BioOne COMPLETE

Bacterial Removal Efficiency of Moringa stenopetala and Cadaba farinosa From Surface Water: Laboratory-Based Cross-Sectional Study

Authors: Tagele Haligamo, Demamu, Ejeso, Amanuel, and Mengistie Beyene, Embialle

Source: Environmental Health Insights, 16(1)

Published By: SAGE Publishing

URL: https://doi.org/10.1177/11786302221111842

Bacterial Removal Efficiency of *Moringa stenopetala* and *Cadaba farinosa* From Surface Water: Laboratory-Based Cross-Sectional Study

Demamu Tagele Haligamo[1] (iD), Amanuel Ejeso[2] (iD)
and Embialle Mengistie Beyene[2] (iD)

[1]Department of Environmental Health, Arba Minch University, Arba Minch, Ethiopia. [2]Department
of Environmental Health, Hawassa University, Hawassa, Ethiopia.

ABSTRACT

BACKGROUND: Consumption of polluted surface waters are leading to waterborne diseases, especially in developing countries, which results in the deaths of millions of people annually around the world. Ethiopia, like the rest of developing countries, suffers a lot of water-associated health problems. Chemical disinfectants are in use to disinfect water with some drawbacks like expensiveness, unavailability, and detrimental effect on human health. Researchers are on the search for non-expensive and locally available methods, and natural plants are the ones in the study. Thus, this study is designed to test *Escherichia coli (E. coli)* removal efficiency of *Moringa stenopetala* (*M. stenopetala*) and *Cadaba farinosa* (*C. farinosa*) from surface water.

METHODS: A cross-sectional study was conducted from June to July 2021. A 14 L water sample was collected from Lake Hawassa. A 30, 60, and 100 mg weights of the leaf and seed powder dosages of *M. stenopetala* and *C. farinosa* at contaminant settling times of 30, 60, and 90 minutes were used. Each 1-L water sample was treated with each of the dosages. *E. coli* count, temperature, pH and turbidity were measured using standard methods for water and wastewater analysis. Statistical package for social sciences (SPSS) version.23 was used for analysis. Treatment differences between plant parts and association between variables were also tested.

RESULT: The result indicated that raw water samples having 18 initial *E. coli* colonies per 100 mL of water showed zero *E. coli* colonies per 100 mL of water after treatment with 30 mg dosage of *M. stenopetala* seed, 30 mg dosage of *C. farinosa* seed, and 60 mg dosage of *M. stenopetala* leaf after 90 minutes of settling time, but *C. farinosa* leaf was unable to reduce *E. coli* colonies to 0 per 100 mL of water. *M. stenopetala* leaf showed the highest turbidity reduction of 83.3% at 60 mg dosage. A pH of 7.30 and 8.50 and a temperature of 20°C to 23.5°C were recorded. There was a significant difference in *E. coli* removal between *C. farinosa* seed and leaf. Turbidity was identified as a factor that positively affects *E. coli* removal during *M. stenopetala* seed and leaf. Dosage and settling time were also identified as predictors of *E. coli* removal.

CONCLUSION: *M. stenopetala* and *C. farinosa* have antimicrobial properties against *E. coli*, but only *M. stenopetala* showed *E. coli*, turbidity, and pH values within the recommended World Health Organization standards. So, we suggest *M. stenopetala* as a promising natural disinfectant that needs attention from organizations working on the water.

KEYWORDS: Bacteria removal, water treatment, natural plants, coagulation, disinfection

Background

Water is a vital natural resource for all life on earth which is not always suitable for all uses.[1] Globally, surface waters are polluted with pathogens in most countries but are widely recorded in the developing world. Consumption of these polluted surface waters leads to waterborne disease outbreaks.[2] Globally, about 663 million people depend on unimproved water sources and about 2.1 billion use contaminated water.[3] According to World Health Organization (WHO) report, only 15% to 20% of the world's population have access to safe drinking water.[4] It was also reported that more than 700 million people, which are mainly living in developing countries, are without access to improved and adequate water.[5] Each year, unsafe drinking water contributes to more than 25 million cases and 250 000 deaths from enteric fevers (typhoid and paratyphoid).[6,7]

In sub-Saharan African countries, only 24% of the population uses safely managed water sources[8] and there is high morbidity in the age group below 5 years ranging from 10% to 35% which is mainly caused by a lack of safe water.[9,10] In Ethiopia, communities are highly affected by waterborne diseases due to a lack of access to a potable water supply that obliges them to use unimproved and unsafe sources of water for domestic purposes.[11] Providing safe, affordable and reliable water is an essential goal, yielding optimal health gains and contributing to the international targets for poverty reduction, nutrition, childhood survival, and environmental sustainability.[7] Household water treatment methods like chlorine-based disinfectants, filtration, solar disinfection, and boiling[12] are best for sub-Saharan African countries.[13] However, only a small number of households are using them, and their consistency in use is dropping over time.[14,15]

A millennium development goal (MDG) was aimed to halve the proportion of people without sustainable access to safe drinking water by 2015. However, Ethiopia achieved 57% of the goal[16] which still needs more effort to provide safe drinking water. The country also launched a health extension program with water supply components in 2003 but was still unable to address what was planned.[17]

Chemical water treatment methods are in use, but behind their advantages, there are also disadvantages like expensiveness, unavailability, detrimental effect on health and unskilled labor to handle chemicals.[1,18-21] Aluminum sulfate (alum) has been indicated to be a causative agent in neurological diseases and the induction of Alzheimer's disease. Chlorine and its compounds are also known to produce trichloromethane, a cancer precursor.[1,20,22,23]

Scientists are on the search for water treatment methods which are non-expensive and can be easily handled by the local community.[24] Natural plants are the ones in the study and some were identified as effective in reducing agents causing waterborne diseases and turbidity of water. They are also stated as having good advantages like biodegradable sludge production, being virtually toxin-free, relatively cheap to obtain, and being locally available.[19,22,25] These advantages currently attracted the attention of many researchers to study them for water treatment purposes as a coagulant, disinfectant or heavy metal removal.

This study was used to test the treatment efficiency of *M. stenopetala* and *C. farinosa*. A study conducted at Jimma University in Ethiopia reported that a 93% reduction in fecal coliform was recorded after treatment with 60 mg weight of *C. farinosa* root powder.[24] It was also reported that crushed seeds of *M. stenopetala* were effective in reducing bacterial contamination by 90% to 99.9%.[1]

There are factors identified by different scientific researches like turbidity, pH, contact time, settling time, stirring time, temperature, and dosage/concentration that might affect the treatment efficiency of natural plants in reducing microbes or turbidity or heavy metals from water.[1,22,24,26-28] The effect of turbidity, pH, settling time, temperature and dosage were considered in this study. Mixing/stirring time plus settling time is equal to contact time-based on this study.

