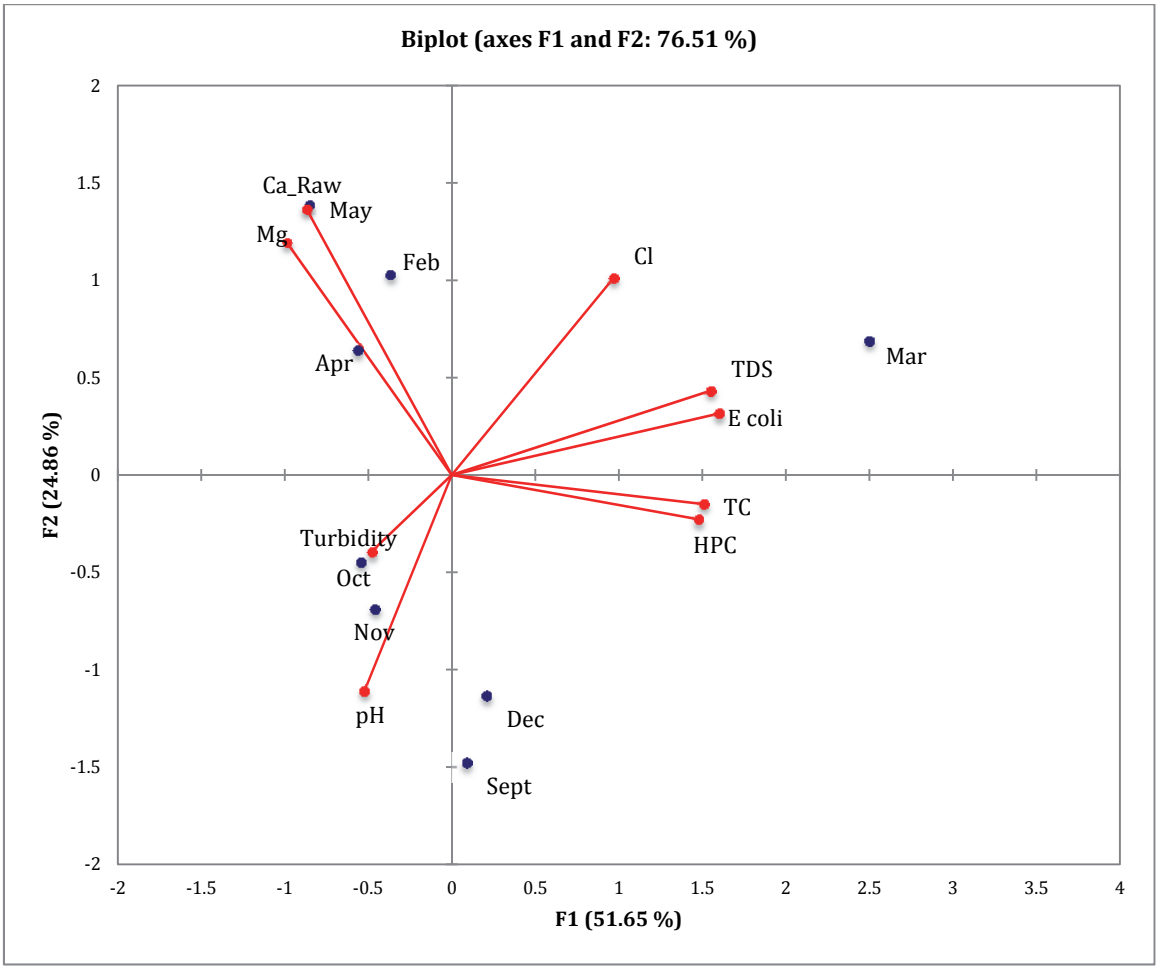

Figure 6: PCA biplot indicating the relationship of physico-chemical and microbiological data of the source water at DWPF labelled C measured over a one year period.

4.4.2 Final drinking water quality

No results were available for January, June and November (Table 11). What was of concern was that the turbidity was higher in the drinking water compared to the source water (Tables 9 and 11). The TDS and some of the other parameters were also generally low but in some cases slightly higher in the drinking water compared to the source water. Faecal indicator microbiological parameters (E. coli and total coliforms) were not detected. Strong significant correlations between levels of TDS, magnesium and calcium were observed (Table 12).

Table 11: Physical, chemical and microbiological parameters of drinking water from the DWPF C

	pH	Cl mg/ℓ	Ca µg/ℓ	Mg mg/ℓ	TDS mg/ℓ	Turbidity NTU	*E. coli* cfu/100 mℓ	Total coliforms cfu/100 mℓ	HPC cfu/mℓ
Feb	8.03	5	52	30	292	1.5	0	0	1
March	7.96	5	43	24	269	2.4	0	0	400
April	8.06	5	52	30	303	1.3	0	0	7
May	7.69	5	55	35	282	0.2	0	0	0
July	8.4	5	48	27	301	0.4	0	0	0
Sept	8.39	7	43	25	283	1.6	0	0	8
Oct	7.88	5	60	33	327	0.2	0	0	15
Dec	8.38	6	57	32	307	1.3	0	0	12

Table 12: Correlation matrix (Pearson (n)) of physico-chemical and microbiological data of the raw drinking water from DWPF labelled C.

Variables	*pH*	*Cl*	*Ca_Final*	*Mg*	*TDS*	*Turbidity*	*HPC*
pH	**1**	0.624	-0.358	-0.506	0.105	0.233	-0.206
Cl	0.624	**1**	-0.331	-0.321	-0.144	0.285	-0.190
Ca_Final	-0.358	-0.331	**1**	**0.925**	**0.763**	-0.651	-0.520
Mg	-0.506	-0.321	**0.925**	**1**	0.521	-0.701	-0.566
TDS	0.105	-0.144	**0.763**	0.521	**1**	-0.592	-0.572
Turbidity	0.233	0.285	-0.651	-0.701	-0.592	**1**	0.667
HPC	-0.206	-0.190	-0.520	-0.566	-0.572	0.667	**1**

Values in bold are different from 0 with a significance level alpha=0.05

In Figure 6 it is demonstrated that October and April were impacted by calcium and magnesium, September and July by pH and chloride, and March by HPC levels.

