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Full Length Research Paper

Survival of enteric bacteria in source-separated human urine used as fertiliser: Effects of temperature and ammonia

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To promote the use of eco-toilets is an alternative to the lack of sanitation and high cost of artificial fertilisers in developing countries. Human urine is the fraction of excreta containing most nitrogen, phosphorus and potassium which are essential nutrients for plant growth. The major concern related to the use of the urine as fertiliser is the possible presence of enteric pathogens. The reduction in the numbers of *Escherichia coli*, *Enterococcus faecalis*, *Salmonella* sp., *Staphylococcus aureus*, *Clostridium perfringens* spores during urine storage was studied in diluted and undiluted urine samples. These experimental samples were stored in ambient air and at temperatures of 25, 35 and 42°C. The initial concentration of ammoniacal nitrogen reduced from 8260 to 1070 mg/L in quarter-diluted urine (1:3), while the pH values remained stable at 8.6 (± 0.2). *E. faecalis* survived for 35 days in the quarter-diluted urine at 25°C. Survival times of the tested bacteria were more shortened in ammonia concentrated samples, on storage at 35, 42°C. Except *C. perfringens* spores, *E. faecalis* was the least sensitive to storage conditions as compared to *S. aureus* and the two Gram-negative bacteria tested. Therefore, temperature and ammonia acted synergistically to remove bacteria in the stored urine. Likewise, *C. perfringens* spores were the most persistent bacteria, with only 1-$\log_{10}$ reduction within 100 days storage at ambient temperature. This study shows that a period of at least 30 days of undiluted urine storage at temperatures ≥ 25°C is sufficient to sanitize urine, considering only vegetative bacteria. Since urine from eco-toilets may contain other enteric organisms (viruses, protozoa, etc) appearing more persistent in external media, storage time must be lengthened for a satisfactory sanitation.

Key words: Human urine, storage, enteric bacteria, ammonia, temperature, sanitation, fertilizer.

INTRODUCTION

Separating the human excreta at the source can contribute to effective management of wastewater. To do that, urine diversion dry toilets (UDDTs) are the best indicated systems, because they are designed to separately collect urine and faeces (Mnkeni and Austin, 2009; Münch and Winker, 2009; Dagerskog and Bonzi, 2010). Thus, to promote the use of these eco-toilets, UDDTs represents an alternative to the lack of sanitation

and high cost of artificial fertilisers that affect many people in developing countries. To this end, since 2006, large-scale ecological systems were implemented in many areas in Burkina Faso (Dagerskog and Bonzi, 2010).

Human urine is the fraction of excreta which contains more nutrients, mainly, nitrogen, phosphorus and potassium (Heinonen-Tanski and van Wijk-Sijbesma, 2005), which are essential nutrients for plant growth. Also, fresh urine can contain microorganisms. In individuals suffering from typhoid fever, *Salmonella* can be found in their urine, only in cases of septicemia (Feachem et al., 1983). Recycling urine nutrients in agriculture therefore seems easier and more profitable as compared to faeces.

For health risks associated with the use of urine as fertiliser, the great concern is about enteric pathogens resulting from environmental samples or faecal cross-contamination during urine collection (Esrey et al., 2001; Schönning et al., 2002). In view of that, it is possible to assume that the source-separated human urine can contain enteric organisms. It is possible that applying in soil the untreated urine, may introduce another pathogens transmission route in the environment.

In order to sanitize urine from UDDTs, several treatment options have been proposed (Maurer et al., 2006; Pronk and Koné, 2010). The urine storage in closed containers and then exposed to the ambient air can remove microorganisms. The storage seems to be an appropriate method for the urine sanitation, as its realization is very simple. Apart from pathogens removal, the urine treatment by storage may preserve the useful nutrients for plant growth (Jönsson and Vinnerås, 2007; Pradhan et al., 2009).

The parameters which may influence the survival of microorganisms in urine during storage are mainly temperature, ammonia and pH. The temperature depends on the storage conditions and the climate. Because of high temperatures regularly reported in tropical regions especially in Sahel (Ouédraogo et al., 2007), urine storage time can be shortened, as compared to temperate regions, where storage times of 2-6 months were recommended to sanitize urine satisfactory (at 20°C or higher), including a wide range of bacteria, protozoan (oo) cysts and viruses (Höglund et al., 2002; WHO, 2006; Vinnerås et al., 2008; Chandran et al., 2009).

