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Evaluation of the clarifying and antibacterial power of *Moringa oleifera* seeds on drinking water in the town of Parakou in Benin

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ABSTRACT: Around the world, water has always been a mysterious element. Designated as a natural drink, it is and remains a major constituent of our body. Aware that thousands of people do not have access to drinking water, this study aims to develop an organic suspension, extract of *Moringa oleifera* seeds capable of clarifying and partially disinfecting turbid and contaminated water intended for consumption. The study method initially consisted in collecting plant drugs in order to produce a suspension (extract of seed of *Moringa oleifera*). Secondly to study the physico-chemical (clarifying) properties followed by a microbiological (bacteriological) study before and after application of this suspension at different concentrations on crude water samples meticulously sampled from some wells in Parakou city. The experimental results have shown a strong improvement in the physico-chemical parameters involved, in particular color, turbidity and conductivity with clarification rates of 84.33% for color and 87.58% for turbidity; followed by a considerable reduction in the bacterial flora estimated at 66.46% of the total Coliforms; 81.19% of mesophilic aerobic germs; 77.78% of Escherichia coli, then a total elimination of *faecal Streptococci* with 100% and Staphylococcus aureus with 100%, thus confirming the improvement in the organoleptic and sanitary quality of drinking water by extracts of seeds of *Moringa oleifera.*

KEYWORDS: Drinking water, *Moringa oleifera* seeds, organic suspension, clarification, disinfection.

1 INTRODUCTION

In many South countries including Benin, access to water is a problem that people face on a daily basis. In many cases, they only have access to poor quality water, unsuitable for drinking or domestic use. These issues are of course found in the North countries as well but these developed countries have technological and financial resources to treat water and make it drinkable. This is not the case for the poorer countries of the southern hemisphere which do not have the resources to import or develop these technologies. In this situation, the search for a lasting solution at a lower cost would be beneficial for everyone in need. Several techniques exist to improve the quality of water at a lower cost. Many scientific teams are working on inexpensive methods that would allow many people to have access to suitable water. (Jahn, Samia Al Azharia, 1999), (Foidl *et al*., 2002).

Besides the chemicals such as chlorine, aluminum sulfates used, natural plant extracts can be used. *Moringa Oleifera* is a plant which has not only many nutritious properties, but also can improve water quality. The grains of this plant, originally from Asia but now found all over the tropical world, contain a protein and a positive electrical charge which gives them flocculating properties: ability to clarify turbid water. (Folkard, 1997).

This study aims to evaluate the clarifying and disinfecting power of a suspension from aqueous extract of *Moringa oleifera* grains on water sampled from large diameter wells in Parakou city. Optimal conditions and effectiveness of the treatment were assessed through a monitoring of physico-chemical and bacteriological parameters at different times and treatment concentrations.

2 MATERIALS AND METHODS

2.1 LOCATION OF THE STUDY

The study was conducted in the third district of Parakou city, located in Borgou prefecture, the commune of Parakou bounded in the North by the commune of N'Dali, in the South, East and West by the commune of Tchaourou.

2.2 DESCRIPTION OF THE SAMPLING AREA

Water samples were taken from large diameter open wells located in the Swinrou, Amaouignon and Zongo II neighborhoods in the city of Parakou. The geographic coordinates of the 3 sampling sites are as follows:

- Well No. 1 (P1): Swinrou located at 2 ° 36’49.248 “ E longitude and 9 ° 23’43.398” latitude N at an altitude of 7 m;
- Well No. 2 (P2): Amaouignon located at 2 ° 38’33.486 “ E longitude and at 9 ° 22’32.19 “ N latitude at an altitude of 7 m;
- Well No. 3 (P3): Zongo II located at 2 ° 37’44.508 “ E longitude and 9 ° 21’59.13 “ N latitude at an altitude of 7 m.

The following figure shows the geographic location of the study sites.

