Field application of the *Micro Biological Survey* method for the assessment of the microbiological safety of different water sources in Horn of Africa and the evaluation of the effectiveness of *Moringa oleifera* in drinking water purification

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Abstract

Water monitoring requires expensive instrumentations and skilled technicians. In developing Countries as Africa, the severe economic restrictions and lack of technology make water safety monitoring approaches applied in developed Countries, still not sustainable. The need to develop new methods that are suitable, affordable, and sustainable in the African context is urgent. The simple, economic and rapid Micro Biological Survey (MBS) method does not require an equipped laboratory nor special instruments and skilled technicians, but it can be very useful for routine water analysis. The aim of this work was the application of the MBS method to evaluate the microbiological safety of different water sources and the effectiveness of different drinking water treatments in the Horn of Africa. The obtained results have proved that this method could be very helpful to monitor water safety before and after various purification treatments, with the aim to control waterborne diseases especially in developing Countries, whose population is the most exposed to these diseases. In addition, it has been proved that *Moringa oleifera* water treatment is ineffective in decreasing bacterial load of Eritrea water samples.

Introduction

Contamination of water is one of the major issues of concern for public safety. It is a serious environmental problem that adversely affects human health and biodiversity in the aquatic ecosystems.[1] Most of the rural communities in developing Countries are poverty-stricken and lack access to potable water supplies. In order to obtain drinking water, the population mainly relies on rivers, unprotected wells and cisterns, which are often highly contaminated with waterborne pathogens. In most cases, water from these sources is used without any preliminary treatment, since the unavailability of energy sources makes simple procedures as boiling or heating water difficult to achieve. A significant part of the population is continuously exposed to water-borne diseases and their potential complications.[2,3] The main risk associated with the consumption of unsafe drinking water is microbiological. Monitoring microbial safety of water is therefore not an option, but an imperative to provide safe water.[4] Traditional methods currently used to test microbiological water safety are laboratory-based assessments that are time consuming and require expensive equipment. Since the number of specialized laboratories is exiguous, water safety control in developing Countries is extremely restricted. In these rural areas microbiological methods of analysis to ensure an effective water safety monitoring, should be portable, low cost, fast and simple to use.[5] In this context, *Roma Tre University*, Italy, developed the colorimetric Micro Biological Survey (MBS) method, which is an easy-to-use and low-cost method for microbiological analysis of food and water.[6,7]

Differently from traditional methods that measure the capability of cells to replicate, creating visible colonies on solid media, the MBS method measures the catalytic activity of redox enzymes of the main metabolic pathways of bacteria allowing an unequivocal correlation between the observed enzymatic activity and the bacteria concentration present in the samples. The MBS analysis is performed in disposable, ready-to-use reaction vials that contain the specific reagent for the analysis to perform. The test results are easy to interpret because they simply require the observation of a color change of the reaction vials, that occurs in times that are inversely proportional to the bacterial charge in the sample: higher is the number of bacteria present into the sample, shorter is the time required for color change. After analyses, the reaction vials can be sterilized by pressing on the perforable cap that releases a bactericide substance that completely sterilizes the vial content in 5-10 minutes. The simple analytical procedure, the reduced labor and automation, positively affect the analytical performance of the MBS method, which displays greater reproducibility and repeatability compared to traditional methods.[8] The effectiveness of the MBS method as an alternative method for microbiological analysis of drinking water was demonstrated in Arienzo *et al.*, 2015.[9] The MBS method was applied to evaluate the microbiological quality of dug and drilled wells in Douala, Cameroon. It was also demonstrated that the simple evaluation of total coliforms in 1 mL of water simples, instead of 100 mL as required by law, could be effective to roughly assess water particularly in developing countries in the absence of specific facilities and instrumentations.[9]

In this study we investigate the possibility to use the MBS method as a point of use test to assess water quality and evaluate the effectiveness of different drinking water treatments in Eritrea, Horn of Africa.

