Research Paper

Urine treatment by solar disinfection for agriculture reuse purpose in a poor rural context: case of Burkina Faso

Drissa Sangare, Alexis L. Brou, Mariam Sou/dakoure and Patricia V. Tagro

ABSTRACT

The study aimed to reduce the storage time of urine treatment and assess the quality of treated urine following the Solar DISinfection (SODIS) method. Microbiological analyses were performed on urine samples taken before each sunlight exposure, between 10am and 4pm at a frequency of 1 h, during which temperature was measured in PET bottles (1.5 L). The initial concentrations of *Escherichia coli* (*E. coli*) and *Salmonella* in unstored urine were 10^6 and 10^3 CFU/100 mL respectively. The combined effect of temperature and UV radiation increased inactivation efficiency of *E. coli* at 5 log units. On the other hand, 98% of *Salmonella* were inactivated in less than 3 h of continuous exposure between 12am and 3pm with temperature varying between 50 and 65 °C in PET bottles. The k values showed that the inactivation rate of *Salmonella* tested was accelerated when the temperature was above 50 °C. Then, the results indicated that the first-order exponential decay model was the best method to predict the inactivation of Salmonella in urine by SODIS. General results showed that after 3 days of exposure to sunlight, urine collected via eco-toilet becomes bacteriologically sanitized and therefore can be used in agriculture.

Key words | *Escherichia coli*, inactivation time, *Salmonella*, Solar DISinfection (SODIS), urine

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HIGHLIGHTS

- *E. coli* was more sensitive to SODIS treatment in human urine than *Salmonella*.
- Higher *Salmonella* inactivation generally occurs with increased temperature.
- SODIS method reduces significantly the urine sanitized time compared to the ECOSAN method before being used as a fertilizer.

INTRODUCTION

Subsistence agriculture is the only source of food provision for vulnerable people in sub-Saharan villages, especially those located in the Sahel. This agriculture is completely dependent on rainy season (3–4 months/year) and its productivity is very low due to poor soil quality and the lack of chemical fertilizer amendment. The production of crops is then recurrently not sufficient, mainly during the hunger gap, making children and elders more vulnerable. It is vital to provide water and fertilizers as agricultural inputs to sustain small farming activities. However, conventional sources of water as well as chemical fertilizers are respectively scarce and too expensive for these poor farmers, so alternative sources of water and fertilizers must be found. In this regard, the reuse of treated greywater has promising potential. Coming mainly from showers, dishwashers, and hand wash basins, greywater represents

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about 70–85% of the total wastewater generated, which could be an important source of water for small farming (Abu Ghunmi *et al.* 2010). On the other hand, human wastes such as urine and feces are sources of fertilizer, which are ubiquitous and inexpensive. On average, one person excretes annually 2.8 kg of nitrogen (N), 0.45 kg of phosphorous (P) and approximately 1.3 kg of potassium (K) (study based on 10 countries in West Africa) (Dagerskog & Bonzi 2010). In urine, the average nutrient content of nitrogen, phosphorus and potassium are 3.0, 0.3 and 1.74 g/L, respectively (Jönsson *et al.* 2004; Ranasinghe *et al.* 2016). Besides, urine is rich in nitrogen (75–90%) and is available in the forms of urea or ammonium. Phosphorus and potassium are in an inorganic form and are directly plant-available (Jönsson *et al.* 2004). Most importantly, urine is an attractive fertilizer because it has a high nutrient content, and is considered as the liquid gold of wastewater (Randall & Naidoo 2018). Additionally, several studies have shown the beneficial effects of applying urine in agriculture (Kassa *et al.* 2018; Sheneni *et al.* 2018). However, both urine and feces are contaminated with pathogenic bacteria and parasites harmful for farmers and consumers (Chandran *et al.* 2009). For this reason, it is necessary to eliminate potential health hazards from the urine prior to agricultural application (WHO 2006).

