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## Influence of *Moringa oleifera* Seed Coagulant and Charcoal-Sand Filter on Bacterial Density of Surface Water

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### Abstract

A complimentary efficacy of Moringa oleifera seed coagulant, charcoal-sand filter and solar heat for surface water treatment was evaluated, using Omoshio stream in Ado-Ekiti. The quality of the treated water was examined with bacteriological and physicochemical analyses. Sensory evaluation of the treated surface water was done using a 20-man panel and the result was statistically analyzed. The mean total bacterial count for the raw sample is 28.2 x 10<sup>o</sup>CFU/ml. the mean total *Salmonella/Shigella* count is 12.0x10<sup>o</sup>CFU/ml and the mean total coliform count is 23.2 x 10<sup>o</sup>cfu/ml. Microbial load was greatly deactivated through the combined effects of moringa treatment, charcoal sand filtration and solar heating, bringing the microbial load to almost 99.9% reduction. It is observed that the mean total bacterial count decreased with 10g compared to 2.5g of moringa treated water sample. 53 (76% of the total) isolates were recovered from the raw water sample with Escherichia coli having the highest occurrence of 47.2% followed by Salmonella spp. with 15.0%, Shigella spp. with 11.3%, Klebsiella spp. with 9.4% while Bacillus spp. and *Proteus* spp. had the least occurrences of 3.8% and 5.7% respectively. After treatment with moringa, only 17 (24%) bacteria were isolated. E. coli reduced from 47.2% to 11.8% and salmonella has no growth. The physicochemical properties of the moringa treated, charcoal sand filtered, solar heated water (MTFSH) met the WHO standard limit for portable water, showing pH of 6.9, TDS (22mg/L), temperature (26°C), hardness (100mg/L), turbidity (0.15NTU) and electrical conductivity (0.14mScm<sup>-1</sup>). The results derived from this study showed that charcoal-sand filtration and solar-disinfection complimented with Moringa oleifera seed coagulation can be recommended as a good means of eradicating water borne pathogens.

### **1. Introduction**

The importance of water as a key to sustainable human productivity, livelihood and development as well as its role in integrating world ecosystems has been stressed over years (1, 2), considering the increasing and competing demands for agricultural, industrial and domestic usage, but increasing pollution that threatens this scarce resource is still a thorn on human flesh (3). Apparently, over 1.1billion people according to World Health Organization, lack access to improved drinking water supply (4), making uncontaminated water scarce in developing countries in Africa and Asia (5). It is not an over statement that over half of population in Nigeria still rely on surface water sources,

which has earlier been reported unfit for usage due to contaminations related to anthropogenic activities (6). Meanwhile, the tendency of these water sources to mediate the spread of different world threatening waterborne infections; such as diarrhea, dysentery, amoebiasis, hepatitis, typhoid, jaundice and so on, is very high, hence has attracted special attention (7). In that regards, environmental risk assessment agency has also given biological contaminants, specifically water-borne microbial pathogens utmost priority among considerable factors in treatment of water for domestic usage (8).

Although varies from place to place, a multi-stage process is required in the treatment of water; all aiming at removing existing contaminants or reducing the concentration to barely minimum level that the water becomes fit for its desired end use (9, 10). Combination of different processes could be applied to achieve water purification, with consideration of the water source, efficiency and cost (11, 5). It is outwardly unsustainable in developing countries like Nigeria, to achieve the conventional method of water purification which requires the use of chemicals to neutralize repelling charges on the colloidal particles in the water, resulting in a faster rate of settling; coagulants (12). This is due to prohibitive cost and scarcity of chemical coagulants and disinfectants. In addition to cost deficiencies, many of these conventional techniques require electricity supply, making it more unattainable in this part of the world (3). Furthermore, it is highly concerning knowing that chemicals like Aluminium sulphate (Alum) used as coagulant/disinfectant in most of the conventional methods from time immemorial has been reported as potential carcinogens (13, 14). The need for simple, cost effective, reliable and effective method is therefore a necessity.