Two microbiological parameters were considered in this study: Total Viable Count at 22°C and total coliforms. Total Viable Count at 22°C is a parameter used to evalu-

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Contributions: GA coordinated the research, participated to the design of the study and critically revised the manuscript for intellectual content. ER and PZ conceived the study and participated to its design. FL and DS performed the experimental work. AA, OS and LM contributed to the interpretation of data. FL and AA drafted the manuscript. All authors read and approved the final version of the manuscript.

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ate the concentration of` heterotrophic bacteria that grow at 20-28°C. This parameter includes all aerobic bacteria that use organic nutrients to grow and are universally present in all types of water, food, soil, vegetation, and air. The Total Viable Count is thus a useful tool to monitor the general bacteriological safety of water samples and to verify the effectiveness of water treatments.[10] A significant presence of heterotrophic bacteria can be considered worrisome for public health, indicating the possible presence of viruses or parasites that are more resistant than bacteria to chemical disinfectants.[11,12] Total coliforms are a group of bacteria that include a wide range of aerobic and facultative anaerobic, Gram-negative, non-spore-forming bacilli. These bacteria can occur in faces of human and other warm-blooded animals, but some can be also found in soil, on various plants, including grains and trees, and in certain industrial wastes. Total coliforms are an indicator of sanitary safety of water and their presence indicates a existing risk of contamination by pathogenic bacteria having oral-fecal transmission. They are moderately sensitive to chemical disinfectants, but they can survive and grow in water distribution systems, particularly in presence of biofilms, so their presence can also be used to assess the cleanliness and integrity of distribution systems.[11,13] The effectiveness of *Moringa oleifera* (*M. oleifera*) in drinking water purification was also investigated. *M. oleifera* is a multipurpose tree native to Northern India that now grows widely throughout the tropics.[14] The active component of the dried crushed seeds (powder) of *M. oleifera* is a soluble protein, a natural cationic polyelectrolyte that causes coagulation. Research over the last four decades has primarily focused on testing *M. oleifera* for the removal of turbidity but there is poor evidence about its efficacy in the reduction of bacterial load in different drinking water sources.[15] In addition, the efficiency of three different household water disinfection treatments already in use in Eritrea was examined: i) filtration and ultraviolet (UV) purification; ii) chlorination using an empirical dosage (about 0.1-0.2 mg/L) and iii) chlorination using sodium hypochlorite produced by an electrolytic device.

Materials and Methods

The study area

Eritrea is situated in the Horn of Africa and lies north of the equator between latitudes 12°22′ N and 18°02′. Eritrea is a country of contrasts with land rising from below sea level to 3000 meters above sea level. In this region climate ranges from hot and arid near the Red Sea, to temperate subhumid in the eastern highlands. The average annual rainfall is of about 380 millimeters (mm/year), varying from less than 50 mm to over 1000 mm. Over 90% of the total area receives less than 450 mm and only 1% receives more than 650 mm of annual rainfalls. Rainfalls are torrential, of high intensity and short duration, and vary greatly from year to year. The rainy season goes from June to September. Mean temperature varies between the agro-ecological zones ranging from 18°C in the highlands to 35°C in the lowlands.[16] Figure 1 shows the map of the study area that was downloaded from Google Maps. The regions of Eritrea under consideration are highlighted: Asmara, Cheren, Akrur and Saganèiti.

Application of the Micro Biological Survey method on field to evaluate the microbiological safety of different water samples

Samples collection

A total of 16 water samples were collected from different water sources and analyzed over a period of fifteen days (October to November, 2014) in Eritrea, Horn of Africa. In particular, the samples were collected in Asmara, Cheren, Akrur and Saganèiti. Out of all samples, 3 were bottled water samples distributed by different Eritrea industries, 2 were tap water samples coming from public distribution systems in Asmara and Cheren, 3 were household treated water samples collected respectively in a house in Asmara, a religious institute in Asmara and an orphanage near Akrur, 5 were water samples coming from public (1 sample) and private cisterns (4 samples) in Saganèiti, Asmara and Akrur and 3 were river water samples collected from the Anseba river in three different sites in Eritrea.

