

Article

Development of A Process For Domestic Wastewater Treatment Using *Moringa Oleifera* for Pathogens and Antibiotic Resistant Bacteria Inhibition under Tropical Conditions

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Abstract: Developing countries are confronted with general issues of municipal wastewater management and treatment. Untreated faecal sludges and wastewaters from septic tanks and traditional toilet are rejected into rivers and sometimes used for urban agriculture without any treatment to reduce the bio-risk. Consequently, there are potential environmental and public health risks. In this study, a wastewater treatment plant prototype coupled with *Moringa Oleifera* seeds treatment was developed to evaluate their effectiveness for the reduction of faecal indicator bacteria and antibiotic resistant bacteria in domestic wastewater. Results indicate that our performed prototype system presents high capacity to reduce bacteria with abatements up to 99.34%. High reductions of bacteria load were obtained after add of *Moringa Oleifera* seeds into waters, with reductions varied from 36.6-78.8% for *E. coli*, 28.3-84.6% for Faecal coliform, 35.3-95.6% for *Vibrio cholerae* and 32.1-92.4% for total flora. Same effects of *Moringa Oleifera* seeds were noted for reducing antibiotic resistant bacteria, Extended Spectrum Beta-lactamases and Carbapenem-Resistant Enterobacteriaceae with abatements up to 98% for *E. coli* and faecal coliform, 100% for *Vibrio cholerae* and 91.96% for total flora. Our results supported high capacity of *Moringa Oleifera* seeds as an excellent alternative for pathogens and antibiotics resistant bacteria reduction/purification from domestic wastewater.

Keywords: domestic wastewater; biological contamination; wastewater treatment plant; *Moringa oleifera*; antibiotic resistance

1. Introduction

Take up the challenges of access to safe drinking water and sanitation remains a global concern. The sustainable development goals establish a program to achieve access to adequate sanitation, eradicate open defecation, halving the proportion of untreated wastewater, increase recycling and safe reuse globally [1]. Sectors such as agriculture are turning to wastewater reuse as an alternative to problems related to water availability. However, the reuse of wastewaters presents risks of contamination of crops, soils, water resources, animals, consumers and agricultural workers, but also risks related to the expansion of resistance to antibiotics [2]. In this century, immediate solutions are required regarding resistance to antibiotics which is considered as the third largest threat to public health [3]. Faced with these concerns, several questions arise when it comes to strengthen wastewater treatment plants in sub-Saharan Africa and to optimise the reuse of liquid and solid waste from individual sanitation facilities in the agricultural sector.

Moringa Oleifera seeds (MOs) as well as other components of the moringa tree are increasingly used in water treatment process as naturel coagulant. Many literatures of the applications of *Moringa oleifera* seeds extract in water treatment indicate that MOs used as a coagulant in wastewaters treatment may be efficient enough to be considered as an alternative to chemicals coagulant use [4]. The most widely used coagulant is aluminium sulphate which presents some inconvenient as aluminium residues that are reported to cause neurodegenerative diseases [5]. In addition, *Moringa Oleifera* seeds are easily accessible for most developing countries and fully biodegradable. A number of researches have reported relevant results by using MOs to reduce faecal indicator bacteria (FIB) [6–8] and improve wastewater treatment using filtration methods [9–11]. It was demonstrated that the glucomoringin presents in MOs remove up to 99.99 % of total coliform [11]. Other compound of the *Moringa Oleifera* tree like leaves have also shown their effectiveness to reduce the antibiotics resistance acquired by bacteria [12].

In this study, we evaluated the capacity of a wastewater treatment pilot (WWTP) prototype to eliminate faecal contamination and antibiotics resistant bacteria from domestic wastewaters. According to the objective of treated wastewaters reuse for agriculture purposes, national and international standards need to be reached. If we consider the French regulation [13], it sets the concentration of *E. coli* in reused wastewater depending on the sanitary quality targeted. In fact, 4 levels of sanitary quality (A, B, C, D) of wastewater are defined in French regulations and requirements vary for each level. After treatment by the bacterial filter the quantity of *E. coli* in wastewaters attained an average of 270 CFU mL⁻¹ which refers to a sanitary quality level C (≤ 1000 CFU mL⁻¹). The level C allowed the reuse of treated wastewaters for nurseries and shrubs and other floral crops, cereal and fodder crops using localized irrigation. It also allowed cultivation of fruit trees only by drip. However, our objective remains to attain the level A which would allow the use of water for market gardening, fruit and vegetables crops not transformed by an adapted industrial heat treatment (except cress culture) and the maintenance of green spaces open to the public. In this context, we propose herein to evaluate the effectiveness of an additional treatment using MOs in order to reach a better sanitary quality of reused wastewaters

The evaluation is based on quantification of faecal indicator bacteria (FIB) including *E. coli*, *faecal coliform*, *faecal streptococcus*, total bacteria flora and *Vibrio cholerae*. The sensibility of bacteria to antibiotics is also tested by adding ampicillin on agar and using counting methods for Extended Spectrum Beta-lactamases (ESBL) and Carbapenem-Resistant Enterobacteriaceae (CRE). The sensibility of FIB, *Vibrio cholerae* and antibiotics resistant bacteria to MOs were tested by adding seeds powder in wastewater samples. To the best of our knowledge, despite the researches carried out on MOs for wastewater pollution removal, this is the first study on the impact of MOs on antibiotics resistant bacteria (ARB) under tropical conditions in a sub-Saharan African country.

2. Materials and methods

2.1. Conception of wastewater treatment plant prototype

The WWTP prototype was built in 2017 in Keur Moussa, located about fifty kilometers east of Dakar (figure 1). The WWTP (figure 2A) built onsite was sized according to French regulations for a house of 12 inhabitants [14]. It is composed by an entry tank (F0) with a useful volume equal to 3 m³, a bacterial filtration tank (F1) which contain 1.6 m³ of filtering porous material composed by basalt with diameters between 3 to 40 mm, and an infiltration well tank (F2) offering a contact area equal to 10 m².

