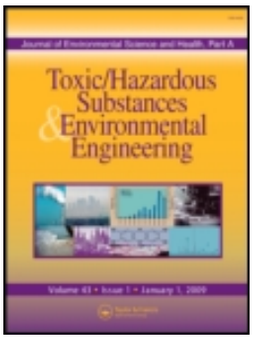

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The effectiveness of sewage treatment processes to remove faecal pathogens and antibiotic residues

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Pathogens and antibiotics enter the aquatic environment via sewage effluents and may pose a health risk to wild life and humans. The aim of this study was to determine the levels of faecal bacteria, and selected antibiotic residues in raw wastewater and treated sewage effluents from three different sewage treatment plants in the Western Cape, South Africa. Sewage treatment plant 1 and 2 use older technologies, while sewage treatment plant 3 has been upgraded and membrane technologies were incorporated in the treatment processes. Coliforms and *Escherichia coli* (*E. coli*) were used as bioindicators for faecal bacteria. A chromogenic test was used to screen for coliforms and *E. coli*. Fluoroquinolones and sulfamethoxazole are commonly used antibiotics and were selected to monitor the efficiency of sewage treatment processes for antibiotic removal. Enzyme Linked Immunosorbent Assays (ELISAs) were used to quantitate antibiotic residues in raw and treated sewage. Raw intake water at all treatment plants contained total coliforms and *E. coli.* High removal of *E. coli* by treatment processes was evident for treatment plant 2 and 3 only. Fluoroquinolones and sulfamethoxazole were detected in raw wastewater from all sewage treatment plants. Treatment processes at plant 1 did not reduce the fluoroquinolone concentration in treated sewage effluents. Treatment processes at plant 2 and 3 reduced the fluoroquinolone concentration by 21% and 31%, respectively. Treatment processes at plant 1 did not reduce the sulfamethoxazole concentration in treated sewage effluents. Treatment processes at plant 2 and 3 reduced sulfamethoxazole by 34% and 56%, respectively. This study showed that bacteria and antibiotic residues are still discharged into the environment. Further research needs to be undertaken to improve sewage treatment technologies, thereby producing a better quality treated sewage effluent.

Keywords: Pathogens, antibiotics, *Escherichia coli*, coliforms, fluoroquinolone, sulfamethoxazole, chromogenic assay, Enzyme Linked Immunosorbent Assay.

Introduction

Faecal contaminants enter environmental water via various routes. Non-human faecal contamination can occur by domestic animals such as dogs and cats.[1] Other significant sources of faecal contamination to environmental water are via rats, beavers, gulls, waterfowl and pigeons.[2]

Humans and other warm-blooded animals have coliforms as intestinal flora. These coliforms are excreted and are discharged to be treated by municipal sewage treatment plants. However, if the wastewater remains untreated, bacterial pathogens present in the sewage effluents can result in diseases such as dysentery, typhoid, and gastroenteritis upon exposure to the contaminated water.[3]

Inefficient treatment processes result in microorganisms being released with treated effluents in the aquatic environment.[4] The effluents then enter aquatic ecosystems and become a major source of faecal contamination. Faecal contaminants pose a health risk to humans and animals upon exposure to contaminated water.[4] Monitoring faecal contamination of sewage could provide valuable information on urban land uses and potential routes of faecal contamination.[1,5] Indicator organisms to monitor bacteriological quality of water include *Escherichia coli* (*E. coli*) and coliforms.[6]

Various methods can be employed to examine faecal contamination of water sources. The classical methods used to screen faecal contaminants include the multiple-tube fermentation (MTF) technique and the membrane filter technique (MFT). Briefly, the MTF technique is carried out using different dilutions of the water sample in test tubes. After 48 hours of incubation, gas production, acid formation and growth of organisms can then be determined. A confirmatory test for the target organisms then follows a presumptive positive reaction.[6] On the other hand, the MFT technique consists of filtering a water sample using a sterile filter (0.45 μM). This filtering technique traps bacteria on the filter. The filter can then be cultured on selective media and enumeration can be done.[6]

The classical methods used have several advantageous and disadvantageous characteristics. For instance, the

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MTF method allows for semi-quantitative enumeration of coliforms but is labour intensive. The MTF method is also time consuming and a subculture stage for confirmation is needed.[6]

Chromogenic tests to monitor total coliforms and *E. coli* are commercially available. Chromogenic tests are effective and are able to detect total coliforms and *E. coli* in different water sources. In addition, these tests take advantage of enzymatic properties of coliforms. These tests are specific and only total coliforms and *E. coli* that feed on defined substrate nutrients in the medium can release a chromogen or fluorochrome. Chromogen or fluorochrome production indicates the presence of the microbes.[6] These tests are easy and rapid to use and can save on costs.[6]

Modern disease management strategies have resulted in increased pharmaceutical use, particularly the use of antibiotics. Additionally, antibiotics are also used in veterinary medicine.[7] In humans and animals, antibiotics exit via urine or faeces. Antibiotics are not always metabolized and a large amount of biologically active ingredients are discharged with urine and faeces. These unchanged or partially metabolized antibiotics then enter sewage where it may either be eradicated by sewage treatment processes or released with sewage effluents into the aquatic environment.[7] Antibiotic residues in the environment could elicit potential adverse consequences such as bacterial resistance. Moreover, antibiotics and their metabolites could display synergism or additional unintended effects and pose a health risk to aquatic species and consumers of the contaminated water.[8]