The pH of fresh urine is usually around 6 (Haneaus et al., 1996), but this value increases to approximately 9 in the collection and storage tanks due to rapid hydrolysis of urea (Jönsson and Vinnerås, 2007). In aqueous solution, the alkaline pH affects the equilibrium between the uncharged ammonia (NH_3) and ammonium (NH_4^+) in favor of the formation of the NH_3. The microbicidal effects of NH_3 were shown in several matrices (Jenkins et al., 1998; Nordin et al., 2009). The urine dilution can affect the ammoniacal nitrogen concentrations and therefore these uncharged ammonia (Pecson et al., 2007).

The survival capacity of enteric microorganisms in external environment differs according to microbial groups (Vinnerås et al., 2008; Chandran et al., 2009). Viruses, protozoan oocysts seem to be more persistent in the environment than bacteria. That could justify the choice of *Clostridium perfringens* spores as biological tracers to follow the fate of pathogenic viruses and protozoa in the wastewater (Payment et al., 2001).

This study investigated the effectiveness of the urine treatment by storage on determining the reduction numbers of five indicators or potentially pathogenic bacterial species: *Escherichia coli, Salmonella* sp., *Staphylococcus aureus*, *Enterococcus faecalis* and *C. perfringens* spores. The main objective was to evaluate the survival in urine of the above-mentioned bacteria, highlighting the effects of ammonia concentrations and temperatures during storage.

MATERIALS AND METHODS

Isolation of bacteria

Vegetative bacteria were respectively isolated from a polluted soil (*E. coli* and *E. faecalis*), wastewater (*Salmonella* sp.) and pathological exudate (*S. aureus*). To do that, standard methods were used (ISO, 1999, 2000). Isolated bacteria were used in the experiments, for a bacterial suspension. On the other hand, a stock of *C. perfringens* spores was prepared with a dried compost (faeces + soil) taken from an UDDT pit. 30 g of crushed and sieved compost were transferred into 270 mL of saline solution (0.9%). After homogenization, the supernatant was removed and used to prepare decimal dilutions (10^{-1}-10^{-3}), 250 mL each. Clostridia spore numbers in dilutions were determined as the described method (Anonymous, 2005), using Tryptone-Sulfite-Neomycin (TSN) Agar. The dilution containing 10^5 spores/mL was considered as the spore stock solution.

Urine samples preparation

Two-days-old urine from a urinal used by male staff at the Pan African Agency, Water and Sanitation for Africa (WSA) located in Ouagadougou (Burkina Faso) was used to prepare the urine samples. Three (03) matrices were prepared: undiluted urine (1:0), half-diluted urine (1:1) and quarter-diluted urine (1:3). The dilution was done with a sterile water (urine : water).

For the exposure to ambient conditions, each above-mentioned matrix was distributed in 5 L plastic jerry cans (white color) in a volume of 4 L per jerry can. On the other hand, three (03) sets of samples were prepared for storage in an incubator (Binder, Germany) under the following temperatures; 25, 35 or 42°C. Each set of samples was made up of twelve (12) bottles containing in triplicate the three urine matrices (1:0, 1:1, and 1:3) and sterile

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Table 1. Temperatures of the urine during storage at ambient air.

Time* (days)	Temperature per day of measurement (°C)			Temperature in jerry cans (°C)	
				UD = 1:0	UD = 1:1
	Min.	Max.	Mean	Mean ± SD	Mean ± SD
0	22	31.2	25.7	30.0 ± 0.3	29.8 ± 0.2
5	24.2	34	27.7	30.3 ± 0.4	31.0 ± 0.2
10	25.8	36.6	30.8	30.1 ± 0.3	30.1 ± 0.2
15	24	36.5	29.4	30.2 ± 0.1	30.8 ± 0.3
20	24	35.6	29.6	30.4 ± 0.2	30.5 ± 0.0
25	23.2	36.2	29.5	29.5 ± 0.5	30.0 ± 0.1
30	26.7	36.8	30.6	31.0 ± 0.0	30.5 ± 0.2

*Time: Measurements were performed with a time interval of five days, between October 07 and Nov. 06, 2010; UD : Urine diluted.

water (control). These matrices were distributed in 100 mL sterile polyethylene bottles (dark brown color) in a volume of 80 mL each.

