USING *Moringa oleifera* SEED EXTRACT AND SOLAR RADIATION IN THE TREATMENT OF WATER INTENDED FOR HUMAN CONSUMPTION

O EXTRATO DE SEMENTES de Moringa oleifera *E RADIAÇÃO SOLAR NO TRATAMENTO DE ÁGUA DESTINADA AO CONSUMO HUMANO*

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SUMMARY

To evaluate the microbiological quality of water samples in communities that use alternative sources of water for human consumption treated with *Moringa oleifera* seed extract and solar radiation, and to provide subsidies for using these treatments. METHODS: The multiple tube method was used to determine the most probable number of total coliforms and thermotolerant mesophile microorganisms in nine water samples from alternative sources (wells). These samples were obtained in Cruz das Almas, in the Reconcavo Baiano region, state of Bahia, Brazil. RESULTS: The number of water samples treated with *Moringa* seeds and exposed to the sun for two, five and twelve hours showed a reduction in the concentrations of TC/FC 1.52 log (56.51%), 1.88 log (64.83%) and 2.14 log (71.33%), respectively. The reduction rate of mesophile microorganisms after sun exposure for two, five and twelve hours were 0.24 log (11.60%), 0.18 log (10.11%) and 1.25 log (65.78%), respectively. CONCLUSIONS: Although solar radiation was effective in removing bacteria, the concomitant use with *Moringa oleifera* seeds extract was not effective to reduce fecal coliform load to zero. Therefore, only mesophile microorganisms reached the levels required by the legislation.

KEY-WORDS: Coliforms. *Escherichia coli.* Microbiology. Seeds. Solar disinfection. Water quality.

RESUMO

Avaliar a qualidade microbiológica de amostras de água em comunidades que utilizam águas de fontes alternativas para o consumo humano, utilizando extrato de sementes de *Moringa oleifer*a e radiação solar, além de fornecer subsídios para o uso destes tratamentos. MÉTODOS: O método dos tubos múltiplos foi utilizado para determinar o número mais provável de coliformes totais e termotolerantes e a quantificação de microrganismos mesófilos em nove amostras de água provenientes de fontes alternativas (poços). Tais amostras foram obtidas na cidade de Cruz das Almas, localizada na região do Recôncavo, no estado da Bahia, Brasil. RESULTADOS: O número de amostras da água tratadas com sementes de moringa e expostas ao sol por duas, cinco e doze horas apresentaram redução nas concentrações de CT/CF de 1,52 log (56,51%), 1,88 log (64,83%) e de 2,14 log (71,33%), respectivamente. Já a taxa de redução para os microrganismos mesófilos após exposição ao sol de duas, cinco e doze horas foram respectivamente de 0,24 log (11,60%), 0,18 log (10,11%) e de 1,25 log (65,78%). CONCLUSÕES: A radiação solar foi eficiente na remoção bacteriana, porém usada concomitantemente com o extrato das sementes de *Moringa oleifera* não foi eficiente para reduzir a carga de coliformes termotolerantes a zero. Apenas a redução dos microrganismos mesófilos alcançou os níveis determinados por lei.

PALAVRAS-CHAVE: Coliformes. Desinfecção solar. *Escherichia coli*. Microbiologia. Qualidade da água. Sementes.

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INTRODUCTION

The availability of drinking water is important for the development of any country. The protection of water sources and the treatment of water before it reaches the distribution systems have greatly reduced the incidence of these diseases in developed countries (POTGIETER et al., 2002, AMARAL et al., 2003; ZAMXAKA et al., 2004; PRITCHARD et al., 2007; BRETTAR & HÖFLE, 2008).

Moringa oleifera is a plant native to Asia and due to the fact that its seeds are coagulants and have bactericidal properties, it has been widely used to treat water intended for human consumption. In addition to these properties, the use of its seeds does not change either the taste or the pH of water (NDABIGENGESERE & NARASIAH, 1998; AMARAL et al., 2006).

Study about coagulation and bacterial reduction with the seeds of this plant in the waters of the Nile River in Sudan, reported a decrease 80-95% in levels of turbidity and 1 to 4 log (90 to 99.9%) in the bacterial parameters, with microorganisms concentrated in the sediment (MADSEN et al. 1987). A study about the efficacy of seven plant species used to reduce the number of microorganisms present in raw river water, pH 6 to 8, showed that the seed of *M. oleifera* had the second best efficiency (KUMAR & GOPAL, 1999).