Two processes can be considered: evaporation and storage. Evaporation, which mainly focuses on nutrient recovery optimization, is efficient but difficult to implement in a rural context by farmers due to handling risk (Antonini *et al.* 2012). Storage is actually the most widespread process and aims to inactivate pathogen microorganisms. Recognized as efficient to remove a major part of pathogens, its major constraint is, however, the large storage capacity required. Knowing that plastic tanks remain an expensive item for the poor rural population, even to store drinking water, it is highly unlikely that those people could pay for the required numbers of tanks to store urine. Further, storage of urine requires a longer treatment time of 1–6 months between 4 and 20 °C, and even then the product cannot be considered as pathogen free. For production and raw consumption of crops, urine has also to be stored for at least six months ($T > 20$ °C) before application to ensure a high level of pathogen inactivation, according to WHO (2016) guidelines.

To address these gaps, solar disinfection (SODIS) is a simple, environmentally sustainable, low-cost solution for urine treatment at the household level that provides usable end products. SODIS is largely applied in developing countries for drinking water disinfection. SODIS is a simple and inexpensive method that has been proven to be effective in removing pathogens and bacteria in contaminated water (Zinn *et al.* 2018). It consists of filling water in a transparent plastic bottle and exposing it to the sun over 6–48 h (McGuigan *et al.* 2012). This treatment is known to inactivate several species of bacteria, fungi as well as protozoa cysts and helminth eggs by the combined effect of heat and UV radiation. The inactivation of microorganisms depends on exposition time, temperature, turbidity and radiation intensity for drinking water (McGuigan *et al.* 2012). Applied to urine disinfection, pH raising can be also taken into account as an improving factor since it already plays an important role in a 'simple storage' process. This treatment is adequate in sub-Saharan countries where the annual mean temperature is higher than 20 °C, meaning that the recommended storage time could be reduced in these countries. It is important to note that the study site (Ouagadougou) is located in the Sudano-Sahelian climate zone characterized by minimum temperatures of 14–21 °C and maximum of 33–42 °C, with a long dry season from November to April.

The present study aims to treat urine by SODIS, prior to agricultural application. Specific objectives were to: (i) identify the exposition time required to reach a satisfactory inactivation of *Escherichia coli* (*E. coli*) in laboratory and field scale; (ii) assess the effectiveness of temperature, and temperature-UV radiation on *E. coli* inactivation; and (iii) identify the best bacterial indicator (*E. coli* and *Salmonella*) during the SODIS treatment.

MATERIAL AND METHODS

Study area

Twenty litres of urine were collected weekly in two cans from pilot Urine Diverting Toilets installed at households in the villages of Kologoudiessé (12.64°N, 1.23°W), and Kamboinsin (12.46°N, 1.55°W), located 30 and 18 km respectively from Ouagadougou, the capital city of Burkina Faso.

Laboratory experiment

The objective of the laboratory experiments was to understand the effect of temperature on *E. coli* inactivation. First, the collected urine was sterilized in an autoclave (Model systec VE-150) at 121 °C for 15 min. Then, 500 mL of sterilized urine was inoculated with a purified strain of *E. coli* with a concentration of about 6 $\log_{10}$ CFU/100 mL. The sample was separated into 10 samples of 50 mL each. Nine of them were stored at 4 °C before use whereas the last sample was used to check the initial concentration of *E. coli*. *E. coli* concentration was determined every 30 min for four samples and every hour for the remaining five samples, by using the spread plate technique. The microbial analyses were conducted in a laboratory located within the site of the International Institute for Water and Environmental Engineering (2iE) campus at Ouagadougou, Burkina Faso. The experiment was performed at 45 and 50 °C. The parameters analyzed and related medium and analysis procedure are summarized in Table 1.

