Journal of
Water, Sanitation & Hygiene for Development

PUBLISHING

 Journal of Water, Sanitation and Hygiene for Development Vol 12 No 9, 659 doi: 10.2166/washdev.2022.100

Research Paper

Effects of *Moringa oleifera* seeds on the physicochemical properties and microbiological quality of borehole water from Botswana

Tiroyamodimo Semanka, Eyassu Seifu* and Bonno Sekwati-Monang
Department of Food Science and Technology, Botswana University of Agriculture and Natural Resources, Private Bag 0027, Gaborone, Botswana
*Corresponding author. E-mail: eseifu@buan.ac.bw; eyassu.b@gmail.com

ABSTRACT

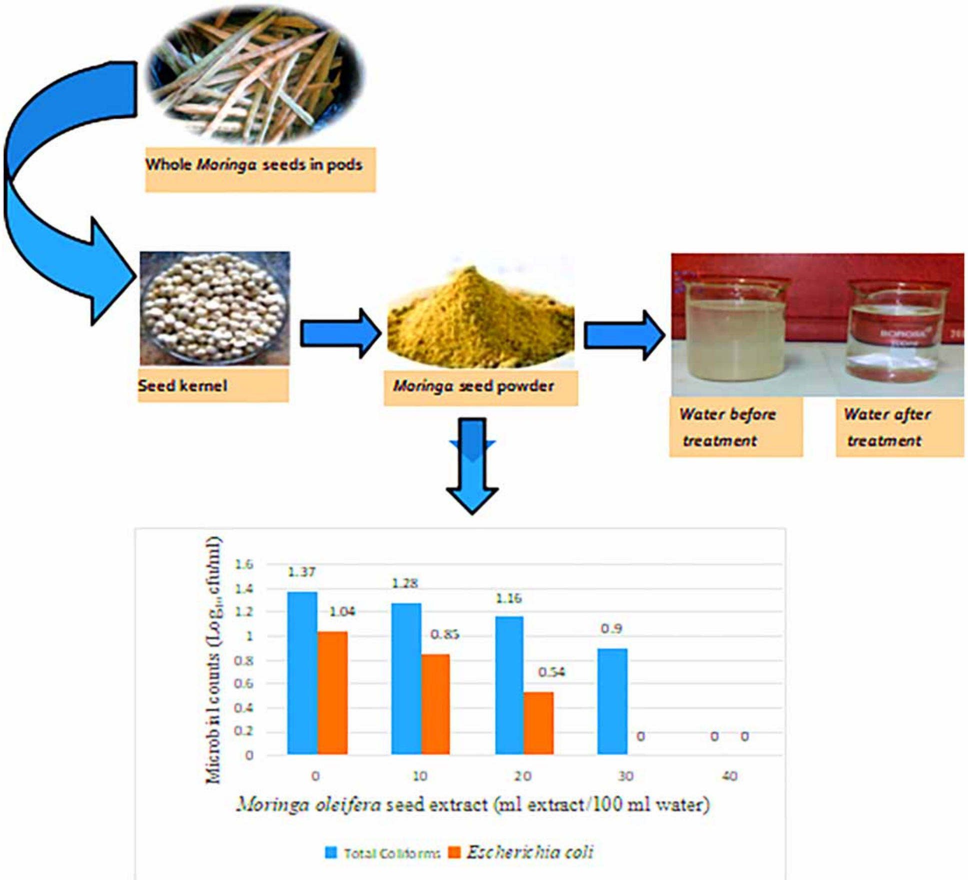
This study was conducted to determine the effects of *Moringa oleifera* seeds on the physicochemical properties and microbiological quality of borehole water collected from Thamaga village in Botswana. Borehole water samples were subjected to two different *Moringa* seed treatment methods: *M. oleifera* crushed seed powder and *Moringa* seed extract. Five treatment levels (0, 10, 20, 30, 40 g or mL extract) were used for each treatment method. Borehole water treated with *Moringa* seed extract had significantly lower ($p < 0.05$) total dissolved solids and conductivity as compared with water treated with crushed *Moringa* seed powder. However, borehole water treated with *Moringa* seed extract had significantly higher ($p < 0.05$) pH and total suspended solids as compared with water treated with crushed *Moringa* seed powder. *Escherichia coli* and total coliforms were completely inhibited by *Moringa* seed extract after 2 h of treatment at a concentration level of 40 mL extract. It was observed that *Moringa* seed extract at a concentration level of 40 mL was the most effective in treating borehole water compared with *Moringa* crushed seed powder. In conclusion, *Moringa* seed extract can be used as an alternative and affordable source of borehole water treatment in rural Botswana.

