

## Determining the limits of flocculation of fecal germs with *Moringa oleifera* seed powder used in the treatment of drinking water in Wawata-Abomey-Calavi (Benin)

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### ABSTRACT

Populations of Wawata in Benin like a large part of those in countries located in the south of the Sahara do not have significant financial resources. For them, the use of appropriate natural methods of disinfection of drinking water at a lower cost constitutes a challenge to be taken up in their rural areas. This study aims to improve the quality of drinking water in these localities. The physicochemical and microbiological characteristics of raw water samples, taken from some traditional wells and from a modern borehole (control) were determined according to standard methods as well as those of water treated with *Moringa oleifera* seed powder. Regarding the results obtained, only the nitrate ion contents exceed the World Health Organization guidelines for certain samples of the raw well water. On the other hand, microbiological analyzes indicate the presence of fecal contamination germs in these waters. They also reveal that the treatment of water samples with 1.2 g/L of *M. oleifera* seed powder eliminated approximately 96% of *total coliforms*, 100% of *Staphylococci* spp., and *thermotolerant coliforms*.

**Key words:** Traditional wells, Fecal contamination, Waterborne diseases, Wawata, Benin.

### 1. INTRODUCTION

Water is a vital good, essential for life. It can also be a source of disease. Because of its involvement in many cases of human disease and environmental pollution, its quality is increasingly monitored. The consumption of unclean water is one of the causes of morbidity and mortality in the world. Indeed, 5 million deaths were attributable to waterborne diseases such as cholera, typhoid fever and diarrhea [1]. If having drinking water poses practically no major problems in Western countries, this constitutes a crucial problem that handicaps the sociocultural and economic development of many African countries. The National Water Company of Benin, in charge of the distribution of drinking water to the population, is unable to cover their drinking water demand. In addition, the performance of this public service is currently insufficient in rural areas of the country and the low rate of installation of drinking water supply structures leads people to resort to sources such as traditional wells, cisterns, and private water points. These water sources often do not meet the country's drinking water quality standards and expose people to the risk of waterborne diseases. Faced with this situation, the populations of certain localities use groundwater as drinking water through traditional wells without realizing the great dangers to which they are exposed. The present study aims to assess the effectiveness of *Moringa oleifera* seed powder in the treatment of the Wawata's wells water in the municipality of Abomey-Calavi in Benin. Indeed, the seeds or cakes of *M. oleifera*, by means of the basic polypeptide flocculant which is a set of cationic polyelectrolytes, are capable of neutralizing the colloids of turbid waters thanks to the formation of bridges [2].

### 2. MATERIALS AND METHODS

#### 2.1. Description of the Site

Wawata is located in Zinvie (Abomey-Calavi/Benin). Its population is around 18200 inhabitants and its area is 5107 ha. Figure 1 presents the study area and the sampling sites.

#### 2.2. Sampling

Water samples were collected in glass bottles. Green flacons have been used for those intended for physical and chemical analyses to prevent the invasion of solar rays. Before sampling, they were rinsed three times with the sampled water. The bottles were sterilized and closed with similarly sterilized aluminum foil for water samples for microbiological testing. The bottles were ballasted outside with a counter-mass, using a similarly sterilized rope and were filled 4/5 to allow the microorganisms to breathe. All of the samples were stored in coolers at approximately 4°C for