The turbidity removal by sedimentation using *Moringa* seeds has a positive effect on the water disinfection process by solar energy since in waters with high turbidity (higher than 200 NTU) less than 1% of the incident ultraviolet radiation penetrates deeper than the 2-cm surface, thus diminishing its germicidal action. The inactivation of *E. coli* has been reported in water samples with low turbidity after 7 hours of exposition to sunlight (WEGELIN, 1994; PULGARIN & RINCON, 2003). In similar work, Amaral et al. (2006) treated water with *Moringa* seeds and exposed it to sunlight for two, five and twelve hours and reported a decrease of *E. coli* MPN of 74.3%, 94.1% and 100.0%, respectively.

Based on the above and the little information available on the subject, the objective of this study is: (a) to evaluate the use of *Moringa oleifera* seed extract and solar radiation exposition to treat water from alternative sources in the Reconcavo region, Bahia; and (b) to provide support for these treatments in communities that use alternative water sources for consumption.

MATERIAL AND METHODS

The study was conducted in Cruz das Almas, located in the Reconcavo region, Bahia. Water from wells located in rural farms was previously investigated regarding its microbial load. Thus, water from nine wells, with the highest microbial load (coliforms and mesophilic microorganisms), was used for the investigation in the study.

The water was collected, according to Apha (1998) in 10-L carboys and transported to the Laboratory of Animal Microbiology, Universidade Federal do Recôncavo da Bahia (UFRB).

Using *Moringa oleifera* seeds for water physical treatment and disinfecting the clarified water by solar radiation

The sedimentation process consisted of mixing the extract of three seeds in the water samples from the wells, stirring rapidly for one minute and then slowly for five minutes. Subsequently, the water samples were allowed to stand for 24 hours, the ideal time for effective sedimentation. It is emphasized that the initial color and turbidity values of the water ranged, respectively, from 0.01 to 1.19 NTU and from 0 to 20 Uhazen (AMARAL et al., 2006).

After sedimentation, samples of the supernatant were collected to determine turbidity and color, as well as concentrations of total coliforms, fecal and mesophilic coliforms (Apha, 1998; AMARAL et al., 2006).

Finally, the clarified water samples were exposed to sunlight for two, five and twelve hours and analyzed to determine how solar radiation affected the microorganisms studied. For the sun exposure, the water samples were placed in transparent; 2,000-mL PET bottles (Polyethylene Terephthalate). One group of bottles were laid horizontally on the ground under the sun from 07:00 to 19:00, taking advantage of the period during which the highest sun incidence is verified, between 09:00 and 15:00. We used 30 bottles, of which 15 were kept in the dark (controls), and 5 were analyzed after 2h (09AM); another 5 after 5h (12AM); and the remaining 5 after 12h (19PM) of sun exposure. To this end, the 30 bottles were filled and the controls for each exposure time and those that were exposed to the sun were randomly selected. A control was analyzed for each treated sample. This protocol was repeated six times to obtain 30 samples from each time with their respective controls (AMARAL et al., 2006).

The temperature in the three exposure times was measured with a Celsius scale (°C) mercury thermometer and checked with data provided by the National Institute of Meteorology (INMET), from the Estação Automática de Cruz das Almas, BA.

Concentrations of coliforms (total and thermotolerant) and mesophilic microorganisms, as well as turbidity and color were recorded for both the control and sun treated water samples.

Detection of microbiological concentrations

The Most Probable Number (MPN) of total coliforms (TC) and thermotolerant coliform (FC) was determined using the multiple-tube, according to APHA (1998), with sets of ten tubes and dilutions of 10°. For the confirmation, the specific TC detection was performed from positive tubes containing Lauryl Sulfate Tryptose broth. By reading the broth tubes with Brilliant Green Bile 2% to gas had cloudiness and the Most Probable Number (MPN) per 100 ml total coliforms were determined by an appropriate dilutions inoculated MPN table.