Field experiment

Eight households were selected to conduct the field experiment. Six of the households were located in a rural area (village of Kologoudiessé) whereas two were from a semi-urban area (village of Kamboinsin). In each household (site), eight 1.5 L PET bottles were filled with untreated urine and placed on the household's roof. Four bottles were covered with an aluminium sheet to test the effect of temperature on bacteria inactivation (Figure 1). The experiments were repeated four times at each site, except sites 7 and 8 during the hot dry period between April and May (Table 2). During that period, the monthly global solar radiation varies from 5.82 to 6.32 kWh m^2 j^{-1} (NASA 2009). The increase of the urine temperature in the PET bottles was monitored from 8am to 6pm. We have observed that the temperature increased significantly between 10am and 4pm during this experiment period. So, this period was chosen to follow pathogen inactivation during the present study.

Table 1 | Microbial analysis conditions

Parameter[a]	Medium	Incubation time and temperature
E. coli	Chromocult coliform Agar	24 h at 44.5 °C
Salmonella sp.	Rappaport Vassiliadis	48 h at 37 °C

[a]Both parameters were analyzed by spread plate technique.

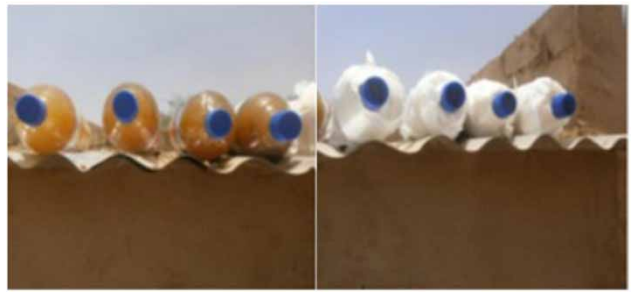

Figure 1 | Photograph of field experiment of urine solar disinfection.

Assessment of inactivation time of *Salmonella*

The experiment was conducted during 3 weeks in February. As analyses were repeated twice at each site, a total of 14 samples were collected three times a week in all sites.

Before solar exposure, a small sample of urine was analyzed to determine the initial concentration of *Salmonella*. A colony of *Salmonella* obtained from these analyses was then insulated and cultivated in a nutritive medium (Rappaport Vassiliadis) at 37 °C to have an inoculum with concentration of *Salmonella* similar to that found in the original urine samples (10^2–10^3 CFU/100 mL). The inoculum was used later to contaminate sterilized urine samples.

After that, urine samples were autoclaved at 121 °C for 15 min, and then transferred into two 1.5 L PET bottles. Three mL of the previous inoculum with an average *Salmonella* concentration of 10^2–10^3 CFU/100 mL was added to each bottle. The bottles were finally subjected to solar exposure. Samples were taken every hour from 10am to 4pm to determine the fate of *Salmonella*.

The inactivation of *Salmonella* followed the first-order reaction in previous studies (Sossou *et al.* 2016), expressed as follows:

$$N = N_0 e^{-kt}$$

where N_0(CFU/100 mL) and N(CFU/100 mL) are the concentration of *Salmonella* in the urine at time 0 and t,

Table 2 | Field experiments of SODIS test performed on eight sites

Number of sampling	site 1	site 2	site 3	site 4	site 5	site 6	site 7	site 8	
1	x	x	x	x					
2					x	x			
3					x	x			
4							x	x	
5	x	x	x	x					
6	x	x	x	x					
7					x	x			
8	x	x	x	x					
9					x	x	x		
10								x	
Total/site	4	4	4	4	4	4	2	2	28

respectively; k (h^{-1}) is the inactivation rate constant and $t(h)$ is exposure time.

Statistical analysis

EXCEL tools were used to calculate the arithmetic average and non-linear regression for *Salmonella* concentrations.

RESULTS AND DISCUSSION

Effect of temperature on *E. coli* inactivation

Figure 2 shows the rate of *E. coli* inactivation within 6 hours of exposure to 45 and 50 °C, respectively. When exposed to a continuous temperature of 45 °C, it was observed that only 50% of *E. coli* was inactivated after 6 hours of exposure, whereas *E. coli* was completely inactivated when exposed to a temperature of 50 °C after 6 hours.

