Pathogens and pharmaceuticals in source-separated urine in eThekweni, South Africa

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Abstract

In eThekweni, South Africa, the production of agricultural fertilizers from human urine collected from urine-diverting dry toilets is being evaluated at a municipality scale as a way to help finance a decentralized, dry sanitation system. The present study aimed to assess a range of human and environmental health hazards in source-separated urine, which was presumed to be contaminated with feces, by evaluating the presence of human pathogens, pharmaceuticals, and an antibiotic resistance gene. Composite urine samples from households enrolled in a urine collection trial were obtained from urine storage tanks installed in three regions of eThekweni. Polymerase chain reaction (PCR) assays targeted 9 viral and 10 bacterial human pathogens transmitted by the fecal-oral route. The most frequently detected viral pathogens were JC polyomavirus, rotavirus, and human adenovirus in 100%, 34% and 31% of samples, respectively. *Aeromonas* spp. and *Shigella* spp. were frequently detected gram negative bacteria, in 94% and 61% of samples, respectively. The gram positive bacterium, *Clostridium perfringens*, which is known to survive for extended times in urine, was found in 72% of samples. A screening of 41 trace organic compounds in the urine facilitated selection of 12 priority pharmaceuticals for further evaluation. The antibiotics sulfamethoxazole and trimethoprim, which are frequently prescribed as prophylaxis for HIV-positive patients, were detected in 95% and 85% of samples, reaching maximum concentrations of 6800 µg/L and 1280 µg/L, respectively. The

27 antiretroviral drug emtricitabine was also detected in 40% of urine samples. A sulfonamide antibiotic
28 resistance gene (*su1*) was detected in 100% of urine samples. By coupling analysis of pathogens and
29 pharmaceuticals in geographically dispersed samples in eThekweni, this study reveals a range of human and
30 environmental health hazards in urine intended for fertilizer production. Collection of urine offers the
31 benefit of sequestering contaminants from environmental release and allows for targeted treatment of
32 potential health hazards prior to agricultural application. The efficacy of pathogen and pharmaceutical
33 inactivation, transformation or removal during urine nutrient recovery processes is thus briefly reviewed.

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35 **Keywords:** urine nutrient recovery, health hazards, risk, sustainable sanitation

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44 **1 Introduction**

45 Decentralized sanitation technologies that separate human urine from feces at the toilet user interface have
46 been implemented in low-, middle- and high-income countries, though primarily at pilot scales. Source-
47 separation of urine has the advantage over traditional toilets of sequestering nutrients primarily excreted in
48 urine, namely nitrogen, potassium, and phosphorus, while enhancing the dehydration of separately stored
49 feces in dry toilets. Collected urine can be used directly as a fertilizer in agriculture after a period of storage
50 or processed into concentrated powder or liquid products. The use or sale of urine-derived fertilizers could
51 offset the purchase of costly synthetic fertilizers or generate a source of revenue for sanitation service
52 providers. Such a sanitation system would also reduce nutrient loads to treatment facilities or other urine
53 discharge locations (Larsen et al., 2013).

54

55 The eThekweni Water and Sanitation (EWS) municipality in the KwaZulu-Natal province of South Africa serves
56 the greater eThekweni region including Durban. EWS installed over 80,000 urine diverting dry toilets (UDDTs)
57 in historically underserved rural and peri-urban areas of eThekweni, providing an ideal test case for the scale-
58 up of urine collection and use. Currently in this system, source-separated urine is diverted to soak pits, and
59 feces are collected in vaults for drying. EWS would like to incorporate nutrient recovery from urine into the
60 system. The VUNA (Valorisation of Urine Nutrients in Africa) project is designing, testing and optimizing urine
61 collection and nutrient recovery technologies to produce a commercially viable fertilizer. Pilot-scale
62 technologies have been developed to produce a solid phosphate-based fertilizer (struvite) as well as a
63 concentrated liquid fertilizer via biological nitrification and distillation (Udert et al., 2015). Urine-derived
64 fertilizers, and their production processes, need to be safe with respect to environmental and public health
65 concerns. This study thus aims to identify potential human and environmental health hazards in source-
66 separated urine, targeting microbial and chemical contaminants. The ultimate goal is to promote the
67 hygienic handling of urine and to design fertilizer production technologies that allow for its safe end use as a
68 fertilizer.

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70 The most pressing human health concerns associated with source-separated urine stem from the presence
71 of human pathogens. While enteric pathogens are primarily excreted in feces rather than urine, the cross-
72 contamination of source-separated urine with feces is well established (Schönning et al., 2002). Diseases
73 transmitted by the fecal-oral route are therefore relevant in the design of urine treatment and recycling
74 systems. Pathogen reduction can be partially achieved through storage, which allows inactivation by heat or
75 ammonia (Decrey et al., 2015; Nordin et al., 2013). The World Health Organization (WHO) recommends
76 storage for >6 months at >20°C to achieve sufficient pathogen inactivation in urine intended for agricultural
77 use for unprocessed food crops (WHO, 2006). While feasible in rural areas with ample space, this storage
78 time can be prohibitive for urine collection at scale in dense urban environments due to storage volume
79 requirements. Long term storage can also cause odor problems and lead to loss of recoverable nutrients by
80 ammonia volatilization. These challenges highlight the need for alternative urine treatment technologies.
81 Urine-derived fertilizers can be produced after short-term urine storage and provide a treatment benefit.
82 However, implementation of this strategy necessitates investigation of the fate of locally relevant pathogens
83 in the fertilizer production processes.

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85 While pathogens are primarily human health hazards, the primary concerns for the presence of trace organic
86 compounds (TrOCs) in the environment stem from their potential ecosystem health hazards (Kolpin et al.,
87 2002). A meta-analysis of 212 pharmaceuticals revealed that an average of 64% of the consumed mass is
88 excreted in urine (Lienert et al., 2007). The use and excretion of antiretroviral (ARV) drugs may be of
89 particular interest in South Africa where HIV prevalence is high, particularly in the KwaZulu-Natal province
90 (16.9% of the population in 2012; Shisana et al., 2014). South Africa has rolled out the largest ARV treatment
91 program in the world – in 2012, 31.2% of eligible people living with human immunodeficiency virus (HIV) or
92 Acquired Immune Deficiency Syndrome (AIDS) had been exposed to ARV treatment (Shisana et al., 2014).
93 While the benefits of this program are clearly demonstrated through the reduction of AIDS deaths in the
94 country, antiviral pharmaceuticals were predicted to be amongst the most hazardous therapeutic drug
95 classes for several ecotoxicological end-points (Sanderson et al., 2004). Commonly prescribed antibacterial

compounds with high excretion rates may also be prevalent in source-separated urine. Sulfamethoxazole and trimethoprim, for example, are frequently prescribed together as a broad-spectrum prophylaxis against bacterial pathogens and protozoa for HIV-positive patients (Zachariah et al., 2007). One potential human health consequence of such widespread use of antibiotics for infectious disease therapy is the accelerated propagation of antibiotic resistance, especially in low-income countries (Okeke et al., 2005).