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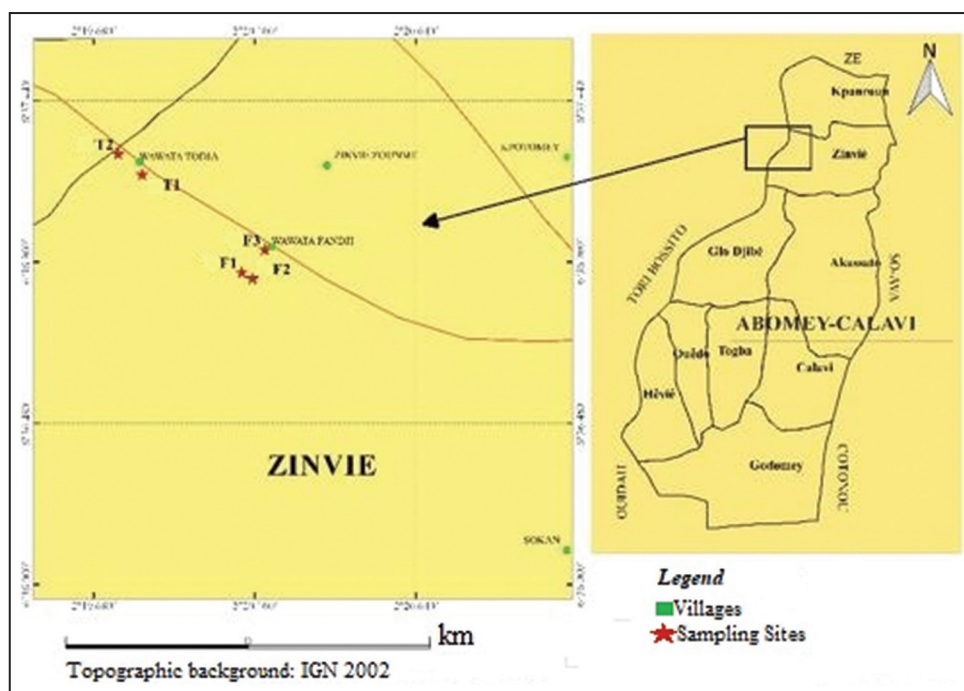


Figure 1: Sampling sites.

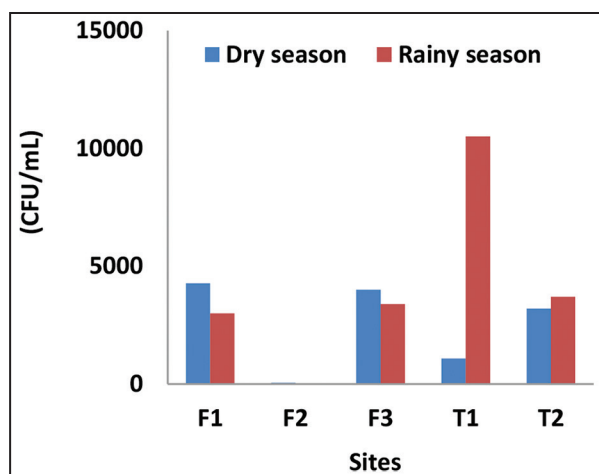


Figure 2: Variations of the common germs rates in raw water.

transfer to the laboratory. The sampling was carried out during both the rainy and dry seasons.

### 2.3. Analytical Methods

The dosage of nitrite ions was carried out by the diazotization method in drinking water (Method 8039\_DR 2800), that of nitrate ions by the cadmium reduction method (method 8039\_DR2800), and that of ammonium ions by the Nesler method. The measurements of lead and cadmium contents in water were carried out in basic solution, by reaction, respectively between lead ions and cadmium ions, with Dithizone to form a Lead-Dithizone complex and a Cd-Dithizone complex, both specific color (red to pink) but obtained differently. Chloroform was used to extract the complex and it was assayed by colorimetry on the molecular absorption spectrophotometer at 515 nm. Copper was determined by method 8506\_DR2800 using Bicinchoninate [3].

Standard methods such as filtration, dilution, seeding, and enumeration were used to measure the microbiological parameters. Common

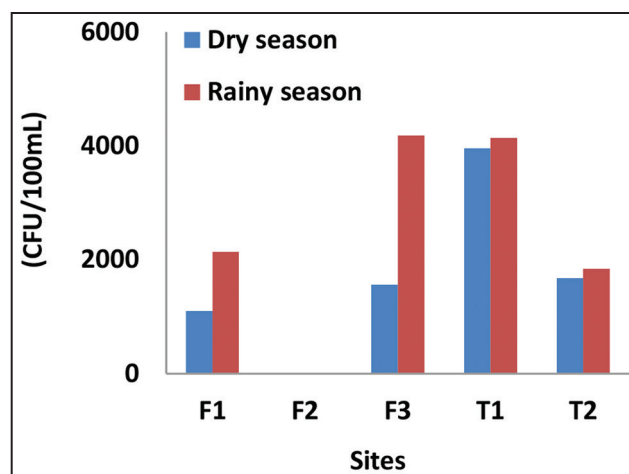


Figure 3: Variations in the rates of thermotolerant germs in brute waters.

germs, thermo-tolerant coliforms, *Escherichia coli*, fecal streptococci or enterococci were counted and identified in all samples. Coliforms were detected using lactose broth with brilliant green and bile by the most probable number method according to standard ISO 4831. Their confirmation and identification were carried out by isolation on agar with eosin methylene blue. The total mesophilic flora was sought with standard agar for enumeration, plate count agar according to the ISO 4830 standard. Fecal streptococci were sought by inoculating broth containing ethyl violet and sodium azothidrate according to the ISO standard [3].