The inactivation rate of *E. coli* increased exponentially with the increase in temperature. The elevated temperatures irreversibly inactivate enzymes of bacteria, protozoa and helminths, thereby resulting in cellular inactivation (Wichuk & McCartney 2007). Furthermore, the present study showed that higher temperature (50 °C) causes bacteria inactivation in 6 h. This finding is in broad agreement with the earlier work of Wegelin *et al.* (1994) who reported that exposing a PET bottle to strong sunlight for a minimum of 6 h enhances

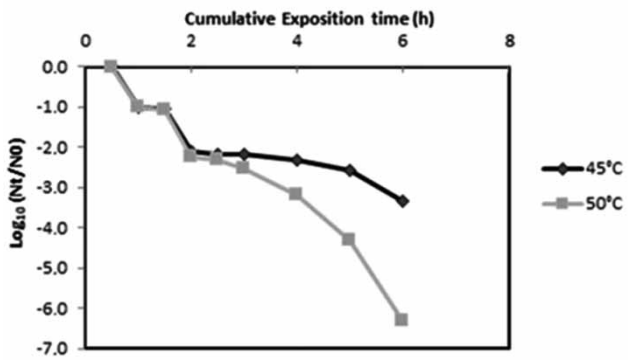

Figure 2 | Inactivation kinetics of *Escherichia coli* during SODIS simulation at constant temperature.

the inactivation of bacteria. Additionally, AL-Gheethi *et al.* (2013) showed that SODIS can reduce numbers of *thermotolerant coliform*, *Salmonella* spp. and *S. aureus* by more than 4 $\log_{10}$ CFU/100 mL after 6 h. Based on these results, 6 h of inactivation time were considered for field experiments.

Comparative effectiveness of temperature only and temperature and UV radiation on *E. coli* inactivation

Figure 3 represents the *E. coli* inactivation rate in aluminum sheet-covered and uncovered PET bottles after 6 hours of sun exposure. The different points represent the 56 SODIS tests performed in all sites. Twenty-eight points represent *E coli* inactivation in covered and uncovered PET bottles, respectively. It was observed that only nine tests out of 28

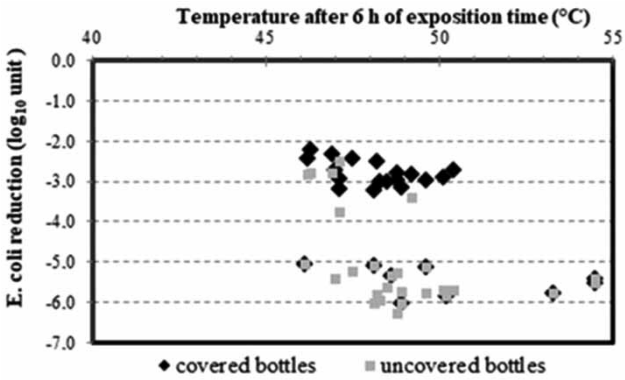

Figure 3 | *E. coli* inactivation in urine by SODIS tested in field conditions.

have achieved a reduction unit higher than 5 Ulog when the PET bottles were covered with an aluminium sheet.

The lowest *E. coli* inactivation rates measured in covered PET bottles showed that the urine temperatures achieved in this experiment cannot sanitize within a reasonable time. The present experiment has clearly demonstrated that the low *E. coli* inactivation in covered bottles can be mainly attributed to the UV absence, irrespective of the strength of sunlight. Similar low inactivation rates were also observed in covered reactors over the 6 h measurement period, where there was less UV (Davies *et al.* 2009).