The application of different alternative approaches in achieving a purified water have recently been reported in many part of Africa, including the use of phytocoagulants such as seeds of *Moringa oleifera* (15, 16, 17); *Jatropha curcas* (14); *Garcinia kola* and *Carica papaya* (3), the use of sand filter media (3); charcoal-sand filtration (18), and the use of solar heating (18). The present study is therefore aimed at studying the potential effect of using combination of phytocoagulants (*Moringa oleifera*), solar-heating and charcoal-sand filtration to purify water sample from Omoshio stream in Ado-Ekiti metropolis as a case study, there by presenting their consortium application as an alternative technology that is low-tech, effective, attainable and affordable for the rural dwellers.

### 2. Materials and Methods

### 2.1. Study Site and Samples Collection

Surface water samples were obtained from Omoshio stream, located along Ekiti State University, Ado-Iworoko road, Ado Ekiti, Ekiti State ( $7.6211^{\circ}$  N,  $5.2214^{\circ}$  E). The water samples ( $25L \times 9$ ) were collected at the depth of the surface

water for three period of collection and transported to the laboratory for the research work.

## 2.2. Preparation of Charcoal-Sand Filter and Solar Reactor

The procedure of Oluyege and Omoyajowo (18) with little modification was adopted in preparing the charcoal-sand filter reactor. The filter column height-90cm, length-70cm and diameter-58cm. The charcoal was ground into powder and the sand was sieved (1.18mm). The charcoal sand filter was prepared by placing the stones with mean diameter of 41.5mm at the bottom of the tank to a high of 20cm. This was followed by layer of fine sand of height between 20cm-35cm (15cm), followed by another layer of charcoal with high between 35cm-45cm (10cm). This procedure continue by placing another layer of sand with the high between 45-55cm (10cm), followed by charcoal of 55cm-65cm (10cm) and then finally followed by sand of height 60cm-70cm (5cm). Each layer of the filter media were separated using sterile muslin cloth. The solar heating reactor was a black painted wooden box (64.0cm x 57.4cm x 43.0cm) with an opening sliding door through which filtered water are loaded. The box is covered with a transparent glass for penetration of sun rays.

# 2.3. Preparation of Phytocoagulant (Plant Seed)

Dried 'drumstick' seeds of *Moringa oleifera* that were in good state (not rotten) were selected and the wing and coat were removed. The seeds were ground using a sterile blending machine, after which were sieved through a  $600\mu m$  sieve. Varied amounts (2.5g, 5g and 10g) of moringa seed powder were weighed and wrapped separately in white muslin bags which were used directly as coagulants.

### 2.4. Treatment of Water with Phytocoagulant, Filtration and Solar Heating

The water samples were directly and separately flocculated with three different portions (2.5g, 5g and 10g) of *Moringa oleifera* seed powder and were allowed to treat the water samples for about 30mins. Flocculated and unflocculated raw water samples (25 litres) were filtered through the charcoal sand filter media at a flow rate of 1.5L/hour. Filtered water samples in 0.5L-sized transparent PET-bottle (19) were exposed to sunlight at a mean temperature of 45°C for 6hours at latitude of 5°20'E and longitude of 7°45'N in the constructed black painted wooden box. Water samples were obtained at every stage of treatment for bacteriological and physicochemical analyses.

### 2.5. Determination of Bacterial Density

The bacterial density of raw and treated water samples were determined using standard plate count techniques. Tenfold dilutions of the water samples were done and 1.0ml of dilutions  $10^{-2}$ ,  $10^{-3}$  and  $10^{-5}$  were inoculated in molten agar

media: Nutrient agar (NA) for total bacterial count, MacConkey agar (MAC) for total coliform count, Salmonella/Shigella agar (SSA) for total Salmonella/Shigella count and Eosin methylene blue agar (EMBA) for total E. coli count (20). Determination of bacterial load of the water samples were done in triplicates. Plates were allowed to set and incubated inverted at 37°C for 24h. The plates were counted after incubation using colony counter (Gallenkamp, England) to obtain the total bacterial counts, which were calculated by multiplying the number of colony per plate by the dilution factor and recorded in colony forming unit per ml (CFU/mL) (21). Pure cultures of isolates were kept on nutrient agar slants at 12°C until used. The isolates were identified on the basis of cellular morphology following Gram stain and results of biochemical testing; including catalase production, growth in 6.5% NaCl broth, haemolytic activity and motility (22). The isolates were named in reference to the Bergey's manual of determinative bacteriology (23).