The objective of this study was to evaluate the presence of pathogenic organisms that are causative agents of diarrheal disease as well as pharmaceuticals in source-separated urine collected from throughout eThekweni. Based on a review of enteric pathogens present in South Africa, polymerase chain reaction (PCR) assays targeting 9 viral and 10 bacterial pathogens transmitted by the fecal-oral route were selected for a screening of human pathogens in the collected urine. An initial screening of 41 TrOCs in the urine facilitated selection of 12 priority pharmaceuticals for further quantitative evaluation. The presence of a sulfonamide antibiotic resistance gene (ARG) in bacteria from urine was also evaluated. By coupling analysis of pathogens and pharmaceuticals, this study seeks to identify a range of priority human and environmental health risk determinants in urine that is intended for fertilizer production and agricultural applications.

2 Materials and Methods

2.1 Sampling

Samples were collected at four different times between 2010 and 2013. Samples from 2010 and 2011 were used for TrOC method validation and to select priority compounds for further monitoring. These initial urine samples were collected from two UDDTs on 10 November 2010, and four composite samples consisting of urine from 29 UDDTs were obtained between 28 April and 25 May 2011. The composite samples were prepared by adding urine collected daily over one week to a 1500 L black polyethylene tank. At the end of

121 the week, the tank was stirred and sampled into triplicate 50 mL centrifuge tubes. The tank was then
122 emptied in preparation for the subsequent composite sample.

123

124 Samples collected in 2012 and 2013 were geographically distributed in the EWS service area. From
125 December 2012 to May 2013, nine urine storage tanks of 1000 to 1500 L capacity were temporarily installed
126 in three distinct regions of the EWS service area in peri-urban Durban as part of an economic study on the
127 collection of urine using incentives (Tilley, 2015). Three urine tanks were installed per region, and each tank
128 was accessible for urine deposits by approximately 90 to 150 nearby households that were provided with
129 urine collection jerry cans. Tanks received deposits of urine carried by individuals from their homes to the
130 tanks during opening hours one day per week. Urine tanks were emptied by the municipality when full. The
131 nine storage tanks were sampled on December 19, 2012 and subsequently on several days from April 12-29,
132 2013 for combined TrOC and microbiological assessments. In December, two additional urine tanks (UT)
133 located at the University of KwaZulu-Natal (UKZN) were also sampled. The study plan was approved by the
134 UKZN Biomedical Research Ethics Committee (BE147/13). Specific locations of the sampling sites are omitted
135 as stipulated in the project protocol.

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137 **2.2 Chemical and physical urine characteristics**

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139 The urine pH, temperature, conductivity, total ammonia and orthophosphate concentrations were measured
140 as described in the Supporting Information.

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142 **2.3 Analytes**

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144 **2.3.1 Bacteria**

145 The presence of bacterial pathogens was monitored by detection of bacterial DNA. Bacteria targets in this
146 study were: *Clostridium perfringens*, *Yersinia enterocolitica*, *Eschericia coli* O157:H7, verocytotoxin producing

147 *Eschericia coli* (VTEC), *Aeromonas* spp. (*A. salmonicida*, *A. sobria*, *A. bivalvium*, *A. hydrophila*), *Vibrio* spp. (*V.*
148 *cholera*, *V. parahaemolyticus*, and *V. vulnificus*), *Clostridium difficile* toxin B, *Salmonella* spp. (*S. bongori* and *S.*
149 *enterica*), *Shigella* spp. (*S. flexneri*, *S. boydii*, *S. sonnei* and *S. dysenteriae*) and *Campylobacter* spp. (*C. jejuni*
150 and *C. coli*). Multiplex end-point PCR was conducted using two bacterial Seegene ACE Diarrheal detection
151 kits (BÜHLMANN Laboratories AG, Schönenbuch, Switzerland). Details of sample processing procedures are
152 available in the Supporting Information. Each of the urine tanks were also evaluated for total viable
153 heterotrophic bacteria (April 2013) as an indicator of the total bacteria concentrations in source-
154 separated urine. One ml of each of three dilutions of sampled urine (at least 100-fold dilution in
155 phosphate buffered saline, 5mM PO₄, 10mM NaCl, pH 7.4) from each urine tank was aliquoted on a 3M
156 Petrifilm dryplate (3M, St. Paul, MN, USA) and handled as per manufacturer instructions. Plates were
157 incubated for 48 hrs at 35°C prior to enumeration, and results are presented as colony forming units
158 per ml urine sampled (CFU/ml).

159

160 **2.3.2 Viruses**

161 The presence of viruses was monitored by detection of viral RNA or DNA. The viral targets were human
162 adenovirus (HAdV), rotavirus (RoV), norovirus (NoV) GI and GII, human astrovirus (HAstV), enteroviruses
163 (EV), hepatitis A Virus (HAV) and JC Polyomavirus (JCPyV). While not typically associated with fecal
164 contamination, JCPyV was included herein because it has been detected consistently in wastewater
165 worldwide (Bofill-Mas et al., 2000). Unlike the other viruses evaluated, JCPyV is excreted indefinitely in the
166 urine of healthy individuals following infection. As such, this virus was expected to be ubiquitously present in
167 urine, and served as an internal process control for virus extraction and detection. Bacteriophage MS2
168 (DSMZ 13767) was used for optimization of the nucleic acid extraction procedure and PCR method. HAdV,
169 RoV, NoV GI and GII, and HAstV were analyzed by viral Seegene ACE Diarrheal detection kits (BÜHLMANN
170 Laboratories AG, Schönenbuch, Switzerland), which included positive controls. EV and MS2 were analyzed by
171 one-step reverse-transcriptase (RT)-PCR with SYBR Green using a Rotor-Gene 3000 thermocycler. HAV and

JCPyV were analyzed by RT-PCR with selective Taqman® probes. Further method details are available in the Supporting Information.