Survival tests of bacterial species

Samples stored at ambient

Each sample in jerry can was contaminated either with a bacterial suspension (in which initial concentrations were 10^8-10^9 CFU/mL for *E. coli*, *Salmonella* sp. and 10^7-10^8 CFU/mL for *S. aureus*, *E. faecalis*) or with a Clostridia spores solution (10^5 cells/mL). Inocula were distributed at a rate of 40 mL per jerry can (1% [vol/vol]). Experiments were performed in duplicate. Contaminated samples were immediately exposed to ambient air (completely out in the sun, at Ouagadougou University site), for 05 months (September 2010-January 2011). Physicochemical and bacteriological parameters in samples were analyzed at regular time intervals (5, 10 days for vegetative bacteria and spores, respectively). Simultaneously, ambient temperatures were recorded daily during the exposure period.

Samples stored in an incubator

Samples in bottles were contaminated with a bacterial suspension (as above-mentioned) at a rate of 1.6 mL per bottle (2% [vol/vol]). Each set of 12 bottles contaminated samples was immediately placed in the incubator under one of these temperatures 25, 35 or 42°C. Bacterial parameters in samples were analyzed within 45 days, for 4 h and 5 days as regular times, respectively for rods and cocci tested bacteria.

Laboratory analyses

Physicochemical analysis

The pH and temperature values in stored samples were measured *in situ* using a multifunctional Inolab 340i pH/Thermo-meter (WTW, Germany). The ammoniacal nitrogen (NH_4-N) was analysed by the Nessler method, using a DR 2400 spectrophotometer HACH (Loveland, Co. USA).

Bacteriological analysis

Stored samples taken at regular time intervals were analyzed for microbial quantification. Clostridia spores were activated by heat (80°C for 10 min) and then counted in Tryptone-Sulfite-Neomycin (TSN) Agar, according to the method previously described (Anonymous, 2005). *E. faecalis* and *S. aureus* were enumerated respectively in Slanetz & Bartley and Mannitol Salt Agar, after incubation at 37°C for 24 to 48 h (APHA, 2005). *E. coli* and *Salmonella* sp. were counted respectively in Eosin Methylene Blue (EMB) and Salmonella-Shigella (SS) Agar (ISO, 1993, 2001).

Data analysis

For drawing graphs and calculating geometric means, standard deviations, minimum and maximum values of data obtained, the Microsoft Excel package was used.

RESULTS

Storage at ambient

Temperature values

During the samples exposure in the sun, ambient temperatures were recorded. The results showed that minimal and maximal temperatures respectively ranged from 13.9-24.9 and 30.7-38.8°C. The high thermal amplitudes were recorded significantly from November (~20°C), indicating the gaps between daytime and nighttime temperatures in the study area at this period of the year.

Table 1 presents values of daily temperature (sampling days) and those measured in the stored urine, exposed to ambient air. The data show that the differences in temperature between the two matrices of urine are not important. Temperatures in the urine storage containers depend on ambient temperature.

Reduction of vegetative bacteria

The graphs of Figure 1A and B show the reduction of bacterial numbers during samples exposure at ambient