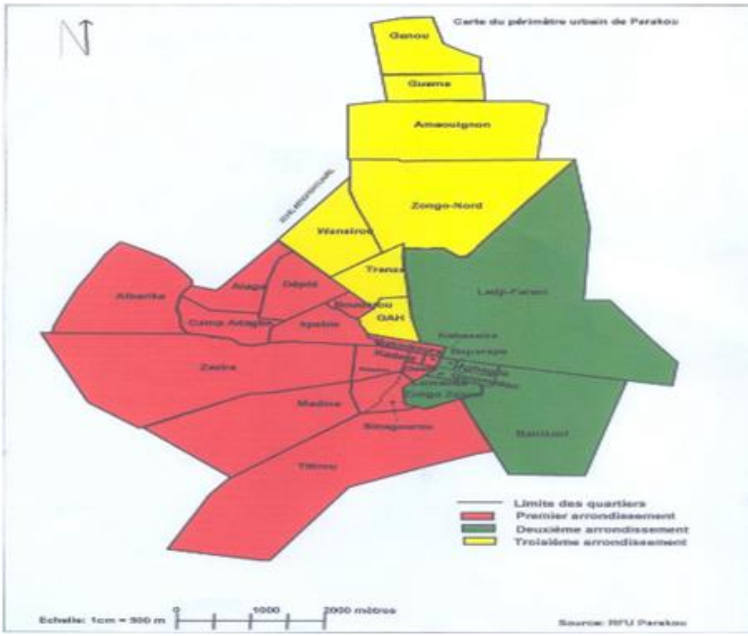

Fig. 1. Geographic map of the town of Parakou

Source: RPU Parakou, 2013.

2.3 BIOLOGICAL MATERIAL

The biological material used consists in ripe and dry seeds of *Moringa oléiféra*, harvested in the garden of the agricultural technical secondary school of Kika located in the commune of Tchaourou, prefecture of Borgou. (Figure 2 and 3).

Fig. 2. Moringa oleifera tree

Fig. 3. Seeds of Moringa oleifera

2.4 METHODS

2.4.1 SAMPLING

The water samples were taken from large open-pit wells located in the Swinrou, Amaouignon and Dopkarou neighborhoods. The samples were taken in sterile jars with a capacity of 1 liter each for each well. The samples judiciously taken using the drips, packaged in the sterilized jars provided for this purpose, were labeled, kept in a cooler at 04 ° C in accordance with the requirements of the parameters to be assayed and then immediately convey to the Laboratory for Water Analysis of Parakou, division of the Borgou Water Service which is a regional structure in the field of water quality. It is located within the confines of the Departmental Direction of Energy and Water of Borgou (DDEE) and has as its intervention area the departments of northern Benin, namely: Borgou-Alibori and Atacora-Donga.

2.4.2 TREATMENT

The water treatment method consisted in preparing the suspension of seeds of *Moringa oleifera*, from which various quantities are taken and then mixed with the crude water samples.

- **Production of the *Moringa oleifera* suspension**

Moringa suspension is a solution (the supernatant) obtained after decanting a mixture of *Moringa* seeds powder with water.

The method of FOLKARD *et al.* (2002) was used to produce a suspension from a mixture of *Moringa oleifera* seeds powder and chilled sterilized water. Thus, after discarding the *Moringa* seeds of their pericarp, the almonds obtained were dehydrated by drying and then reduced to powder. 20g of this powder was dissolved in 1 liter of sterilized water and then homogenized. After 24 hours (diffusion time), the solution obtained was centrifuged in order to remove debris and the maximum amount of oil (which having a proportion of 35% in the seeds could reduce the flocculation power of the seeds). The *Moringa* suspension thus obtained was packed. The diagram below is an illustration of the production process for this suspension.