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2.3.3 Trace Organics

A method initially developed to analyze pharmaceuticals in hospital wastewater (Kovalova et al., 2012) was validated on 2010 and 2011 samples. Samples were screened for a group of 41 TrOCs (see Supporting Information) using liquid chromatography tandem mass spectrometry (LC-MS/MS). A group of 12 priority compounds were selected for further monitoring in the 2012 and 2013 sampling campaign. Concentrations are reported as averages with 95% confidence intervals (CI) unless otherwise noted. Details of the analytical method as well as the process for selecting priority pharmaceutical analytes are available in the Supporting Information. Methods applied for the analysis of the sulphonamide antibiotic resistance gene *sul1* are described in the Supporting Information.

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3 Results

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3.1 Urine tank conditions

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Characteristics of urine in the storage tanks during 2012 and 2013 sampling campaigns are presented in

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Table 1. The average temperature for all urine tank samples in December 2012 was 25.9 ± 0.7 °C, ranging from 24.8 to 34.3 °C at the time of sampling, and the pH was 8.72 ± 0.10 . The average temperature for all tank samples in April 2013 was 20.4 ± 2.0 °C, ranging from 15.5 to 27.1 °C at the time of sampling, with pH 8.90 ± 0.09 . Average ammonia measured in urine tanks was 2240 ± 1180 mg/L $\text{NH}_4\text{-N}$ (range of 400 – 8550 mg/L $\text{NH}_4\text{-N}$) and average phosphate was 240 ± 20 mg/l $\text{PO}_4\text{-P}$ (range of 170-290 mg/l $\text{PO}_4\text{-P}$).

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Table 1. Characteristics of urine in storage tanks.

Region	Sample Collection	Ammonia (mg/L NH ₄ -N) Avg. ± 95% CI (range, n)	Conductivity (mS/cm) Avg. ± 95% CI (range, n)	Temperature (°C) ¹ Avg. ± 95% CI (range, n)	pH Avg. ± 95% CI (range, n)
1	December 2012	-	30.3 ± 3.4 (25-34.3, 6)	27.6 ± 0.3 (27.2-28.1, 6)	8.65 ± 0.06 (8.58-8.75, 6)
	April 2013	1500 ± 530 (400-2100, 6)	-	20.3 ± 0.5 (19.8-20.8, 3)	9.02 ± 0.08 (8.95-9.08, 3)
2	December 2012	-	25.7 ± 0.7 (24.8-26.8, 6)	24.5 ± 0.5 (23.7-24.9, 6)	8.88 ± 0.04 (8.81-8.9, 6)
	April 2013	2250 ± 640 (1600-2650, 3)	-	19.2 ± 3.8 (15.5-27.1, 6)	8.91 ± 0.13 (8.68-9.08, 6)
3	December 2012	-	27.5 ± 2.3 (25.5-31.3, 6)	25.6 ± 1.0 (24.1-26.5, 6)	8.63 ± 0.24 (8.26-8.90, 6)
	April 2013	3650 ± 4820 (800-8550, 3)	-	22.8 ± 1.6 (21.3-24.2, 3)	8.75 ± 0.15 (8.60-8.86, 3)

3.2 Pathogen detection in source-separated urine

The detection frequencies of pathogenic bacteria and viruses in the urine storage tanks during the 2012 and 2013 sampling campaigns are presented in Table 2. Up to six pathogens were detected in a single urine sample.

3.2.1 Bacteria occurrence

The following bacteria were detected in at least two urine tank samples: *C. perfringens*, *Y. enterocolitica*, *E.coli* 0157:H7, verocytotoxin-producing *E.coli* (VTEC), *Aeromonas spp.*, *Vibrio spp.*, and *Shigella spp.* At least one diarrheal bacterium species was detected in each of the 18 samples (100%), and an average of three bacteria species were detected in each sample. Two samples contained five bacteria species each, the maximum number of diarrheal bacteria species detected in a single sample. Region 2 had the most total detects followed by Region 3 and Region 1 (Table 2). The highest frequency detected bacteria were *Aeromonas spp.* (94%); *C. perfringens* (72%); and *Shigella spp.* (61%). Three of the bacteria were not

¹ In Durban, the average low temperature is 20°C in December and 18°C in April; the average high temperature is 28°C in December and 27°C in April.

213 detected in any samples: *C. difficile*, *Salmonella spp.*, and *Campylobacter spp.* Heterotrophic plate counts
214 ranged from 1×10^5 to 7×10^6 CFU/ml across all urine tanks sampled, with an average concentration of 2×10^6
215 CFU/ml.

216

217 **3.2.2 Virus occurrence**

218 The following viruses were detected in at least one urine sample from Durban: JC polyomavirus, human
219 adenovirus, rotavirus, hepatitis A virus, and norovirus GII. Three to five virus species were detected in each
220 of the sampling regions, and up to three virus species were detected in a single sample. The three most
221 frequently detected viruses were JCPyV, HAdV and RoV detected in 100%, 34% and 31% of samples,
222 respectively. In the present study, NoV GII was detected in only one sample, which was collected in April
223 2013. HAstV and NoV GI were not detected.

224

225 **3.3 Pharmaceuticals in source-separated urine**

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227 Concentrations of pharmaceuticals from the priority list for samples taken from urine storage tanks in
228 December 2012 and April 2013 are shown by region in Table 2 and discussed below. Up to 10 of 12
229 pharmaceuticals assessed were detected in a single urine sample. Results from the 2010 and 2011 screening
230 are available in the Supporting Information.