Wastewaters coming from the house are conducted through a 110 mm PVC pipe. At the entrance of the tank there is a 50 cm high deflection wall (Figure 2B) to redirect the waters to the bottom. Another deflection wall was placed before the exit of the tank to regulate the outgoing flow. There is a difference of 15 cm height between the inlet pipe and the outlet pipes to prevent water from backing up to the house. Water coming from

the tank goes into the bacterial filter through 3 pipes (figure 2C), perforated at their bottoms. The pre-treated effluents are distributed on the bed of filtering materials made of basalt. There is a vertical percolation which is followed by a collection of waters at the bottom of the bacterial filter by 3 other pipes, perforated upwards. The lower pipes ensure the delivery of filtered water to the infiltration well over a second layer of basalt (figure 2D) after which there is the natural ground composed of sand. WWTP prototype does not work in continuous flow but rather in overflow. In fact, when the water depth inside the tank reaches 1.5 m, there is an evacuation of the supernatant which is dispersed into the natural ground after filtration.



Figure 1. Google Map indicating the location of the rural community of Keur Moussa (studied site) in Senegal.

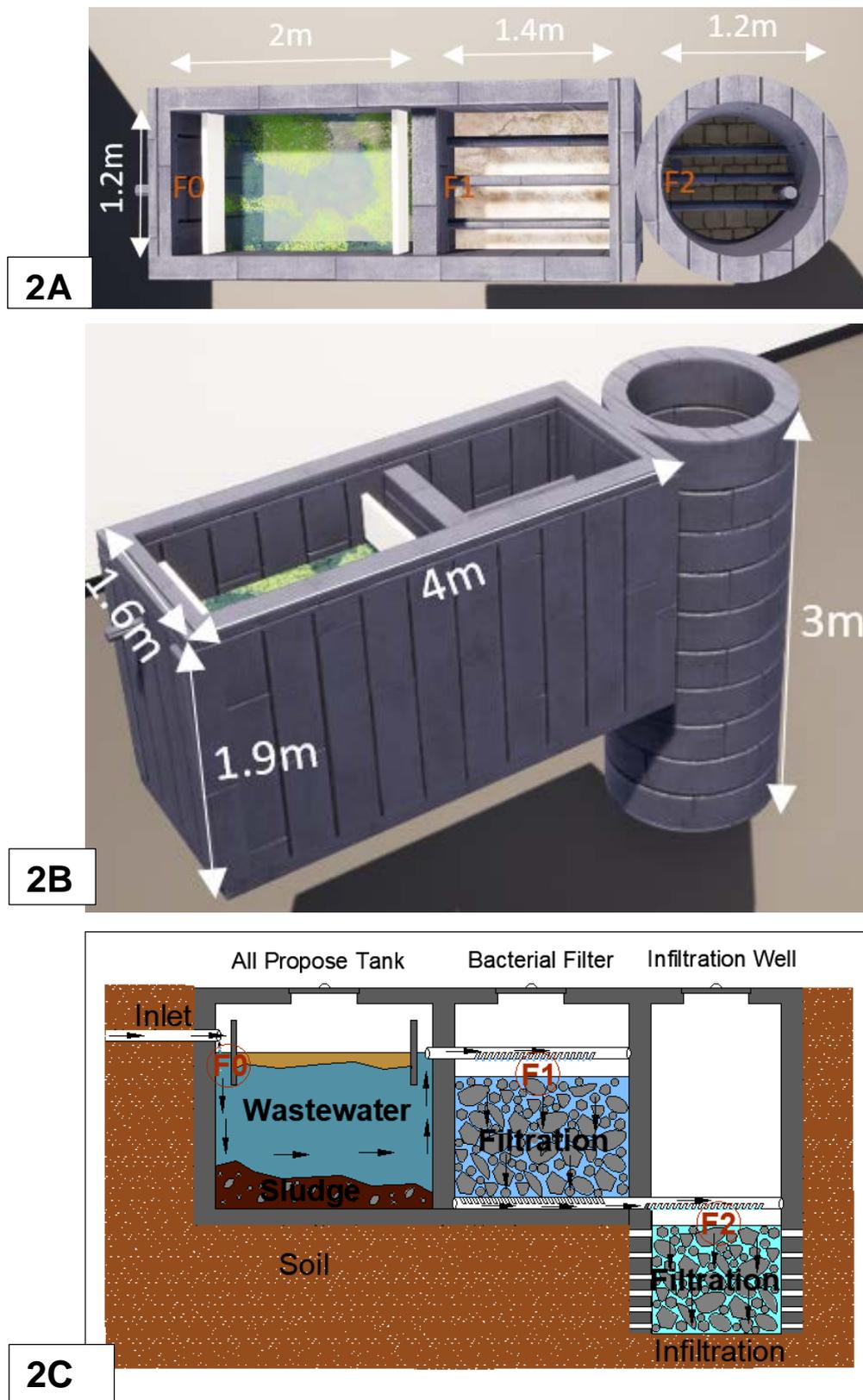


Figure 2. 3D dimensions of the Wastewater Treatment Plant (WWTP) Prototype. 2A: Top view of the WWTP prototype; 2B: Longitudinal view of the WWTP prototype; 2C: Operating outline of the WWTP prototype.

2.2. Sampling procedure

Wastewaters from the WWTP prototype were collected aseptically after homogenization in November 2020 with temperatures around 29 ± 1 °C. Wastewaters were collected at 3 points: entrance of the all-propose tank (F0), entrance of the bacterial filter (F1) and entrance of the infiltration well (F2) and then filled into 250 ml plastic bottles previously cleaned. After sampling, samples were stored in an icebox at 4 °C and transported to the Department F.-A. Forel of the University of Geneva for analysis within 48h.

2.3. Samples treatment with *Moringa Oleifera* seeds

Seeds of *M. oleifera* used in the study were purchased from a plantation in Gaaya, Saint Louis, Senegal. Dry *Moringa* seeds were first removed from their teguments and husk. White kernels were reduced in fine powder using a pestle and a mortar and sieved through a 1mm sieve. The powder was immediately used for downstream applications. Subsequently, we put in contact wastewaters with MOs on the basis of a protocol pre-established by Abdallah LY in 2016. Essays of 250 ml of wastewater F0, F1 and F2 were prepared in glass bottles. MOs powder was added in each assay to a final concentration of 300, 50 and 50 mg L⁻¹ for F0_{MOS}, F1_{MOS} and F2_{MOS} respectively. The incubation was set in 3 times: rapid stirring for 90 seconds to destabilize the colloids, slow stirring for 5 minutes to make micro-flocs, decantation for 2 hours 53 minutes and 30 seconds on a multi-position magnetic stirrer. The supernatant was centrifuged at 4000 rpm for 10 minutes for downstream applications.