- ***Moringa* suspension production technology diagram**

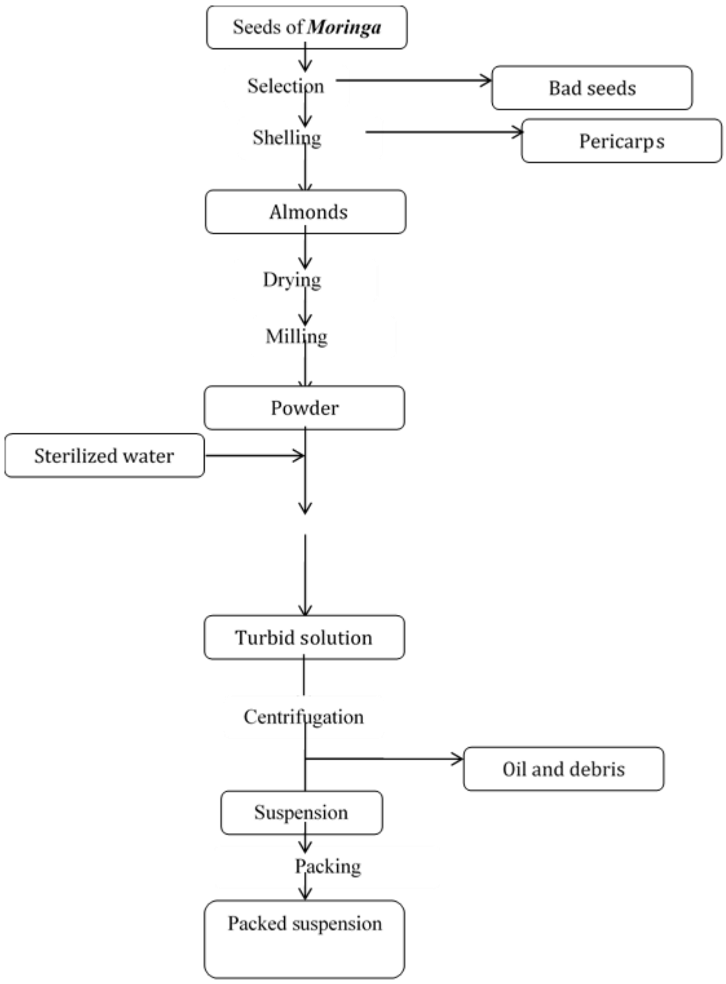

Diagram 1: Technological diagram of the production of the seeds of Moringa oleifera suspension

- **Sample treatment**

At laboratory, the samples were treated in triplicate (with increasing volumes of suspension 0 ml, 10 ml and 20 ml) in order to be able to determine the doses suitable for treatment. To carry out the jar tests, 250 ml of each water sample were introduced into beakers of a six-station electrically controlled flocculator (FC6S Jar Test Velp Scientifique) followed by the addition of different volumes of suspension.

- **Physico-chemical analysis**

Conductivity, temperature, pH, color and turbidity were measured on water samples before and after their treatment with *Moringa* suspension at different concentrations respectively after 1 hour of decantation and after 24 hours of decantation.

The pH, temperature and conductivity were measured by the electrochemical method. As for color and turbidity, they were measured using the DR / 2400 spectrophotometer (HACH). The methods used are summarized in the following table.

Table 1. Physico-chemical parameters analyzed and methods used

Parameters	Units	Reagents/Methods/Equipments	References
pH	-	pH-meter	HANNA
T	°C	Thermometer	H 13292 ATC Conductivity
Conductivity	µS/cm	Conductimeter	H 13292 ATC Conductivity
Color	PtCo	Platine-Cobalt method, DR 2400	Standard method
Turbidity	UTN	Nephelometric method, DR 2400	Norme NF EN ISO 7027 (mars 2007)

- **Microbiological analysis**

The bacteriological analysis (*common germs, total coliforms, E. coli, faecal streptococci and Staphylococcus aureus*) were carried out on the same samples before and after treatment with the suspension of seeds of *Moringa* at different concentrations respectively after 1 hour of decantation and after 24 hours of decantation. The methods used are summarized in the following table.

Table 2. Characteristics and methods of detection of bacteria

	Aerobic Mesophilic Germs	*Total Coliforms*	*E. coli*	*Fecal Streptococci*	*Staphylococcus aureus*
Medium used	plate Count Agar at 37°C	Chrom-agar ECC at 37°C	Chrom-agar ECC at 37°C	Slanetz and Bartley agar	Mannitol agar with sodium chloride and phenol red
Incubation time	48 hours	24 hours	24 hours	48 hours	48 hours
Technics used	Inoculation in agar	Direct inoculation on surface	Direct inoculation on surface	Direct inoculation on surface	Direct inoculation on surface
Observation	All colonies	Red colonies	Blue colonies	Red or brown colonies	Yellow colonies with halo
References	AFNOR NF standard EN ISO 6222	AFNOR NF Standard EN ISO 9308-3	AFNOR NF Standard EN ISO 9308-3	-	-

3 Results And Discussions

Physico-chemical parameters: Figure 4 illustrates the photos of the crude samples of the wells coded P1, P2, P3 compared with those of the first treatment experiment coded E1P1, E1P2, E1P3 then with those of the second treatment experiment coded E2P1, E2P2, E2P3.