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232 **3.3.1 Antibacterial detection**

233 The antibacterial pharmaceuticals tested in this study were: sulfamethoxazole (SMX), trimethoprim (TMP),
234 and clarithromycin. N4-acetylsulfamethoxazole (N4-AcSMX), a human metabolite of SMX, was also tested.
235 SMX and its conjugate N4-AcSMX as well as TMP were frequently detected at high concentrations. SMX, N4-
236 AcSMX and TMP were detected in 95%, 90% and 85% of samples, respectively, with concentrations in urine
237 reaching 6800, 3500 and 1300 $\mu\text{g/L}$, respectively. In contrast, clarithromycin was detected in only 20% of
238 samples and at relatively lower concentrations in this study ($17 \pm 29 \mu\text{g/L}$). The sum of SMX and N4-AcSMX

239 (Σ SMX) concentrations varied widely from less than 2 $\mu\text{g/L}$ to 8500 $\mu\text{g/L}$, with an average of 2700 ± 1100
240 $\mu\text{g/L}$. A weak correlation was observed between TMP concentrations and Σ SMX ($R^2 = 0.34$), such that when
241 Σ SMX was found in very high concentrations, TMP was also typically measured at elevated concentrations.
242

243 **3.3.2 Antiviral detection**

244 Among the four analyzed antivirals, only emtricitabine and ritonavir were detected, in 40% and 70% of
245 samples, respectively. The concentration of emtricitabine averaged to $100 \pm 97 \mu\text{g/L}$ but was highly variable
246 among storage tanks with a maximum concentration of more than 900 $\mu\text{g/L}$. Ritonavir concentrations were
247 typically low, with an average concentration of $1.5 \pm 0.5 \mu\text{g/L}$ and a maximum concentration of 5 $\mu\text{g/L}$.
248

249 **3.3.3 Other pharmaceuticals**

250 The anti-inflammatory drug diclofenac was detected consistently in 100% of the samples, though typically at
251 low concentrations ($30 \pm 10 \mu\text{g/L}$; max = 72 $\mu\text{g/L}$). Hydrochlorothiazide, a diuretic drug often prescribed to
252 treat high blood pressure, measured on average $42 \pm 18 \mu\text{g/L}$ and was found in 80% of samples. The beta-
253 blocker atenolol and its metabolite atenolol acid were detected in 55% and 75% of samples, respectively,
254 with average concentrations of 31 ± 33 and $98 \pm 110 \mu\text{g/L}$. Atenolol acid is a human metabolite of both
255 atenolol and metoprolol, neither of which were detected in the initial 2010 and 2011 screening.
256

257 **3.4 Antibiotic Resistance**

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259 The *sul1* antibiotic resistance gene was present in 100% of bacterial extracts ($n = 18$) and not detected in the
260 two field blanks analyzed. A two-tailed pairwise t-test indicates that duplicate analyses of the same samples
261 were not significantly different ($p > 0.3$). Of 36 samples tested, six were above the highest standard (10^6
262 gc/reaction); one of these was excluded as an outlier because it was several orders of magnitude higher in
263 concentration than replicate analyses of the sample using the same DNA extraction method (Method A, see

264 SI), as well as a different extraction method (Method B, see SI). The number of *su1* genome copies detected
265 per mL of urine sample ranged from 1×10^8 to 4×10^{10} .

266 **Table 2.** Detection of pathogens, pharmaceuticals, and antibiotic resistance genes in source-separated urine.

	Region 1		Region 2		Region 3		UT ¹	All Samples
	Dec. '12	Apr. '13	Dec. '12	Apr. '13	Dec. '12	Apr. '13	Dec. '12	
Virus	Detection Frequency		Detection Frequency		Detection Frequency		Det. Freq.	Freq. % (n = 29)
JC polyomavirus	3	6	3	6	3	6	2	100%
Adenovirus	2	2	1	0	2	3	0	34%
Rotavirus	3	0	3	1	0	0	2	31%
Hepatitis A virus	0	0	0	1	0	1	0	7%
Norovirus GII	0	0	0	1	0	0	0	3%
Astrovirus	0	0	0	0	0	0	0	0%
Echovirus	0	0	0	0	0	0	0	0%
Enterovirus	0	0	0	0	0	0	0	0%
Norovirus GI	0	0	0	0	0	0	0	0%
Bacterium	Detection Frequency		Detection Frequency		Detection Frequency			Freq. % (n = 18)
<i>Aeromonas</i> spp.	-	5	-	6	-	6	-	94%
<i>C. perfringens</i>	-	2	-	6	-	5	-	72%
<i>Shigella</i> spp.	-	2	-	5	-	4	-	61%
<i>Vibrio</i> spp.	-	2	-	2	-	0	-	22%
<i>Y. enterocolitica</i>	-	0	-	0	-	2	-	11%
<i>E.coli</i> O157:H7	-	1	-	1	-	0	-	11%
VTEC	-	0	-	2	-	0	-	11%
<i>Campylobacter</i> spp.	-	0	-	0	-	0	-	0%
<i>C. difficile</i> toxin B	-	0	-	0	-	0	-	0%
<i>Salmonella</i> spp.	-	0	-	0	-	0	-	0%
Pharmaceutical	Concentration Range (µg/L)		Concentration Range (µg/L)		Concentration Range (µg/L)		Conc. Range (µg/L)	Freq. % >LOQ (n=20)
Diclofenac	3.2 – 72	20 – 62	1.6 – 45	16 – 44	13 – 56	16 – 44	27 – 30	100%
Sulfamethoxazole	<2 – 300	320–5200	800–5000	1000–6800	560–4800	1600–6400	5 – 12	95%
N4 Acetyl-SMX	<1 – 560	280 – 720	60 – 3500	9.6 – 280	8 – 520	34 – 174	<1 – 2	90%
Trimethoprim	<2 – 3.6	22 – 380	4.3 – 56	260–1300	<2 – 300	100 – 440	<2	85%
Hydrochlorothiazide	<3 – 19	52 – 94	17 – 120	18 – 64	20 – 134	52 – 94	<3	80%
Atenolol acid	4.2 – 100	<4 – 100	<4 – 11	<4	8.0–1100	20 – 98	130 – 175	75%
Ritonavir	<1 – 2.8	<1 – 1.5	<1 – 1.7	<1 – 1.4	1.9 – 4.6	<1 – 1.0	2.8	70%
Atenolol	<1 – 2.9	<1 – 300	<1	<1	1.2 – 4.3	20 – 184	26 – 29	55%
Emtricitabine	<6	<6	<6 – 920	<6 – 240	<6 – 16	<6 – 380	26 – 120	40%
Clarithromycin	<1	<1	<1 – 1.1	<1	<1	4.4 – 300	<1	20%
Atazanavir	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	0%
Darunavir	<1	<1	<1	<1	<1	<1	<1	0%
Antibiotic Resistance Gene	Detection Frequency		Detection Frequency		Detection Frequency			Freq. % (n = 18)
sul1	-	6	-	6	-	6	-	100%

¹ UT refers to the two urine collection tanks at the University of KwaZuluNatal (UKZN).