The 4-Quinolones and synthetic pharmaceuticals such as fluoroquinolones, quinolones and quinolone carboxylic acids are used extensively as antibiotics in human and veterinary medicine.[9] Fluoroquinolones have a broad spectrum of activity and enhanced pharmokinetic properties.[10] Some of the flouroquinolone antibiotics include ciprofloxacin, ofloxacin, levofloxacin and norfloxacin.[10] Fluoroquinolones have been found in raw and treated sewage effluents.[11,12] The release of fluoroquinolones into the environment can have adverse effects on aquatic microorganisms.[13]

The sulphonamides are components of sulfanilamide.[14] One of the sulphonamide antibiotic residues includes sulfamethoxazole. Sulfamethoxazole is used extensively as an antimicrobial in animals and humans.[15] Sulfamethoxole can be discharged into the environment, via sewage effluents, where it remains persistent.[16]

Many countries are monitoring the presence, removal and fate of contaminants in raw wastewater and treated sewage effluents.[17,18] In South Africa several studies have focussed on the presence of bacteria in sewage effluents.membrane bioreactor plants are higher Little is known about other contaminants in wastewater and treated effluents from sewage treatment plants in South Africa. The National Water Act of South Africa (Act no. 36 of 1998) consists of several chapters. In particular, Chapter 3, Part 4, deals with pollution prevention. Certain requirements need to be implemented by the owner of the properties where activities or processes occur that can result in pollution of a water source. Measures include containing and preventing the release of pollutants into the environment, eliminating any sources of pollutants and to remedy the effects of the pollution.

The South African constitution also has several acts that pertain to environmental rights. Section 24 (a) states that: "Every human has the right to an environment that is not harmful to human health or well-being." The constitution further states in Section 24 (b) that: "Everyone has the right to have the environment protected." Water is a scarce commodity and needs to be protected to ensure sustainable usage. The aim of this study was to determine the occurrence of faecal bacteria, and antibiotic residues in raw and treated sewage effluents from three different sewage treatment plants in the Western Cape, South Africa. Coliforms and *E.coli* were used as bioindicators for faecal bacteria. Fluoroquinolone and sulfamethoxazole are commonly used antibiotics and were used to monitor the efficiency of sewage plants to remove antibiotics.

Materials and methods

Site description and water collection

Raw wastewater and treated sewage effluents were collected from three different sewage treatment plants in the Western Cape, South Africa. The treatment plants investigated are on the same river system. Sewage treatment plant 1 and 2 use older technologies to treat wastewater. Sewage treatment 3 has been upgraded and new technologies were incorporated in the treatment processes. Sewage treatment plant 2 and 3 receives domestic effluents only. However, sewage treatment plant 1 receives both domestic (85% flow intake) and industrial raw wastewater (15% flow intake).

A detailed description of sewage treatment technologies for the different sewage treatment plants are as follows. The older technologies used at the sewage treatment plants can be divided into three processes, namely:

(i) Primary treatment which includes pre-treatment of raw wastewater intake by coarse and fine screens for grit removal. This process uses sedimentation tanks to allow the heavier organic particles to settle.
(ii) Secondary treatment of raw water using activated sludge. This process uses aerated biological digestion by bacteria to remove remaining suspended and dissolved material. In addition, nitrification and denitrification of wastewater is also used as treatment processes within the sewage treatment plants. Thereafter, the wastewater enters the secondary sedimentation tank to allow separation of the liquid and solid phase. After secondary sedimentation the wastewater enters maturation ponds for further pathogen removal.

(iii) Tertiary treatment is the final step in the conventional activated sludge system used by sewage treatment plant 1 and 2. Ultraviolet light (used only at sewage treatment plant 1) or chlorine (used only at sewage treatment plant 2) are the disinfection processes used, before the treated sewage effluent are released from plants.

Sewage treatment plant 3 uses an additional treatment technology (membrane bioreactor) concurrently with conventional or older treatment technologies. The membrane bioreactor technology consists of microporous membranes. These micro-filtration and ultra-filtration membranes separate liquid and solids.

Water collected from the Eerste River in Jonkershoek, Stellenbosch, South Africa was used as a negative control. This site is situated in the Stellenbosch mountains, and there is no human activity upstream from this area. Samples were collected in pre-cleaned 1 Liter (1 L) plastic bottles and transported to the laboratory in a cooler.

Monitoring of total coliforms and E.coli in wastewater samples

Raw wastewater and treated sewage effluents from all sewage treatment plants were collected over a four week sampling period. Total coliforms and *E. coli* in wastewater samples were monitored by using the Readycult Coliforms 100 (Merck, Germany). The test was performed according to the manufacturer's instructions. The Readycult Coliforms 100 is a chromogenic test that simultaneously detects total coliforms and *E. coli*. Tests for total coliforms and *E.coli* were done using 10 mL, 1 mL or 0.1 mL of water samples. Raw wastewater and treated sewage effluent samples were incubated overnight at 37°C, before analysis. Coliforms are indicated by a yellow to blue-green colour change of medium, while fluorescence under U.V. light is indicative of *E. coli* in the sample.

Solid-phase extraction of water samples

Samples were filtered with filter paper (Munktell, 15 μM, 240 mm) (Lasec, SA) before extraction. Water samples were then extracted using C-18 columns (Sigma Aldrich, South Africa). Columns were conditioned with 2 mL of Phase B mixture (45% methanol, 40% hexane and 15% propanol), then 2 mL ethanol and lastly 4 mL distilled water. After the washing step, 100 mL of water sample was passed through the column.

The columns were then dried using a vacuum pump (PALL vacuum pump, LifeSciences, 60 Hz, 1.92 Amperes, 220–240 Volts). The hydrophobic analytes attached to the resin were eluted with 2 mL of Phase B mixture. The eluates were dried under a stream of air. The dried eluate was reconstituted with dimethyl sulfoxide (DMSO) to make a 1000 times concentrated sample stock solution. Extracts were diluted in 10% methanol at a ratio of 1:100 for the fluoroquinolone ELISA.