Fig. 4. Comparison of raw and treated water samples for each well

Table 3 below presents the results of analysis carried out on the crude samples and treated from the wells after 2 hours and 24 hours of treatment.

Table 3. Average values of the physico-chemical parameters of the samples before and after treatment

		Suspension of seeds of Moringa oleifera								
		WELL 1			***WELL 2***			***WELL 3***		
Duration	**Physicochemical parameters**	**0 mL**	**10ml**	**20mL**	**0 mL**	**10ml**	**20mL**	**0 mL**	**10ml**	**20mL**
2 hours	pH	6,3±0,0	6,3±0,0	6,3±0,0	6,4±0,0	6,4±0,0	6,4±0,0	6,3±0,0	6,3±0,0	6,3±0,0
	Temperature (°C)	29,6±0,0	29,6±0,0	29,6±0,0	29,4±0,0	29,4±0,0	29,4±0,0	28,8±0,0	28,8±0,0	28,8±0,0
	Conductivity (µs/Cm)	42	87	131	103	200	203	87	134	74
	Turbidity (NTU)	323	121	71	294	126	48	83	100	160
	color	2150	715	379	1520	485	427	329	440	514
24 hours	pH	6,3±0,0	6,3±0,0	6,3±0,0	6,4±0,0	6,4±0,0	6,4±0,0	6,3±0,0	6,3±0,0	6,3±0,0
	Temperature (°C)	29,6±0,0	29,6±0,0	29,6±0,0	29,4±0,0	29,4±0,0	29,4±0,0	28,8±0,0	28,8±0,0	28,8±0,0
	Conductivity (µs/Cm)	42	87	131	103	200	203	87	134	74
	Turbidity (NTU)	323	146	42	294	14	32	83	111	108
	color	2150	417	337	1520	307	87	329	225	378

The physico-chemical analysis carried out on the samples from wells 1 and 2, as indicated in the table above show that after treatment of the water samples, there was variation of all the parameters evaluated, this in order to improve the water quality. Compared to the different doses of the suspension used, the 20 ml dose gave better results for all the parameters analyzed. With respect to time, a great clarifying activity was obtained after 24 h. we find that the higher the dose the more effective the suspension. These results indicate the clarification of the treated water as shown in figure 4.

The decrease in color depending on the dose and the treatment time at the two wells P1 and P2 confirms that the *Moringa* suspension greatly improves the quality of turbid water through its clarification. These results are in agreement with those of KATRE *et al.,* 2008; NDABIGENGESERE *et al.,* 1995; OKUDA *et al.*, 1999, which affirm that the seeds of *M. oleifera* contain a basic polypeptide, more precisely a set of active cationic polyelectrolytes of 12-14 kDa. These positively charged poly electrolytes neutralize colloids in turbid waters because the majority of these colloids have a negative charge (FOILD *et al.,* 2002). From all the results, it appears that the effectiveness of water treatment with *M. oleifera* seeds varies from one water to another. With regard to turbidity, the results of analysis of wells 1 and 2 reveal that the effectiveness of the suspension of *Moringa* on drinking water depends on the settling time and the dose of suspension used. The higher the dose, the faster the flocculation and clarification. These results are consistent with those of FOLKARD (1997), who asserts that samples heavily loaded with organic matter (turbidity> 1000 NTU) require very high doses of M. oleifera seeds. Likewise FABY and ELELI (1993); FOLKARD (1997) attest that the doses required in the treatment of *M. oleifera* seeds water vary according to the level of organic matter present in the water, the initial turbidity of the latter and the nature of the elements to be flocculated, in particular that of clays. For conductivity, it has been found to increase depending on the amount of suspension used during the treatment. The higher the dose, the more the conductivity increases. This could be due to the contribution of ions and other elements (molecules) by the suspension which derives from a plant matter. These results are approved by Kabore *et al.* (2013) who report that the seeds of *M. oleifera* are rich in minerals, especially nitrates and sulfates, and in organic matter. This could lead to an increase in the concentration of these minerals and organic matter in the treated water. In contrast to the results from wells 1 and 2 at Swinrou and Amaouignon, analysis of data from well 3 at Zongo II reveals an increase in color, turbidity and conductivity. This is explained by the fact that the crude sample from well No. 3 was initially clear and less turbid than the previous samples. Moringa's suspension did not improve the color and turbidity of initially clear and limpid water regardless of the concentration and duration of treatment. These results corroborate those of Kabore *et al*. (2013); on the raw waters of Ouaga 3 which presented a low initial turbidity (9.06 NTU) due to the absence of colloids and suspended solids, in which the introduction of the coagulant resulted in an increase in their turbidity followed by a slow settling of particles. In all samples (treated or not), the pH and the temperature are constant, between 6.4 and 6.9 respectively and between 28 and 29 ° C. The pH, close to neutral, shows that the suspension has no effect on the pH and the temperature of the treated water. The chemical composition (pH, conductivity) of the water changes shortly after treatment with *M. oleifera* seeds (FOLKARD, 1997). This is in agreement with the results obtained which indicate that the treatment has little influence on the pH of the water, the variation of which is not statistically significant. A comparative analysis of the results of the physico-chemical parameters of the three wells P1, P2 and P3 reveals that the more turbid the water, the more efficient the Moringa suspension.