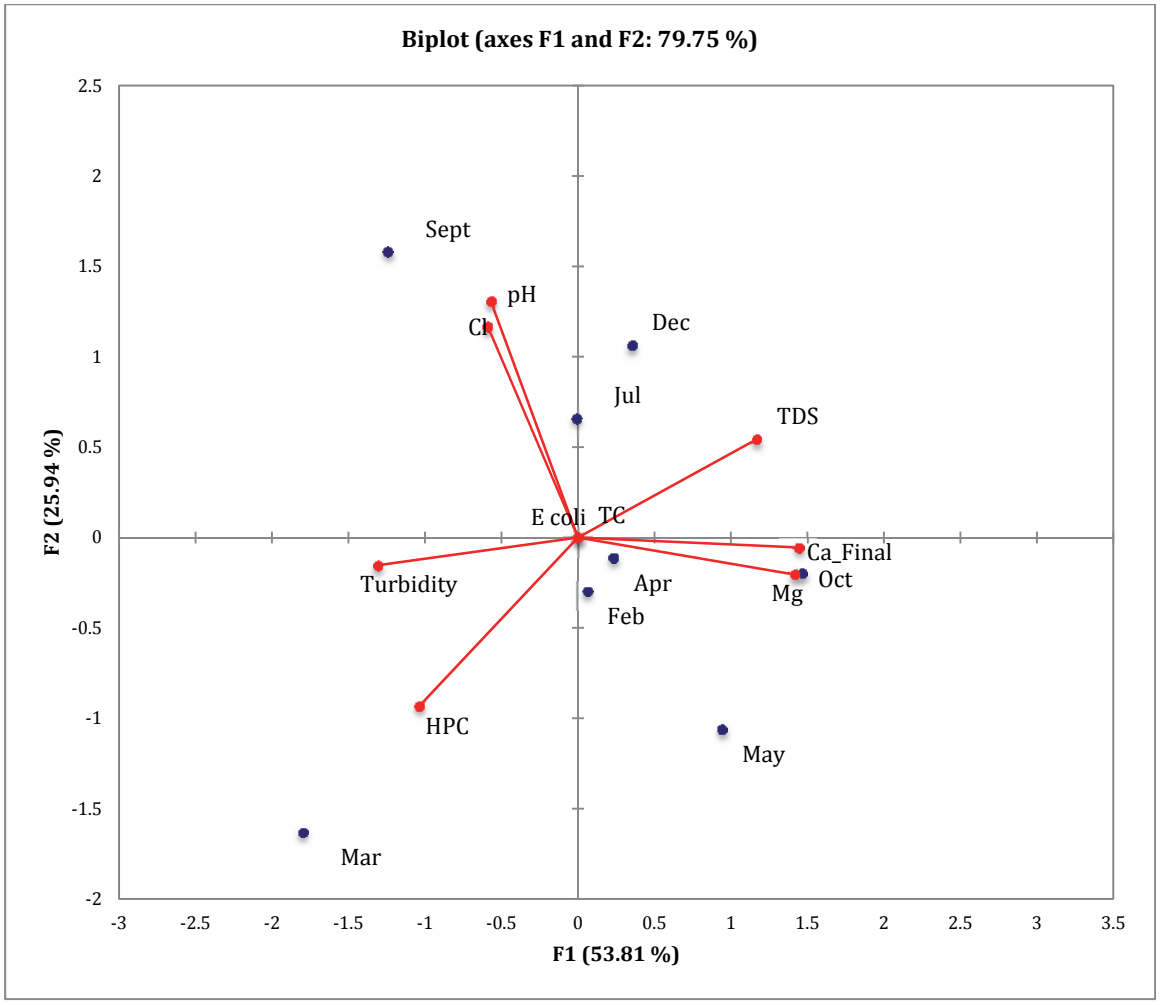

Figure 7: PCA biplot indicating the relationship of physico-chemical and microbiological data of the drinking water at DWPF labelled C measured over a one year period.

4.5 LEVELS OF ANTIMICROBIALS, AGROCHEMICALS AND PPCPs IN RAW AND DRINKING WATER

The levels of antimicrobials, agrochemicals and PPCPs in raw and drinking water were only studied in DWPF A. The list of antimicrobial substances, pharmaceutical personal care products and agrochemicals that were detected and quantified in source and drinking water of DWPF A is provided in Table 13. Some of the compounds were tested for but could not be analysed or the levels were below the limit of detection, and these included; ampicillin, penicillin G, neomycin, oxy-tetracycline, streptomycin, vancomycin, amphotericin B and chlorpyriphos.

From the results presented in Table 13 it is evident that a cocktail of organic substances which include agrochemicals, antimicrobials and pharmaceutical personal care products enter DWPF A. These were detected in the source water and levels ranged from 0.00040 to 0.9700 µg/ℓ. These levels were generally reduced by the DWPF treatment processes. In some cases the levels were reduced to below the lower limits of detection. Two of the antibiotics (chloramphenicol and trimethoprim), the biocide triclosan and the bactericide/fungicide (2-chlorophenol and 2,4-dichlorophenol) was detected in the source water, final water and in the bulk water in the distribution system.