A study conducted in Ethiopia also stated that *M. stenopetala* leaf had 28.44% protein.[29] Another study reported that *M. stenopetala* seed contains 42.6% of protein.[30] *C. farinosa* seed contains 26.72% protein.[31] Also, *C. farinosa* leaf contains 15.2% to 18.2% of protein, crude fiber and ash, respectively.[32] Protein composition (especially water-soluble proteins) in plant parts has been predicted as removing microorganisms by serving as a coagulant or acting directly as growth inhibitors of the microorganisms.[1] Because of this, *C. farinosa* (leaf and seed) and *M. stenopetala* (leaf and seed) with high protein composition[30-33] were selected for this study. There is no previous study conducted on bacterial removal efficiency of *C. farinosa* (leaf and

seed) and *M. stenopetala* leaf and little attention has also been given in research world to *M. stenopetala* in treating water. Thus, the aim of this study is to identify the most efficient plant part in removing *E. coli* from surface water between *C. farinosa* (leaf and seed) and *M. stenopetala* (leaf and seed).

Previous studies were used as a base to select appropriate dosages and settling times that were used to treat surface water under this study and explained as follows. A study in Nigeria on the antimicrobial efficiency of *M. oliefera* seed in treating water used 3 dosages such as 50, 100, and 150 mg. It showed a total coliform reduction of 68.33%, 85%, and 97.5% at 50, 100, and 150 mg dosage per liter of water, respectively. The study also shows 96.3%, 96.6%, and 97.4% turbidity reduction at 50, 100, and 150 mg dosage per liter of water, respectively.[24] A study conducted at Jimma University, Ethiopia on contaminant removal of water with *C. farinosa* root and leaf powder was used 60, 100, 300, 500, and 1000 mg dosages, and 15, 30, 45, 60, 75, 90, and 150 minutes of settling times. It showed that 60 mg dosage was efficient in removing fecal coliform by 93% with both *Moringa oleifera* (*M. oleifera*) seed and *C. farinosa* root powder. It also shows 98.6% and 99.4% total coliform reduction by *C. farinosa* root powder dosages of 60 and 100 mg, respectively. It also indicated that there was 90% and 99% turbidity reduction in 30 and 90 minutes of settling time, respectively, after treatment with 60 mg *C. farinosa* root powder.[34] It was revealed that 100 mg/L *M. stenopetala* seed is equally effective in water clarification when compared with 200 mg/L of *M. oliefera* seed.[1] So, based on these studies, 3 dosages such as 30, 60, and 100 mg, and 3 settling times such as 30, 60, and 90 minutes were selected for this study.

The water samples used for this study were collected from Lake Hawassa. It is the smallest Lake and the highest in altitude among the Ethiopian Rift valley closed basin lakes, located between 06°58′ to 07°14′ North latitudes and 38°22′ to 38°28′ East longitudes with an elevation of 1697 m above sea level. Its annual rainfall average is 969 mm.[35] The lake is a shallow lake with an average depth of 13.3 m and a maximum depth of 23.4 m.[36] It has a surface area of 90 km[2] and a drainage area of 1250 km[237] and a water storage capacity of the lake is 1.36 km[3].[38]

Methodology

Study area

This study was conducted in the environmental health laboratory at Hawassa University, College of Medicine and Health Science in Hawassa City of Sidama region in Ethiopia.

M. stenopetala and *C. farinosa* are plants used for this study and were collected manually for free from farmers' land around Holte Town of Derashe special Woreda in southern Ethiopia from June 17 to 21, 2021. The plants were identified for collection based on their botanical, biological, and ecological description.[39-43] Each 50 g weight of 1 plastic bag of *M. stenopetala* leaf, *C. farinosa* leaf, and *C. farinosa* seed were collected

Figure 1. *M. stenopetala* and *C. farinosa* seed pod and leaf, respectively, Holte Town in southern Ethiopia, 2021.

Figure 2. *M. stenopetala* and *C. farinosa* seed and leaf powder, respectively, Arba Minch city in southern Ethiopia, 2021.

separately. Also, 20 matured seed pods of *M. stenopetala* were collected and made dry. Matured seeds were collected from the 2 plants as shown in Figure 1. Each seedpod of *M. stenopetala* contains 12 to 35 round seeds[40] and it was noted that 1 seed kernel of *Moringa* treats 1 L of water.[1] So, a total of 10 pods of *M. stenopetala* containing the required seeds were used for this study. They were collected in the morning starting from 6:30 Am to 8:30 Am and taken to the laboratory immediately. Figure 1 shows *M. stenopetala* seed pod, *C. farinosa* seed fruits, *M. stenopetala* leaf, and *C. farinosa* leaf, respectively.

Study design and period

A cross-sectional study was conducted to assess the bacterial removal efficiency of *M. stenopetala* and *C. farinosa* (leaf and seed) from surface water from June 30 to July 06, 2021.

Preparation of M. stenopetala *and* C. farinosa *seed and leaf powder*

The *M. stenopetala* seeds with shells were removed from the pods and air-dried for 2 days. The shells were removed and the seed kernels were dried in a plastic bag at ambient temperatures (23°C-25°C) for 5 days before milling. Similarly, the collected leaves of *M. stenopetala* were also shaded to complete dryness at ambient temperatures (23°C-25°C) for 5 days before milling.[23] The seed kernels and dried leaves were ground into a medium-fine powder with a local mill to achieve solubilization of active ingredients in the seed.[26] These crushed kernels and leaves were sieved using a sieve size of <300 μm.[34] Then, the powders of the leaf and seed were packed in a small plastic bag separately for safety (as seen in Figure 2) and kept in the

laboratory under an ambient temperature of 22°C to 25°C and stored in a desiccator to create and maintain a dry environment until treatment and analysis.

C. farinosa seeds with very thin coats/fruits were collected from the trees in a plastic bag. The coats were removed from the seeds. Then after, the seeds were dried in a plastic bag at ambient temperatures (23°C-25°C) for 5 days before milling. The collected leaves of *C. farinosa* were also shaded to complete dryness at ambient temperatures (23°C-25°C) for 5 days before milling.[23] Then after, the same procedure was followed as used for *M. stenopetala* seed and leaves. Figure 2 shows powders prepared from *M. stenopetala* seed, *C. farinosa* seed, *M. stenopetala* leaf, and *C. farinosa* leaf, respectively.

Water sample collection

Water samples for all the tests were collected from Lake Hawassa which is found encircling one side of the city. The reason why water samples were collected from surface water (Lake Hawassa) is to take natural waters instead of preparing synthetic polluted water in the assumption that the rural communities on the other side of the lake are speculated to use the raw surface waters for domestic purposes. So, the treatment efficiency of the leaf and seed of the 2 plants was tested against the actual water used by the rural community.

