

## PRODUCTION OF BIOFERTILISING BY HYGIENIZATION OF EXCRETA AND EVOLUTION OF THE PARASITE LOAD OF SCHISTOSOMES (CÔTE D'IVOIRE, 2014)

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**ABSTRACT:** Ecological sanitation (Ecosan) consists in valuing human excreta as bio-fertilizer after treatment of feces by adding ash for composting and treatment of urine by storage in hermetically sealed containers. As schistosomiasis is endemic in the study area, the question is whether hygienization of schistosome-infected excreta is effective in eliminating them. The objective of the study is to determine the parasitic load of urine and feces, the parameters and the maximum time that influences the hygienization. A prevalence survey helped to identify and know the level of parasitic infestation of sick people. Full urine and ash-treated stool samples collected from infected persons were analyzed daily and every 15 days. The pH and temperature values of each urine sample were recorded. The results show that the eggs of *S. haematobium* and *S. mansoni* disappear respectively after 30 and 15 days of hygienization. The parameters that influence the hygienization are the increase of the pH and the oscillation of the temperature. Ecosan would be a solution to fight against schistosomiasis because the application of sanitized urine and feces breaks the cycle of transmission of the disease.

**KEYWORDS:** Urine, feces, schistosome, bilharziasis, hygienization.

### 1 INTRODUCTION

Ecological sanitation (Ecosan) or productive sanitation (PS) is an alternative to conventional sanitation, which aims to promote sanitation products, particularly as biofertilizers in agriculture [1]. The benefits of Ecosan include the protection of the environment, the water table, health, the improvement of soil fertility, the increase of agricultural production, the improvement of the income of the producer and the improvement of food security [2]. Around the world, Ecosan has been the subject of several publications [3], [4], [5], [6], [7]. In Africa since 2002, CREPA has undertaken research, promotion and training on the Ecosan approach and scientific work has been published by other authors [1], [8], [9], [10]. Specifically in Côte d'Ivoire, the field of research on this approach is still very broad despite some scientific publications [11], [12], [13], [14], [15].

The Marahoué region is an essentially agricultural zone with, for example, a production of 46803 tons of cocoa, 912 tons of coffee, 1090.7 tons of cotton and 10212.79 tons of food products for the year 2014 [16]. In order to perpetuate and optimize this production, fight against soil degradation and protect the environment, the use of excreta as a biofertilizer in the region could be a wise alternative. However, the Marahoué is a well-watered region and the application of urine and feces as a biofertilizer requires special precautions. Poor management of excreta and their use in agriculture can have negative health consequences resulting from the transmission of infectious diseases [17]. Schistosomoses, parasitic diseases due to flat worms (schistosomes or bilharzias), with urinary or faecal transmission, involving intermediate hosts that are freshwater molluscs, are distinguished from those diseases directly related to agricultural development [18]. Of the trematode worms, the eggs of one species, *Schistosoma haematobium*, are mainly excreted in the urine, while the eggs of other species (*S. japonicum*, *S. mansoni* and *S. mekongi*) are excreted in the feces. Eggs hatch in freshwater and larvae (miracidia) infect the intermediate host. Parasites are transformed and multiplied inside the snail, which spreads the next phase of the aquatic larva (cercariae) that can infect humans when in contact with water, penetrating the skin [17].

The Marahoué region has also been identified as a risk area for schistosomiasis [19], [20]. The application of urine and feces contaminated with schistosome eggs as a biofertilizer in agriculture is therefore a source of concern. If the eggs do not reach the bodies of water where the intermediate host lives in a few days, the infectious cycle is interrupted. This is also the case if the urine is stored for several days and used to fertilize the topsoil [21]. On the contrary, the use of fresh or untreated faecal material carries risks if it occurs near sources of fresh water where the intermediate hosts are present and if the eggs of schistosomes remain there. It is therefore important to ensure, before any use, the sanitary quality of excreta to be used as a biofertilizer during the entire period of hygiene in order to minimize health and environmental risks.

The objective of this study is therefore to verify the sanitary quality of the urine and feces contaminated respectively by eggs of *Schistosoma haematobium* and *Schistosoma mansoni* during the entire period of hygienization. Specifically it is:

- determine the parasitic load of urine and faeces hygienized;
- determine the parameters that influence the duration of hygienization;
- know the maximum duration of the hygienization time during the treatment of excreta.

## 2 MATERIAL AND METHOD

### 2.1 STUDY AREA

Located in the transition zone between dense forest and wooded savannah, in the center-west of Côte d'Ivoire, the health district of Bouaflé gathers 5 sub-prefectures with Bouaflé as chief administrative district (Figure 1). Located at 06 ° 58,717 N latitude and 005 ° 44,602 W longitude, it is 310 km from Abidjan, economic capital of the country. The health district of Bouaflé covers a total area of 3980 km<sup>2</sup> and has a population of 409 683 inhabitants [22]. The health center of Blanfa where this study was conducted is located 14 km from Bouaflé. Hot and humid, the climate of Bouaflé health district has two dry seasons (July to August, November to February) and two rainy seasons (March to June, September to October). The health district is crossed by two main rivers that are the White Bandama in the East and the Red Bandama or Marahoué in the West which gave its name to the region [23]. The village of Blanfa is watered by an agricultural dam and small rivers.