There are previous reports of bacteria isolated from surface water in the Gauteng and North West Province that were resistant to some of the antibiotics listed in Table 13 (Bezuidenhout et al., 2013) as well as those that were tested for but not detected. The antibiotic resistance patterns of isolated bacteria will provide more evidence on whether these substances could potentially be responsible for selection of antibiotic resistance phenotypes.

Table 14 is a summary of some of the literature that also reported on the levels of the various organic substances detected in this study. This table and the work of Stackelberg et al. (2004) demonstrate that detecting the various substances in aquatic systems, even tap water is not uncommon. Knowing that these substances could be present in source and drinking water calls for monitoring the levels of these substances as well as determining appropriate mitigation approaches. Another important aspect is to determine seasonal variations as well as finding appropriate methods to remove these as well as other chemical from the source and drinking water.

Table 13: Antimicrobials, pharmaceutical personal care products and agrochemicals detected in source, final and the distribution system of DWPF A.

Substance	Classification	LOD (µg/ℓ)	Source water (µg/ℓ)	Final water (µg/ℓ)	Distribution system (Point 1) (µg/ℓ)	Distribution system (Point 2) (µg/ℓ)
Chloramphenicol	Antibiotic	0.00100	0.00346	0.00328	0.00266	0.00185
Erythromycin	Antibiotic	0.00100	0.21200	N/D	N/D	N/D
Trimethoprim	Antibiotic	0.00010	0.02280	0.00800	0.00212	0.00240
Tolnaftate	Antifungal (Human medicine)	0.00003	0.00272	N/D	N/D	N/D
Triclosan	Biocide (PPCP)	0.00400	0.07660	0.05560	0.03820	0.05740
3,4 methylbenzylidene	UV – Sun screen filter (PPCP)	0.00050	0.00280	0.00122	0.00260	0.00140
N,N-Diethyl-meta-toluamide (DEET)	Insect repellent (PPCP)	0.00010	0.05600	0.01480	0.01020	0.00900
Carbofuran	Insecticide – Carbamate (Agrochemical)	0.00003	0.00040	0.10000	N/D	N/D
BenfuraCarb	Insecticide – Carbamate (Agrochemical)	0.25000	0.97000	0.88000	N/D	N/D
2-chlorophenol	Bactericide, fungicide, intermediate for herbicide, insecticide (Agrochemical)	0.03000	0.60400	0.33400	0.15920	0.28000
2,4 di-chlorophenol	Bactericide, fungicide, intermediate for herbicide, insecticide (Agrochemical)	0.00200	0.07920	0.06180	0.06620	0.03740

N/D refers to levels that were below the limit of detection.

Table 14: Reported environmental levels of various organic substances detected in this study.

Substance	Classification	Concentration range in water systems (ng/ℓ)	Matrix	Reference
Chloramphenicol	Antibiotic	<2-40	Surface water	Birkholz et al., 2014
Erythromycin	Antibiotic	0.5-10 000	Urban wastewater and surface water	Birkholz et al., 2014
Trimethoprim	Antibiotic	<1.5-8 000	Urban wastewater and surface water	Birkholz et al., 2014
Tolnaftate	Antifungal (Human medicine)	-	-	-
-Triclosan	Biocide (PPCP)	ND-300 000	Urban wastewater and surface water	Birkholz et al., 2014
3,4 methylbenzylidene	UV – Sun screen filter (PPCP)	ND-18	Tap water	Birkholz et al., 2014
N,N-Diethyl-meta-toluamide (DEET)	Insect repellent (PPCP)	ND-370	Surface water and tap	Birkholz et al., 2014
Carbofuran	Insecticide – Carbamate (Agrochemical)	949-1671	Surface water	Chowdhury et al., 2012
BenfuraCarb	Insecticide – Carbamate (Agrochemical)	ND-50	River water	Kawamoto & Makihata, 2003
2-chlorophenol	Bactericide, fungicide, intermediate for herbicide, insecticide (Agrochemical)	-	-	-
2,4 di-chlorophenol	Bactericide, fungicide, intermediate for herbicide, insecticide (Agrochemical)	1.1-19960	River water	Gao et al., 2007

4.6 ANTIBIOTIC RESISTANT PROFILES

Bacterial antibiotic resistance profiles were studied in all DWPFs. Tables 15 to 26 show the lists of HPC isolated from the DWPFs. The inhibition zone data is shown in Tables 15, 17, 19, 21, 23 and 25 and the susceptibility/resistance interpretation data is shown in Tables 16, 18, 20, 22, 24 and 26.

4.6.1 DWPF A

In Table 15 (DWPF A period 1) it is evident that 7 of HPC from the source water could be purified and subjected to Kirby-Bauer antibiotic susceptibility testing. The inhibition zones were generally very large, showing that most of these were susceptible to most of the antibiotics. The interpretation data is provided in Table 16. In this table it is indicated that one of these isolates (A1-1R) was resistant to streptomycin, 2 (A1-7R; A1-10R) were resistant or displayed reduced susceptibility to β-lactam antibiotics and one was resistant to erythromycin. Among the 3 HPC from the final water that was tested, one isolate (AF1-1F) was resistant to β-lactam antibiotics, erythromycin and showed reduced susceptibility to chloramphenicol (Table 16).

During sample period 2 (Tables 17 and 18), none of the isolates from the final water showed reduced susceptibility or resistance to any of the antibiotics (Table 17) . However, among the 6 HPC isolates from the source water (raw water), 1 (A2-13R) had reduced susceptibility to chloramphenicol and resistance to β-lactam antibiotics. One of the isolates (A2-11R) was resistant to erythromycin and reduced susceptibility for cephalothin (Table 18).