### 2.4. Treatment of Water by the Powder of *M. oleifera* Grains

The ripe, dry pods of the *M. oleifera* seeds were harvested, then the shells and light “wings” of the seeds were removed. The white seeds obtained were finely ground into powder (Photo 1). Different amounts of powder were used to treat the well and the borehole water. For the experiments, 0.8 mg, 1 mg, and 1.2 mg of *M. oleifera* seed powder were used to treat 10 mL of water.

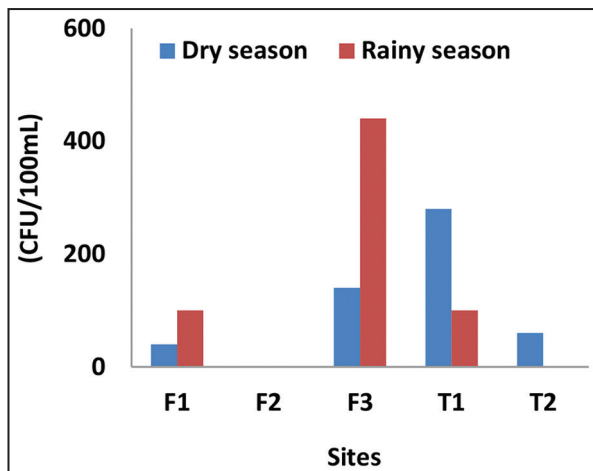


Figure 4: Variation in rates of *Escherichia coli* in brute waters.

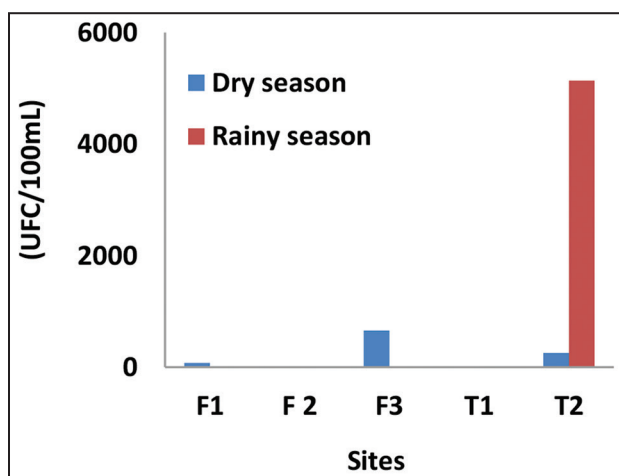


Figure 5: Changes in the raw water levels of *fecal enterococci*.

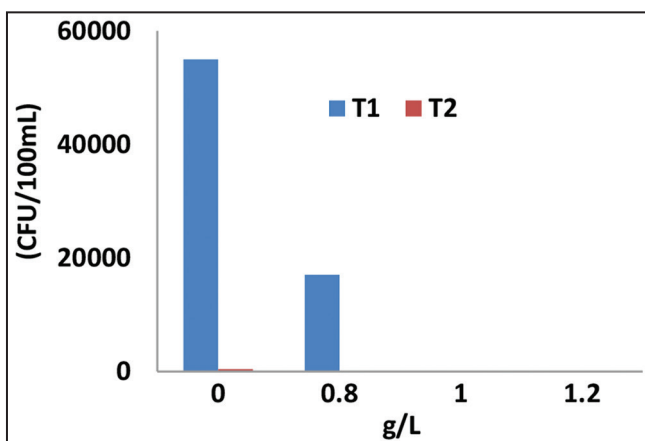


Figure 6: Evolution of the *total mesophilic flora* in T1 and T2 depending on the quantity of *Moringa oleifera* seed powder.