Fluoroquinolone analysis of raw and final treated wastewater extracts

Fluoroquinolone ELISA kits were purchased from Abraxis, Warminister, PA. Samples were analyzed according to the instructions included in the kit. All reagents required were supplied in the kit. The ELISA plate was precoated with antibodies specific to a unique antigenic site on the fluoroquinolone molecule. Samples or standards and fluoroquinolone enzyme conjugate were pre-mixed in an uncoated microplate (100 μL of each solution).

Thereafter, 100 μL of the pre-mixture was transferred per well of the coated plate. The plate was then incubated for 1 hour at room temperature. Thereafter, the wells were washed five times with wash solution and tapped dry. After washing, 100 μLof substrate was added to all wells and incubated for 30 minutes at room temperature. The enzyme reaction was stopped by adding 100 μL of stop solution to all wells. The optical density was read at 450 nM with a microtiter plate reader (Thermo Electron Corporation, Original Multiskan Ex). The 0 μg/L standard results in maximum binding of the enzyme conjugate. All data was expressed as a percentage of 0 μg/L standard. A standard curve was drawn using the results obtained for the standards and the concentrations of the samples were read from this curve.

Sulfamethoxazole analysis of raw and final wastewater

Raw wastewater and treated sewage effluents were sterilized with 0.45 μM sterile filters (Lasec, SA) prior to use in the Sulfamethoxazole ELISA. Sulfamethoxazole ELISA kits were purchased from Abraxis, Warminister, PA. Samples were analyzed according to the instructions included in the kit. All reagents required were supplied in the kit. The ELISA plate was precoated with antibodies specific to a unique antigenic site on the sulfamethoxazole molecule. Samples or standards were added to the precoated microplate (75 μL/well). Thereafter, 50 μL/well of the anti-sulfamethoxazole antibody solution was added to the microplate. The contents of the wells were then mixed for 20–30 seconds. After mixing, the plate was incubated at room temperature for 20 minutes.

After the incubation period, 50 μL/well of the sulfamethoxazole enzyme conjugate solution was added to each well of the microplate. After mixing as before, the plates were then incubated for 40 minutes at room temperature. Thereafter, the wells were washed four times with wash solution and tapped dry. After washing, 150 μL of substrate solution was added to all wells and incubated for 30 minutes at room temperature. The enzyme reaction was stopped by adding 100 μL of stop solution to all wells. The optical density was then read at 450 nm with a microtiter

Table 1. Detection of total coliforms and *E. coli* in raw wastewater and treated sewage effluents from three sewage treatment plants in the Western Cape, South Africa.

		Week 1		*Week 2*		*Week 3*		*Week 4*	
Sample name	*Sample vol. (mL)*	*Coliforms*	*E. coli*	*Coliforms*	*E. coli*	*Coliforms*	*E. coli*	*Coliforms*	*E. coli*
Jonkershoek (control site)	10	Y	N	Y	N	Y	N	Y	N
	1	N	N	N	N	N	N	N	N
	0.1	N	N	N	N	N	N	N	N
STP 1 Domestic Raw water	10	Y	Y	Y	Y	Y	Y	Y	Y
	1	Y	Y	Y	Y	Y	Y	Y	Y
	0.1	Y	Y	Y	Y	Y	Y	Y	Y
STP 1 Industrial Raw water	10	Y	Y	Y	Y	Y	Y	Y	Y
	1	Y	Y	Y	Y	Y	Y	Y	Y
	0.1	Y	Y	Y	Y	Y	Y	Y	Y
STP 1 Treated sewage effluent	10	Y	Y	Y	Y	Y	Y	Y	Y
	1	Y	Y	Y	Y	Y	Y	Y	Y
	0.1	Y	Y	Y	Y	Y	Y	Y	Y
STP 2 Raw water	10	Y	Y	Y	Y	Y	Y	Y	Y
	1	Y	Y	Y	Y	Y	Y	Y	Y
	0.1	Y	Y	Y	Y	Y	Y	Y	Y
STP 2 Treated sewage effluent	10	N	N	N	N	N	N	N	N
	1	N	N	N	N	N	N	N	N
	0.1	N	N	N	N	N	N	N	N
STP 3 Raw water	10	Y	Y	Y	Y	Y	Y	Y	Y
	1	Y	Y	Y	Y	Y	Y	Y	Y
	0.1	Y	Y	Y	Y	Y	Y	Y	Y
STP 3 Treated sewage effluent	10	Y	N	Y	N	Y	N	Y	N
	1	N	N	N	N	N	N	N	N
	0.1	N	N	N	N	N	N	N	N

plate reader (Thermo Electron Corporation, Original Multiskan Ex). The 0 μg/L standard results in maximum binding of the enzyme conjugate. All data was expressed as a percentage of 0 μg/L standard. A standard curve was drawn using the results obtained for the standards and the concentrations of the samples were read off this curve.

Statistical analysis

One way analysis of variance (ANOVA) was used to compare results for the antibiotic assays, with $P < 0.050$ considered as significant. Statistical analysis was done using SigmaPlot Version 11.

Results

The detection of total coliforms and E. coli

The Readycult Coliforms 100 is a chromogenic test that simultaneously detects total coliforms and *E.coli*. A yellow to a green-blue colour change of the culture broth indicated the presence of total coliforms. Fluorescence of the broth under ultraviolet light indicated the presence of *E. coli*. Confirmation of the presence of *E. coli* was further done by addition of Kovac's reagent (Merck, Germany) to the broth. Table 1 shows the recovery of total coliforms and *E. coli* in raw wastewater and treated sewage effluents from all sewage treatment plants over the 4-week sampling period.