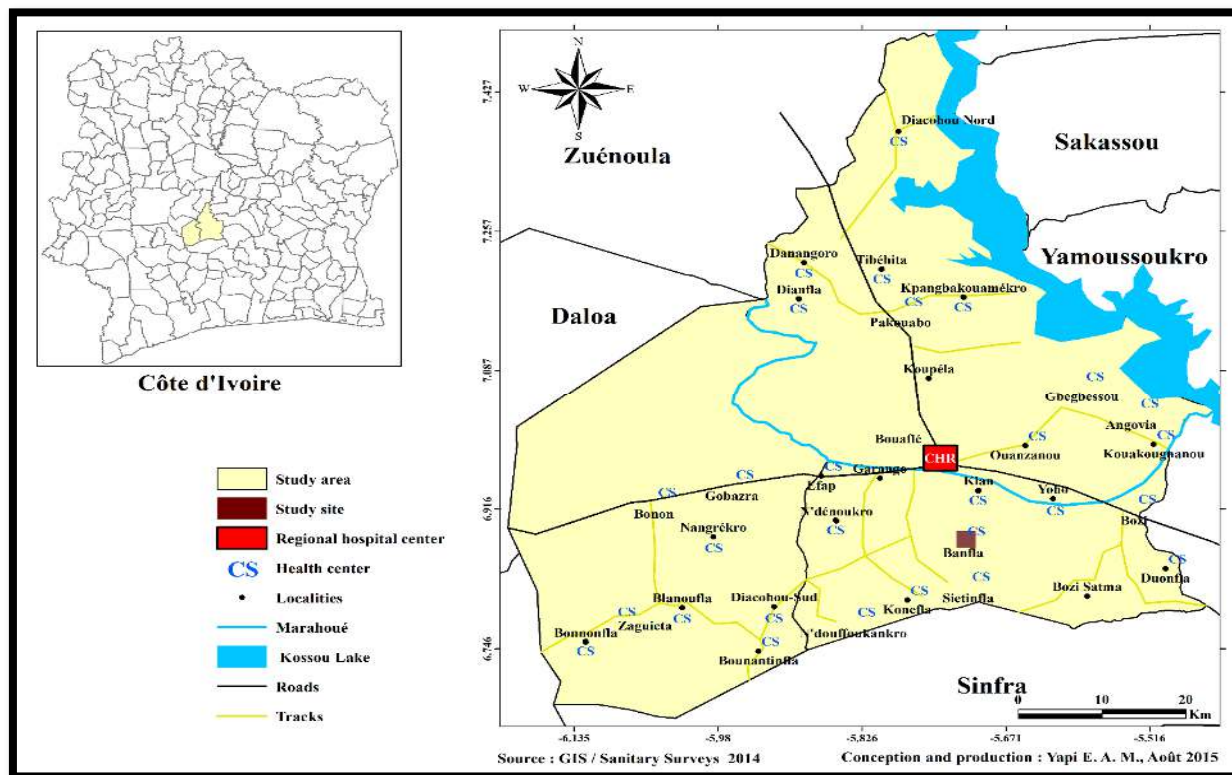


Fig. 1. Map of the Sanitary District of Bouaflé

## 2.2 SAMPLING

This study is a cross-sectional study that took place from June 28 to August 14, 2014 at the Rural Health Center of Blanfla and the Regional Hospital Center of Bouaflé.

The sample size of the prevalence survey plus 10% was calculated using the systematic random sampling method [24]:

$N = (t^2 p(1 - p)) / e^2$  with  $t = 1.645$  (90% confidence level);  $p =$  prevalence at 20%;  $e =$  margin of error at 7%;  $N = 100$  households.

## 2.3 COLLECTION OF URINE AND STOOL SPECIMENS FOR URINARY AND INTESTINAL SCHISTOSOMIASIS SCREENING

To ensure data collection in the field, a team of three people including a community health worker and the village nurse were involved. In each household selected in the village, one person is randomly selected. The inclusion criterion allowed is any consenting person for the study residing at least 6 months in the zone. This person is given two jars of 60 ml to collect his urine and stool. The sampling pots were distributed the day before between 17h and 23h. On a survey form, the person who took part in the study is identified by their first and last name, a unique identification number, the sex, the age and the name of the parent in order to avoid bias on the persons screened. The identification number and the name is marked on the pillbox.

Concerning urinary schistosomiasis, it was recommended a moderate effort to the chosen persons (rapid walk or fast climb of 2 or 3 times a staircase) before the collection of the urine to promote the urination of the eggs and to improve the sensitivity of the examinations of laboratories [25].

With regard to intestinal bilharziasis, the stool collected is the excreta of the first hour of the day. For stool sampling, the pillboxes are filled at  $\frac{3}{4}$ . The pots are recovered the next morning between 6h and 10h in the health center of the said village.

The collected urine and stool samples were collected in two separate boxes and sent to the Bouaflé hospital laboratory.

#### 2.4 COLLECTION OF URINE AND STOOL SAMPLES FOR HYGIENIZATION AND BIWEEKLY STEP ANALYSIS

In order to make urine and faeces biofertilizers without any health risk, all persons screened positive for urinary and intestinal bilharziasis by the preliminary survey were systematically studied in a second study.

Each person tested positive for urinary schistosomiasis received a 60 ml jar to be filled with urine, as recommended [26], the urine jars must be full. Those who tested positive for intestinal schistosomiasis were given a 60 ml jar to be filled at  $\frac{3}{4}$  with stool. Each pot of urine and stool is identified by a number. The collected urine and stool samples were collected in two separate boxes and sent to the Bouaflé hospital laboratory. The hygienization of the samples is carried out at room temperature, sheltered from the sun for 45 days.

#### 2.5 COLLECTION OF URINE SAMPLES FOR HYGIENIZATION AND DAILY ANALYSIS

Apart from the urine samples collected in the 100 selected households, all persons with haematuria in the village were systematically associated with the detection of urinary schistosomiasis. In addition to these people, 60 ml jars were also distributed to collect haematic urine.

These hematoma urine pots were control samples that will allow testing of the exact time of hygienization of urine samples contaminated with *Schistosoma haematobium*.

Control samples (hematic urine), identified by name and number, are collected at the same time as other infected urine samples. The hygienization of these control samples is carried out for 30 days, under the same conditions as the first urine samples contaminated with *Schistosoma haematobium* germs.

#### 2.6 SCREENING FOR URINARY AND INTESTINAL SCHISTOSOMIASIS

Macroscopic examination revealed the presence or absence of hematuria in the urine samples. The quantity and color of the urine contained in the pots are noted on the results sheets.

Microscopic examination of the urine is done after the urinary filtration method used in many studies [27]. The 12  $\mu$ m membrane filters are arranged inside the filter holders 13 mm in diameter. Then, 10 ml of homogenized urine is taken using a 10 cc plastic syringe and filtered. The filter holder is unscrewed, the membrane filter is removed and immediately stained with lugol 5% and the search, observation and enumeration of *Schistosoma haematobium* eggs are performed on the x 40 objective of an Olympus brand microscope. CX 21. The parasite load is defined as the number of eggs per 10 ml. The results obtained are recorded on the results sheet.