The water samples were collected following surface water sampling procedures[44,45] using high-density polyethylene (HDPE) bottles washed 3 times with sample water before collection.[46] First, the water sample was collected into a disinfected 20 L container[34] up to 17 L volume by using a sterilized 300 mL plastic sample bottle by holding the lower part of the bottle and submerging it to a depth of about 20 to 30 cm, with

the mouth facing slightly upwards. Then after, the water sample in the 20 L plastic container was shaken/mixed and added to 48 sterilized 300 mL volume sample bottles. Finally, the sample bottles with sample waters were capped and transported to the laboratory in an icebox within 15 minutes. After reaching the laboratory, the water samples were shaken and analyzed and placed in the refrigerator until treatment.

Experimental phase

Before starting treatment, the raw water sample *E. coli*, pH, temperature and turbidity were measured by using the membrane filtration method, digital pH meter, liquid-in-glass thermometer, and digital turbidity meter, respectively.

During treatment, each 1-L volume water sample was added to 3 beakers for treatment. Then, the beakers were labeled and placed on a magnetic stirrer at a speed of 60 revolutions per minute (rpm). The powders (30, 60, and 100 mg dosages) per each part of *M. stenopetala* and *C. farinosa* were measured on an analytical balance and manually added to the 3 beakers based on the respective dosages labeled on them. The water samples were then mixed with the aid of the magnetic stirrer for 5 minutes.[24,47] After mixing, the water was allowed to settle for 30, 60, and 90 minutes on which analysis was done in each case. Finally, after treatment, the *E. coli*, turbidity, temperature and pH of treated water samples were measured to calculate the improvement from the reading before treatment.

This study followed the methods of a study conducted in Nigeria at Ahmadu Bello University on the antimicrobial efficiency of *Moringa oleifera* (*M. oleifera*) seed in the treatment of greywater.[34]

Bacteriological analysis

A membrane filtration technique was used, both before and after the treatment of the water samples, to determine the amount of *E. coli* in the water. A total of 78 membrane filters with a pore size of 0.45 µm were used. Forty Petri dishes were sterilized and prepared for use on each day of the study, before starting water sample treatment and laboratory analysis. First, a 100 mL volume water sample was filtered on the filtration apparatus through a membrane filter. Then, the membrane filter was taken from the filtration apparatus by sterilized forceps and placed on top of an absorbent pad saturated with the Membrane Lauryl Sulfate Broth (MLSB) media on sterilized petri-dish. Then, the petri-dish lid was replaced and labeled with sample identification. The Petri-dishes were then placed into the incubator (model DHP9052) and incubated for 24 hours at 44ºC. Upon completion of the incubation period, the numbers of *E. coli* were enumerated using a digital colony counter based on the yellow color bacteria colonies formed on the membrane filter. Finally, *E. coli* concentrations were reported as colony-forming units per 100 mL of water (CFU/100 mL).[50-53]

Physicochemical parameters analysis

Temperature and pH of water. Water samples temperature was measured by a liquid-in-glass thermometer and pH was measured by a digital pH meter (Model AD8000)[40] in the laboratory both before and after treatment with the 2 plants' leaf and seed powders. A buffer solution calibrated pH meter was used to measure the pH of the water sample in the laboratory. Accordingly, the results were read from the display.[28]

Turbidity. A digital turbidity meter (MAX electronics (India)) was used to measure the turbidity of the water samples both before and after treatment with the 2 plants' leaf and seed powders. First, the instrument was warmed-up for 2 to 3 minutes and calibrated. Then after, a test tube was filled with the sample water to the 10 mL mark and placed in a tube holder, and a turbidity meter reading was taken directly from the instrument. The results were read directly from the digital turbidity meter display and reported as the nephelometric Turbidity Unit (NTU).[28]

Quality assurance. To ensure data quality, a 1-day training was given for the 2 plants, leaf and seed collectors. The water samples were collected by the principal investigator. Before actual treatment and analysis, laboratory instruments were checked for proper functioning and some were calibrated. Before analysis was undertaken, all the reagents used were checked for expiration dates. All plastics and glassware utilized were disinfected with appropriate detergent and water solution, which was then rinsed thoroughly with distilled water. All containers for bacteriological analysis were sterilized in an autoclave (Model SA-300H) at 1210°C for 15 minutes. A flame was used to sterilize forceps and filtration apparatus in between each use. Triplicate tests were conducted on raw water samples before treatment by the plant parts.

Data analysis

Data from the result of this study were analyzed by using the statistical package for social sciences (SPSS) version.23. The significance of the difference in *E. coli* removal between *M. stenopetala* leaf, *M. stenopetala* seed, *C. farinosa* leaf, and *C. farinosa* seed was tested by using Friedman rank tests. In Friedman, the test is significant when *P*-value is less than .05 at a 95% confidence interval. And, the significance of the differences in *E. coli* removal within plant parts was tested by using the Wilcoxon-signed rank test, which is significant at a *P*-value less than .05. In addition to this, the factors that have an association with *E. coli* removal were determined by using the Kendall's-Tau correlation, which shows a significant correlation/association at *P*-value less than .05.

Ethical consideration

Ethical clearance was obtained from Hawassa University, College of Medicine, and Health Science ethical board

committee. During plant leaf and seed pod collection, the purpose of the study was clearly explained to farmers from which plant leaf and seed were collected. Informed verbal consent was obtained from participants for plant collection.

Result

Treating Lake Hawassa water sample by M. stenopetala *and* C. farinosa

Lake Hawassa water sample with an initial *E. coli* of 18 CFU per 100 mL of water, a pH of 7.91, a temperature of 22°C and turbidity of 12 NTU was treated with 30, 60, and 100 mg dosages of *M. stenopetala* and *C. farinosa* for a settling time of 30, 60, and 90 minutes. After treatment with *M. stenopetala* seed and leaf, there was 0 CFU per 100 mL of water. In reducing *E. coli*, the seed shows maximum efficiency at a minimum dosage of 30 mg after 90 minutes of settling time and the leaf shows maximum efficiency at a minimum dosage of 60 mg after 90 minutes of settling time. *M. stenopetala* leaf also shows the highest reduction in turbidity by 83.3% (from 12 NTU to 2 NTU) at 60 mg dosage after 90 minutes of settling time.

Similarly, in water treatment with *C. farinosa* seed, there was 0 CFU per 100 mL of water. In reducing *E. coli*, the seed was more efficient at a minimum optimum dosage of 30 mg after 90 minutes of settling time compared to a similar amount of leaf. *C. farinosa* leaf showed 62% *E. coli* removal (from 18 CFU to 7 CFU per 100 mL of water) at a minimum optimum dosage of 30 mg after 30 minutes of settling time which shows its inability to reduce *E. coli* to 0 CFU per 100 mL of water.

The pH was recorded between 7.30 and 8.50 at a temperature of 20°C to 23.5°C. The details of the results are shown in Table 1.