2.4. Microbial analysis of water samples

The microbial analysis was performed to evaluate the effectiveness of WWTP prototype for bacterial removal and the impact of *Moringa Oleifera* seeds addition in the process. *Escherichia coli*, Faecal coliforms, *Enterococcus spp.*, *Vibrio cholerae* and Total Heterotrophic bacteria were quantified using the plate pouring method as described by Rodier and al [15]. Microbiological selective media included tryptone bile x glucuronide (TBX) for the quantification of *Escherichia coli*, violet red bile lactose agar (VRBL) for the quantification of faecal coliform, Bile Esculin sodium Azide (BEA) for the quantification of *Enterococcus spp.*, Reasoner's 2A Agar (R2A) for the estimation of the total heterotrophic flora and Thi-sulfate-Citrate-Bile-Saccharose (TCBS) for the quantification of *Vibrio spp.* Samples of wastewater were serially diluted in sterile saline water (0.9 % NaCl). 1 mL of the dilution was placed in the center of a sterile Petri dish plate. Molten cooled selective agar was then poured into the Petri dish and mixed well. After solidification, plates were inverted and incubated. Temperature and time of incubation for each medium are described in Table 1. After incubation, colonies were counted, and results were reported as UFC mL⁻¹ considering the dilution factor. The reproducibility of the experimental procedure was estimated by means of triplicates on samples.

Table 1. Characteristics of nutritive agars used.

Marker	Nutritive Agar	Brand	Reference	LOT	Incubation
<i>E. coli</i>	TBX	Biolife Italiana	402 1562	ML 5507	18 to 24 h at 44 °C
Faecal coliform	VRBL	Biolife Italiana	402 1852	LD7103	18 to 24 h at 44 °C
Faecal streptococcus	BEA	Biolife Italiana	401 0182	FP5303	24 h at 44 °C
<i>Vibrio cholerae</i>	TCBS	Oxoid Ltd	190 0511	CM0333	24 h at 37 °C
Total flora	R2A	Biolife Italiana	401 9962	MC2801	5 days at room T°

TBX: tryptone bile x glucuronide; VRBL: violet red bile lactose agar; BEA: bile esculin sodium azide; TCBS: thiosulfate citrate bile saccharose; R2A: reasoner's 2A Agar.

Antibiotics resistant bacteria (antibiotics resistant *E. coli*, antibiotic resistant Faecal coliform, antibiotic resistant Enterococcus spp, antibiotics resistant *Vibrio cholerae*, antibiotics resistant Total Heterotrophic Flora) were quantified by supplementing selective media with ampicillin (32 mg/L for Enterobacteriaceae and total heterotrophic flora; 16 mg/L for *Enterococcus spp.*), according to CLSI breakpoints (Clinical and Laboratory Standards Institute) M100 sheet [16]. Extended-spectra- β -lactam and carbapenem resistant bacteria were isolated using Chromagar™ ESBL and mSuperCARBA media (Chromagar, Paris, France)

2.5. Data Analysis

Data analysis was performed using Excel software. The WWTP efficiency was estimated considering the abatement rate between the entrance and the exit of the wastewater treatment plant in order to evaluate the performance of the system. Impact of MOs on pollution removal was estimated by abatement rate with and without additional *Moringa* treatment. Statistical analysis was performed using R software (version 4.0.4) and Rcmdr package after log10 reduction. Statistical significance was defined by 95% confidence intervals ($p < 0.05$).

3. Results and discussion

3.1. Abatement of bacteria in WWTP prototype

The purification potential of WWTP prototype was determined by quantifying 5 bacterial markers (*E. coli*, faecal coliform, Enterococcus spp, *V. cholerae* and total heterotrophic flora in water samples before at each step of the treatment process (Table 1). For example, in the tank F0, the average concentration of bacterial markers was $1.97 \times 10^4 \pm 2.52 \times 10^3$, $2.13 \times 10^4 \pm 1.59 \times 10^4$, $3.30 \times 10^3 \pm 4.58 \times 10^2$, $2.63 \times 10^7 \pm 4.35 \times 10^6$ CFU mL⁻¹ for *E. coli*, faecal coliform, *V. cholerae* and total heterotrophic flora (Table 2). However, the abundance of Enterococcus spp bacteria were under the limit of quantification for all samples. After decantation (F1), *E. coli* and total heterotrophic flora abundances increased by 2.5 and 6 times, respectively while faecal coliform and *V. cholerae* abundances decreased by 27.2% and 59.0%, respectively. At a global level, based on the observation between F0 and F1 concerning the increase of total flora load and *E. coli*, the entering tank could be assimilated as a bacteria concentrator due to the environment (temperature between 28.9 °C and 30.10 °C; presence of organic matters) which is favourable to the proliferation of bacteria. Other studies highlighted the growth of *E. coli* inside septic tank as mentioned by Appling and al who reported an increase of *E. coli* by 100-fold into septic tanks under Georgian summer weather with temperatures around 30 °C [17]. Filtration step allow to decrease bacterial abundances from 98.1 to 99.4% between the entrance of the bacterial filter (F1) and the entrance of the infiltration well (F2). ANOVA test showed a significant difference (ANOVA 2, $p < 0.01$) in bacterial abundance after the combined effect of decantation (in the tank) and filtration (in the bacterial filter) which allowed a bacterial purification from 89.9-98.6% for FIB and up to 99.3 % for *Vibrio cholerae*. The number of obtained *Vibrio cholerae* strains was low in comparison with counting number obtained for FIB strains, that could explain the high abatement rate obtained for *Vibrio cholerae*. Consequently, the experimentation with more strains of *Vibrio cholerae* is recommended.

Table 2. Abundance of bacteria and antibiotics resistant bacteria in wastewater.