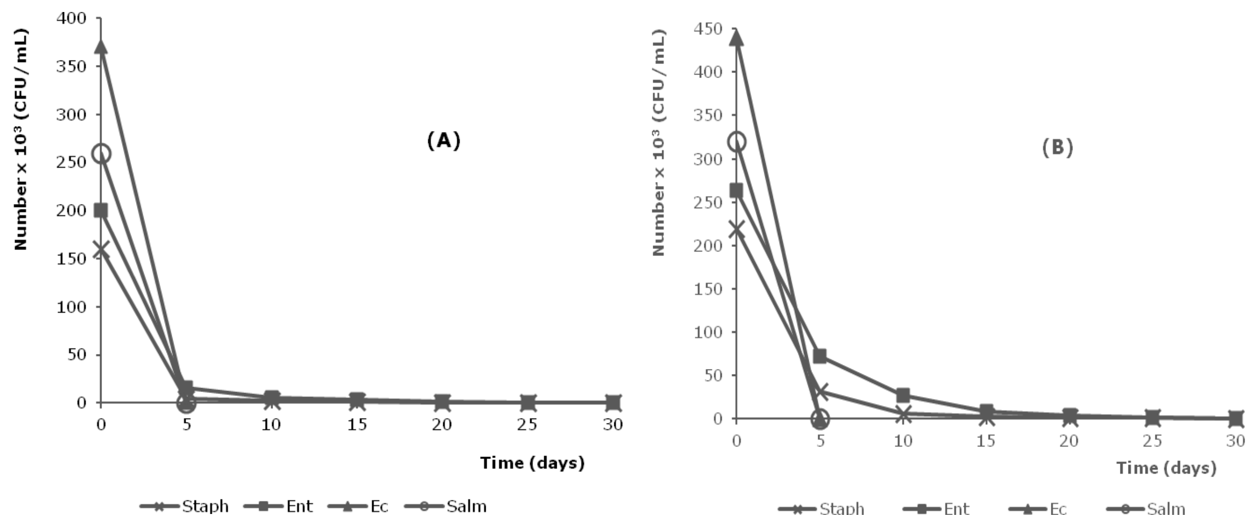

Figure 1. Reduction of bacterial numbers (*E. coli* [▲], *Salmonella* sp. [○], *S. aureus* [X], *E. faecalis* [■]) during samples exposure at ambient temperatures. The initial values were 8260 mg/L NH_4-N (pH 8.8) for the undiluted urine (A) and 4450 mg/L (pH 8.7) for the half-diluted urine (B).

temperatures.

The results show that after five (05) days of samples exposure, *E. coli* and *Salmonella* sp. were eliminated (0 CFU/mL) in both diluted and undiluted urine samples. In the undiluted urine samples, *S. aureus* and *E. faecalis* survived ~10 days (Figure 1A), while in the half-diluted urine, they survived around 15 and 20 days, respectively (Figure 1B).

The above data indicate that Gram-negative rods (*E. coli*, *Salmonella* sp.) were more sensitive in urine samples than Gram-positive cocci (*E. faecalis*, *S. aureus*).

Globally, the survival time for each of bacterial species tested was extended in the diluted samples as compared to the undiluted ones.

For the chemical analysis, a low decline of 0.1 pH units was observed in the corresponding pH on diluting the urine by half, while its ammoniacal nitrogen concentration decreased from 8260 to 4450 mg/L. Then, at the end of the experiments (after 30 days of storage), 7730 and 4060 mg/L were respectively the average concentrations of NH_4-N obtained in the undiluted urine and the half-diluted samples. These decreases of the ammonia concentration in the stored urine can be due to the nitrogen losses (N) occurring during the tests (measurements).

Reduction of Clostridia spores

The results of the reduction of *C. perfringens* spores in urine samples (and control) during the storage are shown in Figure 2. After one hundred (100) days of exposure, 0.85 and 1.07 log_{10} reductions of Clostridia spores were obtained respectively in the half-diluted urine samples (1:1) and undiluted ones (1:0). In the control (water), spore numbers did not decrease.

The reduced number of *C. perfringens* spores was slightly higher in the undiluted urine than in the half-diluted samples. It is well known that bacterial spores are substantially destroyed by heat (≥100°C) and by the tyndallisation process which requires moderate and intermittent temperatures (≤60°C). In fact, a moderate heat for 30 min is sufficient to remove the vegetative cells and to trigger the spores transforming in vegetative cells, and then may be destroyed at the next heating (Stanbury et al., 1995).

Storage in controlled environment

Physicochemical parameters

Means (± standard deviation) for the pH, ammoniacal nitrogen concentrations and temperature values in stored samples are indicated in Table 2.

Mean values of the initial concentrations of ammoniacal nitrogen were respectively 8260, 4450 and 1070 mg/L in the undiluted (1:0), half-diluted (1:1) and quarter-diluted (1:3) urine samples. At the end of the experiments, we found respectively 7980, 4295 and 1040 mg/L as average concentrations of ammonia. Between the start and the end of the experiments, small decreases in NH_4-N concentrations were observed. Apparently, nitrogen (N) losses in the stored urine were lower in the controlled environment (incubator), as compared to the ambient air. The pH was stable, with only a slight decrease (at the most 0.3 pH units) on diluting quarterly the urine, which