For the Jonkershoek negative control sample 1-10 CFU/100 mL of total coliforms was detected at each of the collection times. However, *E. coli* was not found in the Jonkershoek negative control samples. All the raw wastewater samples tested positive with more than 1000 CFU/100 mL total coliforms and *E. coli* detected.

Total coliforms and *E. coli* were detected at levels more than 1000 CFU/100 mL in treated sewage effluent for sewage treatment plant 1. The total coliforms and *E. coli* levels in treated sewage effluents from sewage treatment plant 2 were less than 1 CFU/100 mL. Total coliforms were detected at 1–10 CFU/100 mL in treated sewage effluents from sewage treatment plant 3. This is similar to the levels found in the Jonkershoek negative control water sample. The *E. coli* levels in treated sewage effluents produced by sewage treatment plant 3 were less than 1 CFU/100 mL.

Detection of fluoroquinolones in raw wastewater and treated sewage effluents from the three sewage treatment plants

Raw wastewater and treated sewage effluents from all sewage treatment plants were analysed for the presence of fluoroquinolones. Results for the detection of fluoroquinolones in raw wastewater and treated sewage effluents from all sewage treatment plants are illustrated in Tables 2,

Table 2. Mean concentration (ng/L ± SD) of selected antibiotics found in domestic and industrial raw wastewater and treated sewage effluents for sewage treatment plant 1 ($n = 8$).

	Sewage treatment plant 1					
	Jonkershoek negative control	*Domestic raw wastewater*	*Industrial raw wastewater*	*Calculated value of mixture*	*Treated sewage effluents*	*Percentage reduction (%)*
Fluoroquinolones (ng/L)	2 ± 2	90 ± 24[a]	89 ± 28[a]	90 ± 19[a]	92 ± 29[a]	2 ± 10
Sulfamethoxazole (ng/L)	0 ± 0	111 ± 0[a]	156 ± 12[a]	118 ± 3[a]	121 ± 28[a]	4 ± 1

[a]Statistically different to negative control ($P < 0.050$). Sewage treatment plant 1 uses the conventional activated sludge system as wastewater treatment processes.

Table 3. Mean concentration (ng/L ± SD) of selected antibiotics found in raw wastewater and treated sewage effluents for sewage treatment plant 2 ($n = 8$).

	Sewage treatment plant 2			
	Jonkershoek negative control	*Raw wastewater*	*Treated sewage effluents*	*Percentage reduction (%)*
Fluoroquinolones (ng/L)	2 ± 2	92 ± 11[a]	72 ± 34[a]	21 ± 5
Sulfamethoxazole (ng/L)	0 ± 0	153 ± 7[ab]	101 ± 44[a]	34 ± 9

[a]Statistically different to negative control ($P < 0.050$).
[b]Statistically different to treated sewage effluents ($P < 0.050$).
Sewage treatment plant 2 uses the conventional activated sludge system as wastewater treatment processes.

3, and 4, respectively. Concentrations of fluoroquinolones are represented as Mean ng/L ± Standard deviation (SD). The percentage reduction of fluoroquinolones from raw wastewater to treated sewage effluents are also given in the tables. Very low or undetectable levels of fluoroquinolones were found in the Jonkershoek negative control.

Fluoroquinolones detected in the domestic and industrial raw wastewater from sewage treatment plant 1 were 90 ± 24 ng/L and 89 ± 28 ng/L, respectively (Table 2). The combined concentration of fluoroquinolone for the mixture of domestic and industrial raw wastewater from sewage treatment plant 1 was 90 ± 19 ng/L. Fluoroquinolone concentrations in domestic and industrial raw wastewater and the combined mixture concentration was higher when compared to the Jonkershoek negative control ($P < 0.050$). Fluoroquinolone concentration in treated sewage effluents from sewage treatment plant 1 was 92 ± 29 ng/L.

There was no difference in the fluoroquinolone concentration of the domestic raw wastewater, industrial raw wastewater, the combined mixture and treated sewage effluents from sewage treatment plant 1. Fluoroquinolone concentration in the treated sewage effluents was significantly higher compared to the Jonkershoek negative control ($P < 0.050$). The conventional activated sludge process at sewage treatment plant 1 reduced the fluoroquinolone concentration by 2%.

Fluoroquinolone concentrations detected in raw wastewater from sewage treatment plant 2 was 92 ± 11 ng/L (Table 3). Fluoroquinolone concentrations in the raw wastewater were higher when compared to the Jonkershoek negative control ($P < 0.050$). Fluoroquinolone concentration in treated sewage effluents from sewage treatment plant 2 was 72 ± 34 ng/L. There was no difference in the fluoroquinolone concentration of the raw wastewater and treated sewage effluents from sewage treatment plant 2. Fluoroquinolone concentrations in the treated sewage effluents was significantly higher compared to the Jonkershoek negative control ($P < 0.050$). The

Table 4. Mean concentration (ng/L ± SD) of selected antibiotics found in raw wastewater and treated sewage effluents for sewage treatment plant 3 ($n = 8$).

	Sewage treatment plant 3			
	Jonkershoek negative control	*Raw wastewater*	*Treated sewage effluents*	*Percentage reduction (%)*
Fluoroquinolones (ng/L)	2 ± 2	99 ± 11[a]	68 ± 33[a]	31 ± 3
Sulfamethoxazole (ng/L)	0 ± 0	170 ± 4[ab]	76 ± 23[a]	56 ± 9

[a]Statistically different to negative control ($P < 0.050$).
[b]Statistically different to treated sewage effluents ($P < 0.050$).
Sewage treatment plant 3 uses the newer membrane technology as an additional wastewater treatment process.

conventional activated sludge process at sewage treatment plant 2 reduced the fluoroquinolone concentration by 21%.