In the case of stool, the quality and color of the stool contained in the pots are noted on the results sheets. Microscopic stool examination was performed by the Kato-Kartz method for the identification of *Schistosoma mansoni* [28]. According to this method, cellophane papers of rectangular shape and dimensions of 3 cm x 2 cm are immersed in a solution containing 100 ml of glycerine, 100 ml of distilled water and 1 ml of 50% methylene blue for at least 24 hours. About 41.7 g of collected stool are then spread on an object slide then covered with cellophane paper previously dipped in the previous solution. The preparation is inverted and then crushed over several layers of absorbent paper so as to spread it well. Finally, the slides are examined under an optical microscope after a lightening time of 30-45 min. The number of eggs per gram of fecal matter (pasty stool) is equal to the number of eggs observed x 24 before the correction due to the appearance of the stool. Indeed, [29] indicates that if the stool is not pasty, the correction coefficients are as follows: solid stool: x 0.5; semi-liquid stools: x 1.5; liquid stools: x 2.

#### 2.7 DAILY AND BIWEEKLY ANALYSIS OF URINE AND STOOL SAMPLES DURING HYGIENIZATION

In contaminated urine samples, it is a question of following the mode of disappearance of the eggs of *Schistosoma haematobium* by storage according to the time of hygienization. The parameters monitored by the CX 21 microscope are the parasite load and the state of the eggs of *Schistosoma haematobium*. The urine tests are carried out at time steps of 15 days [2] from the reference period D0 (30 June 2014), D15 (15 July 2014), D30 (30 July 2014) and D45 (14 August 2014). They involved 16 urine samples collected from the 16 people who were positive for urinary schistosomiasis. The analysis technique is the urinary filtration technique

The full hematic urine pots were control samples that would allow verification of the exact time of hygienization of urine samples contaminated with *Schistosoma haematobium*. The analysis carried out on a daily basis concerned 3 samples of urine

hematics collected from 3 people observed. The parasitic load and the state of the eggs of *Schistosoma haematobium* are observed at x10 magnification of the CX 21 microscope during 30 days of hygienization by the urinary filtration technique.

For the stool, the analyzes were carried out also at a time steps of 15 days starting from the reference period D0 (June 30, 2014), D15 (July 15, 2014), D30 (July 30, 2014) and D45 (August 14, 2014). Stool was collected from 27 people who were positive for intestinal schistosomiasis and covered with ash (primary treatment). The search for eggs of *Schistosoma mansoni* and other possible parasites in the faeces is carried out at the level of the samples under experimentation after homogenization. The purpose was to microscopically monitor, every 15 days, the evolution of the parasite load and the condition of the *Schistosoma mansoni* eggs for 45 days. The analyzes were carried out on Days D0, D15, D30 and D45 by the Kato-Kartz technique.

## **2.8 DETERMINATION OF PH AND TEMPERATURE OF URINE SAMPLES DURING HYGIENIZATION**

The pH and temperature of the samples were measured using a PCE-PH 22 brand pH meter. These physical parameters are measured at each urine sample during the step-by-step analysis and daily analysis. To do this, the electrode of the pH meter previously calibrated is immersed in the liquid to be measured and the corresponding values are read on the display screen. The electrode is cleaned after each measurement with distilled water.

## **2.9 DATA PROCESSING AND ANALYSIS**

The collected data are: the urine and faecal hygienization time, the parasite load, the state of the schistosome eggs and the physical parameters such as pH and temperature. This data was processed using Excel and IBM SPSS Statistics Base 22.0. For time-lapse analyzes of urine samples as well as daily analyzes of control urine samples, the Pearson correlation matrices ( $r$ ) between egg condition, parasite load, time of Hygiene, temperature and pH were determined by covariance analysis and ordinal logistic regression.. For time-laps analyzes of stool samples, the Pearson correlation coefficient matrix is established by analysis of variance and binary logistic regression. Parasite load, temperature, and pH parameters are taken as the dependent variable for variance and covariance analyzes, respectively, while egg status is taken as a dependent variable for binary and ordinal logistic regressions. The threshold of significance retained is 5%.

## **2.10 ETHICS AND INFORMED CONSENT**

This study was approved by the National Committee of Ethics and Research of Côte d'Ivoire under No. 74 / MSLS / CNER-dkn, the Departmental Director of Health and the Director of the Regional Hospital Center of Bouaflé. All participants agreed to participate in the study by signing an informed consent form. The children participated in the study after their consent and the informed consent of their parents. The people surveyed are reassured that the names will remain anonymous and that all the information collected during this study will remain strictly confidential. At the end of the study, the populations of the village surveyed benefited from free mass treatment at Praziquantel.

## **3 RESULTS**

### **3.1 POSITIVE TEST SUBJECTS AND EGG STATUS OF URINE AND STOOL SAMPLES AS A FUNCTION OF TIME DURING HYGIENIZATION**

According to Table I, the proportion of persons infected with urinary bilharziasis without hematuria is 15.53% while 2.91% of the subjects are suffering from urinary schistosomiasis with presence of hematuria. Intestinal schistosomiasis affects 27% of the population.

At the beginning of the stepwise hygienization (D0), 100% of the collected urine samples are contaminated with intact and undamaged eggs of *Schistosoma haematobium*. Fifteen days after sanitation (Day D15), 38% of the urine samples have degraded eggs and remain contaminated, while 62% of the samples have a total absence of eggs and are no longer contaminated. The extensive hygienization process at 30 and 45 days (D30 and D45) shows an absence of schistosomes in all (100%) urine samples.

At day (D0) of biweekly step hygienization, all stool specimens (100%) are also contaminated with intact and undamaged *Schistosoma mansoni* eggs. But from day 15, until the end of the hygienization process on day D45, *Schistosoma mansoni* eggs are absent from all these stool samples (100%).

During daily hygienization, the proportion of hygienization and degradation time of *Schistosoma haematobium* eggs of the first control sample is higher (74.18%) than that of the second control sample (58.06%) and the third sample (45.16%). In contrast, the proportion of the absence time of *S. haematobium* eggs is higher in the control sample 3 (54.83%) than the control samples 2 and 1 which have respective proportions of hygienization time of 41.93% and 25.80%.