MICROBIOLOGICAL PARAMETERS

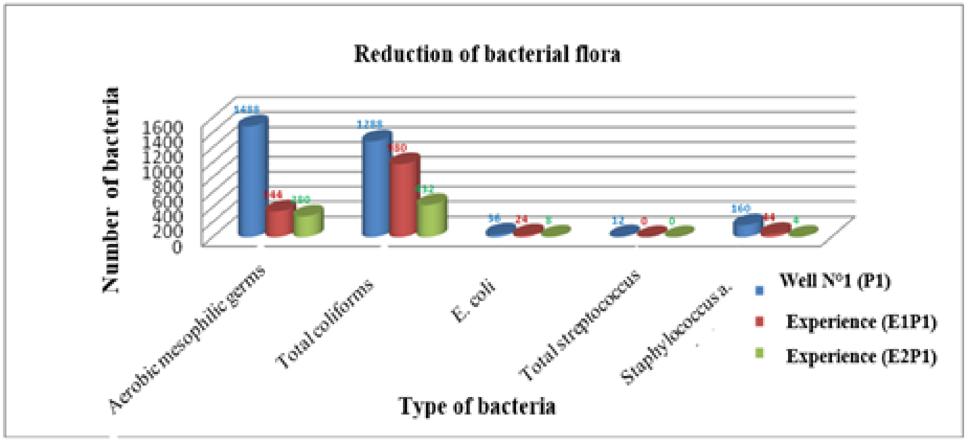

Fig. 5. Evolution of the bacteriological Parameters (well n°1 of swinrou)

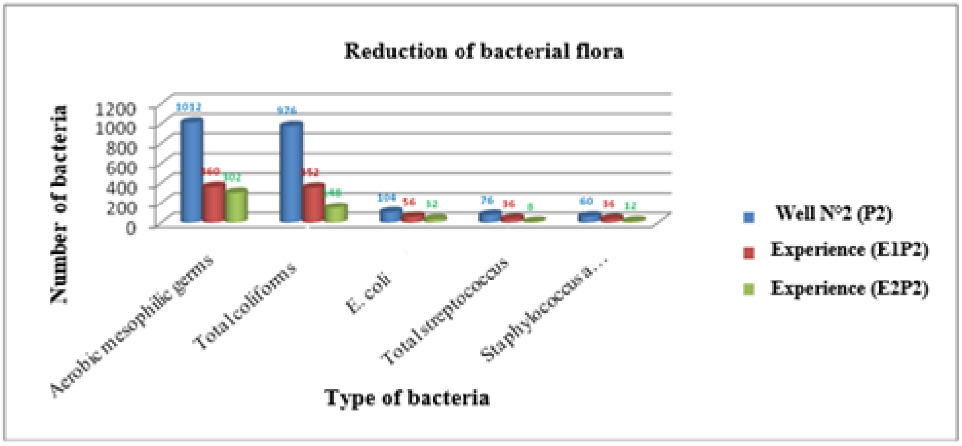

Fig. 6. Evolution of the bacteriological Parameters (well n°2 of Amanouignon)