268 **4 Discussion**

269

270 **4.1 Pathogens in source-separated urine and potential health risks**

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272 The present study is the first of its kind to assess a broad diversity of fecal pathogens in source-separated
273 urine. The urine storage tanks represent a composite of urine from potentially hundreds of individuals. Any
274 urine handled downstream of this set-up is likely to contain a mixture of pathogens originating from fecal
275 contamination. This assumption is corroborated by our findings that demonstrate the ubiquitous presence of
276 a wide spectrum of fecal pathogens in source-separated urine in Durban. The pathogen load and diversity
277 will depend on the extent of fecal contamination of the urine, which in turn depends on the toilet user
278 interface design, usage and maintenance patterns, and disposal processes, in addition to local cultural
279 practices. For example, whether or not children use the UDDTs, especially during an active gastrointestinal
280 infection, will influence the pathogen profile of the collected waste. The detection of rotavirus, a common
281 infection in children, indicates that children likely use the UDDTs in eThekweni.

282

283 The results presented here are based on presence of the pathogen DNA or RNA measured by PCR, and thus
284 do not yield data on the infectivity of detected pathogens. However, the application of PCR to identify
285 pathogens in this study is a conservative approach for the identification of potential health hazards in urine
286 by, for example, addressing the well-known issue of viable but non-culturable pathogens. A safe assumption
287 for the human pathogens detected is that they are infective in fresh feces and therefore also in freshly
288 contaminated urine. Additionally, pathogen monitoring by PCR avoids exclusion of pathogens that were
289 inactivated at the time of sampling but may pose a health risk to individuals in contact with the material
290 prior to inactivation. Finally, several studies have evaluated the inactivation of organisms in source-
291 separated urine, confirming that some bacteria and viruses may remain infective in stored urine for months
292 (Höglund et al., 1998; Vinneras et al., 2008). It is thus reasonable to assume that the detected pathogens
293 were at least in part infective, and thus constitute a potential health risk. A health-risk assessment would

294 benefit from a culture-based analysis of human pathogens to assess the viable and culturable status of
295 detected organisms relative to their total abundance at important exposure points in the urine collection
296 and handling chain.

297

298 When considering the health risks of using source-separated urine for fertilizer, viruses are of particular
299 relevance due to their low infective dose and resistance to inactivation in stored urine (Höglund et al.,
300 2002a). Each of the viruses detected in this study have been identified in various human and environmental
301 matrices in South Africa. Notably, rotavirus was the most frequently detected virus in a 2008 survey of fecal
302 specimens from patients with gastroenteritis in the Pretoria region, followed by adenovirus and norovirus
303 (Mans et al., 2010). Rotavirus may pose a risk in agricultural applications of urine (Höglund et al., 2002b) and
304 continues to be a major contributor to childhood diarrheal disease in South Africa, though the benefits of a
305 vaccine implemented in 2009 are beginning to be demonstrated (Seheri et al., 2012). In contrast to RoV,
306 JCPyV is not expected to increase the public health concerns of urine collection and agricultural application,
307 though it was universally detected in the samples. JCPyV already infects most people asymptotically
308 during childhood, though its pathogenicity emerges in HIV-infected individuals (Major et al., 1992). This
309 consideration demonstrates the importance of considering the risk factors of each viral target, in addition to
310 evaluating virus prevalence, to assess its relative importance to public health.

311

312 Several other viruses that are prevalent in South Africa and transmitted by the fecal-oral route were not
313 detected in the present study. These include enterovirus (Ehlers et al., 2005) and HAstV (Mans et al., 2010;
314 Taylor et al., 1997). The prevalence of these viruses could be low, as was the case for HAstV in South African
315 patients with gastroenteritis (Taylor et al., 1997), yielding concentrations below the detection limit.

316 Alternatively, limited detection could be a result of degradation of the viral genome, an inactivating process
317 expected for single stranded (ss)RNA viruses in stored urine (Decrey et al., 2015). ssRNA viruses are less
318 stable than DNA viruses and double-stranded (ds)RNA viruses, which are expected to retain their infectivity
319 and genome integrity in urine over long periods of time (Decrey, 2015). Correspondingly, the three most

320 frequently detected viruses in this study (JCPyV, HAdV and RoV) are dsDNA or dsRNA viruses. In contrast,
321 even ssRNA viruses with high seroprevalence in South Africa, including HAV and NoV GII (Mans et al., 2010),
322 were detected less frequently. Their limited detection in the present study may be indicative of genome
323 degradation during urine storage.

324

325 Similar to viral pathogens, the bacterial pathogen targets assessed in this study have each been detected in
326 various human and environmental matrices collected in South Africa. For example, *Shigella* spp. was
327 commonly identified in drinking water consumed by individuals with HIV/AIDS in rural households (Samie et
328 al., 2012) and may contribute to the chronic diarrhea commonly experienced by HIV/AIDS patients (Obi and
329 Bessong, 2002). The etiological agent of cholera, *Vibrio cholerae*, which was also detected in our samples,
330 has been regarded as endemic in South Africa and is a common cause of illness and death in the country
331 (Momba et al., 2010). While not detected in this study, *Campylobacter* spp. and *Salmonella* spp., along with
332 other diarrhea-causing bacterial pathogens, are known to circulate in South Africa (Obi and Bessong, 2002).
333 These results illustrate that while country-specific prevalence studies are useful to identify a range of
334 potential pathogen hazards, the local relevance of specific pathogens varies greatly.

335

336 In addition to yielding an overview of the health status of the sample population at the time of
337 measurement, the results indicate potentially problematic levels of fecal contamination in terms of health
338 risk to humans during downstream handling and use of the urine. The actual health risk to humans from this
339 diversity of pathogens will depend on the influent composition, pathogen inactivation rates as well as
340 human exposure routes during urine handling and processing, which requires further evaluation. JCPyV,
341 which is excreted in urine, may be useful as an indicator for tracking the fate of pathogens in source-
342 separated urine or as a treatment process indicator for viruses because of its consistent detection. JCPyV has
343 been proposed as a sewage indicator organism due to its high excretion titer, frequent detection in urban
344 sewage, stability, and absence of animal host (Bofill-Mas et al., 2000).

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4.2 Pharmaceutical excretion in urine and potential health risks

Little public information is available concerning specific pharmaceutical usage in South Africa, rendering estimation of consumption, and excreted concentrations, difficult. Therefore, average measured urine concentrations in South Africa were compared to predicted urine concentrations using pharmaceutical consumption data from the USA, France, Germany and Switzerland in order to identify outliers (Table 3). For example, low to no detection of the antibacterial clarithromycin indicates limited prescription in the study region, contrary to high-income countries (Michael et al., 2013). Similarly, concentrations of the diuretic, hydrochlorothiazide, were relatively low in eThekweni. However, average measured concentrations of SMX and TMP in urine were elevated as compared to predicted concentrations. Concentrations of SMX in particular were markedly high, more than an order of magnitude above the range predicted.