After treatment of water samples by both *M. stenopetala* seed and leaf, there were cell colony bursts on the membrane filter after 24-hour incubation, irrespective of the presence of *E. coli* colonies. The color of the membrane filters was also changed due to the color of seed and leaf powder in addition to the color of Membrane Lauryl Sulfate Broth (MLSB) media used as shown in Figure 3.

E. coli *and turbidity removal efficiency between plants and their parts*

The significance of differences in *E. coli* removal among *C. farinosa* and *M. stenopetala* after treatment of Lake Hawassa water samples was tested by using Friedman rank test. The test is significant at a *P*-value < .05 at 95% confidence interval. And the significant difference in *E. coli* removal within plant parts was tested by using the Wilcoxon-signed rank test, which is significant at a *P*-value < .05.

The result of the statistical analysis revealed that, after treatment of Lake Hawassa water sample, the Friedman rank test shows a significant difference between *C. farinosa* leaf, *C. farinosa* seed, *M. stenopetala* leaf, and *M. stenopetala* seed in

removing *E. coli* (*P*-value = .005, $X^2 = 13.000$, df = 3, N = 9). Comparing plant parts using Wilcoxon-signed rank test, there was no significant difference between *M. stenopetala* seed and leaf in *E. coli* removal (*P*-value = .062). But, there was a significant difference between *C. farinosa* seed and leaf in removing *E. coli* (*P*-value = .011). Based on the positive ranks from the value of the test statistics, *C. farinosa* seed had higher *E. coli* removal efficiency compared to its leaf. *C. farinosa* leaf and *M. stenopetala* leaf also show a significant difference in *E. coli* removal (*P*-value = .017). Based on the value of the test statistics, *M. stenopetala* leaf had higher *E. coli* removal efficiency compared to *C. farinosa* leaf. The test also shows no significant difference between *C. farinosa* seed and *M. stenopetala* seed in *E. coli* removal (*P*-value = .461).

Result from Kendall's Tau correlation

The result of this study discovered that turbidity shows a strong positive significant correlation with *E. coli* removal during *M. stenopetala* seed (*P*-value = .022, correlation coefficient = .647). However, temperature and pH have no association with *E. coli* removal during *M. stenopetala* seed. Turbidity also shows a strong positive correlation with *E. coli* removal during *M. stenopetala* leaf (*P*-value = .009, correlation coefficient = .775). However, temperature and pH have no association with *E. coli* removal during *M. stenopetala* leaf. And similarly, turbidity, temperature and pH have no association with *E. coli* removal after treatment of Lake Hawassa water sample by *C. farinosa* seed and leaf.

E. coli *removal at different doses and settling time*

Figure 4 shows the effect of settling time on *E. coli* removal from Lake Hawassa water samples using *M. stenopetala*. As depicted in the result, there was a successive reduction in *E. coli* as settling time increased both for the leaf and seed. *M. stenopetala* seed was more efficient in removing *E. coli* at a smaller settling time compared to its leaf. The line moved to negative shows speed of removal efficiency.

The effect of dosage on *E. coli* removal from Lake Hawassa water sample by using *M. stenopetala* is displayed in Figure 5. The result found that as the dosage of *M. stenopetala* increased the *E. coli* removal also increased. Treated water with 0 CFU per 100 mL of water was achieved after treatment with *M. stenopetala* seed at a minimum optimum dosage of 30 mg/L after 90 minutes of settling time. *M. stenopetala* seed showed higher *E. coli* removal to 0 CFU per 100 mL of water at a smaller dosage when compared to *M. stenopetala* leaf. It is described in Figure 5.

The effect of settling time on *E. coli* removal from Lake Hawassa water sample by *C. farinosa* is shown in Figure 6. As depicted in the result, there was a reduction in *E. coli* as settling time increased with *C. farinosa* seed treatment. There is no relationship between *C. farinosa* leaf and settling time

Table 1. Water quality result before and after treatment with *M. stenopetala* and *C. farinosa*, Hawassa city in Ethiopia, 2021.

PLANT PART	DOSAGE	SETTLING TIME	PH	TEMPERATURE (°C)	TURBIDITY (NTU)	*E. COLI* (CFU/100ML)	*E. COLI* REMOVAL EFFICIENCY IN %
Before treatment			7.91	22	12	18	
After treatment by *M. stenopetala* Leaf powder	30mg/L	30min	8.10	23	8	15	16.7
		60min	7.80	23	3	6	66.7
		90min	8.24	23	3	3	83.3
	60mg/L	30min	7.68	23	7	10	44.4
		60min	7.74	23	4	6	66.7
		90min	7.95	23.5	2	0	100
	100mg/L	30min	7.77	23	8	7	61.1
		60min	7.82	23	4	4	77.8
		90min	8.07	23	4	0	100
After treatment by *M. stenopetala* Seed powder	30mg/L	30min	7.30	22	12	14	22.2
		60min	8.13	22	12	8	55.6
		90min	7.82	22.5	8	0	100
	60mg/L	30min	7.92	21	12	9	50
		60min	8.15	23	7	0	100
		90min	7.93	22	5	0	100
	100mg/L	30min	8.30	20	8	3	83.3
		60min	8.16	22	5	0	100
		90min	7.92	22	4	0	100
After treatment by *C. farinosa* Leaf powder	30mg/L	30min	8.1	23	8	7	61.1
		60min	8.0	22.5	5	16	11.1
		90min	8.0	23	8	9	50
	60mg/L	30min	8.5	23	11	18	0
		60min	7.8	22.5	8	15	16.7
		90min	8.0	23	6	13	27.7
	100mg/L	30min	8.0	23	12	24	−33.3
		60min	7.8	23	9	17	5.5
		90min	8.0	23	10	13	27.7
After treatment by *C. farinosa* Seed powder	30mg/L	30min	8.5	22.5	12	10	44.4
		60min	8.4	22.5	11	5	72.2
		90min	8.3	23	8	0	100
	60mg/L	30min	7.9	22	8	13	27.7
		60min	8.3	22.5	8	2	88.9
		90min	8.2	23	10	0	100
	100mg/L	30min	7.9	22.5	12	10	44.4
		60min	7.9	22.5	9	3	83.3
		90min	8.2	23	11	0	100