**Table 1. Positive Screening and Egg Status of Urine and Stool Samples as a Function of Time during Hygienization**

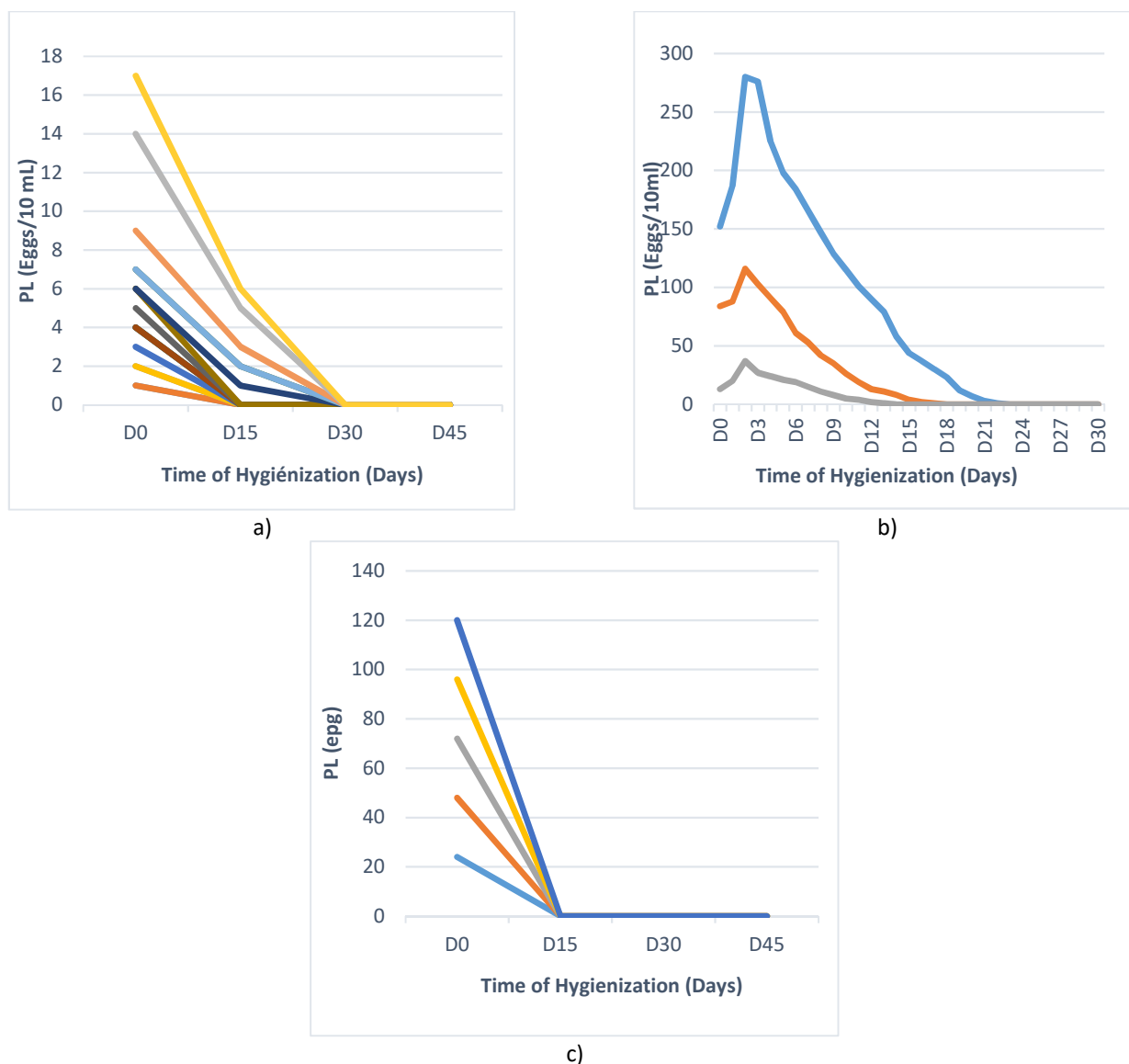
subject	Numbers	Proportion	Type of analysis	Time (Days)	Result	State of the eggs	Frequency	proportion
Positive urine without haematics	16	15,53%	Step of 15 days time	D0	Positive	Not degraded	16	100%
				D15	Positive	degraded	6	37,5%
				D30	Negative	Absence	16	100%
Negative urine	84	81,56%		D45	Negative	Absence	16	100%
Positive Hematic Urines	3	2,91%	Daily Control Sample ET 1	J0-J5	Positive	Not degraded	6	19,35%
				J6-J22		degraded	17	54,83%
				J23-J30	Negative	Absence	8	25,80%
			Daily Control Sample ET 2	J0-J4	Positive	Not degraded	5	16,12%
				J5-J17		degraded	13	41,93%
				J18-J30	Negative	Absence	13	41,93%
			Daily Control Sample ET 3	J0-J2	Positive	Not degraded	3	9,67%
				J3-J13		degraded	11	35,48%
			Total	103	100%		J14-J30	Negative
Positive stools	27	27%	Step of 15 days time	J0	Positive	Not degraded	27	100%
Negative stools	73	73%		J15	Negative	Absence	27	100%
Total	100	100%		J30	Negative	Absence	27	100%
				J45	Negative	Absence	27	100%

### 3.2 EVOLUTION OF THE PARASITE LOAD OF THE SAMPLES ACCORDING TO THE TIME OF HYGIENIZATION

According to figure (2a), the different parasite loads of the 16 urine samples analyzed at time steps change between 1 egg / 10ml and 17 eggs / 10ml. After 15 days of hygienization (D15), the parasite load  $\leq 6$  eggs / 10 ml urine of urine samples become zero. In contrast, parasite loads  $> 6$  eggs / 10 ml of the remaining urine samples cancel after 30 days of sanitation (D30).

The results of figure (2b) show that the parasite load of the three control samples undergoes a growth from D0 to D2, with a more pronounced increase for the control sample 1, then from D3, the different parasite charges decrease until complete cancellation.. The parasite load of sample 3 which is the lowest (13 eggs / 10 ml) vanishes on day D14. Then comes sample 2 of parasite load 84 eggs / 10 ml which becomes zero on day D19, finally that of sample 1 of strong parasitic infestation 152 eggs / 10 ml vanishes on day 23.

According to figure (2c), the parasite loads of the 27 stool samples analyzed vary between 24 opg and 120 opg. From day 15, until the end of the hygiene process on day D45, the parasite loads of all these stool samples (100%) become zero.