Microbiological safety assessment of water samples using the Micro Biological Survey method

Safety assessment of water samples using the MBS method was performed using TVC (Total Viable Count) and COLI (Total coliforms) vials for the quantification of heterotrophic bacteria and Total coliforms respectively. All vials were produced by MBS srl, Rome, Italy. All samples were analyzed in duplicate. Analysis were performed on 1 mL both for Total Viable Count and Total coliforms in accordance to the results obtained by Arienzo *et al*., 2015.[9]

The TVC and COLI vials were filled with 10 mL of sterile distilled water and 1 mL of water samples was added to each vial. After inoculation, vials were incubated for 48 hours at room temperature (22±°C) and for 24 hours at 37±0.5°C in a bench thermostat for TVC and COLI vials respectively. The starting color is blue for TVC vials and red for COLI vials. In the presence of microorganisms, the vials' color changes to yellow, indicating a positive result. The persistence of the initial color after 36 hours for TVC vials and 24 hours for COLI vials indicates the absence of tested microorganisms, and consequently a negative result. The color change was monitored by visual inspection at different times after inoculation. The time for color change was used to determine the bacterial load in the sample, using specific MBS correlation tables between time (expressed as hour) and bacterial concentration (expressed as CFU/mL) (Tables 1 e 2).

Evaluation of the effectiveness of Moringa oleifera seeds in drinking water purification

Samples collection

A total of 9 from 16 water samples collected for the evaluation of the microbiological safety were analyzed also after the treatment with *Moringa oleifera*. Out of all samples, 2 were tap water samples coming from public distribution systems in Asmara and Cheren, 4 were water samples coming from private cisterns in Saganèiti, 3 were river water samples collected from the Anseba river in three different sites in Eritrea.

Water purification treatment using Moringa oleifera seeds

Water samples were treated with 60 g/L of *M. oleifera* seeds, reduced to flour using a mortar. The seeds were obtained from local agricultural productions. The operating procedure used in this study was developed to meet all the requirements of drinking water *on-field* treatment in developing countries, such as the absence of facilities and instrumentations. Water was collected in plastic bottles. The *M. oleifera* flour was added in the bottles that were then immediately shaken for at least 5 minutes to ensure a homogenous distribution and promote the interaction between the active components of *M. oleifera* and bacteria. Water samples were left still in order to allow flocculation and sedimentation and filtered after 1 hour, using a clean cotton cloth.

Application of the Micro Biological Survey method to evaluate the effectiveness of Moringa oleifera seeds in drinking water purification

The concentration of heterotrophic bacteria in water samples, before and after the treatment with *M. oleifera*, was determined

using the MBS method, according to the previously described procedure.

Evaluation of the effectiveness of different water disinfection systems

Samples collection

Different water samples were collected before and after disinfection treatments. Three water samples were collected in a house in Asmara, where water coming from the civil aqueduct is treated with filtration and Ultraviolet (UV) purification before use. Other three water sample were collected in a religious institute in Asmara, where water, coming both from rainfall and from the civil aqueduct, is collected in a cistern and it is empirically treated with chlorine by adding bleaching powder to the cistern (about 0.1-0.2 mg/L). The last three water samples were collected in an orphanage near Akrur, where water is collected from a river, stored in a cistern and chlorinated before use with the electrolytic device Clorel T50.

Application of the Micro Biological Survey method to evaluate the effectiveness of different water disinfection systems

For all water samples, collected before and after disinfection treatments, concentra-

Table 1. Correlation table for TVC vials for the detection of Total Viable Count (22±2°C). Correlation between the time for color change (expressed as hour) and samples contamination (expressed as CFU/mL). In the presence of bacterial load the color of the TVC vials changes from blue to yellow.

Contamination, (CFU/mL)	Total Viable Count (22±2°C)	
	Time for color change (hours)	Final color
$>10^5$	8	Yellow
10^4	14	Yellow
10^3	20	Yellow
10^2	25	Yellow
10	31	Yellow
0	>36	Blue

Table 2. Correlation table for COLI vials for the detection of total coliforms (37±0.5°C). Correlation between the time for color change (expressed as hour) and samples contamination (expressed as CFU/mL). In the presence of coliforms the color of the COLI vials changes from blue to yellow.