Samples	<i>E. coli</i>		Faecal coliform		<i>Vibrio cholerae</i>		Total flora	
	CFU mL ⁻¹	SD	CFU mL ⁻¹	SD	CFU mL ⁻¹	SD	CFU mL ⁻¹	SD
F0	1,97E+04	2,52E+03	2,13E+04	1,59E+04	3,30E+03	4,58E+02	2,63E+07	4,35E+06
F1	5,00E+04	1,00E+04	1,55E+04	1,17E+03	1,35E+03	3,49E+02	1,58E+08	4,05E+07
F2	2,73E+02	3,79E+01	2,97E+02	2,52E+01	2,17E+01	4,93E+00	2,65E+06	1,37E+05

Samples	AREC		ARFC		ARVC		ARTF	
	CFU mL ⁻¹	SD						
F0 _{MOS}	5,47E+03	2,08E+02	3,77E+03	6,66E+02	1,12E+03	5,36E+02	7,00E+06	1,48E+06
F1 _{MOS}	1,06E+04	7,21E+02	1,11E+04	2,54E+03	5,90E+01	2,21E+01	1,20E+07	3,17E+06
F2 _{MOS}	1,73E+02	3,51E+01	4,57E+01	9,02E+00	1,40E+01	6,24E+00	1,80E+06	3,46E+05
F0	1,49E+04	2,16E+03	1,41E+04	2,32E+03	4,30E+02	1,23E+02	1,92E+06	1,18E+07
F1	1,25E+04	5,03E+02	1,30E+04	8,89E+03	1,47E+01	9,02E+00	1,80E+07	2,00E+07
F2	2,03E+02	7,51E+01	1,70E+02	2,65E+01	4,67E+00	4,04E+00	6,83E+05	1,21E+06
F0 _{MOS}	1,23E+03	5,77E+01	1,10E+03	1,00E+02	2,20E+02	1,11E+02	1,34E+06	7,21E+06
F1 _{MOS}	3,23E+03	8,33E+02	2,67E+02	8,02E+01	1,33E+01	5,77E+00	1,45E+06	5,84E+06
F2 _{MOS}	6,00E+00	3,46E+00	1,03E+01	2,31E+00	0,00E+00	0,00E+00	6,40E+05	1,31E+06

SD: Standard deviation; AREC: antibiotics resistant *E. coli*; ARFC: antibiotics resistant faecal coliform; ARVC: antibiotics resistant *Vibrio cholerae*; ARTF: antibiotics resistant total heterotrophic flora.

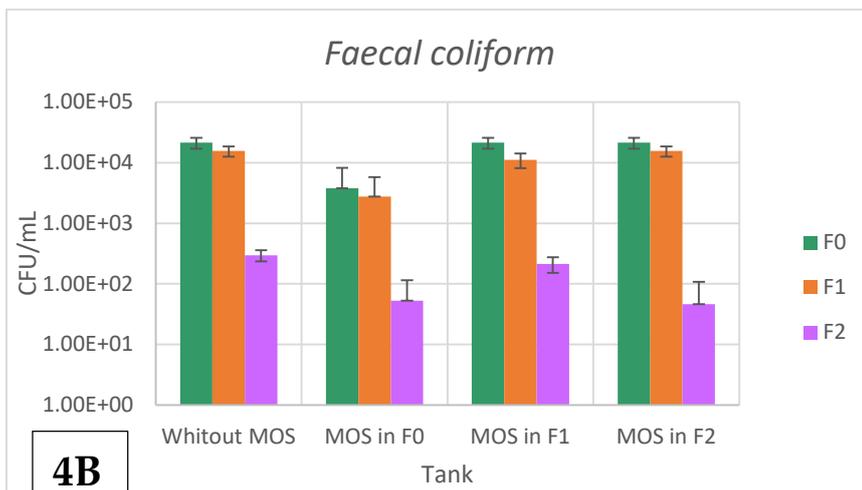
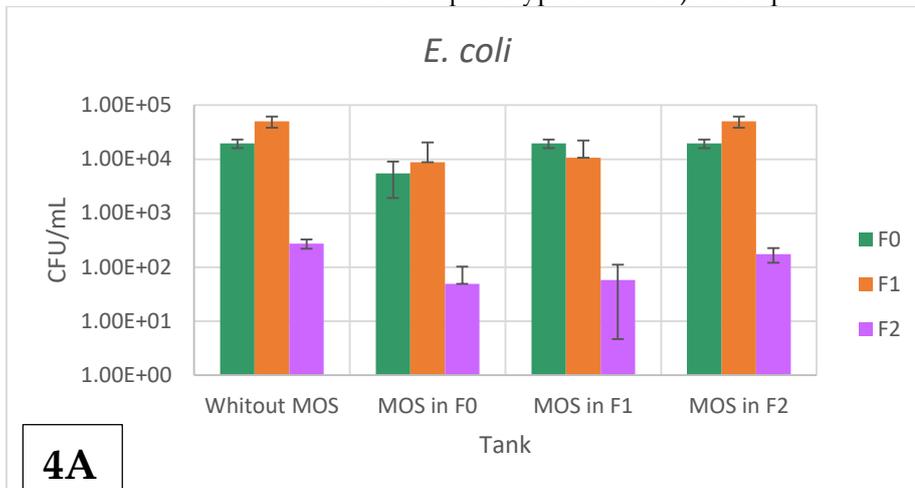
3.2. Impact of *Moringa oleifera* seeds on bacteria abatement

Laboratory experiment was conducted to determine the impact of MOs as natural coagulant during the wastewater treatment process. Results of bacterial abundance in wastewaters (F0_{MOS}, F1_{MOS}, F2_{MOS}) are presented in table 2. The abatements obtained after MOs addition into wastewaters vary significantly according to samples and type of bacteria (ANOVA, $p < 0.01$) (figure 3). The abatements varied from 36.6% to 78.8% for *E. coli*, from 28.3% to 84.6% for faecal coliform, from 35.3% to 95.6% for *Vibrio cholerae*, and from 32.1% to 92.4% for total heterotrophic flora. The highest abatements rates were obtained in samples F1 (at the entrance of the bacterial filter) for *E. coli*, *Vibrio cholerae* and total flora, while for faecal coliform it was obtained in F2.

Our results are found similar to those obtain by Vunain and al. [6] with reduction of 96.6 % in bacteria load, 60.5% for coliform and 97.3% for *E. coli* after using a concentration of 15 g L⁻¹ of MOs and 2 hours of contact time. Lower abatement rate (47 %) for *E. coli* reduction were obtained by Poumaye and al [9] after using a MOs solution stirred into distilled water before to be added in the wastewater, while Delelegn and al [18] obtained greater abatement rate (97 %) using the same method with higher concentration of *Moringa* seed powder. Considering the organic matter contained in MOs which can promote bacterial growth, few studies recommends to complete the MO treatment with a filtration step using sand filter [9,10].