Table 15: Antibiotic inhibition zone data of the various HPC isolates from DWPF A (sampling period 1)

Site	Isolate	Gram reaction	AMP	KF	Ery	Chl	CIP	Kan	Neo	Strep	Van
A raw	A1-1R		18	20	30	30	31	34	31	0	
	A1-3R	-	19	30	15	20	26	28	25	22	
	A1-5R	-	0	10	27	26	28	20	23	22	
	A1-6R	+	25	19	23	20	38	31	35	34	30
	A1-7R	-	13	0	12	21	36	20	23	16	
	A1-10R	-	0	13	21	27	28	20	22	18	
	A1-13R	-	40	50	38	35	35	39	41	31	
A final	AF1-1F	-	12	0	12	17	23	19	22	19	
	AF1-2F	+	28	38	25	27	34	25	27	24	23
	AF1-3F	+	21	44	27	28	34	30	32	29	21

AMP- Ampicillin; KF – Cephalothin; Ery- Erythromycin; Chl – Chloramphenicol ; CIP- Ciprofloxacin; Kan – Kanamycin; Neo- Neomycin ; Strep- Streptomycin; Van- Vancomycin

Table 16: Antibiotic susceptibility patterns of the HPC isolates from DWPF A (sampling period 1)

Site	Isolate	Gram reaction	AMP	KF	Ery	Chl	CIP	Kan	Neo	Strep	Van
A raw	A1-1R		S	S	S	S	S	S	S	R	
	A1-3R	-	S	S	I	S	S	S	S	S	
	A1-5R	-	R	R	S	S	S	S	S	S	
	A1-6R	+	S	S	S	S	S	S	S	S	S
	A1-7R	-	I	R	R	S	S	S	S	I	
	A1-10R	-	R	I	S	S	S	S	S	S	
	A1-13R	-	S	S	S	S	S	S	S	S	
A final	AF1-1F	-	R	R	R	I	S	S	S	S	
	AF1-2F	+	S	S	S	S	S	S	S	S	S
	AF1-3F	+	S	S	S	S	S	S	S	S	S

AMP- Ampicillin; KF – Cephalothin; Ery- Erythromycin; Chl – Chloramphenicol ; CIP- Ciprofloxacin; Kan – Kanamycin; Neo- Neomycin ; Strep- Streptomycin; Van- Vancomycin

(R = resistance, S = Susceptible, I = Intermediate resistant).

Table 17: Antibiotic inhibition zone data of the various HPC isolates from DWPF A (sampling period 2)

Site	Isolate	Gram reaction	AMP	KF	Ery	Chl	CIP	Kan	Neo	Strep	Van
A Raw 2	A2-1R	-	24	40	25	29	30	29	28	21	
	A2-3R	-	28	39	30	23	30	28	28	25	
	A2-8R	+	36	37	43	35	44	32	35	27	32
	A2-10R	-	45	52	50	38	43	38	38	38	
	A2-11R	-	30	17	0	36	31	33	38	0	
	A2-13R	-	0	9	21	15	23	19	20	19	
A Final 2	AO2-3F	+	23	43	24	26	33	20	28	23	24
	AO2-9F	-	41	44	44	36	44	47	35	38	

AMP- Ampicillin; KF – Cephalothin; Ery- Erythromycin; Chl – Chloramphenicol ; CIP- Ciprofloxacin; Kan – Kanamycin; Neo- Neomycin ; Strep- Streptomycin; Van- Vancomycin

Table 18: Antibiotic susceptibility patterns of the HPC isolates from DWPF A (sampling period 2)

Site	Isolate	Gram reaction	AMP	KF	Ery	Chl	CIP	Kan	Neo	Strep	Van
A Raw 2	A2-1R	-	S	S	S	S	S	S	S	S	
	A2-3R	-	S	S	S	S	S	S	S	S	
	A2-8R	+	S	S	S	S	S	S	S	S	S
	A2-10R	-	S	S	S	S	S	S	S	S	
	A2-11R	-	S	I	R	S	S	S	S	R	
	A2-13R	-	R	R	S	I	S	S	S	S	
A Final 2	AO2-3F	+	S	S	S	S	S	S	S	S	S
	AO2-9F	-	S	S	S	S	S	S	S	S	

AMP- Ampicillin; KF – Cephalothin; Ery- Erythromycin; Chl – Chloramphenicol ; CIP- Ciprofloxacin; Kan – Kanamycin; Neo- Neomycin ; Strep- Streptomycin; Van- Vancomycin
R = resistance, S = Susceptible, I = Intermediate resistant).

4.6.2 DWPF B

Table 19 provides the inhibition zone data for the various isolates from drinking water production facility B and Table 20 an interpretation. There were 12 isolates from the source water but only one of the final water could be successfully cultured to be analysed for antibiotic resistance. Isolates from both the compartments were resistant to β-lactam antibiotics (ampicillin and Cephalothin) as well as oxy-tetracyclin and trimethoprim. Among the Gram-positive bacteria, all were also resistant the penicillin G and some were resistant to vancomycin. A large proportion of the source water isolates had reduced susceptibility (intermediate resistance) to erythromycin and kanamycin. Some were also resistant to streptomycin. At least one of the source water isolates were resistant to all three aminoglycosides (kanamycin, neomycin, streptomycin). Multiple resistance (resistance to more than two antibiotic classes) was common among these isolates.

During the second sampling run, a larger number of isolates were obtained (28 from source water and 8 from the final water). The inhibition zone data is provided in Table 21 and the interpretation in Table 22). Resistance and reduced susceptibility (intermediate resistance) was common to ampicillin, erythromycin, kanamycin and neomycin. Some of the isolates were also resistant to trimethoprim. Several of the isolates from the final water had reduced susceptibility to several classes of antibiotics.