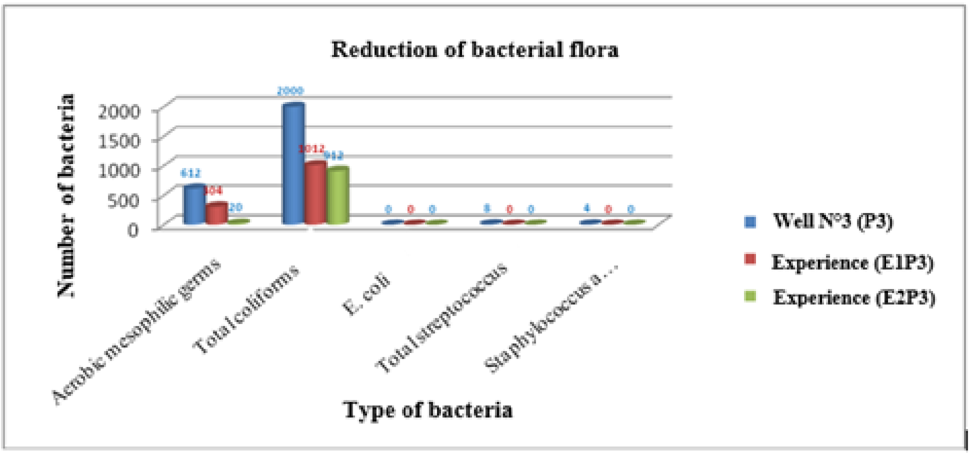

Fig. 7. Evolution of the bacteriological Parameters (well n°3 of Zongo II)

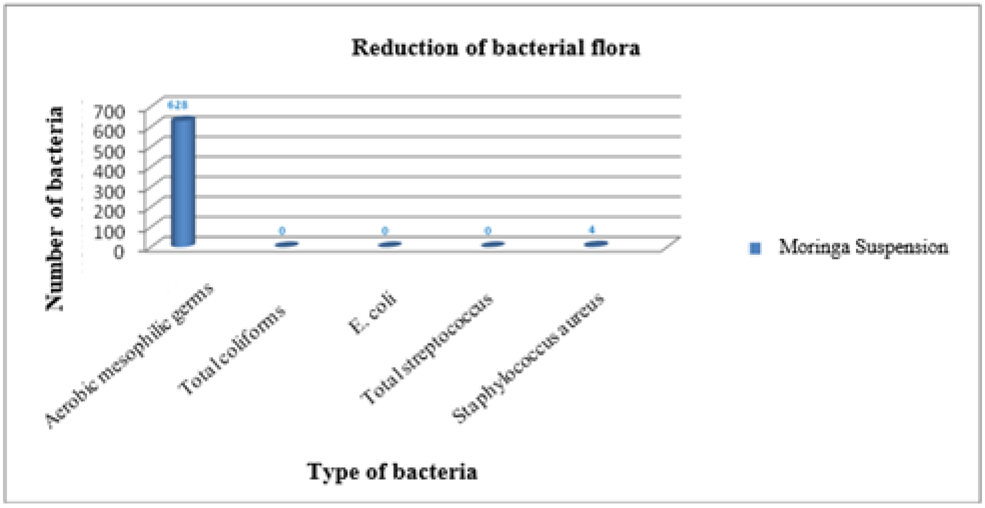

Fig. 8. Evolution of the bacteriological parameters on the Moringa suspension

The results of analysis of the microbiological parameters (*aerobic mesophilic germs; fecal coliforms; E. coli; faecal streptococci and Staphylococcus aureus*) show a very great reduction of certain germs and a total elimination of other types of germs according to their initial content in the raw samples, the concentration of the suspension administered and also the settling time.