**Fig. 2.** Evolution of the parasite load of the samples as a function of the hygienization time: a) Evolution at 15-day time step of the parasite load of the urine samples; b) Daily change in parasite load of urine control samples; c) 15-day evolution of the parasite load of the stool samples

**3.3 CORRELATION OF PARASITE LOAD (PL), TEMPERATURE (T °), pH, TIME OF HYGIENIZATION AND STATE OF EGGS (SE) OF URINE SAMPLES ANALYZED AT BIWEEKLY TIME STEPS**

Table II shows the results of urine samples analyzed at biweekly time intervals (D0, D15, D30, D45). According to the analyzes, between the hygienization time and the state of the egg, the correlation is negative and significant ( $r = -0.828$ ,  $r = 0.003$ ). The correlation between the hygienization time and the respective physical parameters of temperature and pH are positive and significant ( $r = 0.452$ ,  $p = 0.000$  /  $r = 0.844$ ,  $p = 0.000$ )

The correlation between parasite load and temperature and the correlation between parasite load and egg condition are positive and significant ( $r = 0.139$ ,  $p = 0.001$  /  $r = 0.745$ ,  $p = 0.026$ ).

**Table 2. Correlation Matrix of Parasite Load (PL), Temperature (T °), pH, Time of Hygienization and State of Eggs (SE) of urine samples analyzed at biweekly time steps**

	Time	pvalue	T°	pvalue	pH	pvalue	PL	pvalue	SE	pvalue
Time	1		0,452	0,000*	0,844	0,000*	-0,617	0,123	-0,828	0,003*
T°	0,452	0,000*	1		0,331	0,449	0,139	0,001*	-0,080	0,876
pH	0,844	0,000*	0,331	0,449	1		-0,563	0,556	-0,694	0,698
PL	-0,617	0,123	0,139	0,001*	-0,563	0,556	1		0,745	0,026*
SE	-0,828	0,003*	-0,080	0,876	-0,694	0,698	0,745	0,026*	1	

\* Statistically significant; T°: Temperature; SE: State of the egg, PL: Parasitic load

**3.4 CORRELATION OF PARASITE LOAD (PL), TEMPERATURE (T °), pH, TIME OF HYGIENIZATION AND STATE OF EGGS (SE) OF CONTROL URINE SAMPLES ANALYZED DAILY**

**Table 3. Correlation Matrix of Parasite Load (PL), Temperature (T °), pH, Time of Hygienization and State of Eggs (SE) of control urine samples analyzed daily**

CONTROL SAMPLE ET 1										
Variables	PL	pvalue	Time	pvalue	T°	pvalue	pH	pvalue	SE	pvalue
PL	1,000		-0,914	0,008*	-0,846	0,667	-0,867	0,059	0,813	0,562
Time	-0,914	0,008*	1,000		0,929	0,000*	0,874	0,708	-0,900	0,001*
T°	-0,846	0,667	0,929	0,000*	1,000		0,832	0,423	-0,809	0,321
pH	-0,867	0,059	0,874	0,708	0,832	0,423	1,000		-0,809	0,416
SE	0,813	0,562	-0,900	0,001*	-0,809	0,321	-0,809	0,416	1,000	
CONTROL SAMPLE ET 2										
Variables	PL	pvalue	Time	pvalue	T°	pvalue	pH	pvalue	SE	pvalue
PL	1,000		-0,862	0,000*	-0,651	0,008*	-0,815	0,005*	0,855	0,016*
Time	-0,862	0,000*	1,000		0,889	0,011*	0,986	0,000*	-0,916	0,001*
T°	-0,651	0,008*	0,889	0,011*	1,000		0,887	0,478	-0,795	0,552
pH	-0,815	0,005*	0,986	0,000*	0,887	0,478	1,000		-0,916	0,039*
SE	0,855	0,016*	-0,916	0,001*	-0,795	0,552	-0,916	0,039*	1,000	
CONTROL SAMPLE ET 3										
Variables	PL	pvalue	Time	pvalue	T°	pvalue	pH	pvalue	SE	pvalue
PL	1,000		-0,798	0,000*	-0,723	0,924	-0,745	0,000*	0,792	0,107
Time	-0,798	0,000*	1,000		0,963	0,556	0,993	0,000*	-0,874	0,003*
T°	-0,723	0,924	0,963	0,556	1,000		0,967	0,243	-0,813	0,371
pH	-0,745	0,000*	0,993	0,000*	0,967	0,243	1,000		-0,861	0,417
SE	0,792	0,107	-0,874	0,003*	-0,813	0,371	-0,861	0,417	1,000	

\* Statistically significant; T°: Temperature; SE: State of the egg, PL: Parasitic load

The results in Table III show that during daily hygienization, the correlation between the parasite load and the sanitation time is negative and significant for the three control samples 1, 2 and 3 ( $r = -0.914, p = 0.008 / r = -0.862, p = 0.000 / r = -0.7988, p = 0.0001$ ).

The correlation between hygienization time and temperature was positive and significant for control samples 1 and 2 ( $r = 0.929, p = 0.000 / r = 0.889, p = 0.011$ ).

The correlation between hygienization time and egg condition was negative for the three control samples 1, 2, 3 ( $r = -0.900, p = 0.001 / r = -0.916, p = 0.001 / r = -0.874, p = 0.003$ ).

For control sample 2, the correlation between parasite load and temperature was negative and significant ( $r = -0.651, p = 0.008$ ). The correlation between parasite load and pH was significantly negative for control samples 2 and 3 ( $r = -0.815, p = 0.005 / r = -0.745, p = 0.000$ ). For control sample 2 the correlation between egg status and pH was negative and significant ( $r = -0.916, p = 0.039$ ). On the other hand, the correlation between the state of the egg and the parasite load is positive and significant ( $r = 0.855, p = 0.016$ ).



For control samples 2 and 3 the correlation between sanitation time and pH was positive and significant ( $r = 0.986$ ,  $p = 0.000$  /  $r = 0.993$ ,  $p = 0.000$ ).