In addition to being widely used for the treatment of bacterial infections, SMX and TMP are frequently prescribed as prophylaxis to prevent opportunistic infections in patients with compromised immune systems (e.g., for HIV-positive individuals) (Madhi et al., 2002). In a preventative approach, patients receive such pharmaceuticals regularly over an extended period of time, in contrast to targeted antibiotic treatment for acute infections. If 4 – 11% of all HIV infected persons in the KwaZulu-Natal province were taking SMX prophylactic (see SI for estimation), this could explain the observed concentrations of SMX. Therefore, the surprisingly high SMX concentrations detected are reasonable considering the high prevalence of HIV-positive people in eThekweni. Additionally, SMX and TMP prescriptions are often administered together as co-trimoxazole in a 5:1 ratio. Because N4-AcSMX is a metabolite of SMX produced in the human body and deconjugates to SMX under environmental conditions (Göbel et al., 2005), the sum of SMX and N4-AcSMX was expected to correlate with TMP. Correspondingly, TMP was usually found in high concentrations when Σ SMX also occurred in high concentrations, though this prescription ratio was not preserved in the samples.

In addition to widespread use of antibacterial pharmaceuticals in South Africa, nationally, just over 2.0 of 6.4 million estimated people living with HIV in 2012 received anti-retroviral treatment (Shisana et al., 2014). Of

372 four ARV pharmaceuticals tested, emtricitabine and ritonavir were detected. Target antivirals detected at
373 low concentrations or not detected in storage tanks may reflect the low consumption and/or low excretion
374 rates of those compounds. Emtricitabine exhibits a urine excretion rate of 75%, whereas less than 8% of
375 consumed atazanavir, darunavir, and ritonavir are excreted in urine (Table 3). Therefore, low concentrations
376 or lack of detection of antivirals other than emtricitabine in the urine is not necessarily indicative of their low
377 consumption. The presence of feces in urine may also have contributed to the observed ritanovir, as 41% of
378 the consumed drug is excreted in feces. Antivirals not evaluated in this study, such as those recommended
379 for public sector ARV treatment in South Africa (WHO, 2005), may also be present in the urine from
380 eThekweni.

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Table 3. Predicted and measured pharmaceutical concentrations in 2012 and 2013 urine samples, and predicted environmental concentrations in soil following a single application of urine as a fertilizer.

Compounds	Usage	Excretion rate[%] ¹	Predicted concentration in urine (µg/L) ²		Measured concentration in urine (µg/L) (Avg ± 95% CI; n = 20)	PEC _{soil} (µg/kg) ³	
			min	max		min	max
Atazanavir	Antiviral	6	1	-	<2.5	0	0.3
Atenolol	Beta-blocker	37	74	246	31 ± 33	0	7.5
Atenolol acid	(Human) Metabolite of atenolol and metoprolol	ND ⁴	ND	ND	98 ± 110	0	25
Clarithromycin	Macrolide antibacterial	25	99	137	17 ± 29	0	5.4
Darunavir	Antiviral	7.7	2	-	<1	0	0.1
Diclofenac	Analgesic: antiinflammatoxy / antirheumatic	1	0.7	25	30 ± 10	0.2	4.7
Emtricitabine	Antiviral	75	20	-	101 ± 97	0	23
Hydrochlorothiazide	Diuretic	82	526	1118	42 ± 18	0.2	7.1
N ⁴ Acetyl-sulfamethoxazole	(Human) Metabolite of sulfamethoxazole	50	303	468	360 ± 340	0.2	82
Ritonavir	Antiviral	3.5	0.9	2.1	1.6 ± 0.5	0	0.2
Sulfamethoxazole	Sulfonamide antibacterial	20	121	187	2300 ± 1000	11	390
Trimethoprim	Antibacterial	60	2	120	190 ± 140	0.4	39

¹ Excretion rates from Swiss Compendium of Medicines by Documed: www.compendium.ch, last accessed March 10, 2015.

² Predicted minimum and maximum concentrations in urine calculated based on consumption in four countries (CH, DE, FR, USA); see Supporting Information Table S4.

³ Minimum and maximum predicted environmental concentrations in soil (PEC_{soil}) calculated based on measured urine concentrations for a single application of urine as fertilizer for agriculture; see Supporting Information.

⁴ ND = Not Determined.

notably including *Shigella* spp., were isolated from household water stored for use by HIV-positive patients in Limpopo province (Samie et al., 2012). In the present study, the sulfonamide antibiotic resistance gene, *sul1*, was detected in 100% of urine samples. While the *sul1* resistance gene is known to be widespread in the environment (e.g., (Czekalski et al., 2014)) and is not broadly representative of other antibiotic resistance determinants, it is typically detected at lower rates in urine samples than observed in the present study. For example, in a 16-country study of urine collected from patients with urinary tract infections, *sul1* was detected in 31% of 350 uropathogenic *E.coli* isolates (as compared to the sulfonamide resistance gene *sul2* in 48% of isolates). Co-trimoxazole is a common prescription for this infection (Blahna et al., 2006). Further, the concentration of *sul1* gene copies was high, from 10^8 to 10^{10} gc/ml, compared to less than 10^7 gc/ml in hospital and municipal wastewater effluents evaluated in Switzerland (Czekalski et al., 2012), while heterotrophic bacteria concentrations were similar ($\sim 10^6$ CFU/ml) in the source-separated urine to the levels in wastewater evaluated by Czekalski et al. (2012). The ubiquitous detection of *sul1* in the present study thus encourages further evaluation of the prevalence and health risks associated with other clinically relevant ARGs in source-separated urine, and especially those harbored within human pathogens. Despite the potential health challenges from the spread of antibiotic resistance, HIV-infected African adults receiving co-trimoxazole currently still garner consistent benefits in survival even in areas where co-trimoxazole resistance is common (Zachariah et al., 2007).