	Contamination (CFU/mL)Total coliforms (37°C± 0.5°C)	
	Time for color change (hours)	Final color
$>10^5$	3	Yellow
10^4	9	Yellow
10^3	15	Yellow
10^2	21	Yellow
10	27	Yellow
0	>33	Red

Figure 1. Map of the study area: Eritrea, Horn of Africa. Spot A marks Asmara (N15°19'22.332", E38°55'30.1792"); spot B marks Cheren (N15°46'48.0360", E38°27'12.3840"); spot B marks Akrur (N15°3'17.77", E39°15'48.803"); spot C marks Saganèiti (N15°3'38.848", E39°11'26.785").

tions of heterotrophic bacteria and total coliforms were determined using the MBS method, according to the previously described procedure.

Statistical analysis

Statistical analysis of data was performed using Student's t-test.

Results

Application of the Micro Biological Survey method on field in the evaluation of the microbiological safety of different water sources

The primary objective of the study was the application of the MBS method for the assessment of the microbiological safety of different water sources used as drinking water from local population: bottled water, tap water, household treated water, cistern water and river water. Figure 2 shows the level of contamination for both heterotrophic bacteria at 22°C and Total coliforms found in the different water sources analyzed.

Bottled water resulted not contaminated. Tap water samples were not contaminated by coliforms, according to the World Health Organization (WHO) microbiological standards for drinking water. These samples however displayed a Total Viable Count that exceeds the limit of 100 CFU/mL. Household treated water did not result suitable for human consumption. Finally, both cistern and river water samples showed a Total Viable Count that exceeded the standard level of 100 CFU/mL and a very high contamination by coliforms.

Application of the Micro Biological Survey method in the evaluation of the effectiveness of Moringa oleifera seeds in drinking water purification

Natural plant extracts have been used for water purification for many centuries. *M. oleifera* seeds (Figure 2) has been ranked as one of the best plant extracts for water purification.[14] In terms of water treatment applications, *M. oleifera* seeds have proved to be effective at removing suspended material, generate reduced sludge volumes in comparison to alum, soften hard waters and act as effective absorber of cadmium.[17-19] Most studies have considered the removal of turbidity and rarely of bacteria. One study conducted on surface water used for domestic purposes showed a 90-99% reduction in fecal coliform levels. It was found that the reduction in *E. coli* was directly linked to the turbidity removal achieved during coagulation (from 50% up to 97%.).[20]

In this work the effectiveness of *M. oleifera* in the reduction of bacterial load in different water samples was evaluated using the MBS method.

The operating procedure was previously studied in *in vitro* and on field trials in Lazio, Italy: a concentration of *M. oleifera* equal to 60 g/L was chosen as it gave the most promising results (Table 3).

Water samples from different water sources in Eritrea, were treated with *M. oleifera*. Total Viable counts were assessed using the MBS TVC vials before and after the treatment (Figure 3).

Table 4 shows the reduction of bacterial load in water samples collected in Eritrea after treatment with *M. oleifera*.

In the analyzed samples the treatment with *M. oleifera* did not positively affect the microbiological quality in almost all the samples, causing an increase in the bacterial load, on average of 75.5%. The treatment resulted effective in reducing the bacterial load only in freshwater samples collected from the Anseba river. These results completely differ from the ones previously obtained in *in vitro* and on field trials demonstrating the inefficiency of the treatment in Eritrea water samples.

Application of the Micro Biological Survey method on field in the evaluation of the effectiveness of different household water disinfection systems

Considering the inefficiency of *M. oleifera* in drinking water purification, another objective of the study was the application of the MBS method to evaluate the effectiveness of three different systems of household disinfection of water that are already in use in Eritrea: i) filtration and ultraviolet (UV); ii) chlorination using empiric dosage (about 0.1-0.2 mg/l) of the commercially available bleaching powder (mixture of calcium hypochlorite, calcium hydroxide, and calcium chloride); iii) chlorination using sodium hypochlorite produced by an electrolytic device, Clorel T50. For this purpose, the microbiological safety of water was tested before and after treatments, considering both Total Viable Count at 22°C and Total coliforms.