Early field studies confirmed that temperature is not a predominant factor in the elimination of bacteria with sunlight but that it is mainly radiation which determines the efficiency of the SODIS experiment (Martin-Dominguez *et al.* 2005). As in their results, Oates *et al.* (2003) have shown that an exposure period of 48 h or more may be required to achieve inactivation of indicator bacteria to below detectable levels on cloudy days, and sometimes even 2 days are not sufficient (Parsons 2002). However, the tests performed with uncovered bottles gave better results of inactivation. It is suggested that the combined effect of temperature and UV radiation have increased the inactivation efficiency to 79% (i.e. 22 out of 28 tests with reduction unit higher than 5 Ulog). In uncovered bottles, sunlight contains higher levels of UV radiation, which increases *E. coli* inactivation. Indeed, Meierhofer & Wegelin (2002) reported that UV-A radiation produces highly reactive forms of oxygen in the water, such as oxygen free radicals and hydrogen peroxides, which interfere with cell structures and inactivate the pathogens. These results indicate that SODIS harnesses light and thermal energy to inactivate pathogens via a synergistic mechanism (McGuigan *et al.* 2012). Furthermore, Wegelin *et al.* (1994) have also observed that a synergistic effect of UV-A radiation and temperature contributed a 3-log reduction of fecal coliforms at a water temperature of 50 °C during 1-hour sun exposure.

PET bottles were exposed on the roof of the pilot family and reached urine temperatures of between 45 and 50 °C for about 6 h between 11am and 4pm, meaning that laboratory conditions were not achieved during the field test (Figure 4).

Solar inactivation behavior of the two bacteria (*E. coli* and *Salmonella*) under field conditions in uncovered PET bottles

The solar inactivation behavior of the two bacteria is shown in Figure 5. The result showed that *E. coli* is significantly more sensitive to SODIS than *Salmonella*. The order of sensitivity to SODIS batch process is *E. coli* > *Salmonella*. Seventy-nine per cent of inactivation efficiency is reached for *E. coli* (5 log reduction in CFU/100 mL) after 6 h exposure, whereas only 25% of efficient inactivation is observed for *Salmonella*. The result demonstrated that *Salmonella* was more resistant to the SODIS treatment than *E. coli*, also reported by Evison (1988). The inactivation of *Salmonella* by solar radiation was slower than *E. coli* and may be due to the capability of *Salmonella* to adapt to sunlight stress. Consequently, *Salmonella* was deemed to be the most suitable indicator of SODIS effectiveness in urine PET bottles for this present study. These results are in agreement with the works of USEPA (2003), which has reported that *Salmonella* are bacteria of great concern as well as being good representatives of reduction of other bacterial pathogens because they are typically present in higher densities than other bacterial pathogens. Furthermore, Berney *et al.* (2006) showed that *E. coli* may not be the appropriate indicator bacterium to test the effectiveness of SODIS on enteric bacteria. In contrast, other studies have shown that *E. coli* is more resistant to the bactericidal effect of the sun than other bacteria, such as *Campylobacter jejuni*, *S. epidermidis*, *Salmonella typhimurium* and *Salmonella enteritidis* (Boyle *et al.* 2008).

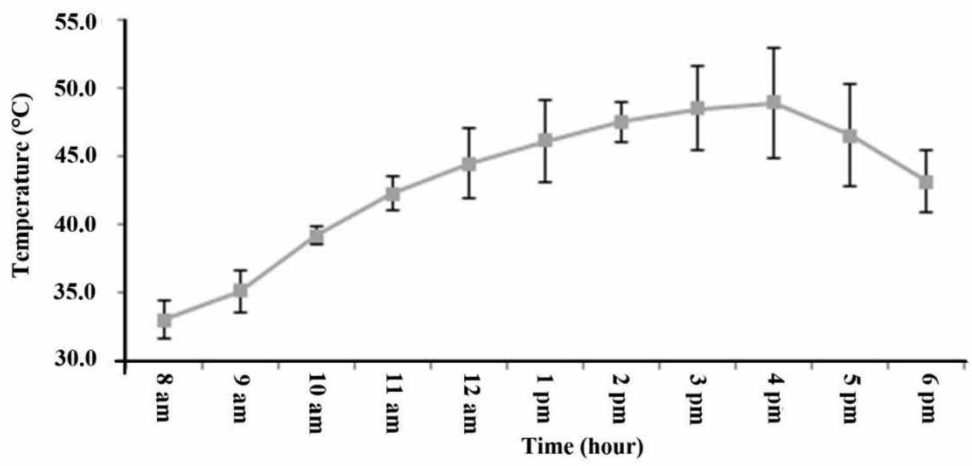

Figure 4 | Temperature in 1.5 L of urine bottle exposed to sunlight (mean of 3 days measurement).