According to the abatement rates obtained with MOs addition, in figure 4 a projection is proposed which gives an overview of the effect that MOs would have if added on-site directly into the tank (F0), into the bacterial filter (F1) or into the infiltration well (F2). The projection was made on the basis of the reductions obtained between F0, F1 and F2 and the reductions obtained after the addition of MOs into samples. The best combination for *E. coli* reduction consists in adding MOs in F0. Adding MOs in the tank (F0) would reduce the quantity of *E. coli* from $1.97 \times 10^4 \pm 2.52 \times 10^3$ CFU. mL⁻¹ to $5.47 \times 10^3 \pm 2.08 \times 10^2$ CFU mL⁻¹. Based on the increase obtained between F0 and F1 (2.5 times) and the reduction effect between F1 and F2 (-99.4%), we could end up with a quantity of *E. coli* of 49 CFU mL⁻¹. *E. coli* abundance would therefore refer to the level B (≤ 100 CFU mL⁻¹) defined by French regulation for wastewater reuse. Level B allowed the use of treated wastewater (with some restrictions) for agricultural proposes as market gardening, fruit and vegetable crops transformed by suitable industrial heat treatment, floral crops, cereal and fodder crops, pasture, etc. However, concerning the other markers studied, the best combinations for bacteria reduction obtained after projection remains add of MOs in F2 for faecal coliform, in F1 for *Vibrio cholerae* and in F1 for total flora. Considering these observations varying

from one marker to another, additional analyses after addition of MOs on-site, would therefore be necessary in order to decide on the best combination of treatment (using our WWTP prototype and MOs) to adopt.



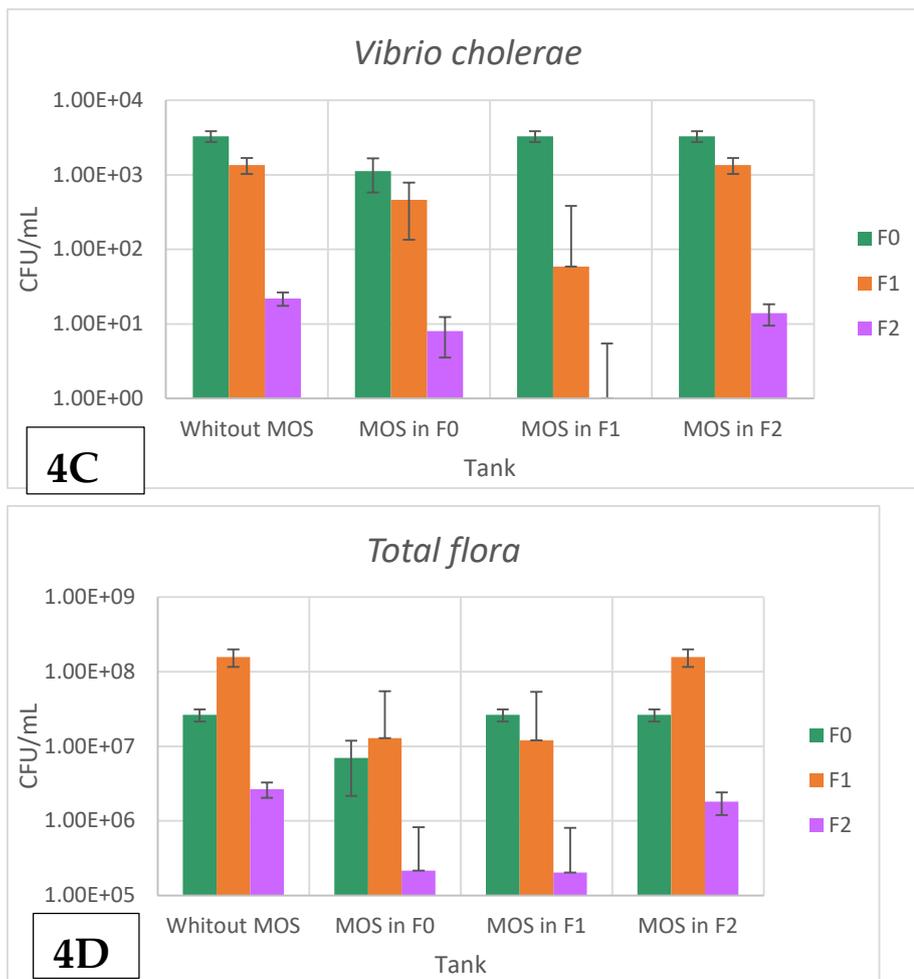


Figure 4. Projection of MOS's effect on the entire treatment plant prototype after MOS addition in F0, F1, F2; 4A: abundance of *E. coli*; 4B: abundance of Faecal coliform; 4C: abundance of *Vibrio cholerae*; 4D: abundance of Total flora.

3.3. Prevalence and abatement of antibiotic resistant bacteria in WWTP prototype

Prevalence of antibiotic resistance bacteria (ARB) was assessed in our WWTP prototype. Results are presented in table 2. The abundance of antibiotic resistant bacteria varies from $4.30 \times 10^2 \pm 1.23 \times 10^2$ to $1.92 \times 10^6 \pm 1.18 \times 10^5$ CFU mL⁻¹ in F0, from $1.47 \times 10^1 \pm 9.02$ to $1.80 \times 10^7 \pm 2 \times 10^5$ CFU mL⁻¹ in F1 and from 4.67 ± 4.04 to $6.83 \times 10^5 \pm 1.21 \times 10^5$ CFU mL⁻¹ in F2. At the entrance of the tank (F0) resistant isolates represented 75.9%, 65.9%, 13%, 7.3% of *E. coli*, faecal coliform, *Vibrio cholerae* and total heterotrophic flora respectively. At the entrance of the bacterial filter (F1) resistant isolates represented 25.1%, 83.7%, 1.1%, 11.4% of *E. coli*, Faecal coliform, *Vibrio cholerae* and total flora, respectively. At the entrance of the infiltration well (F2) resistant isolates represented 75.39%, 57.3%, 21.5%, 25.8% of *E. coli*, Faecal coliform, *Vibrio cholerae* and total flora respectively. Summerlin and al. have recorded 81% of *E. coli* resistant to ampicillin among a total of 140 isolates and this high percentage is in line with the results of this study [19]. From a global level the ratio of antibiotics resistant bacteria represented 7.38% of the total load in F0, 11.43% in F1 and 25.79% in F2. An increase in the percentage of resistant bacteria is observed from F0 to F2 therefore highlighting antibiotics resistance genes (ARG) proliferation in tank microbiome. Similar observation has been published since decades in WWTP. Mao and al. [20]