Figure 4 shows the level of contamination for both parameters observed before and after the treatment. Water treated with filtration and Ultraviolet (UV) radiations resulted suitable for human use, due to the total absence of bacterial load. Water empirically treated with bleaching powder showed a reduction of bacterial load for both parameters, that varied among the samples, with the greatest reduction observed for Total coliforms. Water chlorinated using Clorel T50, was not contami-

Table 3. Reduction of bacterial load in different water samples after treatment with *Moringa oleifera*. The values shown are the means of the results obtained using the Micro Biological Survey method. For each water sample three independent analyses were performed in duplicate. Results are expressed in percentage to analyze the effectiveness of the treatment with *M. oleifera* in relation to the initial concentrations.

Samples	N.	Total Viable Count 22°C (after treatment/before treatment)		
		Mean (%)	Max (%)	Min (%)
Artificially contaminated distilled water samples (ATCC strains)	10	–75.5	–100	–61.1
Freshwater samples (rivers in Lazio, Italy)	12	–48.6	–63.5	–34.1

Table 4. Reduction of bacterial load in Eritrea water samples after treatment with *Moringa oleifera*. The values shown are the means of the results obtained using the Micro Biological Survey method. For each water sample three independent analyses were performed in duplicate. Results are expressed in percentage to analyze the effectiveness of the treatment with *M. oleifera* in relation to the initial concentrations.

Samples	N.	Total Viable Count 22°C (after treatment/before treatment)		
		Mean (%)	Max (%)	Min (%)
Eritrea water samples	12	+75.5	+400	–61.6

nated by Total coliforms. However Total Viable Count did not significantly decrease after the treatment.

Discussion

An adequate and safe supply of water for human use is one of the major prerequisites for a healthy life. Improving water safety is significant for the country's developmental progress in terms of human health, education and gender safety. Access to adequate water is however restricted in developing Countries, resulting in a high incidence of communicable diseases that increases the harshness of daily life.[21] Monitoring the microbiology safety of water for human use is one of the key challenges to ensure safety in developing Countries. Surveillance is typically weakest in these countries, where the access to improved water sources is lowest and the likelihood of contamination is greatest. Considering this scenario the rapid, easy and cheap colorimetric MBS method for microbiological analysis may represent an important tool to increase water monitoring in rural areas. The MBS method is more practical and simple in its execution compared to traditional techniques. It provides reliable results diminishing the time of analysis, favoring procedures and interpretation of data, limiting cost and allowing analysis also in absence of skilled personnel and laboratory. Its features were already exploited for drinking water analysis in the city of Douala, Cameroon.[9]

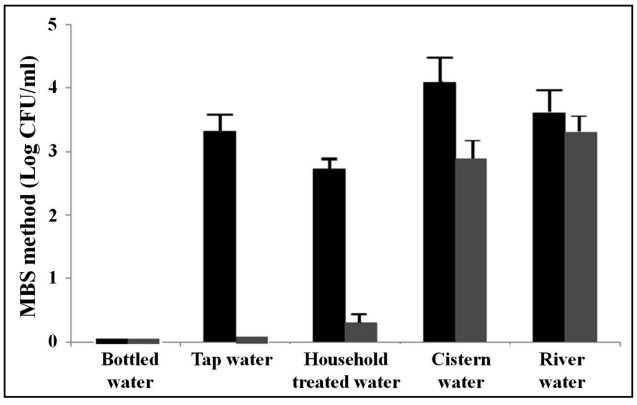

Figure 2. Total Viable Count and total coliforms contamination of different water sources in Eritrea, Horn of Africa. The values shown are the means ± SD of the results obtained using the Micro Biological Survey method (expressed as log of CFU/mL) for different samples of the same type of water source. Black bars show the average of the level of contamination of Total Viable Count at 22°C. Grey bars show the average of the level of contamination of total coliforms. n=3 for bottled water samples; n=2 for tap water samples; n=3 for household treated water; n=5 for cistern water samples; n=3 for river water.