### 3.5 CORRELATION OF PARASITIC LOAD (CP), HYGIENIZATION TIME AND STATE OF EGGS (EO) OF STOOL SAMPLES ANALYZED AT BIWEEKLY INTERVALS

According to Table IV, which presents the analyzes of stool samples from day 0 to day 45, the correlation between the parasite load and the hygienization time is negative and significant ( $r = -0.688$ ,  $p = 0.000$ ) but it is positive and significant between the parasite load and the state of the egg ( $r = 0.888$ ,  $p = 0.000$ ).

**Table 4. Correlation Matrix of Parasite Load (PL), hygienization Time and State of Eggs (EO) of stool samples analyzed at biweekly time steps**

	Time	pvalue	PL	pvalue	SE	pvalue
Time	1	-	-0,688	0,000*	-0,775	1,000
PL	-0,688	0,000*	1	-	0,888	0,000*
SE	-0,775	1,000	0,888	0,000*	1	-

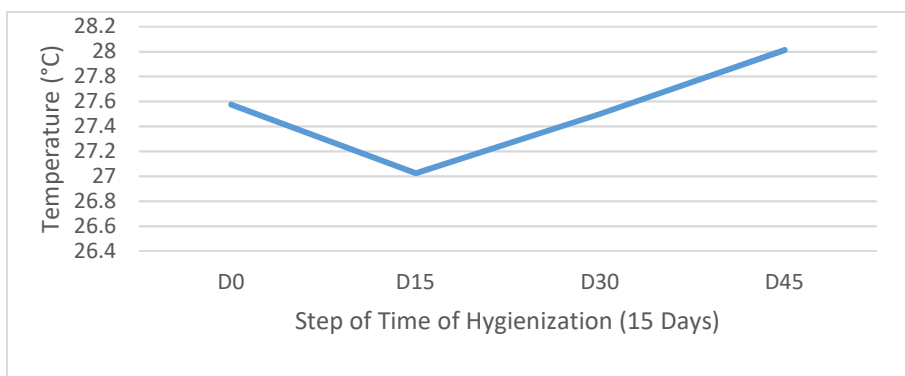
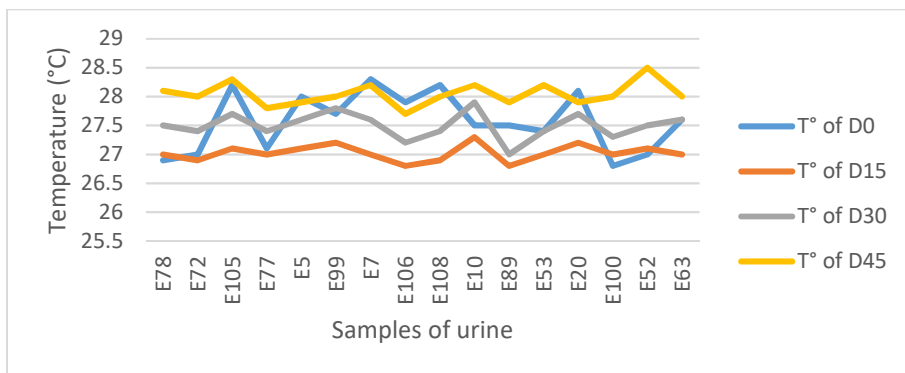
\* Statistically significant; SE: State of the egg, PL: Parasitic load

### 3.6 EVOLUTION OF THE PH, THE TEMPERATURE OF THE 16 SAMPLES AND THE 3 CONTROL URINE SAMPLES ACCORDING TO THE HYGIENIZATION TIME

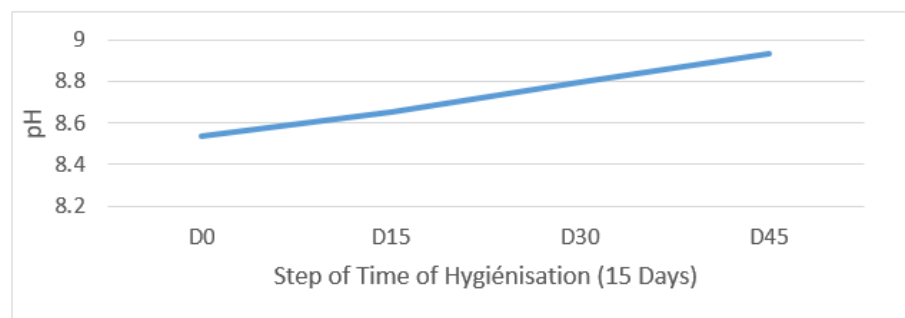
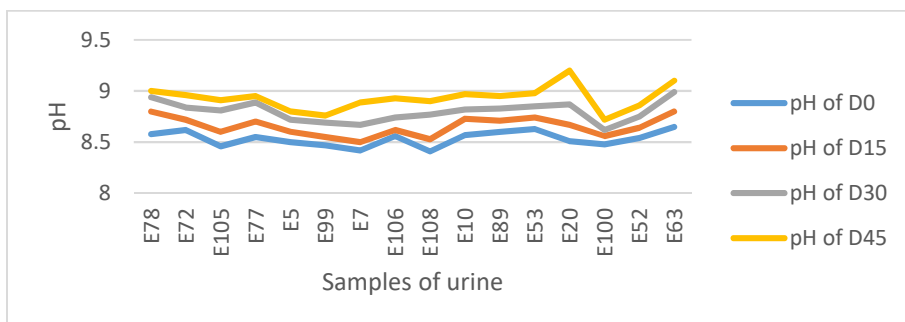
At day D0, temperatures range from 27 ° C to 28.3 ° C. These temperatures decrease between 26.8 ° C and 27.3 ° C to D15 but go back to D30, between 27 ° C and 27.9 ° C and 27.7 ° C and 28.5 ° C to D45 (Figure 3a) . The average temperature curve of the 16 samples illustrates this drop from 27.6 ° C to 27 ° C from day D0 to day D15 and this rise to 28 ° C from day D15 to day D45

At D0, the pH of the samples varies between 8.4 and 8.6 and at D15, it is between 8.5 and 8.8. At day D30, the pH increases from 8.6 to 9 while at day D45, the pH values are between 8.7 and 9.2. The average pH increases from 8.5 to 8.9 from day 0 to day D45 (Figure 3b)

According to Figure (3c), the temperatures and pH values of the three urine samples increase throughout the duration of hygienization. Sample 3 has a temperature of 27.2 ° C to 28.2 ° C and pH values ranging from 8.55 to 8.96 during the 30 days of hygienization. Sample 2 has a temperature between 27.2 ° C and 28 ° C and pH values ranging from 8.43 to 8.9. Sample 1 has a temperature that varies between 26.9 ° C and 28 ° C while pH values between 8.37 and 8.7 are the lowest.