Figure 3. Cell colony bursts after treatment by *M. stenopetala* leaf and seed, Hawassa city in Ethiopia, 2021.
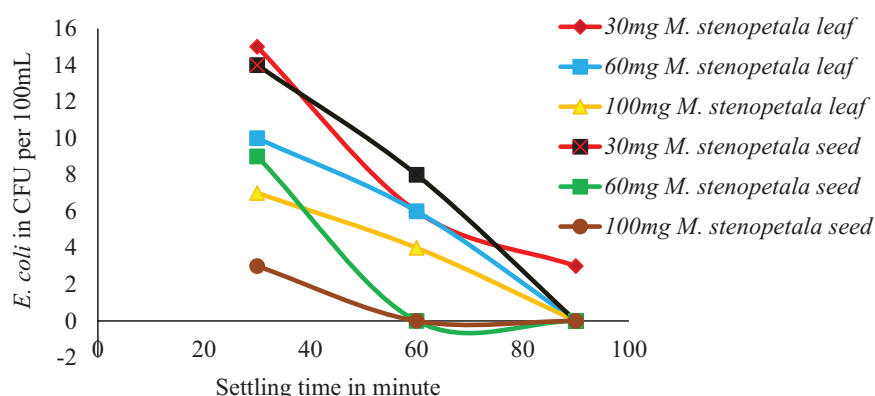
Figure 4. Effect of settling time on *E. coli* removal, Hawassa city in Ethiopia, 2021.
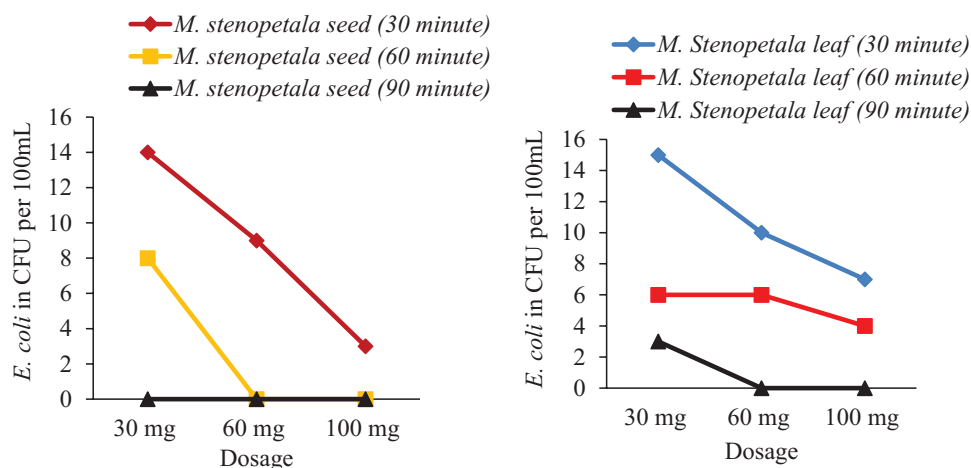
Figure 5. Effect of *M. stenopetala* dosages on *E. coli* removal, Hawassa city in Ethiopia, 2021.

because it shows a slight increment in *E. coli* up to 60 minutes followed by decrement which is not associated with settling time.

Figure 7 illustrates the effect of *C. farinosa* dose on *E. coli* removal from Lake Hawassa water sample. It was found that there was a noticeable reduction in *E. coli* as *C. farinosa* seed dosage increased. The treated water using *C. farinosa* seed has 0 CFU per 100 mL at a minimum optimum dosage of 30 mg after 90 minutes of settling time. On the contrary, *C. farinosa* leaf was not as effective in reducing *E. coli* to 0 CFU per 100 mL of water and there was no relationship between the leaf dosage and *E. coli* removal.

Discussion

One of the good approaches used in the provision of safe water for rural communities of developing countries could be the use of natural plants.[24] Some of such natural disinfectants could be *M. stenopetala* and *C. farinosa*. In this study, the efficiency of *M. stenopetala* and *C. farinosa* in removing *E. coli* from surface water was assessed.
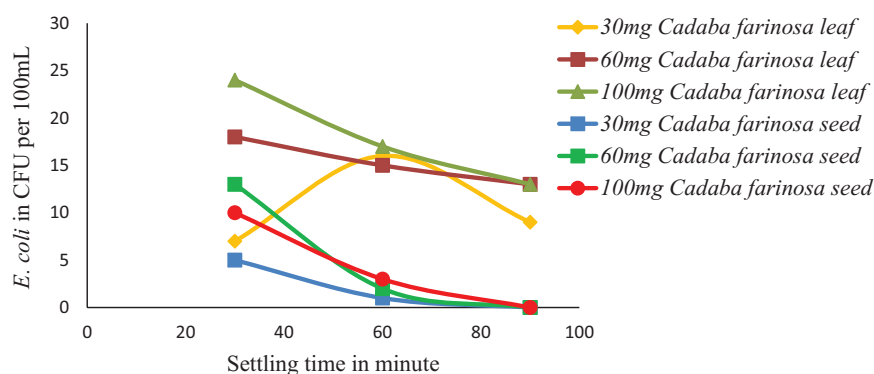
Figure 6. Effect of settling time on *E. coli* removal, Hawassa city in Ethiopia, 2021.
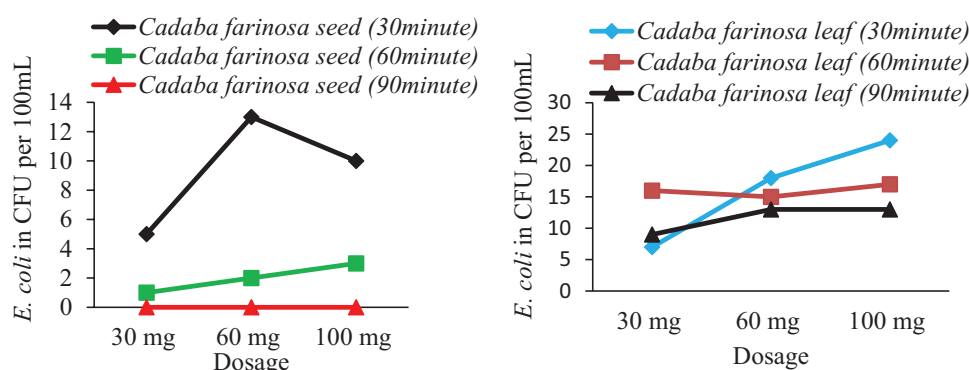
Figure 7. Effect of *C. farinosa* dosages on *E. coli* removal, Hawassa city in Ethiopia, 2021.

The findings of this study show that there is no significant difference in *E. coli* removal between *M. stenopetala* leaf and *M. stenopetala* seed (*P*-value = .062). This might be due to the nature of both plant parts for having water-soluble proteins (active agents) which are stated as removing bacteria through coagulation or acting directly as growth inhibitors of the microorganisms.[1,30,33] This study also shows that there is similar treatment efficiency between *C. farinosa* seed and *M. stenopetala* seed in *E. coli* removal (*P*-value = .461).