Fluoroquinolone concentrations detected in raw wastewater from sewage treatment plant 3 was 99 ± 11 ng/L (Table 4). Fluoroquinolone concentrations in the raw wastewater were higher when compared to the Jonkershoek negative control ($P < 0.050$). Fluoroquinolone concentration in treated sewage effluents was 68 ± 33 ng/L. There was no difference in the fluoroquinolone concentration of raw wastewater and treated sewage effluents from sewage treatment plant 3. Fluoroquinolone concentration in the treated sewage effluents was significantly higher compared to the Jonkershoek negative control ($P < 0.050$). The membrane bioreactor process at sewage treatment plant 3 reduced the fluoroquinolone concentration by 31%.

Detection of sulfamethoxazole in raw wastewater and treated sewage effluents from the three sewage treatment plants

Raw wastewater and treated sewage effluents from all sewage treatment plants were analysed for the presence of the antibiotics sulfamethoxazole. Results for the detection of sulfamethoxazole in raw wastewater and treated sewage effluents in all sewage treatment plants are illustrated in Tables 2, 3, and 4, respectively. Concentrations of sulfamethoxazole are represented as Mean ng/L ± Standard deviation (SD). The percentage reduction of sulfamethoxazole from raw wastewater to treated sewage effluents are also given in the tables. Very low or undetectable levels of sulfamethoxazole were found in the Jonkershoek negative control.

Sulfamethoxazole detected in the domestic and industrial raw wastewater from sewage treatment plant 1 were 111 ± 4 ng/L and 156 ± 12 ng/L, respectively (Table 2). The combined concentration of sulfamethoxazole for the mixture of domestic and industrial raw wastewater from sewage treatment plant 1 was 118 ± 3 ng/L. Sulfamethoxazole concentrations in domestic and industrial raw wastewater, and the combined mixture was higher when compared to the Jonkershoek negative control ($P < 0.050$). Sulfamethoxazole concentration in treated sewage effluents from sewage treatment plant 1 was 121 ± 28 ng/L. There was no difference in the sulfamethoxazole concentration of the domestic raw wastewater, industrial raw wastewater and treated sewage effluents of sewage treatment plant 1. Sulfamethaxole concentrations in the treated sewage effluents was significantly higher compared to the Jonkershoek negative control ($P < 0.050$). The conventional activated sludge process at sewage treatment plant 1 reduced the sulfamethoxazole concentration by 4%.

Sulfamethoxazole concentration detected in raw wastewater from sewage treatment plant 2 was 153 ± 7 ng/L (Table 3). Sulfamethoxazole concentrations in the raw wastewater was higher when compared to the Jonkershoek negative control ($P < 0.050$). Sulfamethoxazole concentration in treated sewage effluents from sewage treatment plant 2 was 101 ± 44 ng/L. Sulfamethoxazole concentrations in raw wastewater was significantly higher than levels found in the treated sewage effluents for sewage treatment plant 2 ($P < 0.050$). Sulfamethaxole concentration in the treated sewage effluents was significantly higher compared to the Jonkershoek negative control ($P < 0.050$). The conventional activated sludge process at sewage treatment plant 2 reduced the sulfamethoxazole concentration by 34%.

Sulfamethoxazole concentration detected in raw wastewater from sewage treatment plant 3 was 170 ± 4 ng/L (Table 4). Sulfamethoxazole concentrations in the raw wastewater was higher when compared to the Jonkershoek negative control ($P < 0.050$). Sulfamethoxazole concentration in the treated sewage effluents from sewage treatment plant 3 was 76 ± 23 ng/L. Sulfamethoxazole concentrations in raw wastewater was significantly higher than levels found in the treated sewage effluents for sewage treatment plant 3 ($P < 0.050$). Sulfamethaxole concentration in the treated sewage effluents was significantly higher compared to the Jonkershoek negative control ($P < 0.050$). The membrane bioreactor process at sewage treatment plant 3 reduced the sulfamethoxazole concentration by 56%.

Discussion

Treated sewage effluents containing residual pollutants are often dishcarged into surface water. These effluents can contribute to the pathogens in the environment.[22] A group of bacteria, known as the coliforms are used to monitor the microbiological quality of water.[23] The occurrence of non pathogenic faecal coliforms in water can indicate the occurrence of pathogenic microorganisms that are of faecal origin.[23] One of the main bacterial indicators of faecal contamination is *E. coli*.[24] Studies have shown that gastrointestinal and respiratory diseases are linked to polluted waters that have increased numbers of indicator bacteria.[25] Monitoring the bacteriological quality of water is an important parameter to limit these diseases.