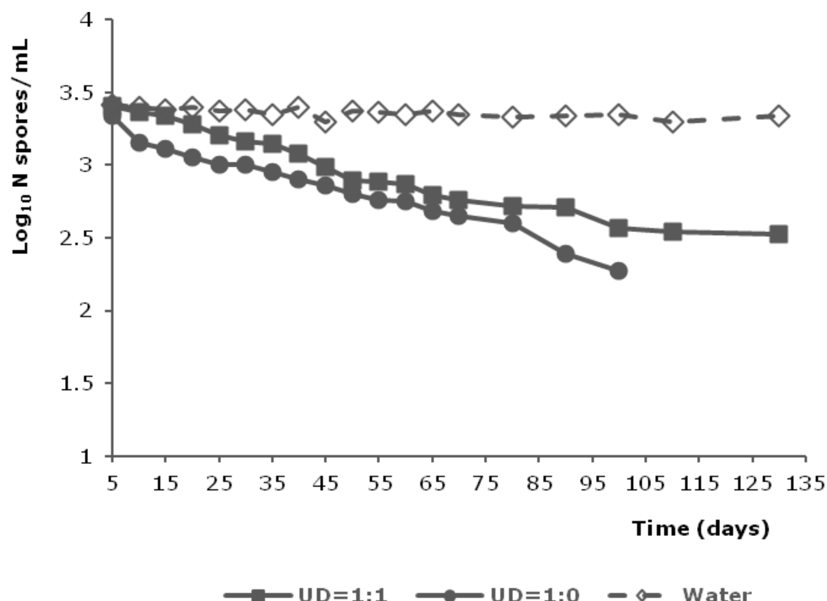

Figure 2. Reduction of *C. perfringens* spores during samples exposure at ambient temperatures. The initial values were 8260 mg/L NH_4-N (pH 8.8) for the undiluted urine (●) ; 4450 mg/L NH_4-N (pH 8.7) for the half-diluted urine (■) and pH 7.4 for the control (◊).

Table 2. Physicochemical parameters of the urine samples under incubating temperatures.

Sample	NH_4-N (mg/L)		Temperature (incubator)					
			25°C		35°C		42°C	
	Start	End	pH	T (°C)	pH	T (°C)	pH	T (°C)
UD=1:0	8260	7980	8.8±0.0	25.1±0.3	8.6±0.0	35.1±0.2	8.6±0.1	41.3±0.6
UD=1:1	4450	4295	8.7±0.0	25.2±0.1	8.4±0.0	35.2±0.2	8.4±0.0	41.7±0.3
UD=1:3	1070	1040	8.3±0.2	25.2±0.2	8.0±0.0	35.1±0.2	8.0±0.0	41.3±0.1
Control	ND	ND	7.4±0.1	25.1±0.2	7.4±0.0	35.1±0.3	7.4±0.2	41.5±0.0

ND: Not determined; Means ± SD; UD: urine diluted.

indicates its buffer property. The results (Table 2) show that temperatures in samples are similar to those shown on the incubator. Globally, a decrease of 0.2 pH units in urine samples was observed over time, under 25, 35 and 42°C.

Reduction of E. coli and Salmonella sp. number

Dark colonies with a metallic green sheen of *E. coli* were enumerated on EMB Agar. For *Salmonella* sp., colorless colonies with black centers were counted on SS Agar. The results in Table 3a and b show, regardless of incubation temperatures, 4 h of storage were sufficient to destroy in the undiluted urine, the two tested bacterial species (0 CFU/mL). The above data indicate that the survival of the two bacteria was better in the quarter-diluted urine (1:3) than in the undiluted (1:0). Similarly, we observed that survival of these bacteria was greater by incubating at 25°C than at 35 and 42°C, since in the case of the quarter-diluted urine samples, *E. coli* survival times were 24, 12 and 4 h respectively at 25, 35 and 42°C of incubation. For the *Salmonella* sp. 28, 16 and 8 h were the survival times obtained respectively in the same conditions as above mentioned. In the control, the reduction of bacterial numbers was particularly fast in function of the incubation temperatures.

Reduction of *E. faecalis and S. aureus* numbers

These bacteria were enumerated firstly, after two days of samples storage and then, at regular time intervals of 5 days. Red to maroon colonies of *E. faecalis* were counted

Table 3a. Reduction *of Escherichia coli* numbers in samples under storage conditions.

Time (h)	Number of *Escherichia coli* x 10^3 CFU/mL											
	25°C				35°C				42°C			
	UD=1:0	UD=1:1	UD=1:3	Water	UD=1:0	UD=1:1	UD=1:3	Water	UD=1:0	UD=1:1	UD=1:3	Water
0	1030±310	1120±670	1200±330	>300	980±211	1000±110	1070±111	>300	990±28	850±41	1010±38	>300
4	0	1.2±0.3	16.7±4.1	>300	0	1.0±0.1	5.9±1.2	>300	0	0.1±0.0	1.8±0.1	>100
8	0	0.1±0.0	13.6±2.5	>300	0	0	1.4±0.1	>100	0	0	0	<100
12	0	0	10.0±2.6	>300	0	0	0.3±0.2	>100	ND	ND	ND	ND
16	0	0	7.5±1.7	>300	0	0	0	>100	ND	ND	ND	ND
20	ND	ND	5.4±2.5	>100	0	0	0	>100	ND	ND	ND	ND
24	ND	ND	0.7±0.6	>100	ND	ND	ND	ND	ND	ND	ND	ND
28	ND	ND	0	>100	ND	ND	ND	ND	ND	ND	ND	ND
32	ND	ND	0	>100	ND	ND	ND	ND	ND	ND	ND	ND

Table 3b. Reduction of *Salmonella sp.* numbers in samples under storage conditions.