Treatment of water with 100 mg *C. farinosa* leaf, seems to slightly increase the *E. coli* from 18 to 24 CFU per 100 mL with a turbidity of 9 NTU. This might be because turbidity above 2 NTU affects the microbiological quality of water by complicating the detection of bacteria or by protecting/shielding pathogens from the action of disinfectants.[54]

This study revealed that after a certain reduction in turbidity, in a few of the samples, the turbidity starts increasing, especially during *C. farinosa* leaf and seed. This might be due to the "charge reversal" reaction which is stated as common in natural plant coagulants in which after subsequent turbidity reduction the formed flock becomes destabilized and result in a successive increase in turbidity.[24,55]

Treatment of the surface water with *C. farinosa* seed in this study resulted in 0 CFU per 100 mL (100% removal efficiency), which is slightly higher than the Jimma University study by *C. farinosa* root which showed 99.4% and 93% total coliform and fecal coliform removal, respectively.[24] This higher efficiency in

the present study might be due to the high protein composition in *C. farinosa* seed (26.72%) compared to the root (4.22%), which was stated as a plant component able to act as a disinfectant.[1,31,56] *M. stenopetala* seed was more efficient in removing *E. coli* from surface water samples up to 0 CFU per 100 mL (100% removal efficiency), which is also confirmed by a study that reported bacterial load reduction of 90% to 99.9% by whole crushed seeds of *M. stenopetala*.[1]

The finding of this study also designated that 0 CFU per 100 mL (100% removal efficiency) was achieved at 60 mg/L dosage of *M. stenopetala* seed powder. The findings of this study are higher than a study conducted in Nigeria using *M. oleifera* seed which showed 85% antimicrobial efficiency at 100 mg/L dosage.[34] A study conducted in Ethiopia also reported that *M. oleifera* seed powder showed the highest bacterial load reduction of 97.5% at 80 mg/L dosage which is slightly lower than the result of this study.[28] This might be due to the high water-soluble protein content in *M. stenopetala* seed (42.6%) when compared to *M. oleifera* seed (23%-27%), which is a component able to act as a disinfectant.[1,30,57]

It was confirmed that 100% *E. coli* removal efficiency can be attained using *M. stenopetala* seed and *C. farinosa* seed at a minimum optimum dosage of 30 mg at 95 minutes of contact time, which is equal in treatment efficiency at 1.5 g per 1 L of water by free chlorine concentration after 30 minutes of contact time. This shows that free chlorine concentration is effective at low concentrations and shortest contact time when compared to

the 2 plants.[58] The result also has similar treatment efficiency with Dolichos lablab (Hyacinth Bean) extract exposed to sunlight after treatment, which showed 100% *E. coli* removal within 2 hours.[25]

The turbidity reduction from 12 NTU to 4 NTU (66.7%) was reached using a 100 mg/L dosage of *M. stenopetala* seed. This result is slightly lower than a study conducted on *M. stenopetala* seed that reported 98.5% turbidity removal by 100 mg/L powder dosage after 60 minutes of settling time. The difference might be due to the variance in the type of raw water used since the present study used surface water while the study in comparison used tannery wastewater.[59] Likewise, a turbidity reduction of 59% was achieved using *C. farinosa* leaf, which is lower than a study conducted at Jimma University which showed a turbidity reduction of 90% by *C. farinosa* leaf.[24] This might be due to differences in the initial turbidity of the raw water used since the initial turbidity of this study was 12 NTU which is lower than the initial turbidity of 938 NTU obtained under the Jimma University study.

The WHO guidelines and Ethiopian water quality standards recommend drinking water must have a maximum permissible turbidity limit of 5 NTU, 6.5 to 8.5 pH and no detectable *E. coli* number per 100 mL of water.[60,61] After treatment of the surface water sample, only *M. stenopetala* seed and leaf dosages showed *E. coli*, pH and turbidity within the recommended standards. For instance, *M. stenopetala* leaf showed *E. coli* of 0 CFU per 100 mL of water, pH of 7.95 and turbidity of 2 NTU after treatment by 60 mg dosage.

Turbidity was identified by this study as a factor that negatively affects *E. coli* removal during *M. stenopetala* seed (*P*-value = .022, correlation coefficient = .647) and leaf (*P*-value = .009, correlation coefficient = .775). As turbidity decreases, there is a subsequent decrease in the number of *E. coli* using *M. stenopetala* seed and leaf. The finding of this study is also supported by the studies done in Nigeria on *M. oleifera* seed that reported a subsequent decrease in total coliform bacteria as turbidity decreases.[23,34] Also, a study conducted at Gondar University in Ethiopia on *M. oleifera* seed reported that there was a decrease in total coliform as turbidity decreased.[28] This might be due to the low turbidity in waters increasing the effectiveness of disinfection[62] and/or it might also be because microorganisms from water can be removed by the action of coagulation.[1]

As confirmed from the result, the dosage has an association with *E. coli* removal using *M. stenopetala* seed, *M. stenopetala* leaf, and *C. farinosa* seed. This means that as dosage increases there was a decrease in the number of *E. coli*. This finding is also supported by studies conducted at Ebonyi State University and elsewhere in Nigeria which reported that as dosage increases there was a decrease in total coliform bacteria.[23,34] A study conducted at Jimma University in Ethiopia also supports the finding as *M. oleifera* seed and *C. farinosa* root powder dosages increased, there was a subsequent decrease in total coliform.[24] This might be because an increase in power

increases the number of active agents in plant parts that act as a disinfectant which in turn results in higher bacterial removal efficiency.

This study exhibited that settling time has a relationship with *E. coli* removal using *M. stenopetala* seed, *M. stenopetala* leaf, and *C. farinosa* seed. As settling time increases there was a consequent decrease in the number of *E. coli* under this study. A study conducted at Jimma University in Ethiopia reported in agreement with the finding of this study that stated an increment in settling time results in a succeeding decrease in fecal coliform during *C. farinosa* root and *M. oliefera* seed.[24] This might be due to an increase in the contact time will provide sufficient opportunity so that the *E.coli* will be accessed by the active agents in the water which made it effective.[44,45]

Conclusion

This study concludes that *M. stenopetala* and *C. farinosa* seeds have the highest *E. coli* removal efficiency from an initial 18 CFU per 100 mL to 0 CFU per 100 mL of water at a minimum optimum dosage of 30 mg at 90 minutes of settling time. *M. stenopetala* leaf was also the second in reducing *E. coli* to 0 CFU per 100 mL of water at 60 mg optimum dosage at 90 minutes of settling time. However, *C. farinosa* leaf was unable to reduce *E. coli* to 0 CFU per 100 mL of water. *M. stenopetala* seed and leaf showed pH, turbidity and *E. coli* values to the recommended standards of WHO guidelines.[23,60,61] Turbidity, settling time and dosage are also found to be the parameter that affects *E. coli* removal using *M. stenopetala* seed and leaf whereas settling time and dosage were also identified as parameters that have a relationship with *E. coli* removal during *M. stenopetala* (leaf and seed) and *C. farinosa* seed.