In this study, total coliforms and *E. coli* were detected in raw wastewater from all sewage treatment plants. Since wastewater from homes, hospitals and commercial buildings collects in sewers and flows to sewage treatment plants, high faecal bacteria counts were expected in the raw sewage.[19]

High loads of total coliforms and *E. coli* present in treated sewage effluents from sewage treatment plant 1, show that the treatment processes and disinfection by the UV light at this plant are ineffective in removing faecal bacteria. The maximum for no risk is 0 CFU/100 mL for faecal coliforms and 10 CFU/100 mL for total coliforms.[26] Consequently these guidelines set out (1998) by the Department of Water Affairs and Foresty of South Africa imply that the treated sewage effluents from sewage treatment plant 1 is of poor microbiological quality.[26] The results of this study confirm data obtained in previous studies that have shown

that U.V. light disinfection of sewage effluent does not reduce microbial populations as effectively as disinfection by chlorine.[27]

Treatment technologies employed by sewage treatment plant 2 is similar to that of sewage treatment plant 1, except at the tertiary treatment where chlorination is used instead of UV disinfection. The treated sewage effluents produced by sewage treatment plant 2 was of acceptable microbiological quality with both total coliforms and *E.coli* below the recommended levels. However, studies have shown that other properties play a role in treatment of wastewater. Conductivity, pH, dissolved oxygen, nitrogen and phosphate content may have an effect on bacterial communities present in sewage.[19,28] Sewage effluent treatment with chlorine may have adverse effects on aquatic life. Chlorination results in the formation of some toxic by-products formation and these can have adverse effects on the aquatic life.[29]

E. coli was not detected in treated sewage effluents from sewage treatment plant 3. This therefore implies that the membrane bioreactor technology employed by the plant was effective in removing *E. coli* from sewage. These results are consistant with studies that showed high removal rates of *E. coli* from sewage upon membrane bioreactor treatment.[30]

The global consumption of antimicrobials is estimated to be between 100,000 and 200,000 tons per year.[31] The occurrence of pharmaceuticals in raw wastewater is dependant on different factors.[11] For instance, the total consumption of antibiotics by different populations and countries may vary.

Fluoroquinolones are the most widely prescibed antibiotics.[10] Results of this study shows that these antibiotics are extensively used in South Africa and high levels of the antibiotics are present in sewage. Fluoroquinolones were detected in all raw wastewater samples. The levels detected ranged from 89 ng/L to 92 ng/L. Seasonal variations in antibiotic levels in sewage can occur. During winter months people are more likely to become sick and therefore increased levels of antibiotics are prescribed. Castiglioni et al.[32] has shown that antibiotic use in winter is considerably more than in summer. However, the levels of antibiotics can differ between sewage treatment plants at different time periods.[33]

This study shows that fluoroquinolones were not effectively eliminated by the treatment processes at the three sewage treatment plants investigated (Tables 2, 3, 4). Treated sewage effluents contained significantly higher fluoroquinolones than the Jonkershoek negative control site ($P < 0.050$). No significant difference in fluoroquinolone concentrations between raw wastewater and treated sewage effluents were found, indicating that sewage treatment processes used by the three plants are inefficient at removing this antibiotic from sewage. The results show that high loads of fluoroquinoloes are discharged into the environment.

The results of this study are consistent with previous studies that showed high levels of fluoroquinolones in effluents from sewage treatment plants.[34] Fluoroquinolones have been measured in sewage treatment plant effluents in European countries such as France (300–500 ng/L); Italy (300–500 ng/L); Greece (500 ng/L) and Switzerland (30–1100 ng/L).[34] The current study shows that the fluoroquinolone concentrations in the sewage effluents from the three sewage treatment plants investigated are similar to levels in Switzerland.

The type of treatment technology used may aid in the removal of antibiotics from wastewater.[35] In this study sewage treatment plant 1 and 2 use the conventional activated sludge process only for treatment. In addition to the conventional activated sludge process, sewage treatment plant 3 also use membrane bioreactor technology for sewage treatment. The percentage reduction of fluoroquinolones differed according to the sewage treatment processes used. A 2% and 21% reduction of fluoroquinoloes for the conventional activated sludge processes at sewage treatment plant 1 and 2 were calculated. For the membrane bioreactor technology at sewage treatment plant 3, a calculated value of 31% was found. These results indicate that despite the different treatment technologies used, elimination of the fluoroquinolones from treated sewage effluents are minimal.

The nature of the drug also plays a role in its removal from wastewater. The fluoroquinolone antibiotics are very hydrophilic compounds.[11] Elimination of fluoroquinolones is mainly via sorption to sludge.[12] In contrast, other studies have shown high removal rates of fluoroquinolones from wastewater.[8] However, this was not evident in this study.

Several other factors need to be taken into consideration. Studies have suggested that the dilution of raw wastewater by heavy rain can result in the reduction of pharmaceutical removal by sewage treatment plants.[17] Other factors such as temperature of the wastewater, the hydraulic and solid rentention time, age of the activated sludge, environmental conditions and characterstics of the raw influent may all play a role in the elimination of pharmaceuticals in wastewater.[11,40] Discharge of these compounds to receiving waters can result in adverse effects to fish species and an eventual health risk to consumers of fish caught in contaminated water bodies.[36,37]

Sulfamethoxazole is an antibiotic used widely in human and veterinary medicine.[14] Sulfamethoxazole is resistant to breakdown and has been found in environmental ecosystems.[38,39] Sulfamethoxazole was detected in all raw wastewater samples analysed in this study (Tables 2, 3, 4). The sulfamethoxazole concentration of the treated sewage effluents were significantly higher than the Jonkershoek negative control ($P < 0.050$), indicating incomplete removal during sewage treatment processes. Sewage treatment plant 1 did not reduce the sulfamethoxazole concentration and the antibiotic was released at very high levels in the treated sewage effluents. These results are similar to those published by Zuccato et al.[40] A significant decrease

of sulfamethoxazole concentration in treated sewage effluents compared to raw wastewater can be seen for sewage treatment plant 2 and 3 ($P < 0.050$).