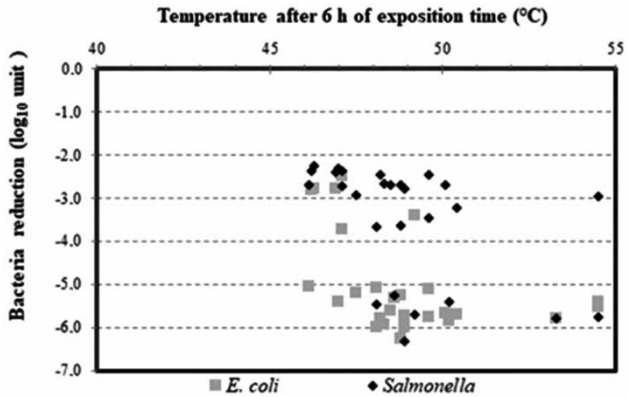

Figure 5 | *E. coli* and *Salmonella* inactivation in urine by SODIS under field conditions in uncovered PET bottles.

Assessment of inactivation time of *Salmonella*

The first-order exponential decay model of inactivation created with the data collected in the experiment worked well within the k values and N_0 (concentration of *Salmonella* in the urine at time 0) used in this study (Figure 6). The linear regression models inactivation based on exponential decay allows estimation of the time required to achieve 98% inactivation of *Salmonella*. The k values showed that the inactivation rate of *Salmonella* tested was fast in bottles at high temperatures above 50 °C. This shows that the elevated temperature may still have increased reaction rates identified by Heaselgrave *et al.* (2006). Ninety-eight per cent of *Salmonella* concentrations are inactivated in less than 3 h of continuous exposure between 12am and 3pm where the temperature varies between 50 and 65 °C inside PET bottles. Inactivation of *Salmonella* rates gradually increased over the course of 3 h. Data indicate that the logistic model provided the best fit for *Salmonella* inactivation. The results indicate that the logistic model may predict correctly the *Salmonella* inactivation in urine by the solar disinfection method. An inactivation rate coefficient, k (h^{-1}), would be useful for developing a management type of model to simulate the behaviour of bacteria in the sunlight. Therefore, the study showed that the time of storage of the urine before use in agriculture is reduced considerably in 3 days of solar exposure, contrary to the ECOSAN method which suggests 30 days of storage under sunlight (Makaya *et al.* 2014; Hijikata *et al.* 2017).

CONCLUSIONS

The SODIS method, usually applied as microbial treatment for drinking water, was tested in the present study to inactivate bacteria contained in urine. The results indicated that the usual recommended storage time from the literature of 1–6 months can be greatly reduced to 5 h. This SODIS effectiveness on pathogen inactivation in urine may be due to a synergy between increasing temperature and UV radiation. The study also highlights that *E. coli* was inappropriate as