The pollution status of surface waters varies across seasons. So, consideration of water samples of all seasons of a year should be considered by researchers' study on surface water treatment.[48,49]

Abbreviations

E. coli: Escherichia coli; CFU: Colony-forming Unit; NTU: Nephelometric Turbidity Unit; WHO: World Health Organization; *Moringa stenopetala: M. stenopetala; Cadaba farinosa: C. farinosa; Moringa oleifera: M. oleifera.*

Authors Contributions

DT and AE conceived the idea of the study. DT carried out water sample collection, treatment and analysis. EM and AE helped and guided during water sample collection, treatment

and analysis. DT and AE carried out the data analysis and statistical portion of the study. DT, EM, and AE are participated and reviewed in data analysis, interpretation of results and manuscript writing. DT, EM, and AE reviewed and finalized the manuscript. The authors read and approved the final manuscript.

Availability of Data and Materials

All available data are found in the research article.

Ethics Approval and Consent to Participate

Ethical approval has been obtained from Hawassa University College of Medicine and Health Science Ethics Committee on Human Research.

Consent for Publication

Not applicable.

ORCID iDs

Demamu Tagele Haligamo https://orcid.org/0000-0002-3592-1024

Amanuel Ejeso https://orcid.org/0000-0002-2447-5620

Embialle Mengistie Beyene https://orcid.org/0000-0002-0790-9087

REFERENCES

1. Abiyu A, Yan D, Girma A, Song X, Wang H. Wastewater treatment potential of *Moringa stenopetala* over *Moringa olifera* as a natural coagulant, antimicrobial agent and heavy metal removals. *Cogent Environ Sci*. 2018;4:1433507.
2. Patel CB, Shanker R, Gupta VK, Upadhyay RS. Q-PCR based culture-independent enumeration and detection of Enterobacter: an emerging environmental human pathogen in riverine systems and potable water. *Front Microbiol*. 2016;7:172.
3. WHO/UNICEF. Joint Monitoring Programme for Water Supply and Sanitation (JMP). 2015. https://www.unwater.org/publications/whounicef-joint-monitoring-program-water-supply-sanitation-jmp-2015-update/
4. World Health Organization. Evaluating Household Water Treatment Options: Health-Based Targets and Microbiological Performance Specifications. World Health Organization; 2011.
5. WHO/UNICEF. *Progress on Drinking Water and Sanitation: 2014 Update*. WHO/UNICEF; 2014.
6. Forstinus N, Ikechukwu N, Emenike M, Christiana A. Water and waterborne diseases: a review. *Int J Trop Dis Health*. 2016;12:1-14.
7. Clasen T. Household water treatment and safe storage to prevent diarrheal disease in developing countries. *Curr Environ Health Rep*. 2015;2:69-74.
8. WHO/UNICEF. Safely managed services: accounting for service levels. In: Grojec A, ed. *Progress on Drinking Water, Sanitation and Hygiene: 2017 Update and SDG Baselines*. World Health Organization (WHO) and the United Nations Children's Fund (UNICEF); 2017.
9. Bado AR, Susuman AS, Nebie EI. Trends and risk factors for childhood diarrhea in sub-Saharan countries (1990-2013): assessing the neighborhood inequalities. *Glob Health Action*. 2016;9:30166.
10. Troeger C, Blacker BF, Khalil IA, et al. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of diarrhoea in 195 countries: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Infect Dis*. 2018;18:1211-1228.
11. Bireda M. *Assessment of Water Access and Prevalence of Waterborne Diseases in Rural Community: A Case of Endegagn Woreda, Gurage Zone, SNNPR, Ethiopia*. St. Mary's University; 2015.
12. Sobsey MD, Stauber CE, Casanova LM, Brown JM, Elliott MA. Point of use household drinking water filtration: a practical, effective solution for providing sustained access to safe drinking water in the developing world. *Environ Sci Technol*. 2008;42:4261-4267.
13. Geremew A, Damtew YT. Household water treatment using adequate methods in sub-Saharan countries: evidence from 2013–2016 demographic and health surveys. *J Water Sanit Hyg Dev*. 2020;10:66-75.
14. Waddington H, Snilstveit B, White H, Fewtrell L. Water, sanitation and hygiene interventions to combat childhood diarrhoea in developing countries. *Sythn Rev*. 2009:17-22.
15. Rosa G, Clasen T. Estimating the scope of household water treatment in low- and medium-income countries. *J Trop Med Hyg*. 2010;82:289-300.
16. USAID. USAID investment connect communities to clean drinking water sources. 2020.
17. Wang H, Tesfaye R, Ramana GNV, Chekagn CT. *Ethiopia Health Extension Program: An Institutionalized Community Approach for Universal Health Coverage*. World Bank; 2016.
18. Peter A, Walter A, Wagai S, Joseph O. Antibacterial activity of *Moringa oleifera* and *Moringa stenopetala* methanol and n-hexane seed extracts on bacteria implicated in water borne diseases. *Afr J Microbiol Res*. 2011;153-157.
19. Bhattacharjee T, Gidde MR, Bipinraj N. Disinfection of drinking water in rural area using natural herbs. *Int J Eng Res Dev*. 2013;5:7-10.
20. Graham N, Gang F, Fowler G, Watts M. Characterisation and coagulation performance of a tannin-based cationic polymer: a preliminary assessment. *Colloids Surf A: Physicochem Eng Asp*. 2008;327:9-16.
21. Vijayaraghavan G, Sivakumar T, Kumar AV. Application of plant based coagulants for waste water treatment. *Int J Adv Eng Res Stud*. 2011;1:88-92.
22. Ugwu SN, Umuokoro AF, Echiegu EA, Ugwuishiwu BO, Enweremadu CC. Comparative study of the use of natural and artificial coagulants for the treatment of sullage (domestic wastewater). *Cogent Eng*. 2017;4:1365676.
23. Alo M, Anyim C, Elom M. Coagulation and antimicrobial activities of *Moringa oleifera* seed storage at 3°C temperature in turbid water. *Adv Appl Sci Res*. 2012;3:887-894.
24. Awoke A, Ambelu A, Legesse W. Treatment of raw water using *forssk farinosa* shrub, a traditional water clarifier by "Tseme Bena" people. *Ethiop J Health Sci*. 2006;16:121-129
25. Unnisa SA, Deepthi P, Mukkanti K. Efficiency studies with Dolichos lablab and solar disinfection for treating turbid waters. *Journal of Environmental Protection Science*. 2010;4:8-12.
26. Gebino G, Govindan N. Use of *Moringa (Moringa stenopetala)* seed extract for removal of some anionic dyes (direct and reactive dyes) in textile wastewater. *Curr Trends Fash Technol Textile Eng*. 2018;4:64-73.
27. Seghosime A, Awudza JAM, Buamah R, Ebeigbe AB, Kwarteng SO. Effect of locally available fruit waste on treatment of water turbidity. *Civil Environ Res*. 2017;9:7-15.
28. Delelegn A, Sahile S, Husen A. Water purification and antibacterial efficacy of *Moringa oleifera* lam. *Agric Food Secur*. 2018;7:1-10.
29. Tekle A, Belay A, Kelem K, W/Yohannes M, Wodajo B, Tesfaye Y. Nutritional profile of *Moringa stenopetala* species samples collected from different places in Ethiopia. *Eur J Nutr Food Saf*. 2015;5:1100-1101.
30. Seifu E. Physicochemical properties of *Moringa stenopetala* (Haleko) seeds. *J Biol Sci*. 2012;12:197-201.
31. IIAtiku MK. Effect of processing method on the nutrients and antinutrients contents of *Cadaba farinosa* Forssk seeds. *Nigeria Food J*. 2017;35:1-8.
32. El-Kamali HH. *Cadaba farinosa* (PROTA) by H.H. El-Kamali from Botany department, faculty of science and technology, Omdurman Islamic University (https://uses.plantnet-project.org/en/Cadaba_farinosa_(PROTA). 2014 edition.
33. Melesse A, Bulang M, Kluth H. Evaluating the nutritive values and *in vitro* degradability characteristics of leaves, seeds and seedpods from *Moringa stenopetala*. *J Sci Food Agric*. 2009;89:281-287.
34. Alfa M, Igboro S, Ajayi S, Dahunsi S, Ochigbo B. Assessment of the antimicrobial efficiency of *Moringa oleifera* seed extracts in the treatment of grey water. *Curr J Appl Sci Technol*. 2014;4(3):558-567.
35. Wind H. Rift Valley Lakes Basin Integrated Resources Development. Master Plan Study Project, Draft Phase 2 Report Part II Prefeasibility Studies, Halcrow Group Limited and Generation Integrated Rural Development (GIRD) consultants. Unpublished report. Addis Ababa. 2008. http://open_jicareport.jica.go.jp/pdf/12066403_03.pdf. Accessed 20 December 2021
36. Belete MD. The Impact of Sedimentation and Climate Variability on the Hydrological Statur of Lake Hawassa, South Ethiopia. Photometer Instructional Manual for Palin Test. 2013. https://www.palintest.com/en/products/photometer-7100. Accessed 17 December 2021
37. Tilahun G, Ahlgren G. Seasonal variations in phytoplankton biomass and primary production in the Ethiopian Rift Valley lakes Ziway, Awassa and Chamo – the basis for fish production. *Limnologica*. 2010;40:330-342.
38. Ayenew T. Water management problems in the Ethiopian rift: challenges for development. *J Afr Earth Sci*. 2007;48:222-236.
39. Oe AL. *Cadaba farinosa*: Agroforestry Database. 2009. http://apps.worldagroforestry.org/treedb2/speciesprofile.php?Spid=1781
40. Leone A, Spada A, Battezzati A, Schiraldi A, Aristil J, Bertoli S. *Moringa oleifera seeds* and oil: characteristics and uses for human health. *Int J Mol Sci*. 2016;17:2141.
41. Ashfaq M, Basra SMA, Ashfaq U. Moringa: a miracle plant for agro-forestry. *J Agric Soc Sci*. 2012;8:115-122.