### 3. RESULTS

#### 3.1. Overview of the Physical and Chemical Qualities of Raw Water

The results obtained reveal that only the nitrate contents exceed the World Health Organization (WHO) guidelines [4] for certain samples of the raw well water as noted at Pobe by Lagnika *et al.* [5]. They also identified nitrogen pollution resulting by high levels of nitrates and ammonium in more than 50% of twelve wells studied. The values of the other parameters

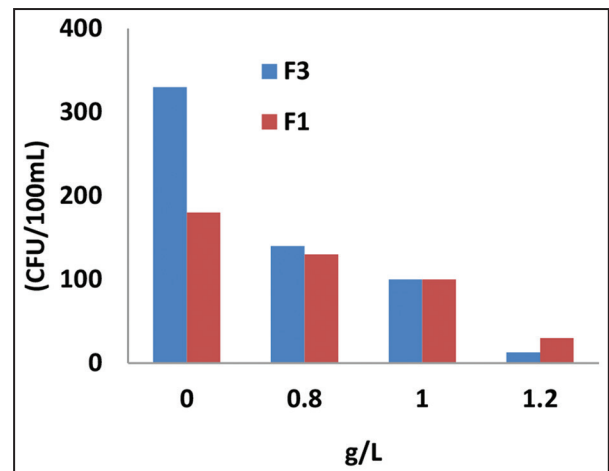


Figure 7: Evolution of *total coliforms* levels in F1 and F3 depending on the quantity of *Moringa oleifera* seed powder.

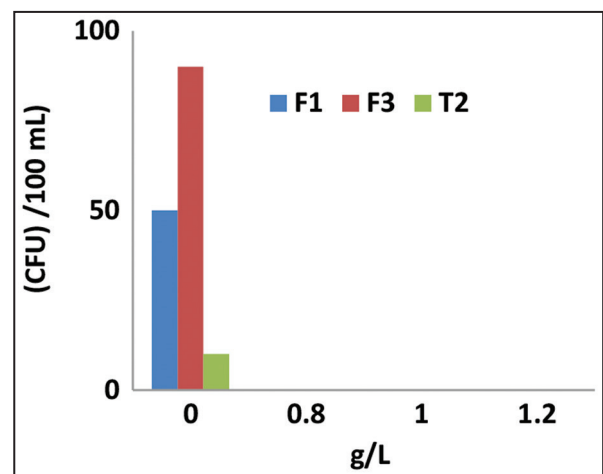


Figure 8: Evolution of *total Staphylococcus* spp. levels in F1, F3, and T2 depending on the quantity of *Moringa oleifera* seed powder.

measured are lower than the Beninese standards indicated in Decree N° 2001-094 of February 20, 2001, setting the quality standards for drinking water as well as the WHO guidelines for water of consumption.

#### 3.2. Overview of the Microbiological Quality of Raw Water

##### 3.2.1. Common germs counting

The microbiological results [Figure 2] reveal that, except borehole water (F<sub>2</sub>), all of the well's water common germs rates exceed standards which value is 50 CFU/mL for non-disinfected water in Benin. However, these values are lower than those obtained by Akodogbo [6] in Porto-Novo in Benin for well's water. The presence of these germs would result from the lack of hygienic conditions due to insanitary around the wells.

##### 3.2.2. Measurement of thermotolerant coliforms levels

All of these values [Figure 3] exceed the standard (0 CFU/100 mL) in Benin. They exceed also those published by Assogba (2020) in the commune of Zogbodomey in Benin [7]. He did not detect the presence of any microorganisms in the well's water. The recorded values are in line with the ones Kpinsoton [8] obtained in Togba in Abomey-Calavi in Benin, which range from 2 to 4800 CFU/100 mL. The presence of these thermotolerant *coliforms* in the well's water would mean organic pollution, a degradation of the bacterial quality of the water and a vulnerability of wells to the intrusion of external materials.