analysed the abundance of 23 ARGs along WWTPs process and highlighted ARGs enrichments ratio from 8 to 268. Summerlin et al. noticed an increase of ciprofloxacin resistant strains prevalence throughout treatment process. Wastewaters are reported in other studies to be a source of antibiotic resistance and WWTP to contribute to the prevalence of anti-microbial resistance, antibiotics resistance bacteria and antibiotics resistance genes [21,22]. Despite the antibiotic resistance enrichment throughout the treatment process, our WWTP prototype help to decrease significantly antibiotic resistant bacteria abundance by 98.6% for *E. coli*, 98.8% for faecal coliform, 98.9% for *Vibrio cholerae*, and 64.35% for total heterotrophic flora (ANOVA, $p < 0.01$).

Resistance to antibiotics was further investigated for ESBL (extending-spectra- β -lactamase) and carbapenem resistance in Enterobacteriaceae. In F0, the abundance of *E. coli* and KESC group (e.g. *Klebsiella*, *Enterobacter*, *Serratia* and *Citrobacter*) resistant to beta-lactam were 279 ± 47 and 3851 ± 315 CFU mL⁻¹ respectively while, the abundance of *E. coli* and KESC resistant to carbapenem were 13 ± 8 and 1714 ± 157 CFU mL⁻¹. Resistance to antibiotics were lower in F1 with an abundance of 14 ± 0 and 101 ± 28 CFU mL⁻¹ for *E. coli* and KESC resistant to beta-lactam while the abundance of *E. coli* and KESC resistant to carbapenem were 1 ± 0 and 384 ± 29 CFU mL⁻¹. No resistance to beta-lactam or carbapenem were detected in F2. ESBL *E. coli* (ESBLEC) prevalence of 1.4% was 10 times higher than data published by Bréchet and al. [23]. However, our WWTP prototype deserve 12 inhabitants and ESBLEC prevalence in the WWTP prototype cannot be representative of the prevalence of ESBLEC in the population.

3.4. Effect of *Moringa oleifera* seeds on antibiotic resistant bacteria abatement

Moringa oleifera seed powder was used as natural coagulant in F0, F1 and F2 to evaluate its effect on bacteria load but also its effect on antibiotic resistant bacteria. Measured abatements after MOs treatment in samples varied from one marker to another. The reductions of antibiotic resistant bacteria in F0 were on average 91.7% for antibiotics resistant *E. coli* (AREC), 92.2% for antibiotics resistant faecal coliform (ARFC), 48.8 % for antibiotics resistant *Vibrio cholerae* (ARVC) and 30.09% for antibiotics resistant total heterotrophic flora (ARTF). In F1, reductions of 74.2%, 98%, 9.1% and 92% were obtained respectively for AREC, ARFC, ARVC and ARTF. Variable abatements rate was also noted in F2 with reduction of 97% for AREC, 93.9% for ARFC, 100% for ARVC and 6.3% for ARTF. Figure 5 shows the impact of MOs addition in wastewater on antibiotic resistant bacteria removal. The effectiveness of moringa treatment on ARB removal varied according the bacterial species and the step of wastewater purification (ANOVA, $p < 0.01$). For AREC, MOs addition was the most effective in F2 with a 1.53 log reduction, whereas for ARFC and ARTF, the addition was most effective in F1 with a respective 1.68 and 1.09 log reduction. The effectiveness of MOs addition on ARVC was relatively low (0.04-0.29 log reduction), probably due to the low prevalence of *V. cholerae* in the system. Based on these reductions and the abatement calculated between F0-F1 and F1-F2, projections were made and are given in figure 6 with the aim to determine in which tank the addition of MOs will be more effective for the improvement of the entire treatment process. Projections show that adding MOs at the entrance of infiltration well is more effective for AREC and ARTF, while for ARFC and ARVC the best combination is the addition of MOs in F1 (after decantation in the tank).