Time (h)	Number of *Salmonella sp.* x 10^3 CFU/mL											
	25°C				35°C				42°C			
	UD=1:0	UD=1:1	UD=1:3	Water	UD=1:0	UD=1:1	UD=1:3	Water	UD=1:0	UD=1:1	UD=1:3	Water
0	240±34	330±27	217±56	>300	280±85	341±71	490±141	>300	320±132	460±133	547±169	>300
4	0	1.4±0.4	22.0±5.0	>300	0	1.4±0.1	23.0±5.3	>300	0	0.2±0.0	17.6±4.7	>100
8	0	0.1±0.0	17.9±5.7	>300	0	0	5.0±0.7	>300	0	0	3.5±0.8	>100
12	0	0	11.0±3.8	>300	0	0	1.2±0.2	>300	0	0	0	>100
16	0	0	7.3±3.2	>100	0	0	0.2±0.0	>100	ND	ND	ND	ND
20	ND	ND	3.1±1.9	>100	ND	ND	0	>100	ND	ND	ND	ND
24	ND	ND	0.6±0.3	>100	ND	ND	ND	ND	ND	ND	ND	ND
28	ND	ND	0.1±0.0	>100	ND	ND	ND	ND	ND	ND	ND	ND
32	ND	ND	0	>100	ND	ND	ND	ND	ND	ND	ND	ND

on Slanetz & Bartley agar. For *S. aureus*, yellow colonies with yellow zones were enumerated on Mannitol Salt Agar. Data obtained (Table 3c and d) show that the two tested bacteria have survived at least 5 days in the undiluted urine samples (1:0) under 25°C of incubation, however they could not survive for up to two days at 42°C in the same samples as mentioned before. In the control, bacterial removal was faster at 42°C than at 25°C. Considering the incubation at 25°C, *E. faecalis* survival time in the quarter-diluted urine was 35 days approximatively, however it was less than 5 days in the undiluted urine samples. For the *S. aureus*, survival times were 15 and 5 days, respectively in the quarter-diluted and the undiluted urine samples. Extended times in the survival of the tested bacteria were observed by incubating in the diluted urine samples.

Table 3c. Reduction of *Enterococcus faecalis* numbers in samples under storage conditions.

Time (days)	Number of *Enterococcus faecalis* x 10^3 CFU/mL											
	25°C				35°C				42°C			
	UD=1:0	UD=1:1	UD=1:3	Water	UD=1:0	UD=1:1	UD=1:3	Water	UD=1:0	UD=1:1	UD=1:3	Water
0	96±17	117±14	105±11	>300	103±36	99±15	120±10	>300	86±12.7	97±18	102±40	>100
2	0.8±0.2	9.7±2.2	59±10	>300	0.3±0.1	5.4±0.9	22±7	>300	0	0.4±0.1	2.3±0.6	>100
5	0.2±0.0	4.5±0.4	28.0±1.3	>300	0	1.2±0.4	11.5±0.6	>300	0	0	0.9±0.2	>100
10	0	0.1±0.0	23.0±2.6	>300	0	0.2±0.0	1.5±0.4	<300	0	0	0	99
15	0	0.04±0.0	19.0±2.0	>300	0	0	0.5±0.0	244	ND	ND	ND	ND
20	0	0	5.1±3.2	>100	ND	ND	0	191	ND	ND	ND	ND
25	0	0	1.6±0.7	<100	ND	ND	ND	ND	ND	ND	ND	ND
30	ND	ND	0.2±0.0	<100	ND	ND	ND	ND	ND	ND	ND	ND
35	ND	ND	0.15±0.0	<100	ND	ND	ND	ND	ND	ND	ND	ND
40	ND	ND	0	<100	ND	ND	ND	ND	ND	ND	ND	ND
45	ND	ND	0	26	ND	ND	ND	ND	ND	ND	ND	ND

UD: Urine diluted.