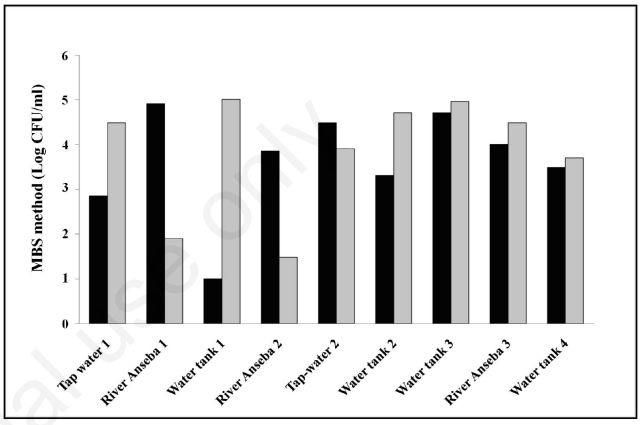

Figure 3. Effectiveness of the treatment with *Moringa oleifera*. Total Viable Count in Eritrea water samples before and after 1 and 31 hours from the treatment with M. oleifera. The values shown are the means of the results obtained using the Micro Biological Survey method (expressed as log of CFU/mL). For each water sample three independent analyses were performed in duplicate. Black bars show the average of the level of contamination of Total Viable bacteria in the samples before treatment. Grey bars show the average of the level of Total Viable bacteria 1 hour after the treatment with *M. oleifera*.

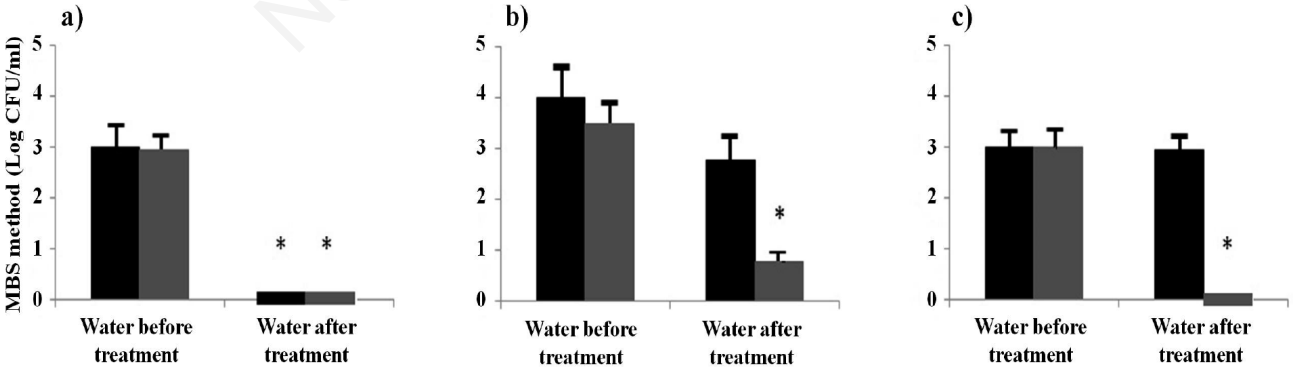

Figure 4. Effectiveness of three different household systems for water disinfection used in Eritrea. a) Filtration and ultraviolet (UV) purification; b) Chlorination using empiric dosage (about 0.1-0.2 mg/l) of the commercially available bleaching powder (mixture of calcium hypochlorite, calcium hydroxide, and calcium chloride); c) Chlorination using Clorel T50. The values shown are the means ± SD of the results obtained using the Micro Biological Survey method (expressed as log of CFU/mL) for three samples of the same type of water analyzed in duplicate (*P<0.01, Student's t test). Black bars show the average of the level of contamination of Total Viable Count. Grey bars show the average of the level of contamination of Total coliforms.