It appears from the analysis of well No. 1 in Figure. 5 that the aerobic mesophilic germs and total coliforms have been reduced considerably, going from 1488 aerobic mesophilic germs counted initially to 344 germs at the first dose then to 280 germs at the second dose for aerobic mesophilic germs and from 1288 fecal coliforms initially to 980 coliforms at the first dose then to 432 coliforms at the second dose. The same effects are also noted on E. coli and Staphylococci with respective reductions from 36 *E. coli* to 08 *E. coli* and from 160 *Staphylococcus aureus* to 04 *Staphylococcus aureus*. By account, a total elimination of *faecal Streptococci* was noted. The results of well No. 2 in Figure. 6 indicate a strong reduction in all the germs highlighted, meaning that a significant reduction in aerobic mesophilic germs; total coliforms of *faecal Streptococci* and *Staphylococcus aureus*. In well N ° 3 (figure 7) which waters were less colored and less turbid compared to the others, there were a very great reduction of germs and a total elimination of Streptococci and Staphylococci as well. Although the suspension of Moringa could not improve the physico-chemical characteristics of the water from well No. 3 of Zongo II, it had a positive effect on the reduction and on total elimination of certain germs identified.

Figure. 8 shows an overview of the results of bacteriological analyzes performed on the pure suspension of produced *Moringa oleifera* seeds. We note that this suspension is free from any contamination except for a few unknown colonies of aerobic germs which call into question the technic of its preservation.

In view of the results from the analysis of the treated samples from wells P1, P2 and P3, it appears that the Moringa suspension is provided with antibacterial and bactericidal power for certain germs such as fecal Streptococci and Staphylococci. Its antibacterial power is more noticeable on certain bacteria such as aerobic mesophilic germs and total coliforms. The bactericidal power is noticed on other types of bacteria which are indicators of sanitary state such as *E. coli, fecal Streptococci and Staphylococci aureus.* These powers and efficiency depend not only on the initial rate of germs identified but also on the concentration (dose) of suspension used during treatment and also on the settling time. These results are consistent with those of Kabore *et al.* (2013) which, after microbiological analysis of the supernatants of the samples of water treated with Moringa seeds, showed very significant elimination of all the germs after two hours of decantation. Likewise, Folkard and Sutherland (1992) then Faby and Eleli (1993) also showed that the effectiveness of treatment on microorganisms is a function of the initial degree of pollution of the water. Moreover, microbial reductions are a function of the initial physico-chemical and microbiological characteristics of crude water and proportional to the reduction in turbidity (BRATBY, 2006).

From all of the above, it appears that from the physico-chemical and bacteriological point of view, the very principle of flocculation considerably improves the characteristics (color and turbidity) of turbid water and greatly reduces the rate of germs (especially the *E. coli; faecal Streptococci* and *Staphylococcus aureus*) because a large majority is eliminated with the suspended solids, when these precipitate and are separated from clear water. The reduction in turbidity constitutes a partial purification. Moringa seed extract is therefore an alternative flocculant to the chemicals currently used when drinking water is made potable. However, it could replace all clarification and purification techniques if better technology is applied when it is obtained.

The experimental results showed a strong improvement in the physico-chemical parameters involved, in particular the color, the turbidity and the conductivity with respective clarification rates of 84.33% for the color and 87.58% for the turbidity; followed by a considerable reduction in the bacterial flora evaluated at 66.46% of the total Coliforms; 81.19% of mesophilic aerobic germs; 77.78% of Escherichia coli then, a total elimination of *faecal Streptococci* at 100% and *Staphylococcus aureus* at 100%, thus confirming the improvement in the organoleptic and sanitary quality of drinking water by extracts of *Moringa oleifera* seeds.

4 CONCLUSION

In Abstract, the suspension of *Moringa oleifera* seeds is effective in the treatment of cloudy and infected water both from the physico-chemical and bacteriological point of view, passing through the organoleptic aspect. It greatly improves the physico-chemical characteristics such as color; turbidity without varying the pH or temperature. It also considerably decreases part of the bacterial flora and completely eliminates others types of bacteria. *Moringa oleifera* seeds considerably improve the physico-chemical quality and disinfect drinking water from wells. This approach is ecological and respectful of the environment. It is inexpensive, simple and beneficial for rural populations. In addition, *Moringa oleifera* is a plant taxon that is particularly easy to cultivate intensively and is adapted to the tropical climate of Africa.