Beyond the human health concerns associated with antibiotic resistance determinants, pharmaceutical usage and environmental release pose potential ecotoxicological risks. Urine discharged from UDDTs or urine collected and applied directly as fertilizer may contribute to such risks. However, recommendations regarding environmentally safe concentrations of pharmaceuticals are limited, especially for soil applications of human-waste derived fertilizers (e.g., wastewater sludge, manure, or urine). Predicted no effect concentrations in soil ($PNEC_{soil}$) were previously estimated for SMX, TMP, and diclofenac using aquatic PNECs and compound physiochemical properties (Martín et al., 2012). For comparison to these values, we estimated a range of predicted environmental concentrations (PEC_{soil}) and risk quotients ($RQ = PEC_{soil}/$

PNEC_{soil}) based on urine application as fertilizer in agriculture (Table 3 and Table S5). The estimated soil concentrations were based on a one-time direct urine application to achieve 150 kg N/ha (see SI for calculation), assuming no degradation of the target compound in the soil. While the RQs for TMP and diclofenac (RQ<<1) indicated no significant environmental risk, the RQ for SMX for a range of scenarios evaluated (RQ>1) suggests a potential ecotoxicological risk from this compound due to its high concentration in urine and relatively low PNEC_{soil}. The actual SMX concentration in soil, and associated risk quotients, would depend on urine and water application rates, the soil characteristics, the degradation of SMX in soil, and measured PNEC_{soil} values. However, the results presented herein suggest that further detailed study on SMX ecotoxicity in these scenarios is warranted.

The breadth, high frequency and sometimes high concentrations of pharmaceuticals detected in source-separated urine in this study highlight the importance of evaluating pharmaceutical fate during urine collection and fertilizer production. Sequestering these compounds via urine diversion and collection prevents their release into the environment and provides an opportunity to remove or transform the compounds via urine treatment.

4.3 Fate of pathogens and pharmaceuticals in treatment processes for urine-derived fertilizer production

Due to the widespread detection of pathogenic viruses and bacteria as well as high concentrations of several pharmaceuticals in source-separated urine in eThekweni, thorough consideration of the downstream urine treatment processes is imperative for the protection of individuals handling stored urine and for its use as an agricultural fertilizer. In eThekweni, the primary urine treatment and fertilizer production technologies under development for potential scale up are: (1) urine storage for pathogen inactivation; (2) struvite fertilizer production for phosphorus recovery; and (3) urine nitrification for ammonia stabilization followed by distillation to recover all nutrients in a concentrated liquid product (Udert et al., 2015). In each of these technologies, processes promoting the degradation, inactivation, or removal of pathogens and TrOCs may occur. Below, available data from literature is interpreted in the context of eThekweni to evaluate the

450 treatment benefits of nutrient recovery processes in reducing environmental loads of human and
451 environmental health risk determinants from the application of source-separated urine, or fertilizers
452 produced from that urine, at scale.

453

454 **4.3.1 Urine storage**

455 The efficiency of pathogen indicator organism inactivation during the storage of urine under a range of
456 conditions has been extensively evaluated (Höglund et al., 2002a; Nordin et al., 2013; Vinneras et al., 2008).

457 Uncharged ammonia (NH_3), a volatile compound produced during biological urea hydrolysis, acts as an *in situ*
458 sanitizer in stored urine (Decrey et al., 2015; Vinneras et al., 2008). Measured ammonia concentrations in
459 this study were similar to those reported in urine storage tanks in Sweden (Höglund et al., 2000). To
460 maximize its biocidal effect, NH_3 losses from stored urine should be minimized. Therefore, dilution of urine
461 or the use of unsealed tanks or aeration during pumping of urine to transport facilities, which could lead to
462 NH_3 volatilization, should be limited. Ammonia losses should also be avoided to limit occupational inhalation
463 or dermal exposure to ammonia, by which deleterious health effects may be experienced (ATSDR, 2004), as
464 well as potential detrimental environmental effects (Galloway and Cowling, 2002).

465

466 Besides NH_3 , high storage temperature is conducive to pathogen inactivation in urine. Measured urine tank
467 temperatures depend on the external temperature, sun exposure, tank color, and time of day that samples
468 were taken. Fluctuating temperatures within urine storage tanks due to diurnal heating from the sun may
469 further enhance inactivation (Nordin et al., 2013). Durban is characterized by a mild subtropical climate, with
470 average maximum temperatures of 28 or 29°C occurring between January and March and 23 or 24°C in
471 winters from June to August.¹ Given these high temperatures year-round, urine storage in Durban may
472 achieve significant inactivation of a range of important pathogens. Specifically, of the most frequently
473 detected bacteria in the present study, the gram negative bacteria *Aeromonas spp.* and *Shigella spp.* are

¹ Durban Yearly Weather Summary : <http://www.worldweatheronline.com/Durban-weather-averages/Kwazulu-Natal/ZA.aspx>, accessed January 28, 2014.

474 expected to be inactivated rapidly in undiluted, stored urine with a temperature above 20°C. *Aeromonas*
475 *hydrophila*, for example, loses greater than 90% of infectivity per day in urine at 20°C (Höglund et al., 1998).
476 This is commonly observed for other gram negative bacterial pathogens and fecal indicator organisms. *C.*
477 *perfringens*, in contrast, is likely to remain infective in stored urine for extended periods of time (Höglund
478 and Stenström, 1999).

479
480 Viruses in stored urine exhibit a wide range of inactivation rates that are typically slower than those of gram
481 negative bacteria. As discussed above, dsDNA viruses are the most persistent in urine at urine storage
482 temperatures expected in Durban (Decrey, 2015). The dsDNA viruses most frequently detected in this study,
483 JCPyV and HAdV, are thus likely to remain infective in undiluted or diluted stored urine for several weeks or
484 longer. In contrast, ssRNA viruses such as HAV and NoV are expected to inactivate more rapidly than dsRNA
485 or dsDNA viruses due to susceptibility of the genome to degradation.

486
487 While the efficiency of pathogen and surrogate inactivation during the storage of urine under a range of
488 conditions has been extensively evaluated, data is lacking regarding the behavior of pharmaceuticals during
489 urine storage. Forthcoming results (Özel Duygan et al., 2015) indicate little benefit of urine storage for the
490 transformation of pharmaceuticals detected in eThekweni, as 11 out of 12 compounds were very stable; only
491 hydrochlorothiazide was degraded substantially. Nevertheless, source-separation coupled with urine
492 collection has the potential to sequester excreted drugs and reduce the diffuse discharge of pharmaceuticals
493 to the environment.