**Table 1:** Sampling sites coordinates.

Side ID	Reason for site selection	Geographic coordinates
F <sub>1</sub>	Its proximity to a tributary of the Oueme river which covers the area	N 06°37'45" E 002°19'04,1" Altitude 23 m
F <sub>2</sub>	Control sample: Modern borehole fitted with a human-powered pump	N 06°37'43,9" E 002°19'04,9" Altitude 22.2 m
F <sub>3</sub>	Its proximity to a traditional latrine	N 06°37'47,8" E 002°19'09,6" Altitude 27.8 m
T <sub>1</sub>	Presence of porcs farm, traditionnal latrine and an agricultural activities area	N 06°37'58,4" E 002°18'48,9" Altitude 47.7 m
T <sub>2</sub>	Located within 15 m from a traditional latrine	N 06°38'01,7" E 002°18'44,5" Altitude 33.5 m

### 3.2.3. Analyzing the quantity of *E. coli*

As per the WHO guidelines, the water from the borehole is free of *E. coli* in both dry and rainy seasons [Figure 4]. This is not the case in the dry season in T<sub>1</sub> (280 CFU/100 mL) and in the rainy season in F<sub>1</sub> (100 CFU/100 mL) and F<sub>3</sub> (440 CFU/100 mL). The values obtained are comparable to those recorded at Kpomasse in Benin for the well's water, which range from 0/100 mL to 320 UFC/100 mL.

### 3.2.4. Quantifying fecal enterococci

As it was observed for *E. coli*, [Figure 5] the borehole water is free of *faecal enterococci* in both dry and rainy seasons, as recommended by WHO guidelines, like for the T<sub>1</sub> sample, and only in the rainy season for F<sub>1</sub> and F<sub>3</sub>.

## 3.3. Evaluation of the Impact of Flocculation by *M. oleifera* Grains Powder on the Microbiological Quality of Water

### 3.3.1. Mesophilic aerobic flora's case

It emerges from the analysis of figure 6 that the powder of the *M. oleifera* seed allowed the flocculation of 97.5% of the germs of sample T<sub>2</sub> and that of almost 100% of those of sample T<sub>1</sub>.

### 3.3.2. Case of total coliforms

The analysis of figure 7 reveals an elimination of 83.44% of total coliforms in F<sub>1</sub> and 96.1% in F<sub>3</sub> with 1.2 g/L of the use of *Moringa* seed powder.

### 3.3.3. Case of *Staphylococcus* spp.

Anlysing the results of figure 8 shows a total flocculation of *Staphylococcus* spp. In water with already 0.8 g/L of the use of *Moringa* seed powder.

## 4. DISCUSSION

The microbiological quality standards for water for human consumption in the Republic of Benin prescribe, for non-disinfected water, 0 CFU/100 mL for total coliforms, fecal coliforms and *E. coli*. Apart from the F<sub>2</sub> sample which comes from modern drilling, the values of the microbiological parameters are mostly significantly higher than these standards. These values are also higher than those, between 0/100 mL and 6700 CFU/100 mL, of the well water in Aguegues and Porto-Novo in Benin. The high values of common germs, total coliforms, and fecal coliforms could be explained by the presence of wild dumps of garbage of all kinds in the vicinity of the wells and the poor management of fecal matter in the locality. In fact, most of the population throws feces into the open air in Wawata and the installed latrines, which are

**Photo 1:** *Moringa oleifera* seeds used.

insufficient in number and do not respect the minimum distance of 15 m recommended by the WHO from wells. Furthermore, these are poorly maintained and are in reality dug and paved pits whose depths do not take into account the level of the exploited water table which is also not uniform in the area. These results are consistent with those obtained by Edorh *et al.*, [9] who noted that non-compliance with certain hygiene rules, notably the distance between latrines and wells, the establishment of garbage dumps in flood-prone areas, jeopardizes the quality of the well water.

Observation of all the results obtained indicates that water contents are generally lower in germs in the dry season than those found in the rainy season. This difference is due to the fact that runoff water drains part of the human fecal matter deposited in bulk and even that of latrines as well as that coming from the leaching of animal manure towards wells.

Total coliforms and *E. coli* are used as indicators to measure the degree of pollution and quality of the well water. Recent contamination by human or animal feces represents the main source of pathogens in drinking water and clearly indicates that drinking water from traditional wells at Wawata is not suitable for consumption and justifies the frequency of waterborne diseases recorded in the locality. This requires precautions to be taken for the treatment of drinking water from these wells. The use of *M. oleifera* seed powder at concentrations of 0.8g/L, 1g/L, 1.2g/L respectively made it possible to agglomerate 84%, 96%, or 100% of the germs in the groundwater from Wawata. These results are similar to those obtained by Kabore [10] in Burkina Faso. The agglomeration of germs by *Moringa* powder is due to the presence of active cationic proteins which act as adsorbent, coagulant, and disinfectant agents in this powder. These proteins neutralize colloidal materials and cause the sedimentation of mineral and organic particles by the electrostatic patch mechanism. However, it is important to recognize that this powder does not allow 100% flocculation-coagulation of all contamination germs, and after decantation, fairly thorough filtration on charcoal and well-washed fine sand is recommended.