CF MPN was also determined from the positive tubes containing Lauryl Sulfate Tryptose Broth. By reading the tubes which had EC broth turbidity gas and the Most Probable Number (MPN) per 100 ml total coliforms were determined by an appropriate dilutions inoculated MPN table.

Samples were diluted to 10^0 and 10^2 with 0.1% peptone water to count the strict and facultative aerobic mesophilic microorganisms. Subsequently, 1-mL samples and/or dilutions were poured into petri dishes where the previously melted and cooled PCA agar was added. After the medium solidified, the plates were inverted and incubated at 35 ± 2°C, for 24 to 48 hours. The colony counts were made using a colony counter. The average number of colonies counted on the plates was multiplied by the corresponding dilution factor and the result was expressed as colony-forming units per mL of sample (CFU. mL^{-1}) (APHA, 1998).

Color and turbidity determination

Turbidity was measured using an ADAMO bench turbidimeter, model TB 1000. The values were expressed in NTU.

Color was determined using a Del Lab colorimeter, model DLNH-100. The results were expressed as Uhazen.

Analysis of results

Microbial removal efficiency was based on determination of logarithmic units (LU), in order to avoid over evaluation removal such as 90 and 99.0% of coliform (total and thermotolerant) and mesophilic microorganisms (BARROS et al., 2003).

RESULTS AND DISCUSSION

Figure 1 shows the reduction level of total (TC) and thermotolerant coliforms (CF) in water exposed to solar radiation. It is noteworthy that all CT concentrations detected were characterized as CF. Therefore, this paper will discuss only CF. In addition, it is known that 75% of CF detected is *E. coli* (APHA, 1998).

The water treated with *Moringa* seed extract and exposed to the sun for two hours displayed decreased concentrations of TC/CF (Figure 1) of 1.52 log (56.51%). The microbial reduction of water exposed to the sun for 5 and 12 hours was 1.88 log (64.83%) and 2.14 log (71.33%), respectively. Thus, the highest reduction rate was observed after twelve hours of exposure; however, even these high removal rates did not warrant drinking water within the limits required by the Brazilian law, established by Decree 518 of the Ministry of Health (Brazil, 2011). According to these standards, drinking water is characterized by the complete absence of *Escherichia coli* or coliforms per 100 mL sample.

The removal rates of mesophilic microorganisms (Figure 2) after 2, 5 and 12 hours of sun exposure were 0.24 log (11.60%), 0.18 log (10.11%) and 1.25 log (65.78%), respectively. Unlike *E. coli,* the removal rates of mesophilic microorganisms after sun exposure, especially after twelve hours, was enough to guarantee potable water according to Ordinance 2914 from the Ministry of Health (Brazil, 2011). The limit tolerated by law for this group of microorganisms is 500 CFU. mL^{-1}.

These research findings disagree from those by Amaral et *al.* (2006) who reported that *Moringa oleifera* and solar radiation used to treat water from dams reached up to 99.99% removal rates for *E. coli*, thus characterizing potable water.

The initial levels of water color and turbidity were low but they improved greatly after the treatment with Moringa seed extract and sun exposure, as seen in Table 1. Accordingly, in agreement with Ndabigengesere & Narasiah (1998), it is believed that the introduction of *Moringa* extracts increased the load of organic matter and nutrients, which can be seen by the increased color and turbidity values. This new organic load may have stimulated the multiplication of total and fecal coliforms of saprophytic microbiota, even after twelve hours of sun exposure.

Madsen et al. (1987) used moringa seeds extract to treat the murky waters of the River Nile, in Sudan, and reported that microorganisms were concentrated in the sediment and that within the next twenty-four hours the number of *S.* and *S. typhimurium Soney* and in some cases *E. coli* increased.

The increasing *E. coli* associated with increasing nutrients in the water when *Moringa Oleifera* extract is added may be related to the assertion by Byappanahalli & Fujioka (1998) who reported that *E. coli* may multiply, increasing its number by two logs when nutrients are added to the soil. These researchers also verified that the *E. coli* increases by 2 logs when a minimal amount of wastewater is added in sterilized soil.