In this study, the MBS method was used to assess the microbiological safety of water samples from various sources and to evaluate the effectiveness of different water treatments in Eritrea, Horn of Africa. Total Viable Count at 22°C and Total coliforms were evaluated. Safe water must be characterized from a low level of Total Viable bacteria (<100 mL/CFU) and from the absence of Total coliforms.[11] It has been demonstrated that the presence of heterotrophic bacteria (<100 CFU/mL) indicates the possible presence of viruses or parasites that are more resistant than bacteria to chemical disinfectants,[11,12] while the presence of Total coliforms indicates a existing risk of contamination by pathogenic bacteria having oral-fecal transmission.[13]

Most of the analyzed water samples resulted not compliant with the current standards for drinking water. Only bottled water was found to be always microbiologically pure. Tap water coming from the civil aqueduct, despite the absence of coliforms, showed a high level of heterotrophic bacteria, that exceeded the standard limits. This indicates that, although the presence of a public water distribution system should be an indicator of improved water supplies in a developing country, it should not be assumed that the resulting water is always suitable for human consumption.[22] Also the household treated water resulted not suitable for human consumption, since the amount of heterotrophic bacteria exceeded the standard level and low level of contamination by coliforms was detected. These results highlight the importance of verifying the effectiveness of potabilization treatments. Cistern and river water also resulted not compliant with current microbiological standards for drinking water underlining a potential health risk due to high concentrations of both heterotrophic bacteria and Total coliforms. The use of cisterns is an ancient practice to collect and store water, but they are susceptible to microbiological contamination.[23] Moreover, in some villages, river water is adopted as the main water supply although it can be highly polluted. This contamination is a result of the surrounding environment (*e.g.* contamination from land and fecal material originating from local animals present on the banks of the river).

This study also demonstrated the importance of verifying the effectiveness of several water treatments.

Coagulation is a common process used for removing suspended matter from water. Recently, a resurgence of interest in natural coagulants has emerged. Various plant based materials have been identified as effective coagulants. The major merits of plant-derived coagulant materials when used as point-of-use (POU) technology in water treatment methods are obvious: these are less expensive, do not alter the pH of treated water, and the sludge they produce is less voluminous and readily biodegradable. Of all plant materials investigated, *M. oleifera* has drawn special attention as it treats water by acting both as a coagulant and antimicrobial agent.[24,25] *M. oleifera* is widely available, easy to store, especially in developing countries, and has been reported as an ecofriendly substitute to widely used disinfectants.[26]

In this study, however, the treatment of water with *M. oleifera* seeds did not result effective in reducing the bacterial load in most of the examined water samples. Unexpectedly in 75% of the samples the concentration of bacteria has risen on average of the 80%. This result conflicts with those found in literature and with those previously obtained treating different water samples in in vitro and on field trials in Italy. The reasons behind such discrepancy have been investigated. In vitro experiments excluded the influence of pH, cation and anion concentrations, but further experiments are required to understand this behavior.

In any case, it is important to stress that, after the treatment with *M. oleifera*, water samples were indeed not suitable for human consumption. Apart from the worsened microbiological quality, in fact, water samples displayed a considerable increase of turbidity due to a higher concentration of organic matter, that caused odor, color and taste issues, as previously described.[27]

Regarding the other water treatments examined in this study it appears clear that despite their common use not all of them are effective, at least not following the household protocols usually implemented.

Water treated by national drinking water distribution companies, using filtration and Ultraviolet (UV) radiation, resulted microbiologically acceptable for human use. On the contrary, the chlorination treatment either using empiric dosages (about 0.1-0.2 mg/L) of the commercially available bleaching powder (mixture of calcium hypochlorite, calcium hydroxide, and calcium chloride) or through sodium hypochlorite produced by an electrolytic device, Clorel T50, did not result efficient and the obtained water was not suitable for human use. The amount of chlorine used following the household protocols was not enough to decrease the bacterial load down to acceptable levels. These results highlight that the inefficiency of chlorination is most likely to arise from human error.

Conclusions

In conclusion, the results of this study suggest a need to increase water monitoring in order to effectively improve water quality and thereby reduce incidence of water-related diseases. In this context, the MBS method can be suitable for microbiological water analysis in rural areas by local personnel, operating without a microbiological laboratory.

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