494

495 **4.3.2 Struvite fertilizer production and drying**

496 Struvite ($\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$) can be precipitated from stored urine by adding a magnesium source such as
497 MgO, MgCl_2 or bittern (Etter et al., 2011). The process is completed by filtration and drying of the struvite
498 precipitate, which can be applied directly as a fertilizer.

499

500 In the production of struvite, heterotrophic bacteria in urine accumulate in the solid during struvite
501 precipitation and filtration and are inactivated during drying with decreasing struvite moisture content
502 (Schoger, 2011). However, concentrations of the *in situ* bacteria in struvite may stabilize under mild drying
503 conditions (e.g., 20°C, 80% relative humidity). The inactivation kinetics of pathogenic bacteria during struvite
504 drying has not been considered specifically, though achieving only partial inactivation could be problematic
505 for attaining high fertilizer end-product quality. Increased desiccation of struvite at elevated temperatures
506 would likely mitigate this challenge, though struvite degrades rapidly above 55°C due to ammonia loss
507 (Bhuiyan et al., 2008). Thorough dessication of struvite at temperatures above ambient conditions is also
508 important to inactivate more resistant pathogens (e.g., helminths), which are also retained in the precipitate
509 during filtration (Decrey et al., 2011). In contrast to bacteria, viruses present in the urine are retained in
510 struvite pore spaces but are not preferentially accumulated (Decrey et al., 2011). Virus inactivation was also
511 found to increase linearly with decreasing moisture content of the struvite.

512

513 Similar to viruses, pharmaceuticals in struvite were contained in the residual urine before struvite drying. For
514 seven pharmaceuticals evaluated, an average of 98% of the analytes remained in urine following struvite
515 precipitation (Ronteltap et al., 2007). Washing struvite with water prior to drying was found to further
516 reduce pharmaceutical concentrations in the end-product by removing the residual urine. Such a procedure
517 would likely reduce pathogen concentrations in struvite as well.

518

519 With proper washing and drying procedures, the production of struvite can thus promote the inactivation or
520 removal of bacterial and viral pathogens in fertilizers produced from source-separated urine and reduce end-
521 product pharmaceutical concentrations. These procedures facilitate the production of urine-based fertilizers
522 with shortened urine storage times. However, pathogens and pharmaceuticals remaining in the urine after
523 struvite precipitation are not treated during this procedure and remain a potential human and
524 environmental health hazard. Longer-term field scale assessments as well as analysis of additional pathogens

and TrOC targets are also required in order to fully assess the fate and transport of contaminants during struvite production.

4.3.3 Urine nitrification for nutrient stabilization

An alternative to struvite production for urine nutrient recovery is the application of biological nitrification to stabilize nutrients in urine (Udert et al., 2003). Recent evaluation of the inactivation of bacterial and viral surrogates during urine nitrification indicates that nitrification is insufficient as a stand-alone technology for pathogen inactivation (Bischel et al., 2015). A relatively short nitrification reactor hydraulic retention time would likely yield only 90% or less inactivation. Viruses are likely to remain infective through nitrification treatment. However, because volatile ammonia is converted to nitrate during nitrification, the nitrified urine product can be distilled to yield a concentrated fertilizer and a pathogen treatment benefit. In a pilot reactor (Udert et al. 2015), nitrified urine is distilled for several hours at 80°C. This is a harsher treatment than recommended for sanitization of compost (70°C for 30 min; US EPA, 2003), and can be expected to yield a microbially safe product.

Of the 12 pharmaceuticals studied herein, only four were found to degrade substantially during urine nitrification (Özel Duygan et al., 2015). This is in accordance to the behavior of pharmaceuticals in municipal wastewater treatment, as many are still detected in considerable concentrations after wastewater treatment (Michael et al., 2013).

Nitrification of urine alone thus does not provide a significant treatment benefit for pathogens and pharmaceuticals in urine. However, coupling biological nutrient stabilization with the post-nitrification steps of distillation and advanced treatment can improve the quality of the liquid fertilizer by inactivating pathogens and removing pharmaceuticals, respectively. Several treatment processes have been successfully tested for the removal of pharmaceuticals from stored urine: ozonation (Dodd et al., 2008), electrodialysis (Pronk et al., 2006), and nanofiltration (Pronk et al., 2006). Treatment with activated carbon was shown to

be an effective process for pharmaceutical removal from nitrified urine (Özel Duygan et al., 2015).

Nitrification with distillation provides the added benefit over struvite production of complete nutrient recovery for enhanced fertilizer product quality.

4.4 Conclusion

UDDTs provide increased access to sanitation, especially when coupled with resource recovery and reuse, which further creates a financial driver for the sanitation system (Diener et al., 2014). With a large network of dry, urine-diverting toilets in place, the opportunity is ripe in eThekweni to move up the “sanitation ladder” (Mara et al., 2010) by eliminating the practice of urine discharge via soak-away pits. The presence of pathogens, pharmaceuticals and antibiotic resistance determinants in the source-separated urine reveals an important opportunity to reduce discharge of these contaminants to the environment via urine collection. However, the implications of such human and environmental health hazards also require further evaluation. In particular, the results may be contextualized in eThekweni via a quantitative microbial risk assessment of local urine collection practices, when infective pathogen concentrations are expected to be highest and opportunities for accidental human-urine contact are likely greatest.

Beyond urine collection, urine nutrient recovery processes must also yield high-quality products that are protective of human and environmental health and that ensure adequate fertilizer quality for optimal market value. The high content of such unwanted substances in the fertilizer source material may impact the quality, or perceived quality, of end-products. Therefore, this study also highlights how potential environmental and human health concerns could be reduced by incorporating a urine treatment step. Processes to produce urine-derived fertilizers can be designed or modified to inactivate pathogens and transform or remove pharmaceuticals in source-separated urine. The production of the solid fertilizer, struvite, can inactivate pathogens and remove pharmaceuticals from the end-product when implemented with proper washing and drying procedures. Biological nitrification for urine nutrient stabilization can be

coupled with the post-nitrification steps of distillation and advanced treatment to improve the quality of the liquid fertilizer in terms of pathogen and pharmaceutical concentrations.

Further evaluation and optimization of such urine treatment and fertilizer production technologies at field-scales is needed to reduce occupational hazards to staff involved in urine processing and minimize environmental risks associated with urine management. Nevertheless the prevention of pathogen and pharmaceutical discharge to the environment via urine source separation and collection is a considerable advantage to this sanitation technology.

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Urine Diversion

Urine Collection

Health Hazards Assessment