The fact that *E. coli, Klebsiella* sp. and *Enterobacter cloacae* can multiply in river water with 3.2 $mg.L^{-1}$ of dissolved organic carbon and in water treated with concentrations of 0.4 and 0.8 $mg.L^{-1}$ (JORET et al., 1991; Ndabigengesere & Narasiah, 1998.) may explain the presence of total and fecal coliforms, in this study

Conversely Amaral et al. (2006), in the present study (Figures 1 and 2) it was believed that the addition of the extract from the seeds of this plant, in waters where, initially, levels of turbidity and color were low, might have decreased the disinfecting action of radiation solar.

However, this work showed (Figures 1 and 2) that sunlight exposure may be a tool to improve the microbiological quality of drinking water in areas with restrictions on water quantity and quality, as in the semi-arid Northeast of Brazil and Africa.

The solar radiation reduces the number of mesophilic and *E. coli* microorganisms, the latter

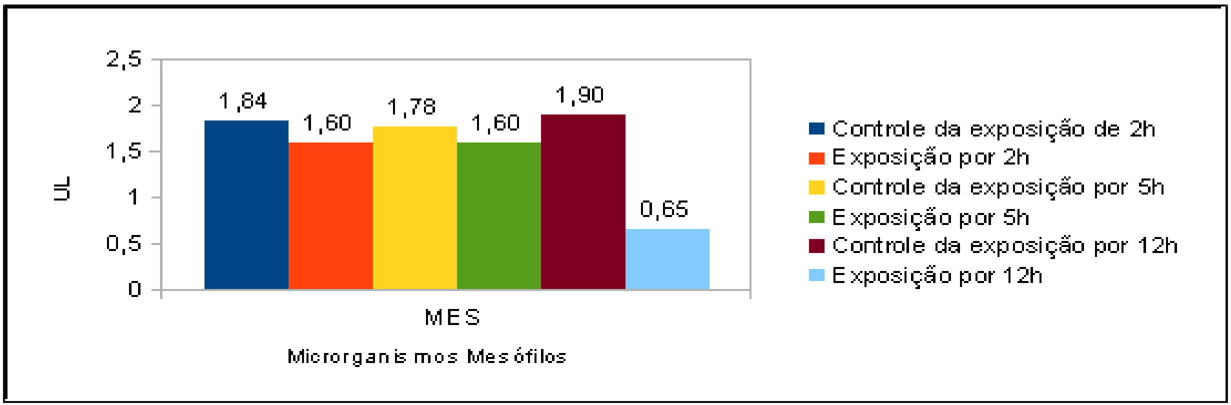

Figure 1 - Means of logarithmic units (LU) for total coliform (TC) and fecal coliform (CF) in water samples from nine wells in the rural area of Reconcavo, in Bahia, treated with moringa seed extract *(M. oleifera)* and exposed to the sun for two, five and twelve hours.

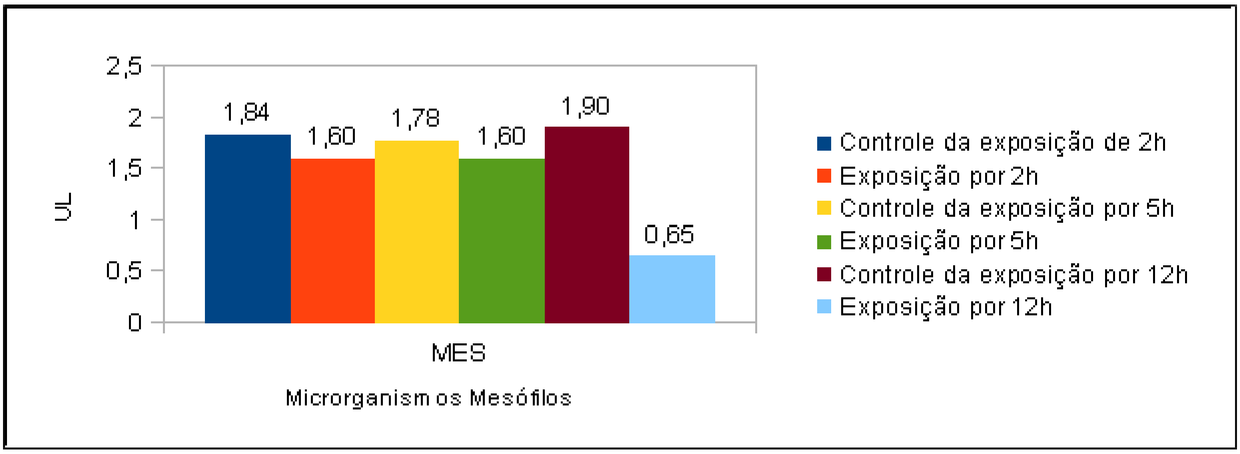

Figure 2 - Means of logarithmic units (LU) of mesophilic (MES) microrganims in water samples from nine wells in the rural area of Reconcavo, in Bahia, added moringa seed extract *(M. oleifera)* and exposed to the sun for two, five and twelve hours.