### 2.6. Determination of Physicochemical Properties of Water Samples

Conductivity, pH and total dissolved solid (TDS) were determined using PH/EC/TDS/Meter model number H19813/0. Total hardness and turbidity of the raw and treated water samples were determined using Lovibond kit 424 (Tintometer Limited, Salisbury, England) and Turbidometer TBN 80120-1(Shanghai China Instrument and meter Limited), respectively.

### 2.7. Determination of Organoleptic Properties of Treated Water

The sensory evaluation of the treated water was done using a Twenty-man panel based on the consent of the members to participate in the evaluation. The test parameters were clarity, taste, odour, appearance and overall acceptability with scores of 0 to 5. The data obtained were subjected to descriptive statistical analyses using one-way ANOVA at  $P \le 0.05$  and Duncan multiple range test. The statistical package used for the analysis was SPSS 11.0.

### 3. Results

The mean total bacterial, *salmonella/shigella* and coliform counts are 28.2 x  $10^{5}$ CFU/mL, 12.0 x  $10^{5}$ CFU/mL and 23.2 x  $10^{5}$ CFU/mL, respectively. There was an observable decrease in microbial load of charcoal sand filtered water as the samples were subjected to filtration. However, the microbial load was still highly unacceptable. The bacterial colonies were later observed to be deactivated as the samples were subjected to solar heating. The total bacterial count decreased to 4.9 x  $10^{5}$ CFU/mL, total coliform count decreased to 3.8 x  $10^{5}$  CFU/mL, total *salmonella/shigella* count decreased to 3.8 x  $10^{5}$  CFU/mL, total *salmonella/shigella* count decreased to 5.9 x  $10^{5}$  CFU/ml and total *E. coli* count decreased to 4.2 x  $10^{5}$  CFU/mL (Table 1).

	al density	(CFU/mI	<i>.</i> )									
Sample	TBC			TCC			TSSC			TEC		
	10 <sup>2</sup>	10 <sup>3</sup>	10 <sup>5</sup>	10 <sup>2</sup>	10 <sup>3</sup>	10 <sup>3</sup>	10 <sup>2</sup>	10 <sup>3</sup>	10 <sup>5</sup>	10 <sup>2</sup>	10 <sup>3</sup>	10 <sup>5</sup>
	68	26	12	56	22	-	26	14	-	36	12	-
Raw water Filtered water	52	20	10	55	21	5	26	13	-	30	17	-
	40	17	9	33	15	2	20	9	-	25	15	-
	34	20	6	25	17	8	21	1	-	22	6	-
	30	17	4	25	13	5	19	11	3	16	3	-
	25	15	-	19	10	3	13	7	-	12	3	-
	12	5	-	7	5	-	21	11	-	22	6	-
Solar heated and filtered	11	5	-	9	3	1	7	4	-	3	-	-
	9	2	-	7	2	-	6	4	-	3	-	-

 Table 1. Bacterial density of raw water, filtered water and solar heated filtered water.

Key: TBC - Total Bacterial Count, TCC - Total Coliform Count, TSSC - Total Salmonella Shigella Count, TEC - Total E. coli Count

The microbial load has been greatly deactivated through the combined effects of moringa treatment, charcoal sand filtration and solar heating, bringing the microbial load to almost 99.9% reduction. It is observable that the mean total bacterial count decreased with 10g compared to the one treated with 2.5g of moringa. There were no microbial growth at the third run of filtered surface water sample and solar heated water sample due to the combine effect of solar reactor and moringa on the surface water. Raw water sample treated with moringa (2.5g) has total bacteria load of 7 x  $10^2$ 

CFU/mLand reduced to 3 x  $10^2$  CFU/mLafter filtration while raw water sample with moringa 10g has the total bacterial load of  $3x10^2$ CFU/mLand has no growth after filtration has taken place (Table 2).