The percentage reduction of sulfamethaxazole for sewage treatment plant 2 and 3 was 34% and 56%, respectively. Watkinson et al.[41] has shown that the mean removal rate of sulfamethoxazole in conventional activated sludge plants was 92%; however, concentrations in the ng/L range are still present in treated sewage effluents. In contrast, removal rates of sulfamethoxazole in membrane bioreactor plants are higher.[42]

Conclusion

The present study indicated the occurrence of faecal bacteria in raw wastewater and treated sewage effluents from certain sewage treatment plants. UV light disinfection showed inefficient removal of faecal bacteria compared to chlorination. Newer technologies such as the membrane bioreactor technology in sewage treatment plant 3 reduced the faecal bacteria in treated sewage effluents. However, other factors such as pH and conductivity of wastewater may play a role in bacterial communities that survive.

The results of this study also show that due to inefficient removal by treatment processes, antibiotic residues are still present in treated sewage effluents. Therefore, wastewater with high raw influent concentrations of antibiotics will require some form of additional treatment to reduce their concentration in treated sewage effluents. This study also showed that membrane bioreactor technology could potentially be helpful in reducing the amount of contaminants released into the environment.

The National Water Act of SA (Act no. 36 of 1998) needs to be strictly enforced by government to ensure the conservation of our water sources. Further research needs to be undertaken to improve sewage treatment technologies, thereby producing a better quality treated sewage effluent.

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References

[1] Whitlock, J.; Jones, D.; Harwood, J. Identification of the sources of faecal coliforms in an urban watershed using antibiotic resistance analysis. Water Res. **2002**, *36*, 4273–4282.

[2] Siewick, T.; Pullaro, T.; Pan, W.; McDaniels, S.; Glenn, R.; Stewart, J. Models of total and presumed wildlife sources of faecal coliform bacteria in coastal ponds. J. Environ. Man. **2007**, *82*, 120–132.

[3] Rose, J.; Dickson, L.; Farrah, S.; Carnahan, R. Removal of pathogenic indicator microorganism by a full-scale water reclamation facility. Water Res. **1996**, *30*(11), 2788–2795.

[4] George, I.; Crop, P.; Servais, P. Fecal coliform removal in wastewater treatment plants studied by plate counts and enzymatic methods. Water Res. **2002**, *36*, 2607–2617.

[5] Young, K.; Thackston, E. 1999. Housing density and bacterial loading in urban streams. J. Environ. Eng. **1999**, *125*(12), 1177–1180.

[6] Rompré, A.; Servais, P.; Baudart, J.; de-Roubin, M.; Laurent, P. Detection and enumeration of coliforms in drinking water: current methods and emerging approaches. J. Micro. Meth. **2002**, *49*, 31–54.

[7] Hirsch, R.; Ternes, T.; Haberer, K.; Kratz, K. Occurrence of antibiotics in the aquatic environment. Sci. Tot. Environ. **1999**, *225*, 109–118.

[8] Gulkowska, A.; Leung, H.; So, M.; Taniyasu, S.; Yamashita, N.; Yeung, L.; Richardson, B. Removal of antibiotics from wastewater by sewage treatment facilities in Hong Kong and Shenzhen, China. Water Res. **2008**, *42*, 395–403.

[9] Martinez, M.; McDermott, P.; Walker, R. Pharmacology of the fluoroquinolones : A perspective for the use in domestic animals. Vet. J. **2006**, *172*, 10–28.

[10] Picó, Y.; Andreu, V. Fluoroquinolones in soil-risks and challenges. Anal. Bioanal. Chem. **2007**, *387*, 1287–1299.

[11] Vieno, N.; Tuhkanen, T.; Kronberg, L. **2007**. Elimination of pharmaceuticals in sewage treatment plants in Finland. Water Res. **2007**, *41*, 1001–1012.

[12] Golet, E.; Xifra, I.; Siegrist, H.; Alder, A.; Giger, W., 2003. Environmental exposure assessment of fluoroquinolone antibacterial agents from sewage to soil. Environ. Sci. Technol. **2003**, *37*(15), 3243–3249.

[13] Lindberg, R.; Björklund, K.; Rendahl, P.; Johansson, M.; Tyskling, M.; Andersson, B. Environmental risk assessment of antibiotics in the Swedish environment with emphasis on sewage treatment plants. Water Res. **2007**. *41*, 613–619.

[14] Zhang, H.; Wang, S. Review on Enzyme-linked Immunosorbent assays for sulphonamide residues in edible animal products. J Immun. Meth. **2009**, *350*, 1–13.

[15] Peng, X.; Wang, Z.; Kuang, W.; Tan, J.; Li, K. A preliminary study on the occurrence and behaviour of sulphonamides, ofloxacin and chloramphenicol antimicrobials in wastewaters of two sewage treatment plants in Guangzhou, China. Sci. Tot. Environ. **2006**, *371*, 314–322.

[16] Hong, C.; Ying, H.; LeZheng, W.; Bing, S. 2008. Occurrence of sulphonamide antibiotics in sewage treatment plants. Chin. Sci. Bull. **2008**, *53*(4), 514–520.

[17] Ternes, T. Occurrence of drugs in German sewage treatment plants and rivers. Water Res. **1998**, *32*, 3245–3260.

[18] Ternes, T.; Kreckel, P.; Mueller, J. Behaviour and occurrence of estrogens in municipal sewage treatment plants II. Aerobic batch experiments with activated sludge. Sci. Tot. Environ. **1999**, *225*, 91–99.

[19] Samie, A.; Obi, C.; Igumbor, J.; Momba, M. Focus on 14 sewage treatment plants in the Mpumalanga Province, South Africa in order to gauge the efficiency of wastewater treatment. Afr. J. Biotech. **2009**, *8*(14), 3276–3285.