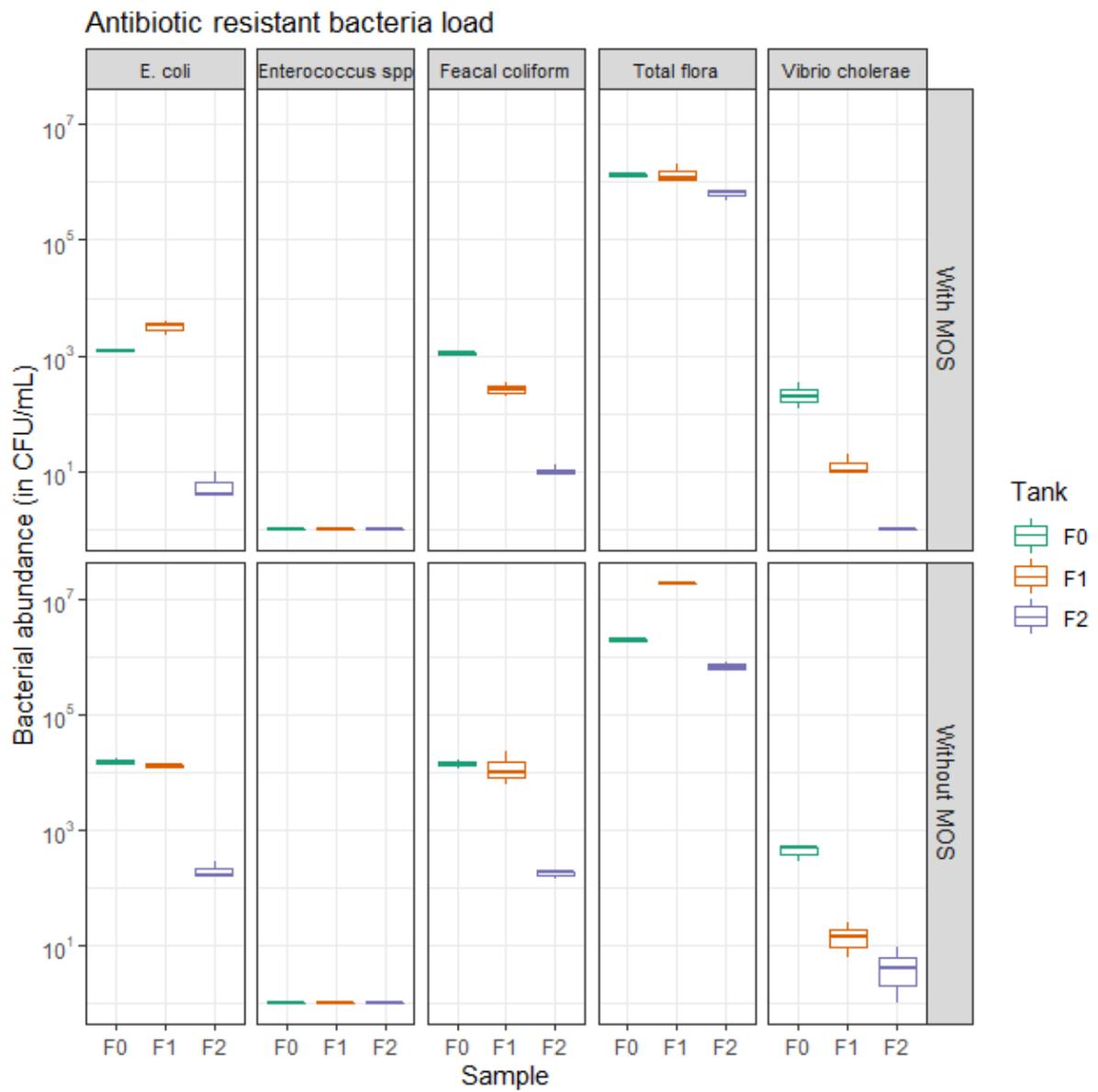
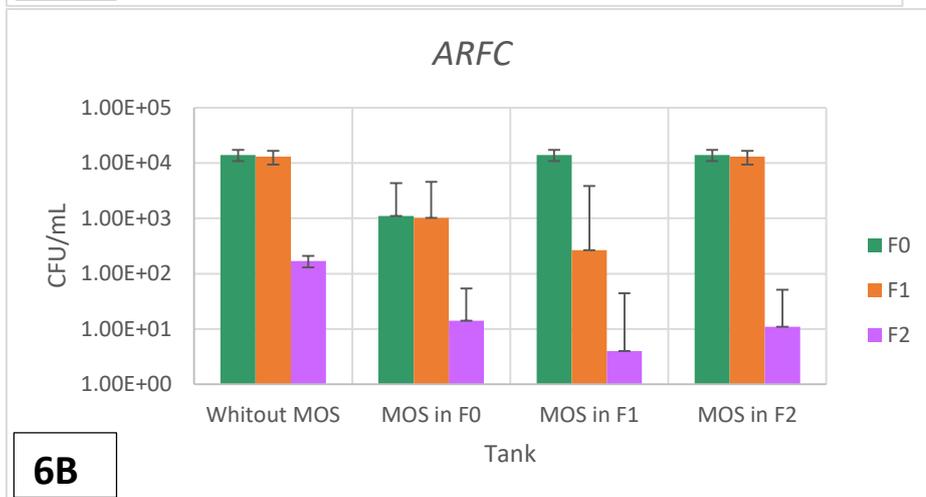
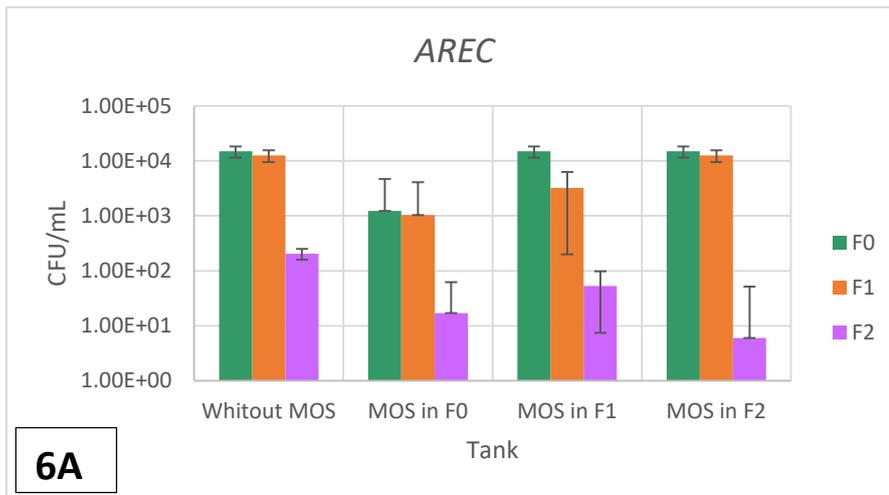


Figure 5. Abundances of antibiotic resistant bacteria in samples taken at the entrance of each tank for evaluation of abatement; counted number of bacteria represented as log 10 reduction