Key words: antibacterial property, *Moringa oleifera* seed, Thamaga village, water treatment

HIGHLIGHTS

- *Moringa* seed extract resulted in complete elimination of *Escherichia coli* and total coliforms from borehole water.
- *Moringa* seed extract improved the physical properties of borehole water.
- *Moringa* seed extract was most effective in improving the quality of borehole water at a concentration level of 40 mL.
- *Moringa* seed extract can be used as an alternative source of borehole water treatment.

GRAPHICAL ABSTRACT



INTRODUCTION

Water is one of the fundamental requirements of human life (Ugwu *et al.* 2017; Rashid *et al.* 2021). It remains one of the scarce resource to most of the developing countries (Hashim *et al.* 2021a). Due to inabilities of governments to meet escalating water demand, most people in rural areas resort to groundwater sources such as borehole as an alternative water source (Central Statistic Office 2009). Although groundwater is the major water source in rural areas of many countries, it is susceptible to contamination from different sources (Sharma *et al.* 2006; Al-Hashimi *et al.* 2021). The quality of groundwater in Botswana is highly variable and groundwater pollution is reported to be caused by manmade or natural factors (Mmereki 2018). Some of these factors that cause pollution of groundwater include farming, mining, and domestic and industrial waste disposal, which render the groundwater unsuitable for human consumption (Department of Water Affairs 2016). The chemical, physical, as well as microbiological properties of groundwater used for human consumption in developing countries is a significant, but neglected public health issue (WHO 2008). The supply of potable water has been a recurrent problem which seriously need urgent attention in many of the developing countries around the world, with worrisome situations in both rural and urban settlements (Bakare *et al.* 2017; Hashim *et al.* 2021a).

A comparison of urban and rural villages in Botswana showed that 99.5% of the population in cities/towns have access to tap water, while in rural villages, the proportion is 84.1% (Central Statistics Office 2009; UNDP 2012). Groundwater is the main source of potable water supply in Botswana and much of the country (about 66%) depends entirely on underground water (Central Statistics Office 2009). All of the western part of Botswana depends largely on underground water for both human consumption and livestock (Central Statistics Office 2009; UNDP 2012). Despite access to drinking water, the country still faces challenges to address water supply in rural and informal settlements (Central Statistics Office 2009; UNDP 2012). Like many other countries in Africa, the country relies on groundwater as a major source of drinking water supply for its dispersed rural population. Most rural areas such as Thamaga village, where most of the water sources is from the Malwelwe wellfields in Kweneng District depends entirely on groundwater (Department of Water Affairs 2016). With high population estimated at around 23,000 according to 2011 population census, the village often encounters water scarcity (Statistics Botswana 2011). Some areas in the village do not have access to adequate water supply and some households are not connected to water distribution pipelines (UNDP 2012). In cases where there is no water supply, Water Utilities Cooperation (WUC) provides the villagers with water using its water bowsers. Even though WUC has been trying hard to alleviate water shortage, this is not enough to cover the whole village therefore most people opt to use water from nearby boreholes directly for various domestic purposes without any treatment. The water supply system in most rural areas of Botswana especially rural villages in north-eastern and central parts of Botswana is mainly based on boreholes (Central Statistics Office 2009; UNDP 2012). Water-borne diseases such as diarrhea remain to be the major cause of morbidity and mortality in children under 5 years of age in Botswana (Statistics Botswana 2017).

In many cases, people living in rural areas drink water from those untreated sources such as rivers and boreholes (Department of Water Affairs 2015). According to Al-Bari *et al.* (2006), the escalating life-threatening infections are caused by drinking of untreated water and it is an important cause of mortality in developing countries. Various conventional methods have been utilized for wastewater treatment, such as coagulation–flocculation, adsorption, membrane filtration, reverse osmosis, and ion exchange resins, which are applied before the water is distributed to consumers (Ugwu *et al.* 2017; Rashid *et al.* 2021). Despite the significant role of synthetic and chemical coagulants in water purification, they are associated with several neurotoxic and carcinogenic effects due to their leftover residuals in the treated water, such as aluminum (Shan *et al.* 2017).