Table 3d. Reduction *of Staphylococcus aureus* numbers in samples under storage conditions.

Time (days)	Number of *Staphylococcus aureus* x 10^3 CFU/mL											
	25°C				35°C				42°C			
	UD=1:0	UD=1:1	UD=1:3	Water	UD=1:0	UD=1:1	UD=1:3	Water	UD=1:0	UD=1:1	UD=1:3	Water
0	88±16	107±24	94±20	>100	102±31	100±15	94±30	<300	84±6	90±13	109±14	<100
2	0.6±0.2	09±0.2	3.6±0.9	>100	1.0±0.3	1.6±0.2	3.2±0.9	<300	0	0.2±0.0	0.9±0.2	<100
5	0.1±0.0	0.3±0.1	1.2±0.1	>100	0.2±0.1	0.5±0.1	1.3±0.2	<300	0	0	0.2±0.0	40
10	0	0	0.3±0.0	<100	0	0.1±0.0	0.4±0.0	<100	0	0	0	0
15	0	0	0.1±0.0	<100	0	0	0.1±0.2	<100	ND	ND	ND	ND
20	ND	ND	0	<100	ND	ND	0	<100	ND	ND	ND	ND

UD: Urine diluted.

Overall, Gram-positive cocci particularly *E. faecalis,* were less sensitive in the above described conditions (samples, incubation temperatures) as compared to Gram-negative rods (*E. coli*, *Salmonella* sp.).

DISCUSSION

The chemical results were that ammoniacal nitrogen (NH_4-N) concentrations in samples decreased in function of the urine dilution rate (Table 2), while pH values remained almost stable, even during the storage. Other previous studies indicated a biocidal effect in urine due to the uncharged ammonia (Jenkins et al., 1998; Nordin et al., 2009). Note that in aqueous solution,

ammoniacal nitrogen is present in both uncharged (NH_3) and ionized (NH_4^+) forms. The uncharged ammonia concentration is proportional to the NH_4-N concentration and then, can be calculated, using chemical equations (Erickson, 1985).

Our findings on microbial behaviour show that the destruction of the tested bacteria was faster in the urine samples greatly concentrated in ammonia (Figure 1 and Table 3a to d), thus explaining the biocidal properties of this urine component. It is well known that uncharged ammonia can cross easily biological membranes as compared to ions (NH_4^+, H_3O^+, OH^-). Nordin et al. (2009) indicated that inside microorganism, the uncharged ammonia acts by increasing the internal pH to deleterious levels, so destroys cell by causing the dysfunction of the metabolism. Previous studies showed that microbicidal effects of alkaline pH in media (~8.8 for the urine) were less significant than these due to the uncharged ammonia (Jenkins et al., 1998; Vinnerås et al., 2008; Nordin et al., 2009). Moreover, a link has been found between the NH_3 membrane permeability and the external temperature (Pecson et al., 2007; Vinnerås et al., 2008). In fact, these authors observed the removal of the NH_3 microbicidal properties at low external temperatures (~4°C) because of the inhibition of its membrane permeability. However, inverse effect was observed by increasing temperature. Likewise, it was shown in this study that the reduction of the bacterial numbers was more rapid in samples incubated at 42°C than those placed at 25°C (Table 3a to d). Accordingly, these results show that temperature and urine intrinsic components, particularly the ammonia act synergistically to sanitize the urine samples.

Moreover, the sensitivity of bacteria during sample storage varied depending on microbial group since, Gram-positive cocci have survived more than Gram-negative rods. The presence or not of a large peptidoglycan layer in the wall of the tested bacteria can explain the difference in bacterial behaviour. In fact, Gram-negative bacteria possess in their wall, a slim peptidoglycan layer, that make them more sensitive to environmental factors like heat shock and ionic strength (Sinton et al., 1994; Madigan and Martinko, 2006).

Usually, to assess the environmental risks due to enteric pathogens, *E. coli* is the common faecal indicator (Finney et al., 2003). Because of its rapid removal in the urine, *E. coli* would not be a suitable faecal indicator for the urine from UDDTs. All the same, *E. faecalis* and *C. perfringens* spores were suggested as suitable indicators of the effectiveness of wastewater treatment (Payment and Franco, 1993; Ashbolt et al., 2001). We have found, for *E. faecalis,* a survival time of 10 days in undiluted urine at 26.5°C as the average of the ambient temperatures. And then, their reduction was faster when temperatures increased (Table 3c). As urine from UDDTs can contain enteric microorganisms such as viruses and protozoan oocysts which are very resistant to the treatment by storage (Höglund et al., 2002; Nordin et al., 2009), so, *E. faecalis* could not be considered as reliable indicator of the effectiveness of the urine treatment by storage.