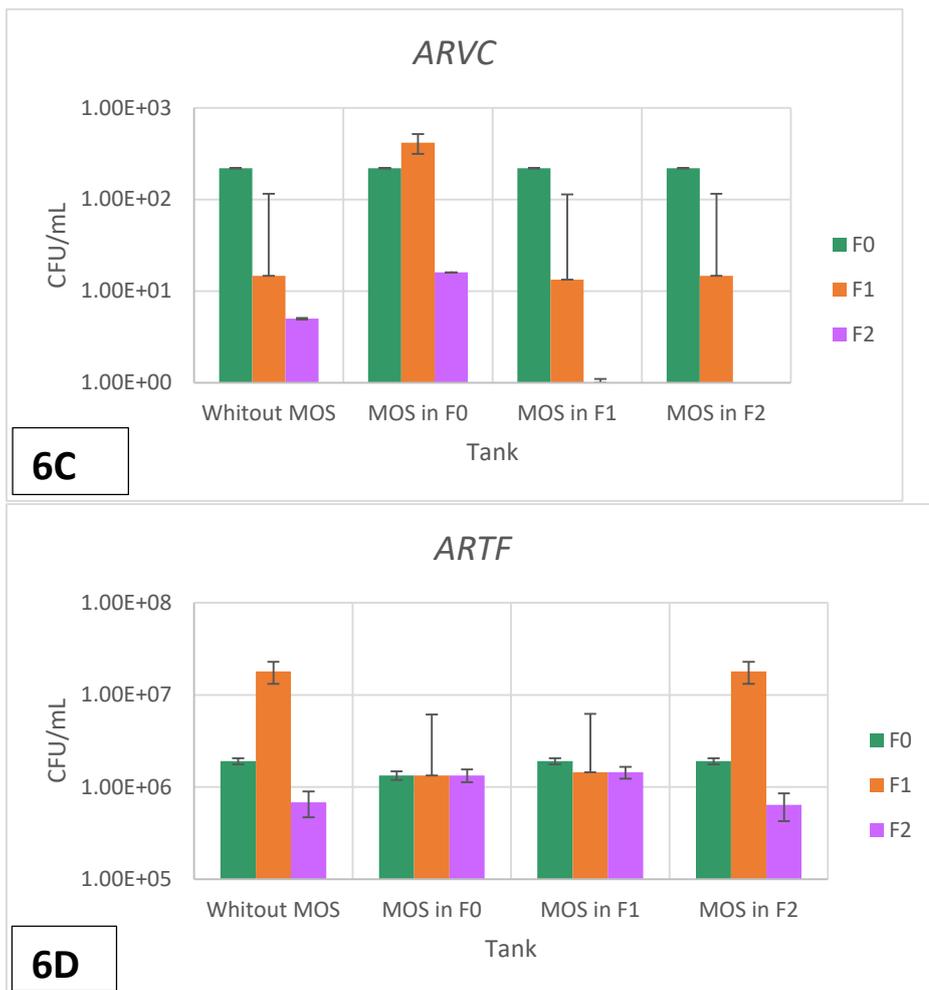


Figure 6. Projection of *Moringa Oleifera* seeds (MOs)'s effect on the entire treatment plant prototype after MOs addition in F0, F1, F2; 6A: abundance of antibiotics resistant *E. coli* (AREC); 6B: abundance of antibiotics resistant faecal coliform (ARFC); 6C: abundance of antibiotics resistant *Vibrio cholerae* (ARVC); 6D: abundance of antibiotics resistant total heterotrophic flora (ARTF).

Additional observation was made on ESBL and carbapenem resistant bacteria. After incubation, abundances of ESBLEC and CR *E. coli* were 7 ± 0 and 1 ± 0 CFU mL⁻¹ in F0_{MOS}; 7 ± 0 and 1 ± 0 CFU mL⁻¹ in F1_{MOS} and not detected in F2_{MOS}. The abundance of ESBL and carbapenem resistance in the KESC group was 69 ± 19 and 440 ± 28 CFU mL⁻¹ in F0_{MOS}; 34 ± 0 and 7 ± 0 CFU mL⁻¹ in F1_{MOS} and not detected in F2_{MOS}. The combined effect of the WWTP prototype with the use of MOs as a natural coagulant in F2 helped to reduce 100 % of resistant KESC and *E. coli* in the WWTP prototype.

Additional studies on the effect of MOs on antibiotic resistance genes (ARG) need to be carried out because the elimination of resistant bacteria does not necessarily include the elimination of ARG, which implies health risks if we consider a possible reuse of treated wastewater for irrigation. In a study conducted in 2013, Fahrenfeld and al. suggests that recycled water maybe an important reservoir of ARGs and ARG amplification is often detected during the distribution process while using wastewater for irrigation. They also demonstrated that chlorination step did not have any impact on ARGs elimination after wastewater treatment [24].

4. Conclusion

The aim of this study was to evaluate the effectiveness of well managed wastewater treatment plant on reducing bacterial pollution and antibiotic resistant bacteria but also to assess the combine effect of the treatment plant with *Moringa Oleifera* seeds use as a natural coagulant. After treatment by the WWTP prototype, the abundance of bacteria has decreased about percentage from 89.9 to 98.6% for FIB and up to 99.3% for *Vibrio cholerae* while reduction from about 64.35% to 98.8% were noted for antibiotics resistant FIB and up to 98.9% for antibiotics resistant *Vibrio cholerae*. Therefore, the counting number of *Enterococcus* spp and antibiotics resistant *Enterococcus* spp were under the limit of quantification. The results indicate the strong efficiency of our WWTP prototype to reduce microbiological load contained in domestic wastewaters. Similar results were obtained by Eregno and Heistad [25], with a reduction of around 93 % and 91 % of total coliform and *E. coli* by onsite greywater treatment plant. An additional treatment with stratified filter medias compound by Filtralite, fine sand, and till soil were applied for a reduction of 3–4 log₁₀ of remaining total coliforms and *E. coli*. At a global level our WWTP prototype allowed a reduction of 90% of total bacteria load which is higher than the reduction obtained by Jałowiecki and al. after using onsite wastewater treatment plant compound by 2 septic tanks and a compact filter [26]. After the addition of MOs into samples, the abatements of bacteria load varied from about 28.3% to 92.4% for FIB and 35.3% to 95.6% for *Vibrio cholerae* while the percentages varied from 6.34% to 97.95% for antibiotics resistant FIB and from 9.1% to 100 % for antibiotics resistant *Vibrio cholerae*. The results highlight here the positive impact of MOs coagulation during domestic wastewater treatment processing. MOs are effective in reducing antibiotic resistant bacteria and faecal indicator bacteria despite the persistence and enrichment of ARB after wastewater treatment. *Moringa oleifera* seeds are traditional used for their antibacterial properties, others studies recommend its exploitation by pharmaceutical industries as an antibacterial treatment that can also decrease resistance to antibiotics [27,28]. The efficiency of the WWTP prototype could be improved by an onsite addition of MOs either at the entrance of bacterial filter (F1) or at the entrance of infiltration well (F2). It would be also important to identify the mechanisms which made possible the abatements such as flocculation observed by additional studies, and an insight into on the components and chemical properties of the materials used in this study. Author's recommendations are to highlight, the effectiveness of affordable unitary WWTP and its possible deployment for African developing countries, the benefits of using *Moringa Oleifera* seeds as an alternative to chemical coagulants for wastewater treatment and the prevalence of antibiotics resistant bacteria in WWTP.

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