In addition to the conventional water treatment methods, nowadays, different new and innovative methods have been used to remove organic and inorganic contaminants from water. Some of the innovative water treatment methods used include electromagnetic radiation-electrocoagulation technology which is used to remove organic matter and metal ions from water (Hashim *et al.* 2021b), and use of fresh bottom ash from power production plants as a cost-effective and eco-friendly alternative (adsorbent) for phosphate removal from water (Hashim *et al.* 2021a).

Moreover, in recent years, there has been a growing interest to use plant-based coagulants to treat water as alternative to conventional water treatment techniques. Plants are a cost-effective and environmentally friendly way to breakdown, absorb, metabolize, and detoxify various pollutants. A recent study by Karaghool *et al.* (2022) showed that sweet basil (*Ocimum basilicum*) has the potential to absorb and remove methylene blue dye from wastewater discharged from textile industries. Zedan *et al.* (2022) reported that walnut seed extract can be used as a coagulant for removal of water turbidity.

Among the plant-based coagulants, the use of *Moringa* seeds for water treatment stands out. The seeds of *Moleifera oleifera* have traditionally been used to clarify muddy water in some African countries (Jahn 1991; Mayer & Stelz 1993). *M. oleifera* seeds present a turbidity removal efficiency comparable to industrial coagulants and flocculants (Alo *et al.* 2012). Reports indicate that Moringa seed powder can be used to remove heavy metals such as cadmium and lead from water and industrial wastes (Mataka *et al.* 2006, 2010). The value of *M. oleifera* seed in water/wastewater treatment lies on its biodegradability, low index of residual sludge formation, non-toxicty and non-corrosive nature, and the absence of notable drawbacks compared with conventional coagulants (Alo *et al.* 2012; Shan *et al.* 2017). In terms of cost, plant-based natural coagulants including Moringa seed are reported to be less expensive options for water treatment as compared with conventional methods such as the use of alum (El Bouaidi *et al.* 2022). A field trial study conducted in Malawi to assess water treatment efficiency of Moringa seed in comparison to the conventional coagulant alum revealed that the cost of water treatment using Moringa seed (75 Malawi Kwacha (MK) per 1,000 m^3 of water treated) was much less than the cost of using alum (501 MK) for water treatment (MK 10.07 = £1 sterling in March 1993) (Sutherland *et al.* 1994). In addition to its role in water purification, Moringa seeds exhibit antimicrobial effect against some microorganisms (Eilert *et al.* 1981; Ugwu *et al.* 2017). An *in vitro* study showed that an aqueous extract prepared from seeds of *M. oleifera* is effective against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli* (Fuglie 1999; Shan *et al.* 2017).

There is no documentation available regarding the use of *M. oleifera* seed as alternative source of water purification method despite its huge potential and wide distribution in Botswana (Seifu & Teketay 2020). The objectives of this study were to determine the effects of crushed seed powder and seed extract of *M. oleifera* on the physicochemical properties of borehole water; determine effects of crushed seed powder and seed extract of *M. oleifera* on the microbial load and safety of borehole water; and determine the most effective concentration levels of crushed seed powder and seed extract of *M. oleifera* on the quality of borehole water.

MATERIALS AND METHODS

Collection of *Moringa* seed samples

M. oleifera seeds were collected from different households in Gaborone city. About 2 kg of *Moringa* seeds were collected and only seeds in dry pods were used for the study. The seeds were removed from the pods and stored with winged seed covers at room temperature and transported to the Department of Basic Science, Biochemistry Laboratory at Botswana University of Agriculture and Natural Resources (BUAN). Prior to commencement of the experiment, the winged seed cover was removed and the kernel was grounded to a fine powder using a sterilized mortar and pestle.

Preparation of *M. oleifera* seed powder

The *Moringa* seeds were de-shelled and dried at ambient temperatures (23–25 °C) for a period of 5 days before milling. The white kernels were milled into a fine powder using a mortar and pestle then sieved through a 2 mm sieve to get the fine powder. The powder was then stored in a sterile bottle with cap (Sharma *et al.* 2006).