Table 1 - Mean color and turbidity values of water samples from nine wells in the rural areas of Reconcavo, in Bahia.

Seed treatment	Without	with
Turbidity (NTU)	1.19	18.50
Color (UHazen)	9.58	37.80

belonging to the group of faecal coliforms and is superior to other traditional indicators of faecal pollution since it survives a shorter time in the environment similar to intestinal origin pathogens. Total coliforms during the warmer seasons can multiply in water, providing false positive results (BAUDISOVA, 1997).

The positive action of solar radiation as a disinfectant of water was reported by Conroy et al. (1999) while studying 349 children of the Maasai community in Africa. They reported that the consumption of water treated by sun exposure significantly decreased the risk of developing diarrhea, when compared with those who consumed water without exposure to the sun.

Pinfold (1990) in a study conducted in the Philippines, examined the relationship between bacterial indicators of water quality and diarrhea in children, and found that children who drank highly polluted water (> 1,000 *E. coli*/100 mL) had a significantly higher occurrence of diarrhea ($p < 0.01$) than those who consumed water with lower pollution levels.

Although complete inactivation of thermotolerant coliforms was not reached, the results obtained in this study show that simple and low cost solutions are likely to prevent waterborne diseases, responsible for many deaths, especially of children in developing countries. In semi-arid regions of Brazil, it is common to use water from alternative sources, such as wells, springs and dams; however, these waters have a higher risk of contamination, whether environmental or from animals. But most developing countries (including Brazil) are in regions of high insolation, between 35°N and 35°S latitudes, which favors the use of solar radiation for various purposes, including water disinfection (WEGELIN, 1994).

In Brazil, there is a concern about the quality of the water consumed in urban areas, as well as sanitary and political efforts to adopt measures that ensure good water quality for the population, but in rural areas this concern is nonexistent. This is dangerous because, in terms of public health, the quality control of drinking water should result from a joint action of different health professionals in order to promote and prevent waterborne diseases (AMARAL, 2001).

Finally, it appears that a massive effort must be done to monitor the quality of water used in rural areas and to program actions for enlightening the consumer population.

CONCLUSION

Solar radiation was effective in bacterial removal, but together with the seeds extract of *Moringa oleifera* was not effective to reduce the load of fecal coliform to zero. Only the mesophilic microorganism removal reached levels determined by law. The use of moringa seed extract in this work has possibly reduced the disinfecting power of sunlight, because it increased the organic load.

The poorest communities are the most affected regarding consumption of unsafe water and, therefore, should be the focus of this type of study. These findings corroborate previous studies regarding the use of *Moringa oleifera* seeds and solar radiation to treat water, at only a fraction of the cost of conventional chemical treatment. Thus, these two tools are an alternative of great importance, both economic and scientific, as they combine low cost, quality as well as feasible and legal results.

REFERENCES

AMARAL, L. A. A água como fator de risco para saúde humana e saúde animal em propriedades leiteiras situadas na região nordeste do Estado de São Paulo, 2001. Post-Doctoral Thesis – Faculdade de Ciências Agrárias e Veterinárias da Universidade Estadual Paulista, Jaboticabal-SP, 2001.

AMARAL, L. A.; NADER FILHO, A.; ROSSI JUNIOR, O. D.; FERREIRA, L. A.; BARROS, L. S. S. Água de consumo humano como fator de risco à saúde em propriedades rurais. Revista de Saúde Pública, v.37, n.4, p.510-514, 2003.

AMARAL, L. A.; ROSSI JÚNIOR, O. D.; BARROS, L. S. S.; LORENZON, C. S.; NUNES, A. P. Tratamento alternativo da água utilizando extrato de semente de *Moringa oleifera* e radiação solar. Arquivos do Instituto Biológico, v.73, n.3, p.287-293, 2006.