With respect to our results on *C. perfringens* spores, overall no or small reduction was observed in samples (Figure 2) during the storage. These findings may confirm the statement according to a conservative effect of bacterial spores in alkaline media (Venczel et al., 1997; Gaillard et al., 1998), in so far as the urine pH is ~9.

Only ~ 1-$\log_{10}$ reduction of Clostridia spores was observed within 100 days of storage. These small reduction in spore numbers may be due in part to the low activation of spores. In fact, the occurrence by activating spores is the wall rip, which may exhibit these spore-forming bacteria which has deleterious effects on the environment. Note that spore activating agents can be mechanical (shock), physical (heat) or chemical (acidity). In our case, *C. perfringens* spores were placed in alkaline samples (urine) and then, exposed to ambient (26.5°C). Under these conditions, spore activation cannot occur physically or chemically. Therefore, the mechanically treatment of samples before analyses (homogenization), could be the main cause of spores activation. *C. perfringens* spores have been showed as suitable tracers for determining the fate of viruses and protozoan oocysts in wastewater treatment (Payment et al., 2001; Ashbolt et al., 2001). But, for the treatment of the urine by storage, more data on viruses and protozoan behaviour in this matrix are needed in order to confirm the use of Clostridia spores as tracers in the urine sanitation. Nevertheless, it had been shown that Rotavirus and *Cryptosporidium parvum* oocysts remain viable in urine after 6 months of storage, under very low temperatures (Höglund and Stenström, 1999; Höglund et al., 2002; WHO, 2006).

The ammonia concentrations used for experiments (Table 2), are the same found in the urine collected in fields, by considering large-scale systems (Jönsson and Vinnerås, 2007; Dagerskog and Bonzi, 2010). In these conditions, low NH_4-N concentrations observed, can be due to the urine-water mixture that occurred when the UDDTs are used for washing bodies.

On taking into account only the samples incubated at 25°C, the survival times for *E. faecalis* (less sensitive bacteria), were 5, 15 and 35 days respectively in undiluted, half-diluted and quarter-diluted urine. Based on these data, storage times of 30-45 days recommended to sanitize urine from large-scale systems in Ouagadougou (Dagerskog and Bonzi, 2010), seem to be sufficient since in this Sahelian zone, annual average temperatures are above 25°C. Unfortunately, urine collected via UDDTs can contain other enteric microorganisms more persistent in environment than vegetative bacteria.

Also, the volumetric capacities in liquid samples can influence the microorganisms survival. Previous studies had shown that the sensitivity of microbes to the external factors (UV-light, temperature) was more important in the

small volumes than in the high volumes (Lemunier et al., 2005; Pecson et al., 2007; Niwagaba, 2009). Consequently, the urine volumes in containers must be considered for determining the storage time needed to sanitize urine. Although, the effects linked to the matrix volumetric were not studied here, however the ways for the urine collection via UDDTs and the climate variability must be integrated into the process of the urine sanitation by storage, before been applied in soils as fertiliser.

Conclusion

Experimental results have shown that the survival times of the tested bacteria increased during the storage with the urine dilution for which, the direct effect was the reduction of the ammonia concentration. For an effective treatment by storage, adding water into the urine collection containers must be avoided. Also, our findings showed that ammonia and temperature act synergistically to reduce or remove microorganisms in urine, during storage. That is why climatic factors are decisive to estimate the exposure time needed to sanitize urine. On top of that, analyses have shown that the survival capacities of bacteria in the urine, varied depending on bacterial group. Gram-positive cocci, particularly *E. faecalis* which were less sensitive in urine as compared to Gram-negative rods, because of the difference in peptiglycan layer size. The use of *E. faecalis* as indicator of the effectiveness of the urine treatment seems inappropriate since, *C. perfringens* spores are more resistant to the storage conditions. If the *C. perfringens* spores are suitable tracers for the fate of viruses and protozoan oocysts in wastewater treatment, their use as tracers for the urine treatment (storage) may be possible, in view of their stability. Nevertheless, it would be important to elucidate before the survival potential of viruses and protozoan oocysts in the urine during storage.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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