Table 19: Antibiotic inhibition zone data of the various HPC isolates from DWPF B (sampling period 1)

Site	Isolate	Gram reaction	AMP	KF	Ery	Chl	CIP	Kan	Neo	Strep	Van	Pen-G	O-T	TM
B-Raw	B1-2R	+	0	0	19	12	28	0	29	0	10	12	10	12
	B1-3R	-	0	9	17	26	18	0	10	9	N/A	N/A	12	0
	B1-4R	+	13	7	12	14	27	23	27	14	0	0	23	0
	B1-5R	+	0	0	19	0	23	23	24	0	0	0	11	0
	B1-6R	-	0	0	19	-	22	0	25	0	N/A	N/A	22	12
	B1-8R	-	22	32	24	27	26	27	25	19	N/A	N/A	9	36
	B1-9R	+	0	8	18	24	13	17	12	15	11	0	19	0
	B1-11R	-	19	32	23	21	30	25	30	24	N/A	N/A	15	21
	B1-13R	-	0	12	25	20	20	14	21	18	N/A	N/A	21	0
	B1-14R	+	10	16	22	30	25	20	25	25	20	13	10	0
	B1-15R	-	10	12	25	25	25	22	15	25	N/A	N/A	21	0
	B1-16R	-	13	19	22	32	32	0	20	25	N/A	N/A	_	0
B-Final	B1-1F	+	8	12	12	21	24	15	23	20	18	0	10	0

Table 20: Antibiotic susceptibility patterns of the HPC isolates from DWPF B (sampling period 1)

Site	Isolate	Gram reaction	AMP	KF	Ery	Chl	CIP	Kan	Neo	Strep	Van	Pen-G	O-T	TM
B-Raw	B1-2R	+	R	R	I	R	S	R	S	R	R	R	R	I
	B1-3R	-	R	R	I	S	I	R	R	R	N/A	N/A	R	R
	B1-4R	+	R	R	R	I	S	S	S	I	R	R	S	R
	B1-5R	+	R	R	I	R	S	S	S	R	R	R	R	R
	B1-6R	-	R	R	I	-	S	R	S	R	N/A	N/A	S	I
	B1-8R	-	S	S	S	S	S	S	S	S	N/A	N/A	R	S
	B1-9R	+	R	R	I	S	R	I	R	S	R	R	S	R
	B1-11R	-	S	S	S	S	S	S	S	S	N/A	N/A	I	S
	B1-13R	-	R	R	S	S	S	I	S	S	N/A	N/A	S	R
	B1-14R	+	R	I	I	S	S	-	S	S	S	R	R	R
	B1-15R	-	R	R	S	S	S	S	I	S	N/A	N/A	S	R
	B1-16R	-	R	S	I	S	S	R	S	S	N/A	N/A	-	R
B-Final	B1-1F	+	R	R	R	S	S	I	S	S	S	R	R	R

AMP- Ampicillin; KF – Cephalothin; Ery- Erythromycin; Chl – Chloramphenicol ; CIP- Ciprofloxacin; Kan – Kanamycin; Neo- Neomycin ; Strep- Streptomycin; Van- Vancomycin; Pen-G – Penicillin G; O-T – Oxy-tetracycline; TM – Trimethoprim. (R = resistance, S = Susceptible, I = Intermediate resistant; N/A not applicable;).

Table 21: Antibiotic inhibition zone data of the various HPC isolates from DWPF B (sampling period 2)

Site	Isolate	Gram reaction	AMP	KF	Ery	Chl	CIP	Kan	Neo	Strep	Van	Pen-G	O-T	TM
A raw	B2-1R	-	14	28	17	24	27	33	26	28	N/A	N/A	27	0
	B2-2R	-	10	18	18	29	27	11	18	21	N/A	N/A	21	0
	B2-3R	-	14	31	25	12	27	31	14	33	N/A	N/A	21	20
	B2-4R	-	19	28	9	25	32	0	9	30	N/A	N/A	27	34
	B2-6R	-	0	30	14	0	0	0	0	33	N/A	N/A	28	33
	B2-7R	-	12	19	0	15	34	0	0	33	N/A	N/A	20	23
	B2-9R	-	28	32	37	46	48	22	37	32	N/A	N/A	22	17
	B2-10R	-	24	39	19	21	22	27	34	0	N/A	N/A	35	0
	B2-11R	-	23	24	NG	35	35	13	27	13	N/A	N/A	25	0
	B2-12R	-	29	30	24	40	41	12	12	24	N/A	N/A	21	0
	B2-13R	-	27	30	24	40	24	21	24	25	N/A	N/A	26	23
	B2-14R	-	0	18	0	14	16	0	0	20	N/A	N/A	19	30
	B2-15R	-	29	30	22	30	30	31	22	30	N/A	N/A	30	34
	B2-16R	-	13	12	16	34	12	25	30	48	N/A	N/A	55	20
	B2-17R	-	24	0	NG	34	23	33	NG	22	N/A	N/A	NG	16
	B218R	-	15	35	NG	33	27	45	23	21	N/A	N/A	35	20
	B2-19R	-	34	34	NG	28	34	NG	26	16	N/A	N/A	33	24
	B2-20R	-	16	23	28	16	15	34	25	30	N/A	N/A	26	33
	B2-21R	-	0	29	22	29	34	NG	27	24	N/A	N/A	23	37
	B2-22R	-	41	30	29	37	32	NG	33	NG	N/A	N/A	23	40
	B2-23R	+	20	0	0	24	18	0	0	0	17	0	15	17
	B2-24R	+	36	44	40	27	33	26	40	25	17	30	34	0