APHA. Standard Methods for the Examination of Water and Wastewater 1998 20th edition, American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC, 1998.

BARROS, L. S. S.; AMARAL, L. A.; JUNIOR, J. L. Monitoramento sanitário de um sistema integrado de tratamento de águas residuárias da suinocultura. Revista Panam Salud Publica, v.14, n.6, p.385-393, 2003.

BAUDISOVA, D. Evaluation of *Escherichia coli* as the main indicator of fecal pollution. Water Science and Technology, v.35, n.11, p.333-338, 1997.

BRASIL. Portaria n° 2.914, de 12 de dezembro de 2011. O Ministério da Saúde aprova normas e padrões de potabilidade da água destinada ao consumo humano. Diário Oficial, Brasília, 26 mar. 2004, Seção 1, p.266-70.

BRETAR, I.; HÖFLE, M. G. Molecular assessment of bacterial pathogens- a contribution to drinking water safety. Current opinion in Biotechnology, v.19, p.274-280, 2008.

BYAPPANAHALLI, M. M.; FUJIOKA, R. S. Evidence that tropical soil environment can support the growth of *E. coli*. Water Science and Technology, v.38, n.12, p.171-174, 1998.

CONROY, R. M., MEEGAN, M. E., JOYCE, T., McGUICAN, K.; BAMES, J. Solar disinfection of water reduces diarrhoeal disease: an update. Archives of Disease in Childhood, v.81, n.4, p.337-338, 1999.

JORET, J. C., LEVI, Y.;VOLK, C. Biodegradable dissolve organic carbon (BDOC) content of drinking water and potential regrowth of bacteria. Water Science and Technology, v.24, n.2, p.95-101, 1991.

JOYCE, T. M.; McGUICAN, K. G.; ELMORE-MEEGAN, M.; CONROY, R. M. Inactivation of fecal bacteria on drinking water by solar heating. Applied Environmental Microbiology, v.62, n.2, p.399-402, 1996.

KUMAR, S.; GOPAL, K. Screening of plant species for inhibition of bacterial population of raw water. Journal of Environmental Science Health, v.34, n.4, p.975-984, 1999.

MADSEN, M.; SCHLUNDT, J.; OMER, E. F. Effect of water coagulation by seeds of *Moringa oleifera* on bacterial concentrations. Journal of Tropical Medicine and Hygiene, v.90, n.3, p.101-109, 1987.

NDABIGENGESERE, A.; NARASIAH, K. S. Quality of water treated by coagulation using *Moringa oleifera* seeds. Water Research, v.32, n.3, p.781-791, 1998.

PINFOLD, J. V. Faecal contamination of water and fingertiprinses as a method for evaluating the effect of low cost water supply and sanitation activities on faecal-oral disease transmission. II.A hygiene intervention study in rural northest Thailand. Epidemiology and Infection, v.105, p.377-380, 1990.

POTGIETER, N.; OBI, C. L.; BESSONG, P. O.; MATSAUNG, G. Assessment of the microbial quality of river water sources in rural venda communities in South Africa. Water SA, v.28, n.3, p.287-291, 2002.

PRITCHARD, M.; MKANDAWIRE, T.; O'NEILL, J. G. Biological, chemical and physical drinking water quality from shallow wells in Malawi: Case study of Blantyre, Chiradzulu and Mulanje. Physics and Chemistry of the Earth, v.32, p.1167-117, 2007.

RINCON, A. G.; PULGARIN, C. Photocatalytical inactivation of *Escherichia coli*: effect of light intensity and of TiO_2 concentration. **Applied Catalysis B: Environmental,** v. 44, p.263-284, 2003.

WEGELIN, M. Solar water disinfection: scope of the process and analysis of radiation experiments. **Journal of Water SRT-Acqua,** v.43, n.3, p.154-169, 1994.

ZAMXAKA, M.; PIRONCHEVA, G.; MUYIMA, N.Y.O. Microbiological and physico-chemical assessment of the quality of domestic water sources in selected rural communities of the Eastern Cape Province, South Africa. **Water SA**, v.30, n.3, p.333-340, 2004.