Fifty three (76% of the total isolates) were recovered from the raw water sample with *Escherichia coli* having the highest occurrence of 47.2% followed by *Salmonella* spp. with15.0%, *Shigella* spp. with 11.3%, *Klebsiella* spp. with 9.4% while *Bacillus* spp. and *Proteus* spp. had the least occurrences of 3.8% and 5.7% respectively. After treatment with moringa, only 17 (24%) bacteria were isolated. *E. coli* reduced from 47.2% to 11.8% and *salmonella* has no growth. Treatment of the water sample with moringa, filtration and

solar disinfection removes virtually all the bacterial in the water sample (Table 3).

		Micro	bial den	sity (CF	U/mL)								
Sample	weight of	TBC			TCC			TSSC			TEC		
	moringa (g)	10 <sup>2</sup>	10 <sup>3</sup>	10 <sup>5</sup>	10 <sup>2</sup>	10 <sup>3</sup>	10 <sup>5</sup>	10 <sup>2</sup>	10 <sup>3</sup>	10 <sup>5</sup>	10 <sup>2</sup>	10 <sup>3</sup>	10 <sup>5</sup>
	2.5	7	2	-	5	-	-	7	5	-	5	3	-
Raw water	5	5	2	-	3	-	-	1	-	-	6	-	-
	10	3	-	-	5	-	-	-	-	-	3	-	-
	2.5	3	-	-	2	-	-	5	-	-	4	-	-
Moringa treated filtered water	5	3	-	-	-	-	-	-	-	-	-	-	-
	10	-	-	-	-	-	-	-	-	-	-	-	-
	2.5	2	-	-	1	-	-	3	-	-	2	-	-
Moringa filtered treated and Solar	5	3	-	-	-	-	-	-	-	-	-	-	-
neated water	10	-	-	-	-	-	-	-	-	-	-	-	-

Table 2. Microbial load of Moringa treated water samples.

Key: TBC – Total Bacterial Count, TCC – Total Coliform Count, TSSC – Total Salmonella Shigella Count, TEC – Total E. coli Count

Table 3. Percentage occurrence of bacterial isolates from raw and treated water.

	Untreat	ed Water Sa	nple		Treated	Water Sample		
Organisms	RW	CSF	SH	Percentage occurrence	MT	MT-CSF	MTFSH	Percentage occurrence
Klebsiella spp.	2	1	2	5 (9.4)	4	1	-	5 (29.4)
Proteus spp.	2	-	-	2 (3.8)	1	-	-	1 (5.9)
Staphylococcus spp.	2	2	-	4 (7.5)	1	1	-	2 (11.8)
Salmonella spp.	5	2	1	8 (15.0)	-	-	-	0 (0)
Shigella spp.	5	1	-	6 (11.3)	2	2	-	4 (23.5)
Bacillus spp.	2	1	-	3 (5.7)	2	1	-	3 (17.6)
Escherichia coli	11	8	6	25 (47.2)	2	-	-	2 (11.8)
Total	29	15	9	53	12	5	0	17

Key: RW = Raw Water, CSF = Charcoal sand filtered water, SH = Solar heated water,

MT = Moringa treated, MT-CSF = Moringa treated filtered water,

MTFSH = Moringa treated, filtered solar heated water

The pH decreased from 7.45 in the untreated raw water sample to 7.04 after solar heated. It was found to increase from 6.20 in the moringa treated water sample to 6.9 in the solar heated moringa treated sample. Total dissolved solid decreased from 64mg/ml in untreated raw water to 44mg/ml in solar heated, while it decreased from 60mg/ml in moringa treated water to 22mg/ml in solar heated water. The hardness decreased from 65.3mg/ml in raw of untreated sample to 20mg/ml in solar heated moringa treated water. Turbidity was found to reduce to 0.15NTU in solar heated moringa treated sample from 2.25NTU in the untreated raw water. Electrical conductivity decreased from 0.19mScm<sup>-1</sup> to 0.14mScm<sup>-1</sup> (Table 4).