Ethanol extraction of *M. oleifera* seeds

The *M. oleifera* seeds were ground and solubilized in 90% ethanol at 50 g 100 mL^{-1}. The mixture was kept on a 120 rpm incubator shaker at 30 °C for 24 h and then filtered using Whatman No. 1 filter paper. The filtrate was collected and evaporated using a rotary evaporator N-1200 BS series from EYELA (Shanghai, China) at 50 °C and using a water bath at 50 °C until a thick extract was obtained (Hikmawanti *et al.* 2021). During the evaporation process, the volume of the mixture changed until the final volume reached about 2–3 mL of the plant material extract.

Water sampling

Bottles (250 mL) used for storing water samples for microbiological and physicochemical analysis were sterilized by using autoclave and sodium thiosulfate (0.1 mL), respectively. The borehole water was then pumped to fill the bottles leaving an air space of 2.5 cm to create space for oxygen such that organisms do not die before testing in the laboratory. The bottles were marked for identification using labels. The bottles were then transported to the National Food Control Laboratory in a cooler box containing ice packs to prevent external factors such as high temperatures from changing some of the water parameters. Analysis was done within 12 h of sampling (APHA 1998).

Experimental design and treatment of borehole water

Water samples were collected from an identified borehole in Thamaga village. A total of 12 water samples were collected into 250 mL sterilized bottles each for microbiological and physicochemical analyses and then stored in cooler box with ice and transported to the National Food Control Laboratory. Out of the 12 water samples used for physicochemical analysis, 4 samples were treated with 4 different concentrations of *Moringa* crushed seed powder and another 4 samples were treated with 4 different concentrations of *Moringa* seed extract. The remaining four water samples were not treated and used as a control. The same procedure was followed for the microbiological analysis as described above. In order to determine the optimum level of the crushed seed powder and/or the crude extract, each treatment (*Moringa* seed type) was divided into four levels (10, 20, 30, and 40 g or mL extract). After treating water with *Moringa* seed, the water was allowed to stand undisturbed for 2 h for microbiological analysis and 5 h for chemical analyses. The experiment was conducted in triplicates. The experimental design and concentrations of *M. oleifera* crushed seed powder and crude seed extract used to treat borehole water samples are indicated in Table 1.

Physicochemical analysis of borehole water samples

The physicochemical parameters of the water samples were determined prior to and after treatment with *M. oleifera* crushed seed powder and *M. oleifera* seed extract using recommended methods. The parameters analyzed are indicated below.

Determination of pH

The pH of the water samples was measured using the method 4500-H + B, Electrometric method (APHA AWWA & WEF 2017). Firstly, the pH meter (Crison pH meter, Cloverland) was calibrated using distilled water. Then, 10 mL sample of the experimental water was placed into a 10 mL beaker and the probe was dipped into it until the reading stabilizes. The measurement was repeated twice.

Table 1 | Experimental design and concentrations of *Moringa oleifera* crushed seed powder and crude seed extract used to treat borehole water samples

Water source	*Moringa* seed concentrations (g or mL extract)	Treatment with crushed seed powder (CS)	Treatment with crude seed extracts (CE)	Untreated control (UC)
Borehole	10	CS1	CE1	UC
Borehole	20	CS2	CE2	UC
Borehole	30	CS3	CE3	UC
Borehole	40	CS4	CE4	UC

CS, *Moringa* crushed seed powder; CE, *Moringa* crude seed extract; UC, untreated control.

Total suspended solids

Total suspended solids (TSS) were determined by weighing a filter paper on weighing beam balance. The water sample was filtered using a Whatman No. 1 filter paper. The wet paper was then dried in a hot air oven at 103–105 °C, after which the filter paper was removed and reweighed. The increase in weight was read and recorded in mg/L as the TSS in the water sample (McAlister & Ormsbee 2005).

Total dissolved solids (TDS) and electrical conductivity

These parameters were determined using a multimeter analyzer (HACH, Cloverland) that has a software application that can inter-change to read different parameters when the 'mode' button is pressed. For determination of TDS, ISO 7888:1985 (ISO 1985) was used. The instrument was calibrated by setting the approximate apparatus. Then, 10 mL of water sample was taken in a beaker and the electrode was dipped into it until the reading was stabilized. The measurement was repeated twice to get the values. Total dissolved solids (TDS) were recorded in mg/L and electrical conductivity was recorded in μs/cm.