	B2-26R	-	0	27	7	34	31	30	7	14	N/A	N/A	26	43
	B2-27R	-	22	25	14	25	37	23	22	35	N/A	N/A	26	21
	B2-28R	+	0	26	12	38	20	30	12	23	0	26	23	33
	B2-29R	+	29	29	23	28	37	32	23	23	30	28	30	16
	B2-30R	-	12	43	30	27	33	24	28	23	N/A	N/A	20	30
	B2-31R	+	10	20	26	30	29	10	26	18	0	23	21	33
A final	B2-2F	+	23	28	24	25	37	40	29	29	19	12	27	31
	B2-3F	+	22	21	18	30	0	30	33	20	0	20	20	22
	B2-4F	+	13	37	17	15	16	30	32	20	0	30	17	20
	B2-5F	-	13	16	32	15	17	12	34	11	N/A	N/A	25	23
	B2-6F	+	11	18	20	20	12	14	26	36	0	23	20	0
	B2-7F	+	25	30	30	38	14	0	50	33	12	30	34	22
	B2-8F	-	24	13	12	30	30	17	30	27	N/A	N/A	27	20
	B2-10F	+	13	15	22	23	36	37	34	25	15	24	37	25

AMP- Ampicillin; KF – Cephalothin; Ery- Erythromycin; Chl – Chloramphenicol ; CIP- Ciprofloxacin; Kan – Kanamycin; Neo- Neomycin ; Strep- Streptomycin; Van- Vancomycin; Pen-G – Penicillin G; O-T – Oxy-tetracycline; TM – Trimethoprim. (R = resistance, S = Susceptible, I = Intermediate resistant; N/A – not applicable; NG – no growth).

Table 22: Antibiotic susceptibility patterns of the HPC isolates from DWPF B (sampling period 2)

Site	Isolate	Gram reaction	AMP	KF	Ery	Chl	CIP	Kan	Neo	Strep	Van	Pen-G	O-T	TM
A raw	B2-1R	-	I	S	I	S	S	S	S	S	N/A	N/A	S	R
	B2-2R	-	R	S	I	S	S	R	S	S	N/A	N/A	S	R
	B2-3R	-	I	S	S	R	S	S	R	S	N/A	N/A	S	S
	B2-4R	-	S	S	R	S	S	R	R	S	N/A	N/A	S	S
	B2-6R	-	R	S	R	R	R	R	R	S	N/A	N/A	S	S
	B2-7R	-	R	S	R	I	S	R	R	S	N/A	N/A	S	S
	B2-9R	-	S	S	S	S	S	S	S	S	N/A	N/A	S	S
	B2-10R	-	S	S	I	S	S	S	S	R	N/A	N/A	S	R
	B2-11R	-	S	S	NG	S	S	R	S	I	N/A	N/A	S	R
	B2-12R	-	S	S	S	S	S	R	R	S	N/A	N/A	S	R
	B2-13R	-	S	S	I	S	S	S	S	S	N/A	N/A	S	S
	B2-14R	-	R	S	I	I	I	R	R	S	N/A	N/A	S	S
	B2-15R	-	S	S	S	S	S	S	S	S	N/A	N/A	S	S
	B2-16R	-	R	R	I	S	R	S	S	S	N/A	N/A	S	S
	B2-17R	-	S	R	NG	S	S	S	NG	S	N/A	N/A	NG	S
	B2-18R	-	I	S	NG	S	S	S	S	S	N/A	N/A	S	S
	B2-19R	-	S	S	NG	S	S	NG	S	S	N/A	N/A	S	S
	B2-20R	-	I	S	S	I	15	S	S	S	N/A	N/A	S	S
	B2-21R	-	R	S	I	S	S	NG	S	S	N/A	N/A	S	S
	B2-22R	-	S	S	S	S	S	NG	S	NG	N/A	N/A	S	S
	B2-23R	+	S	R	R	S	R	R	R	R	S	R	S	S
	B2-24R	+	S	S	S	S	S	S	S	S	S	S	S	R

	B2-26R	-	R	S	R	S	S	S	R	I	N/A	N/A	S	S
	B2-27R	-	S	S	I	S	S	S	S	S	N/A	N/A	S	S
	B2-28R	+	R	S	R	S	I	S	R	S	R	S	S	S
	B2-29R	+	S	S	S	S	S	S	S	S	S	S	S	S
	B2-30R	-	R	S	S	S	S	S	S	S	N/A	N/A	S	S
	B2-31R	+	R	S	S	S	S	R	S	S	0	S	S	S
A final	B2-2F	+	S	S	S	S	S	S	S	S	S	R	S	S
	B2-3F	+	S	S	I	S	0	S	S	S	R	S	S	S
	B2-4F	+	R	S	I	I	I	S	S	S	R	S	S	S
	B2-5F	-	R	I	S	I	I	R	S	R	N/A	N/A	S	S
	B2-6F	+	R	S	I	S	I	I	S	S	R	S	S	R
	B2-7F	+	S	S	S	S	I	R	S	S	R	S	S	S
	B2-8F	-	S	R	R	S	S	I	S	S	N/A	N/A	S	S
	B2-10F	+	R	I	I	S	S	S	S	S	S	S	S	S

AMP- Ampicillin; KF – Cephalothin; Ery- Erythromycin; Chl – Chloramphenicol ; CIP- Ciprofloxacin; Kan – Kanamycin; Neo- Neomycin ; Strep- Streptomycin; Van- Vancomycin; Pen-G – Penicillin G; O-T – Oxy-tetracycline; TM – Trimethoprim. (R = resistance, S = Susceptible, I = Intermediate resistant).

4.6.3 DWPF C

In Tables 23 and 24 the inhibition zone data and the interpretation, respectively, for the first sampling period for DWPF C is provided In this system all isolates (10/10) were resistant to β-lactam antibiotics (including penicillin-G for the Gram positive isolates), trimethoprim and also mostly (9/10) oxy-tetracycline. In the latter case the isolate demonstrated reduced susceptibility to O-T. All the Gram positive isolates showed reduced susceptibility to vancomycin. One (C1-27F) was resistant to the latter antibiotic and had intermediate resistance to neomycin and streptomycin. One isolate from the distribution system (C1-22D) also had reduced susceptibility towards the latter aminoglycoside resistance.