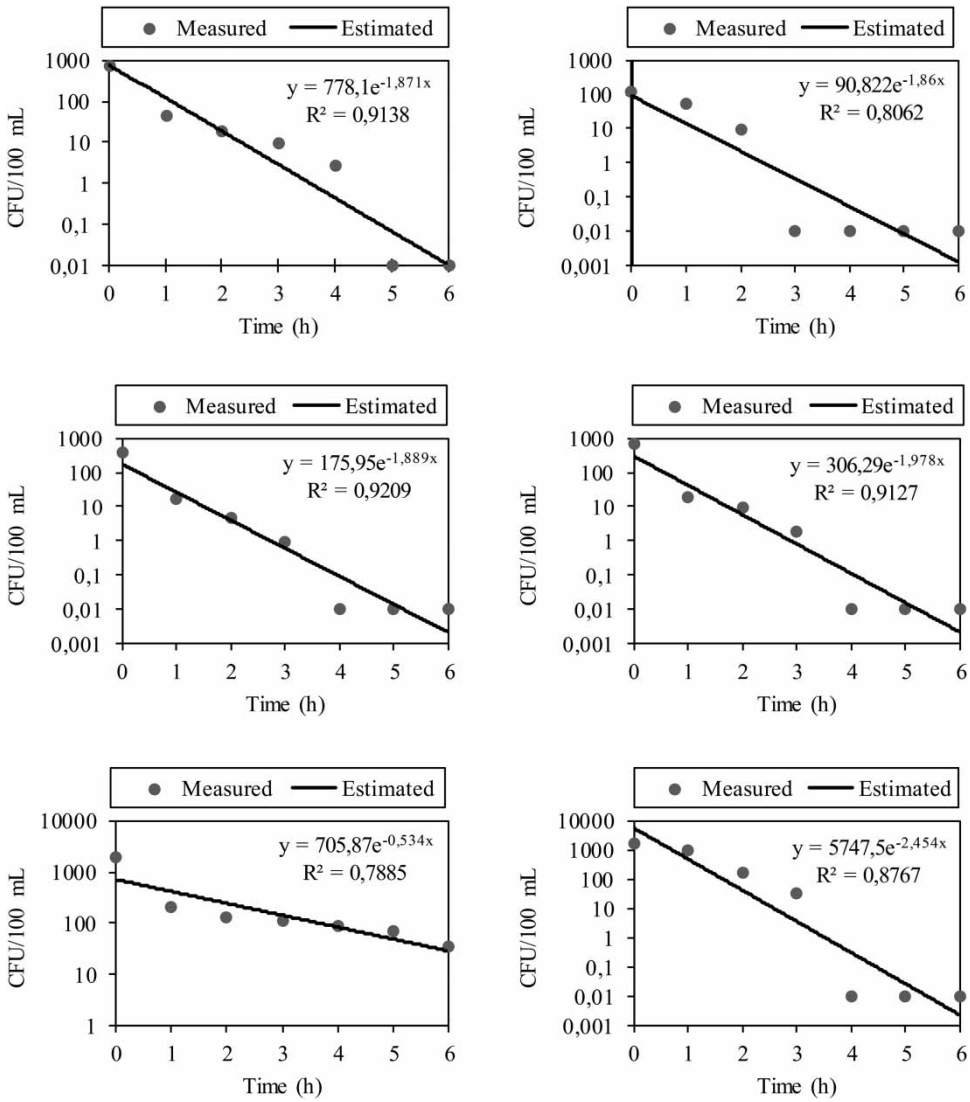

Figure 6 | Graphic representation of model of *Salmonella* inactivation.

a fecal indicator of Gram negative bacteria. On the other hand, the complete inactivation of *Salmonella* (98%) was achieved between 12am and 3pm, which is the high temperature period (e.g. 50–65 °C).

Therefore, the time of storage of the urine before use in agriculture is reduced considerably in 3 days of solar exposure, as opposed to the ECOSAN method which requires 30 days of storage under sunlight. Results obtained in this study confirm that after 3 days of exposure to sunlight, urine collected via an eco-toilet becomes bacteriologically sanitized and can therefore be used in agriculture. To ensure the effectiveness of the method, metal roofs are recommended to meet a 3-day delay. Otherwise, in the absence of metal roofs, for all other materials, sun exposure of up to 6 days would be required for effective bacterial inactivation. Also, it remains the end-user's choice whether to use glass or PET bottles depending on factors such as availability, affordability and portability. Finally, SODIS requires little technical knowledge to operate and can be utilized easily, and it is also very cost-effective because the only resources required are plastic bottles for use in semi-urban and rural areas of developing countries.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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