REFERENCES

[1] Jahn, Samia Al Azharia. (1999), “From clarifying pearls and germs to water coagulation with alum. History, surviving practices and technical assessment.” Anthropos 94. 1999: 419-430.

[2] Foidl N., Makkar H.P.S. and Becker K. (2002), “Potentiel du Moringa oleifera pour les besoins agricoles et industriels. In: Saint Sauveur, Appora, Besse et Fuglie, Potentiel de développement pour les produits du Moringa.” Actes de l’atelier international de Dar es Salaam, 29 octobre-2 novembre 2001, CIRAD/PROPAGE/SILVA, Montpellier, France (disponible aussi sur www.moringanews.org et dans L. Fuglie, 2002. « L’arbre de la vie”, CWS/CTA, Dakar, Senegal). PP 4-5.

[3] Folkard G. (1997), “The development of the Moringa oleifera and stenopetala tree to provide valuable products: coagulant for water/wastewater treatment and vegetable oil.” Rapport à la Commission Européenne, DG 12, projet de recherche n° TS3*CT94-0309, période 1995-1997.

[4] Angela R. M. (2006), “Antanarivo (Madagascar) Moringa Madagascar: Juillet 2006”. PP 3-16.

[5] Jahn, Samia Al Azharia. (1988), “Using Moringa seeds as coagulants in developing countries.” Journal AWWA, June 1988, pp 43-50.

[6] Maurice D. (2011), “Technologie de l’eau”: Cours De Technologie Industrielle /PPEA3. pp 3-6.

[7] OMS (1965) "Potable et politique nationale de l'eau au Benin." Normes internationales pour l'eau de boisson,2eme édition, de l'OMS (Organisation Mondiale de la Santé) 1965, 31p.
[8] Kabore, A., Savadago, B., Rosillon, F., Straore, A. S. and Dianou, D. (2013), "Optimisation de l'efficacité des graines de Moringa oleifera dans le traitement des eaux de consommation en Afrique sub-saharienne: cas des eaux du Burkina Faso." Revue des sciences de l'eau / Journal of Water Science, 26 (3), 209–220. https://doi.org/10.7202/1018786ar.
[9] Bratby J. (2006), "Coagulation and flocculation in water and wastewater treament." Seconde édition, IWA Publishing, Londres, 450 p.
[10] Jahn S.A.A. (1989), "Différents rôles des coagulants naturels dans la clarification de l'eau, dans les technologies appropriées à usage domestique et dans les installations communales d'épuration. Dans: Proceedings of International Seminar on the Use of Natural Coagulants for Water Treatment, Yogyakarta, Indonesia." 2-7 octobre, 11 p.
[11] Faby J.A. and A. Eleli. (1993), "Utilisation de la graine de Moringa, essais de floculation au laboratoire et en vraie grandeur." CIEH/EIER/Oieau, Série hydrolique urbaine et assainissement, Ouagadougou, Burkina Faso, 132 p.
[12] Kabore A. (2011), "Étude du pouvoir floculant et des qualités épuratoires des graines de Moringa oleifera dans le traitement des eaux brutes de consommation en Afrique sub-saharienne: Cas des eaux du Burkina Faso." Mémoire de DEA, Université de Ouagadougou, Ouagadougou Burkina Faso, 54 p.
[13] Folkard G. and J. Sutherland. (1992), "Development of robust water treatment systems incorporating natural coagulants." Field study report, January-March 1992, Thyolo, Malawi 16 p.
[14] Ndabigengesere A., K.S. Narasiah et B.G. Talbot. (1995), "Active agents and mechanism of coagulation of turbid waters using Moringa oleifera." Water Res., 29, pp 703-710.
[15] Okuda T., A.U. Baes, W. Nishijima and M. Okada. (1999), "Improvement of extraction method of coagulation active components from Moringa oleifera seed." Water Res., 33, pp 3373-3378.
[16] Folkard G. et J. Sutherland. (2002), "Development of a naturally derived coagulant for water and wastewater treatment." Water Suppl., 2, pp 89-94.
[17] Katre U.V., C.G. Suresh, M.I. Khan and S.M. Gaikwad. (2008), "Structure-activity relationship of a hemagglutinin from Moringa oleifera seeds." Int. J. Biol.Macromol., 42, pp 203-207.