The data in Tables 25 and 26 are for the second sampling period. From table 26 it is evident that the antibiotic resistance pattern amongst the isolates is generally similar to those of the first sampling period, with respect to two of the β-lactams (ampicillin and cephalothin), oxy-tetracycline and trimethoprim. Resistance and intermediate resistance was also observed among a few individual isolates to erythromycin, chloramphenicol and ciproflaxacin. One of the multiple resistant isolates were resistant to neomycin and streptomycin.

Table 23: Antibiotic inhibition zone data of the various HPC isolates from DWPF C (sampling period 1)

Site	Isolate	Gram reaction	AMP	KF	Ery	Chl	CIP	Kan	Neo	Strep	Van	Pen-G	O-T	TM
Raw water	C1-2R	-	0	0	23	19	27	20	23	24			12	0
	C1-11R	-	0	7	24	21	27	20	24	23			11	0
After chlorination	C1-13F	+	0	0	15	20	25	20	23	23	15	0	0	0
	C1-15F	-	0	0	25	20	25	19	23	23			12	0
	C1-16F		0	0	24	21	26	22	21	21	15	0	12	0
	C1-25F	-	0	0	20	19	24	19	22	20			11	0
	C1- 27F	+	0	0	22	18	21	20	17	16	12	0	0	0
Distribution	C1-19D	-	0	0	20	20	24	19	21	19			12	0
	C1-22D	+	0	0	24	22	27	20	16	16	15	0	11	0
	C1-24D	+	0	0	19	21	23	25	22	24	15	0	13	0

Table 24: Antibiotic susceptibility patterns of the HPC isolates from DWPF C (sampling period 1)

Site	Isolate	Gram reaction	AMP	KF	Ery	Chl	CIP	Kan	Neo	Strep	Van	Pen-G	O-T	TM
Raw water	C1-2R	-	R	R	S	S	S	S	S	S			R	R
	C1-11R	-	R	R	S	S	S	S	S	S			R	R
After chlorination	C1-13F	+	R	R	I	S	S	S	S	S	I	R	R	R
	C1-15F	-	R	R	S	S	S	S	S	S			R	R
	C1-16F		R	R	S	S	S	S	S	S	I	R	R	R
	C1-25F	-	R	R	S	S	S	S	S	S			R	R
	C1- 27F	+	R	R	S	S	S	S	I	I	R	R	R	R
Distribution	C1-19D	-	R	R	S	S	S	S	S	S			R	R
	C1-22D	+	R	R	S	S	S	S	I	I	I	R	R	R
	C1-24D	+	R	R	S	S	S	S	S	S	I	R	I	R

AMP- Ampicillin; KF – Cephalothin; Ery- Erythromycin; Chl – Chloramphenicol ; CIP- Ciprofloxacin; Kan – Kanamycin; Neo- Neomycin ; Strep- Streptomycin; Van- Vancomycin; Pen-G – Penicillin G; O-T – Oxy-tetracycline; TM – Trimethoprim. (R = resistance, S = Susceptible, I = Intermediate resistant).

Table 25: Antibiotic inhibition zone data of the various HPC isolates from DWPF C (sampling period 2)

Site	Isolate	Gram reaction	AMP	KF	Ery	Chl	CIP	Kan	Neo	Strep	Van	Pen-G	OT	TM
A raw	C2-1R	-	20	-	-	-	-	-	41	24	NA	NA	34	46
	C2-2R	-	18	0	0	12	30	0	22	0	NA	NA	9	0
	C2-3R	+	34	36		46		44	34	31	26	30	34	0
	C2-4R	-	0	0	0	21	0	30	16	22	NA	NA	18	0
	C2-5R	+	-	-	-	44	-	-	38	-	-	-	42	20
	C2-8R	+	15	11	31	38	37	27	24	26	24	24	13	0
	C2-9R	-	26	32	42	38	52	40	-	-	NA	NA	24	11
	C2-10R	-	11	15	27	12	0	30	30	21	NA	NA	15	35
	C2-14R	-	28	24	-	-	-	-	-	-	NA	NA	-	0
	C2-15R	+	24	25	43	45	54	33	31	30	32	32	26	0
	C2-18R	+	0	0	26	19	18	13	20	20	19	19	14	0
	C2-20R	-	9	16	32	33	34	23	26	26	NA	NA	18	0
A final	CF2-23F	+	0	0	21	25	27	17	18	20	19	19	8	0
	CF2-24F	+	0	0	23	28	14	18	23	15	21	21	12	0
	CF2-25F	+	16	0	24	30	26	20	24	23	21	21	18	0
	CF2-26F	+	11	15	24	32	27	21	26	21	27	27	16	0

AMP- Ampicillin; KF – Cephalothin; Ery- Erythromycin; Chl – Chloramphenicol ; CIP- Ciprofloxacin; Kan – Kanamycin; Neo- Neomycin ; Strep- Streptomycin; Van- Vancomycin

Table 26: Antibiotic susceptibility patterns of the HPC isolates from DWPF C (sampling period 2)