### 4. Discussions

The result of this study clearly indicates that the Omishio stream is heavily contaminated, since it does not conform to the standard of the World Health Organization for portable water (4, 8). This is in support of the report by Jurdi*et al.* (24), which says 50 - 72% of water sources including stream,

springs, wells and storage tanks do not conform to the WHO standard for drinking water quality. The present results obtained for total bacterial and coliform counts are similar those obtained by Edema *et al.* (25); Banwo (26) and Okonko *et al.* (8). Gram negative being the highest isolated bacteria in this work is in accordance to the report of Suma *et al.* (27). It is also comparable with the result obtained by Barrell *et al.* (28) that claimed most of the microbial pollutants in surface water are coliform bacteria and attributed this to feacal contamination. The report by Prat and Munne (29) also correlates with this study, indicated that most water borne diseases are attributed to presence of coliform.

Charcoal sand filtration is essentially effective in reducing microbial load of raw water. This is observable in Table 1 that showed a wide decrease in the microbial loads of the samples. Further subjection of the charcoal sand filtered water to solar disinfection also results in massive decrease in the microbial load. This could be attributed to the fact that charcoal-sand filter is made up of a number of layers of materials with different textures and filtering capacity: fine sand, coarse sand, charcoal and gravel (12).

Table 4. Physiochemical properties of raw and treated water samples.

Dhysics showing   Dougmotous	Mean Value						WIIO Standard
Fuysicochemical Farameters	RW	CSF	SH	MT	MT-CSF	MTFSH	WHO Standard
pH	7.45	7.26	7.04	6.20	6.8	6.9	6.5-8.5
Temperature	25	24	24	25	25	26	25.30
TDS (mg/L)	64	10	44	0.6	20	22	500
Hardness (mg/L)	65.3	20	20	52.4	20	20	100
Turbidity (NTS)	2.25	1.35	0.65	0.20	0.17	0.15	5.0
EC (mScm <sup>-1</sup> )	0.19	0.15	0.14	0.18	0.38	0.14	1.0

Key: RW = Raw Water, CSF = Charcoal sand filtered water, SH = Solar heated, MT = Moringa treated, MT-CSF = Moringa treated filtered water, MTFSH = Moringa treated, filtered solar heated water, TDS = Total dissolved solid, EC = Electrical Conductivity

<b>Tuble 5.</b> Organolepile properties of treated surface wat	Table 5. (	Organoleptic (	properties of	f treated :	surface wate
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Quality nonemator	Samples	N	Moon	Standard Doviation	Standard Erman	95% Confidence interval for mean			
Quanty parameter	Samples	IN	wiean	Standard Deviation	Stanuaru Error	Lower bound	Upper bound		
Taste	GW	20	3.35	1.725	0.386	2.54	4.16		
	PW	20	2.55	1.395	0.312	1.90	3.20		
	CSF-MTW	20	1.95	1.050	0.235	1.46	2.44		
	SW	20	2.90	0.852	0.191	2.50	3.30		
	BH	20	3.60	0.995	0.222	3.13	4.07		
	Total	100	2.87	1.353	0.135	2.20	3.14		
Odour	GW	20	2.70	1.380	0.309	2.05	3.35		
	PW	20	3.05	0.759	0.170	2.69	3.41		
	CSF-MTW	20	2.65	1.843	0.412	1.79	3.51		
	SW	20	4.00	1.414	0.316	3.34	4.66		
	BH	20	3.60	1.429	0.320	2.93	4.27		
	Total	100	3.20	1.477	0.148	2.91	3.49		
Clarity	GW	20	2.35	1.531	0.342	3.55	3.07		
	PW	20	2.10	1.294	0.289	1.63	2.71		
	CSF-MTW	20	3.55	1.050	0.235	1.49	4.04		
	SW	20	2.35	0.813	0.812	3.06	2.73		
	BH	20	3.20	1.642	0.139	1.97	3.97		
	Total	100	2.71	1.395	0.139	2.43	2.99		
Appearance	GW	20	3.85	0.366	0.082	3.68	4.02		
	PW	20	3.30	0.979	0.219	2.84	3.76		
	CSF-MTW	20	3.10	1.294	0.289	2.49	3.71		
	SW	20	3.65	0.813	0.182	3.27	4.03		
	BH	20	2.30	1.261	0.282	1.71	2.89		
	Total	100	3.24	1.120	0.112	3.02	3.46		
Overall Acceptability	GW	20	3.00	1.338	0.299	2.37	3.63		
	PW	20	2.75	1.333	0.298	2.13	3.37		
	CSF-MTW	20	3.25	0.967	0.216	2.80	3.70		
	SW	20	2.60	1.188	0.266	2.04	3.16		
	BH	20	1.85	0.933	0.209	1.41	2.29		
	Total	100	2.69	1.237	0.124	2.44	2.94		
Overall Quality	GW	20	1.55	0.826	0.185	1.16	1.94		
	PW	20	2.65	1.387	0.310	2.00	3.30		
	CSF-MTW	20	2.95	1.317	0.294	2.33	3.57		
	SW	20	2.60	1.314	0.294	1.99	3.21		
	BH	20	2.00	0.795	0.178	1.63	2.37		
	Total	100	2.35	1.242	0.124	2.10	2.60		