[20] Dungeni, M.; Momba, M. The abundance of *Cryptosporidum* and *Giardia* spp. in treated effluents produced by four wastewater treatment plants in the Gauteng Province of South Africa. Water SA. **2010**, *36*, 4.

[21] Omar, K.; Bernard, T. The occurrence of pathogenic *Escherichia coli* in South African wastewater treatment plants as detected by multiplex PCR. Water SA. **2010**, *36*(2), 172–176.

[22] Ottoson, J.; Hansen, A.; Björlenius, B.; Norder, H.; Strenström, T. Removal of viruses, parasitic protozoa and microbial indicators in conventional and membrane processes in a wastewater pilot plant. Water Res. **2006**, *40*, 1449–1457.

[23] Wutor, V.; Toga, C.; Pletchke, B. Suitability of total coliform β-D-galactosidase activity and CFU counts in monitoring faecal contamination of environmental water samples. Water SA. **2009**, *35*(1), 85–88.

[24] Molleda, P.; Blanco, I.; Ansola, G.; de Luis, L. Removal of wastewater pathogen indicators in a constructed wetland in Leon, Spain. Ecol. Eng. **2008**, *33*, 252–257.
[25] Thurston, J.; Gerba, C.; Foster, K.; Karpiscak, M. Fate of indicator microorganisms, *Giardia* and *Cryptosporidium* in subsurface flow constructed wetlands. Water Res. **2001**, *35*(6), 1547–1551.
[26] Department of Water Affairs and Forestry (DWAF). Quality of domestic water supplies - assessment guide, Department of Water Affairs and Forestry, Pretoria. **1998**, *1*.
[27] Guo, M.; Hu, H.; Liu, W. Preliminary investigation on safety of post-UV disinfection of wastewater: bio-stability in laboratory-scale simulated reuse water pipelines. Desalination **2009**, *239*, 22–28.
[28] Moura, A.; Tacâo, M.; Henriques, I.; Dias, J.; Ferreira, P.; Correia, A. Characterization of bacterial diversity in two aerated lagoons of a wastewater treatment plant using PCR-DGGE analysis. Micro Res. **2007**, doi: 10.1016/j.micres.2007.06.005. Available at http://www.dwa.gov.za/iwqs/AssessmentGuides/AssessmentGuide/AssessmentGuide.pdf (accessed July 11, 2011).
[29] Brungs, W. Effects of residual chlorine on aquatic life. J Water Environ. Life. **1973**, *45*(10), 2180–2193.
[30] Bolzonella, D.; Fatone, F.; di Fabio, S.; Cecchi, F. Application of membrane bioreactor technology for wastewater treatment and reuse in the Mediterranean region: Focusing on removal efficiency of non-conventional pollutants. J. Environ. Man. **2010**, *91*, 2424–2431.
[31] Senta, I.; Terzic, S.; Ahel, M. Simultaneous determination of sulphonamides, fluoroquinolones, macrolides and trimethoprim in wastewater and river water by LC-Tandem-MS. Chromatog. **2008**, *68*, 747–758.
[32] Castiglioni, S.; Calamari, D.; Bagnati, R.; Zuccato, E.; Fanelli, R. Comparison of the concentrations of pharmaceuticals in sewage treatment plants and rivers in Italy as a tool for investigating their environmental distribution and fate, Abstract, SETAC Europe, 14th Annual meeting, 18–22 April, **2004**, Prague.
[33] Tixier, C.; Singer, H.; Oellers, S.; Müller, S. Occurrence and fate of carbamazepine, clofibric acid, diclofenac, ibuprofen, ketoprofen, and naproxen in surface waters. Environ. Sci. Technol. **2003**, *37*, 1061–1068.
[34] Nakata, H.; Kannan, K.; Jones, P.; Giesy, J. Determination of fluoroquinolone antibiotics in wastewater effluents by liquid chromatography–mass spectrometry and fluorescence detection. Chemosphere. **2005**, *58*(6), 759–766.
[35] Xu, W.; Zhang, G.; Xiangdong, L.; Zou, S.; Li, P.; Hu, Z.; Li, J. Occurrence and elimination of antibiotics at four sewage treatment plants in the Pearl River Delta (PRD), South China. Water Res. **2007**, *41*, 4526–4534.
[36] Miranda, C.; Zemelman, R. Antibiotic resistance bacteria in fish from the Concepción Bay, Chile. Mar. Poll. Bull. **2001**, *42*(11), 1096–1102.
[37] Pathak, S.; Gopal, K. Occurrence of antibiotic and metal resistance in bacteria from organs of river fish. Environ. Res. **2005**, *98*, 100–103.
[38] Holm, J.; Rugge, K.; Bieg, P.; Christensen, T. Occurrence and distribution of pharmaceutical organic compounds in the ground-water downgradient of a landfill (Grindsted, Denmark). Environ. Sci. Technol. **1995**, *29*, 1415–1420.
[39] Kummerer, K. Drugs in the environment: Emission of drugs, diagnostic aids and disinfectants into wastewater by hospitals in relation to other sources—A review. Chemosphere. **2001**, *45*, 957–969.
[40] Zuccato, E.; Castiglioni, S.; Bagnati, R.; Melis, M.; Fanelli, R. Source, occurrence and fate of antibiotics in the Italian aquatic environment. J. Hazard. Mat. **2010**, *179*, 1042–1048.
[41] Watkinson, A.; Murby, E.; Costanzo, S. Removal of antibiotics in conventional and advanced wastewater treatment: Implications for environmental discharge and wastewater recycling. Water Res. **2007**, *41*, 4164–4176.
[42] Radjenović, J.; Petrović, M.; Barceló, D. Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment. Water Res. **2009**, *43*, 831–841.