Site	Isolate	Gram reaction	AMP	KF	Ery	Chl	CIP	Kan	Neo	Strep	Van	Pen-G	OT	TM
A raw	C2-1R	-	S	-	-	-	-	-	S	S	N/A	N/A	S	S
	C2-2R	-	S	R	R	R	S	S	R	R	N/A	NA	R	R
	C2-3R	+	S	S	-	S	-	S	S	S	S	S	S	R
	C2-4R	-	R	R	R	S	R	S	I	S	N/A	NA	I	R
	C2-5R	+	-	-	-	S	-	-	S	-	-	-	S	S
	C2-8R	+	I	R	S	S	S	S	S	S	S	S	R	R
	C2-9R	-	S	S	S	S	S	S	-	-	N/A	NA	S	S
	C2-10R	-	R	I	S	R	S	S	S	S	N/A	NA	I	I
	C2-14R	-	S	S	-	-	-	-	-	-	-	NA	-	R
	C2-15R	+	S	S	S	S	S	S	S	S	S	S	S	R
	C2-18R	+	R	S	S	S	I	R	S	S	S	S	R	R
	C2-20R	-	R	I	S	S	S	S	S	S	N/A	NA	I	R
A final	CF2-23F	+	R	R	S	S	S	I	S	S	S	S	R	R
	CF2-24F	+	R	R	S	S	R	S	S	S	S	S	R	R
	CF2-25F	+	I	R	S	S	S	S	S	S	S	S	I	R
	CF2-26F	+	R	I	S	S	S	S	S	S	S	S	I	R

4.7 SUMMARY OF FINDINGS

In two of the DWPFs turbidity was effectively reduced to below recommended drinking water standards (<1 NTU; DWA 2005). However, the levels were not >5 NTU. Microbial levels were generally high in the source water but were effectively reduced to drinking water standards (DWA 2005; SANS 241: 2015). From the available physico-chemical data it is evident that total dissolved solid levels were generally high in source water, except for one of the systems. Over an annual cycle the various DWPFs did not remove the TDS. There were correlations of the high TDS levels and levels of calcium and magnesium. In some of the scenarios TDS levels correlated with the levels of microbial parameters measured. This observation could probably be attributed to the protective capability of calcium and magnesium at high levels. High TDS levels in the northern provinces of South Africa could be due to geology at the source and during the course of the water systems (DWAF, 1996a).

Besides its requirement for growth of microbes and integrity of structural systems, calcium is also important in the regulation of transport of substances across membranes of these microbes. Aich et al. (2012) demonstrated that elevated levels of calcium increase the expression of certain membrane proteins such as OmpC. These proteins are involved in the uptake of DNA directly from aquatic systems (Aich et al., 2012). This takes place particularly when, in addition to calcium, sodium and chloride ions as well as some dissolved organic materials are also present. It was also recently demonstrated that the presence and levels of these two cations (calcium and magnesium) are important in antimicrobial activity of certain antibiotics (Matzneller et al., 2015). Thus including these ions in analyses of aquatic systems may be useful in understanding and explaining antibiotic resistance patterns. The pH of the source and drinking water was generally slightly alkaline and potentially reflected the elevated levels of cations present in the water. However, the pH levels were within drinking water (pH 6 to 9; DWA, 2005) and agricultural (DWAF, 1996ab) use standards. Physico-chemical and microbiological water quality data provided and overview and a context for interpretation of the antibiotic resistance data.

In so far as the specific objectives of the study are concerned, three antibiotics, an antifungal agent and agrochemicals that have antimicrobial effects were detected in the source water of DWPF A. Some of these were also detect in the final water and in the distribution system. The levels of these antibiotics were at sub-therapeutic levels (in the ng/ℓ range).

Antibiotic resistant heterotrophic plate count bacteria were commonly isolated from source and final water. Resistance to β-lactam antibiotics and trimethoprim was common amongst the isolates. In some cases the isolates were also resistant to oxytetracyline, chloramphenicol as well as erythromycin and selected aminoglycosides.

5 CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

Physico-chemical data obtained was used to provide an overview of the general characteristics of the source and drinking water for three drinking water production facilities. The final water quality in each of the DWPF was within SANS 241 (2015) for the various parameters.

One of the objectives was to determine if antibiotic resistant bacteria are present in the drinking water production facilities and distribution system. From the results it is evident that such bacteria are present in these compartments: source and final water. The situation is more pronounced in DWPF C, compared to DWPF A. This trend has been observed in other local studies (egg. Mulamattathil et al., 2014ab). Finding these bacteria in aquatic systems further demonstrates that water systems act as reservoirs of ARBs and ARGs and very low level of antimicrobial substances could result in a selection pressure for such antibiotic resistance patterns (Birkholz et al., 2014; Stackelberg et al., 2004; Watkinson et al., 2009). In the present study it was demonstrated that a cocktail of organic substances, including antibiotics, agrochemicals and PPCPs, were detected in source water and in final drinking water. Although this is not uncommon that potentially provides a selection pressure for the accumulation of antibiotic resistant bacteria.

The detection range of antibiotics and other chemicals in the present study was similar to that of Watson et al (2009) and other international studies. The results presented in the present study suggest a reduction of these antibiotics and other organic substances by the drinking water system. However, as recognised by Bai et al. (2015) and other researchers including Watson et al. (2009), the studied systems were not designed to eliminate antibiotics. Thus finding these substances in extremely low concentrations in the final water when the source water is contaminated should not be regarded as uncommon. However, it could have implications on the spread of antibiotic resistance.

5.2 RECOMMENDATIONS

Based on the finding from this study, the following can be recommended:

- A comprehensive study on antimicrobial substances removal capacity of various drinking water treatment configurations in operation in South Africa. These should also be done under varied flow conditions.
- Sufficient repeats be conducted so that statistical analyses could be done to investigate the relationship between ARB, antimicrobials, agrochemicals and physico-chemical parameters.

- The presence, distribution and dynamics of antibiotic resistance genes in the ARBs be investigated. However, the presence of these genes/genetic materials in bulk water should also be investigated with a focus on the potential for transfer to susceptible bacteria.
- Data from such studies be used to determine if mitigation is necessary and if so which strategies could be used or developed that would be appropriate for local conditions.

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