(a)



(b)

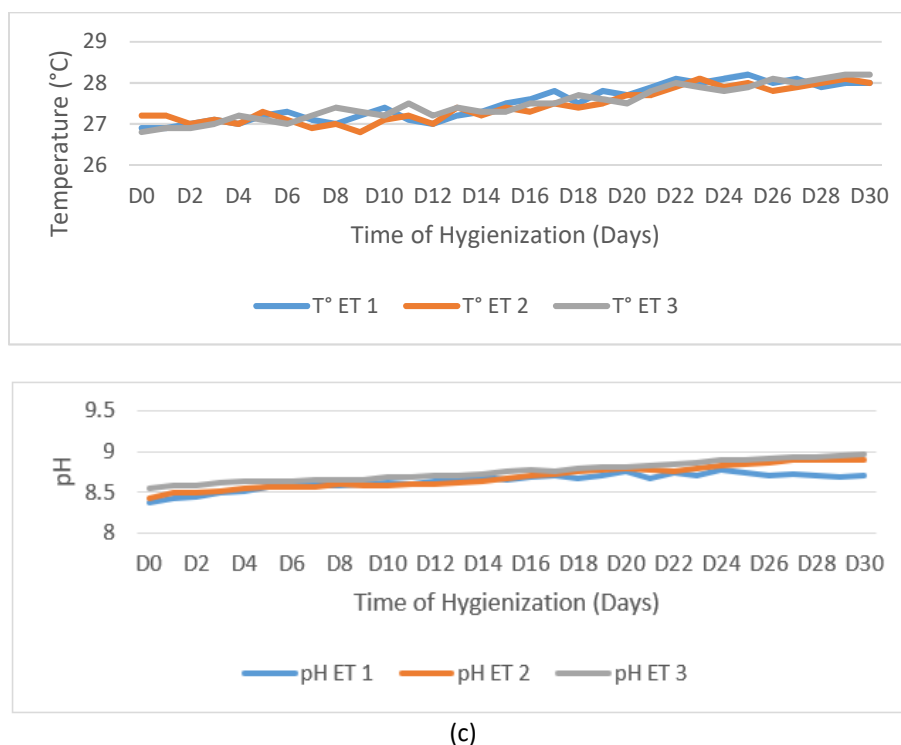


Fig. 3. Evolution of the pH, the temperature of the 16 samples and the 3 control samples of urine according to the time of hygienization

#### 4 DISCUSSION

This study made it possible to check the sanitary quality of the urine and feces, contaminated respectively by the eggs of *Schistosoma haematobium* and *Schistosoma mansoni* during the entire hygienization period. At day D0, at the beginning of the daily hygienization and time steps (D0, D15, D30 and D45), all the urine samples observed are contaminated by the eggs of *Schistosoma haematobium*. At D15, this contamination persists, for 38% of the samples with a parasite load varying between 7 and 17 eggs / 10ml. At day D30 and day D45, almost all the samples are no longer contaminated. At the same time, for the daily analyzes of the control samples, the absence of contamination is found on D14 for the sample of 13 eggs / 10ml, then on D19 with 84 eggs / 10ml and finally on D23 for 152 eggs / 10ml. However, with the control samples, we notice a strong increase of the parasite load in the first days of the hygienization (D0 to D2) then a sudden fall of this parasitic load with the time of hygienization. These results could be explained by the fact that before initiating their total disappearance, the eggs of *Schistosoma haematobium* would release the miracidia which would initiate a polyembryony, then not finding the favorable conditions to continue their cycle, they will begin to degrade until completely disappearing urine samples. In fact, according to [30] and [31], *Schistosoma haematobium* eggs can hatch in the urine and empty shells and free, mobile miracidia are found. However, the hatching of the embryonated egg at egg-laying, requires good conditions (fresh water, good oxygenation, T ° 26-30 ° C etc ...) and the rupture of the shell of the egg would be the result of the absorption of water, stimulated by light and heat [32], [33]. These results show that the absence of contamination or parasitic infestation is a function of the importance of the parasite load and the storage time of the urine samples. This is reflected in the significant correlations of time-laps hygienization (D0 to D45) and during daily hygienization between hygienization time and parasite load. The less contaminated samples (low egg quantities or low parasite load) quickly lose the eggs, unlike those whose contamination is more accentuated (high egg content and high parasite load). In fact, the urine samples with light infestations have 15 days of hygienization, against the urine samples with heavy infestations last 30 days. Thus, can we conclude that the maximum duration of hygienization regardless of the degree of infestation is 30 days. These results are consistent with those of [1], [2], [21] which show that 30 days are sufficient for the hygienization of urine.

The condition of *Schistosoma haematobium* eggs was observed during the daily and time steps analyzes. At day D0, 100% of the samples have intact and undamaged eggs but at day D15, 38% of the samples have their eggs completely degraded and 62% have no trace of schistosome eggs. At day D30 and day D45, all (100%) of the samples have a total absence of eggs. According to the time steps analyzes, the correlation is negative and significant between the state of the egg and the time of

hygienization, ( $r = -0.828$ ,  $r = 0.003$ ) but positive and significant between the state of the egg and parasite load ( $r = 0.745$ ,  $p = 0.026$ ). The analysis of the results of the control samples shows that the degradation time of the eggs increases from low to high levels of infestations and the correlations of the control samples confirm those of the analyzed samples at a time step. In fact, between the state of the egg and the time of hygienization, the correlation is negative and significant for the three control samples ( $r = -0.900$ ,  $p = 0.001$  /  $r = -0.916$ ,  $p = 0.001$  /  $r = -0.874$ ,  $p = 0.003$ ). With control sample 2 the correlation between egg status and parasite load was positive and significant ( $r = 0.855$ ,  $p = 0.016$ ). According to these results, the deterioration of the state of the eggs would be mainly a function of the decrease in the parasite load and the increase in the hygienization time of the urine samples. These results corroborate those of [34] which shows that the hygienization up to day D45 of storage makes it possible to obtain eggs of schistosomes more or less degraded and inactive that can remain in the urine.