This is absolutely in accordance to the report of Oluyege and Omoyajowo (18), which revealed removal of 92 - 97% of total bacteria and coliform respectively from surface water samples after subjected to charcoal-sand filtration combined with solar disinfection. Although, charcoal-sand filtered water could be stored for a longer time, but it cannot instantly purify densely polluted surface water (with 27.3-33.5 NTU). The sand filter container has however been reported to be very efficient in treating well water, borehole and deep stream water with low turbidity (NTU < 10NTU) (3).

It is interesting to present the vast reduction of about 99.9% of bacteria and coliform in the present study after the Omoshio water was coagulated with the seed powder of *Moringa oleifera* and further treated through Charcoal-sand filtration and solar disinfection. This is rather surprising since there are various evidences that have proven the efficiency of plant as a strategic method of purifying drinking water (30). Furthermore, considering recent indications about the possible numbers of water borne pathogens growing resistance to Aluminum sulphate and/or the tendency of serving as a precursor for cancer has alerted reasons for alternative (3).

The observable changes in physiochemical parameters shown in Table 4 supports the findings of Abatneh *et al.* (14), who comparatively analyzed the efficiency of three plant seeds as phytocoagulant and reported *Moringa oleifera* as most effective among others. Total dissolved solid reduced from 64 in the untreated sample to 22 in the treated sample. Turbidity decreased from 2.25 to 0.15, pH from 7.45 to 6.9. The electronic conductivity reduced from 0.195 to 0.14, the reduction in the total dissolved solids, pH and conductivity could also be due to the surface area of activated charcoal which absorbs some chemicals from the water samples (31). Reduction in turbidity is as a result of compatibility in the sand filters.

Statistical analysis based on the outcome of a 20 man panel of judges reveals that the filtered, solar heated and treated water samples are slightly different from the commercially produced water samples in terms of odour, colour, taste and overall acceptability. Thus, it is necessary that all the sources of water used for drinking and other domestic purposes in rural setting should be subjected to adequate treatment when feacal pollution is suspected (32).

### 5. Conclusion

The use of charcoal-sand filter for treatment of water cannot be effective without further treatment with other means of disinfection which makes it more effective. It is possible to have this kind of set up in rural communities where there is no access to portable water. Also, much need is to be done on the part of the health authority to increase the awareness of the general populace on the importance of using water from a good source to prevent the risk of containing water-borne pathogens. Conclusively, to emphasize on this research work, charcoal-sand filter complimented with *Moringa oleifera* seed coagulation can be recommended as a good means of eradicating water borne pathogens.

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