42. Daba M. Miracle tree: A review on multi-purposes of *Moringa oleifera* and its implication for climate change mitigation. *J Earth Sci Clim Change*. 2016;7:1-5.

43. Dubey DK, Dora J, Kumar A, Gulsan RK. A multipurpose tree-*Moringa oleifera*. *Int J Pharm Chem Sci*. 2013;2:415-423.

44. Oshiro R. *Method 1604: Total Coliforms and Escherichia coli in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium)*. US Environmental Protection Agency; 2002.

45. Abayneh A. Lecture note on water supply II. 2004:55.

46. Fadaei A, Sadeghi M. Evaluation and assessment of drinking water quality in Shahrekord, Iran. *Resour Environ*. 2014;4:168-172.

47. Noel Bourke GC, O'Leary G, Crowe M, Page D. Water treatment manual: coagulation, flocculation and clarification. 2002.

48. PCE Americas Inc.: Test Instrument, Tools and equipment for measuring, weighing and control systems. PCE instrument Magnetic stirrer. 2014. www.pce-instruments.com/english

49. Jash M. Instrumental technique presentation for magnetic stirrer. 2016. https://tue.iitm.ac.in/Teaching_and_Presentation/grouppresentations/2016/3.%20Madhuri-Instrumental%20Presentation.pdf. Accessed 29 November 2021

50. Standing Committee of Analysts. The Microbiology of Drinking Water; Part 4–Methods for the Isolation and Enumeration of Coliform Bacteria and *Escherichia coli* (Including *E. coli* O157: H7): Methods for the Examination of Waters and Associated Materials. 2009.

51. NEOGEN. Membrane lauryl sulphate broth (NCM0039): Technical Specification Sheet. 2019.

52. Inc. BDL. Membrane lauryl sulphate (Mlsb) Broth: technical sheet. 2016.

53. Muhammed A, Usman NG, Evita H, Pangaribowo. Determinants of household drinking water quality in rural Ethiopia. *ZEF Discussion Papers on Development Policy*. 2016;220:33.

54. Keegan A, Wati S, Robinson B. Chlor (am) ine disinfection of human pathogenic viruses in recycled waters. *Smart Water Fund*. 2012;62M-2114. https://waterportal.com.au/swf/images/swf-files/62m---2114-chlorine-disinfection-of-human-pathogenic-viruses-_final_report.pdf. Accessed 21 November 2021

55. Worku L. Environmental quality control, biological aspect; Teaching Manual. 2004.

56. Telrandhe UB, Hemalatha S, Modi A. Pharmacognostic and phytochemical investigation of root of *cadaba farinosa forsk*. *Int J Pharma Bio Sci*. 2010;1-13.

57. Amante B, López-Grimau V, Smith T. Valuation of oil extraction residue from *Moringa oleifera* seeds for water purification in Burkina Faso. *Balaban Desalination Publications*. 2016;57:2743-2749.

58. Owoseni M, Olaniran A, Okoh A. Chlorine tolerance and inactivation of *Escherichia coli* recovered from wastewater treatment plants in the Eastern Cape, South Africa. *Appl Sci*. 2017;7:810.

59. Gatew S, Mersha W. Tannery wastes water treatment using *Moringa stenopetala* seed extract. *Wyno Acad J Physic Sci*. 2015;12:29-38.

60. Ethiopian Standards Agency. *Drinking Water–Specification. CES 58, Compulsory Ethiopian Standard, First Edition, ICS: 13.060.20*. Ethiopian Standards Agency. Addis Ababa; 2013.

61. WHO. Guideline for drinking water quality. Incorporating the first and second addenda. Volume 1 recommendations. Geneva. Copyright of journal of Himalayan Earth science is the property of Asianet-pakistan and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use. 2008.

62. WHO. Water quality and health-review of turbidity: information for regulators and water suppliers. 2017.