In the case of *Schistosoma mansoni*, 100% of the samples are contaminated on D0, the eggs are intact, non-degraded, with low to moderate parasite infestation. From D15 to D45, all the samples (100%) are no longer contaminated, hence the total absence of *Schistosoma mansoni* eggs and parasitic infestation. In addition, from day 0 to day 45, the correlation between the parasite load and the hygienization time is negative and significant ( $r = -0.688$ ,  $p = 0.000$ ) but it is positive and significant between the parasite load and the state of the egg ( $r = 0.888$ ,  $p = 0.000$ ). We can conclude that the deterioration of the state of the egg is made as the parasite load decreases and the time of hygienization increases. These results could be due to a rise in temperature and pH in the faeces samples with the addition of ash for preservation. The reference [35] explains that ash is a PK fertilizer with micronutrients and a high pH that contribute to increase the pH effect of the product during storage. Also, according to [36] the reduction of pathogenic germs increases with the rise of the ambient temperature. In the end it is the temperature, the drying, the high pH of the ash and the duration which ensure the death of the germs and therefore the hygienization [2]. These results concerning the hygienization of the faeces are confirmed by [37] for which after the destruction of the pathogenic germs by the dehydration and / or the decomposition, the harmless matter which results from it can be spread on the ground to increase the contents of organic matter

With respect to physical parameters, time-laps hygienization shows that the correlation between pH and hygienization time is positive and significant ( $r = 0.844$ ,  $p = 0.000$ ). Correlations between temperature and hygienization time and temperature and parasite load were positive and significant ( $r = 0.452$ ,  $p = 0.000$  /  $r = 0.139$ ,  $p = 0.001$ ).

With daily hygienization, for control samples 2 and 3, the correlation between pH and hygienization time was positive and significant ( $r = 0.986$ ,  $p = 0.000$  /  $r = 0.993$ ,  $p = 0.000$ ). For control sample 2, the correlations between pH and parasite load and between pH and egg state are negative and significant ( $r = -0.916$ ,  $p = 0.039$  /  $r = -0.815$ ,  $p = 0.005$ ). The correlation between pH and parasite load was negative and significant for control sample 3 ( $r = -0.745$ ,  $p = 0.000$ ). Daily hygienization also shows that the correlation between temperature and hygienization time is positive and significant for control samples 1 and 2 ( $r = 0.929$ ,  $p = 0.000$  /  $r = 0.889$ ,  $p = 0.011$ ). For control sample 2, the correlation between temperature and parasite load was negative and significant ( $r = -0.651$ ,  $p = 0.008$ ). In conclusion of these biweekly and daily analyzes, the increase of the pH and the variation of the temperature influence the degradation of the egg and the decrease of the parasite load with the time of hygienization.

In the end, the correlation between the different parameters could explain the disappearance of the eggs in the urine samples. The pH of the medium is basic and increases, the temperature oscillates, the eggs are not in fresh water and lack of oxygenation, hence their degradation and disappearance with the duration of hygienization. However, the use of a multiparameter (Salinity, pH, Redox, Conductivity, Nitrates, Ammonium, Ammonia, dissolved Oxygen, Temperature) could further explain the phenomenon that occurs during the hygienization of urine. These results concerning parasite absence and storage time were studied by authors such as [38], [39] in temperate climates who indicated the inactivation of pathogens in the urine at around 6 months at 20° C. According to [40] urine also has ovicidal power and Ascaris eggs are killed in a few hours. Older studies of [41] have also indicated the inactivation of *Schistosoma haematobium* in urine. According to [17], temperature, dilution, pH, ammonia and time are the main determinants affecting the persistence of pathogens in the collected urine. Reference [21] also demonstrates that in urine, it is mainly temperature and a high pH combined with ammonia that have been recognized as influencing the inactivation of microorganisms. These results are almost identical to those of [26] for which the pH values increase and affect the urine hygienization time. The work of this author reveals that pH values are basic and promotes the elimination of fecal germs from urine. For the same author, the temperature evolves slightly between 31 ° C and 32 ° C and does not promote the destruction of fecal test germs test. Reference [34] also demonstrates that temperature and pH increase with time and favor the inactivation of *Schistosoma haematobium* eggs.

## 5 CONCLUSION

This study showed that the urine and faecal hygienization time depends on the importance of the parasite load but the maximum duration of hygienization does not exceed 30 days. The parameters involved in the degradation and disappearance of *Schistosoma haematobium* and *Schistosoma mansoni* eggs are pH, temperature and time of hygienization. Application of urine and faeces as a biofertilizer in agriculture in zones endemic for schistosomiasis is without health and environmental danger beyond 30 days of storage. Despite hygienization measures, the health impact of the use of urine and faeces must be verified on harvesting products.

## ACKNOWLEDGMENTS AND OTHER MENTIONS

**Funding:** This work has been achieved thanks to the financial and material support of the Intergovernmental Pan-African Agency Water and Sanitation for Africa through the Sanitation Component of the Millennium Water and Sanitation Program (PHAM) FED / 2012 / 024-147, National Schistosomiasis Control Program, Geo Helminthiasis and Lymphatic Filariasis and Félix Houphouët Boigny University of Abidjan (Côte d'Ivoire)

We express our sincere gratitude to all those involved in carrying out this work for their frank collaboration. These thanks are especially for:

- Dr. Djè Koffi Hyacinthe, Departmental Director of Health of Bouaflé,
- Dr. Kobenan Koffi Tchiré, Director of the Bouaflé Regional Hospital Center,
- Dr. Méité Abdoulaye, Director of the National Program for the Control of Schistosomiasis, Geo Helminthiasis and Lymphatic Filariasis
- Mr. Effoussou Ettièn Luc, Biologist Engineer, Head of the Central Laboratory of the Regional Hospital Center of Bouaflé,
- Mr. N'Doli Konan Honoré, State Graduate Nurse, Head of the Rural Health Center of Blanfla,
- Mr. Ouattara Zié, State Graduate Nurse, Head of Rural Health Center Diacohou North
- to the villagers of Blanfla and Diacohou Nord

**Conflict of interest:** the authors have no conflict of interest to declare.

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