## 5. CONCLUSION

Obtaining good quality drinking water is one of the major concerns of the population of Wawata. This study made it possible to determine the microbiological quality of groundwater in Wawata in Abomey-Calavi in Benin and to quantify the powder of the seed of *M. oleifera* necessary for the improvement of the microbiological quality of this water. The analyzes of the water samples from the traditional wells of this locality indicate that they are polluted by germs of fecal origin, the presence of which is due to the poor maintenance of these generally uncovered



wells, the poor management of solid and liquid waste, the excreta and the lack of good hygienic practices in households. *M. oleifera* seed powder was used to eliminate 84%, 96%, and 100% of germs of microbiological contamination from the water sampled. These seeds, once transformed into powder, facilitate the natural flocculation of microorganisms in polluted water. *M. oleifera* is a significant option for the treatment of drinking water in this locality.

## 6. REFERENCES

1. WHO, (2010) Water for health. In: **WHO Guidelines for Drinking Water Quality, Recommendations**, 3<sup>rd</sup> ed. Geneva: World Health Organization.
2. K. N. Ngbolua, A. L. Pambu, L. S. Mbutuku, H. K. Nzapo, G. N. Bongo, N. B. Muamba, C. M. Falanga, Z. B. Gbolo, P. T. Mpiana, (2016) Comparative study of the flocculating activity of *Moringa oleifera* and *Vetivera zizanoides* in the clarification of pond water from "Plateau de Bateke", Democratic Republic of the Congo, *International Journal of Innovation and Scientific Research*, **24(2)**: 379-387.
3. J. Rodier, B. Legube, N. Merlet, (2009) **Water Analysis**, 9<sup>th</sup> ed. Paris: Dunod. Available from: <https://www.dunod.com> [Last accessed on 2022 Oct 13].
4. WHO, (2011) Nitrate and nitrite in drinking water. Background document for development of WHO. In: **Guidelines for Drinking-Water Quality**, Geneva: World Health Organization.
5. M. Lagnika, M. Ibikounle, J. P. C. Montcho, V. D. Wotto, N. G Sakiti (2014) Physico-chemical characteristics of the well water in the commune of Pobe, Benin. *Journal of Applied Biosciences*, **79**: 6887-6897.
6. H. Akodogbo, *Contribution to Improving the Quality of Water for Domestic Use in the 5<sup>th</sup> Arrondissement of the Municipality of Porto-Novo-Benin*. Available from: <https://www.memoireonline.com/a/fr/cart/show> [Last accessed on 2022 Nov 11].
7. C. C. H. Assogba, (2020) Evaluation of the Natural Recharge of an Aquifer in the Commune of Zogbodomey. *Thesis for Obtaining the 2IE Engineering Diploma with Maste's Degree*. Available from: [https://documentation.2ie-edu.org/cdi2ie/opac\\_css/doc\\_num.php?explnum\\_id=3427](https://documentation.2ie-edu.org/cdi2ie/opac_css/doc_num.php?explnum_id=3427) [Last accessed on 2022 Aug 23].
8. A. P. D. Kpinsoton, (2016) *Study of the Quality of Well Water Consumed in the Commune of Abomey-Calavi: Case of the Village of Togba Maria-Gleta*, Benin, EPAC-UAC.
9. A. Etorh, K. Gnandi, M. B. Elegbede, S. F. Enonhedo, M. Boko, (2007) Groundwater water quality and its impact on the health of the populations of *Kerou*. *Clim. Devel.*, **4**: 27-37.
10. A. Kabore, (2020) Potabilization of the well water by coagulation-flocculation with *Moringa oleifera* cakes coupled with sand filtration in Burkina Faso. *Ewash and Ti Journal*, **4(1)**: 307-314.