Microbial analysis of the borehole water sample

E. coli and coliforms

E. coli/coliforms counts were determined according to ISO 4832-2006 (E) (ISO 2006). 100 mL of water sample was serially diluted from 10^{-1} to 10^{-2} and then 1 mL of each serial dilution was transferred into a Petri dish. A pour plate method using Brilliance *E. coli* medium was performed. Then the media was left to solidify before being incubated at 37 °C for 24 h. The plates were inverted and put into the incubator. After the incubation, the plates were removed and bacterial colonies were counted using a colony counter. Purplish red colonies were counted as coliforms.

Salmonella spp.

Salmonella spp. was determined according to the procedure outlined by ISO 6579:2002. Twenty-five mL of water was pre-enriched in 225 mL of buffered peptone water at 37 °C for 24 h. Then, 1 mL of pre-enrichment water sample was incubated in 10 mL of Selenite Cystine, Tetrathionate (TT), and Rappaport-Vassiliadis Soya Peptone (RVS) broths then incubated at 37 °C for 24 h. About 0.1 mL of two plates of each selective agar Desoxycholate (XLD) and Hektoen Enteric (HE) were streaked to obtain single colonies with inoculum from TT and RVS broths. The plates were then incubated inverted at 37 °C for 24 h. After incubation, presumptive *Salmonella* colonies from the selective medium were inoculated on the Triple Sugar Iron (TSI) agar by streaking the slant in a zigzag motion and end with a stab of the butt of the slant. Lysine Iron Agar (LIA) slant was inoculated with the same colony by stabbing the butt and streaking the slant at last. Both TSI and LIA slants were incubated for 24 h at 37 °C and observation was made after incubation (FDA 2001).

Detection and isolation of pathogenic *E. coli*

A loopful of the positive EC broth was streaked onto Eosin Methylene Blue agar (EMBA) and incubated for 24 h at 37 °C. The test was completed by making a thin smear for Gram staining from the purple colonies and confirmed by biochemical tests (Method 9223A, APHA 1990). *E. coli* colonies were confirmed by the indole test.

Statistical analysis

All statistical analysis was carried out using the Statistical Analysis System (SAS) software version 9.3 (SAS Institute, Cary, NC, USA). For data on the physicochemical properties, a one-way Analysis of Variance (ANOVA) was used to determine differences in mean values between the two Moringa types and among the different concentration levels for each parameter. Mean separation was done using the Least Significant Difference (LSD) technique and significant differences were declared at a 5% significance level. The microbial count data are presented using a bar chart as mean $\log_{10}$ cfu/mL.

RESULTS AND DISCUSSION

Physicochemical properties

The physicochemical properties of borehole water samples treated with *M. oleifera* crushed seed powder are indicated in Table 2. The mean pH values of borehole water treated with various amounts of crushed *Moringa* seed powder ranged from 6.86 $\pm$ 0.010 to 6.48 $\pm$ 0.01 and a decrease in pH value was observed with increasing concentration of crushed *Moringa* seed powder (Table 2). The decrease in pH of water observed in the present study is in line with the findings of Shan *et al.* (2017) and Oluduro & Aderiye (2007) who reported a decrease in pH of water with increase in concentration of Moringa seed cake and Moringa seed extract, respectively. The observed pH values are within the limits (6.5–8.5) of the Botswana (BOBS 2009) and WHO (2008) standard for drinking water. A significant difference ($p < 0.05$) in pH was observed between the treatment groups and the control and CS4 (addition of 40 g *Moringa* seed powder) had the lowest pH value (Table 2). Similarly, *Moringa* seed extract resulted in reduction in the pH of water and a decrease in pH value was observed with increasing concentration of *Moringa* seed extract (Table 2). *Moringa* seed extract caused a slight reduction in pH of water as compared with crushed *Moringa* seed. The pH of water treated with *Moringa* extract was above pH value of 7.0 for all the concentration levels considered. For water samples treated with *Moringa* seed extract, a significant difference ($p < 0.05$) in pH was observed between the treatment groups and the control and CE4 (addition of 40 mL *Moringa* seed extract) had the lowest pH value (Table 2). Comparison between *Moringa* crushed seed powder and *Moringa* seed extract showed that *Moringa* crushed seed powder significantly decreased pH as compared with *Moringa* seed extract which slightly reduced pH (Table 2). The preparation of the Moringa seed extract involved use of ethanol as a solvent which removed fats from the Moringa seed powder. Thus, the decrease in pH of water observed with increasing dose of the crushed Moringa seed powder could be attributed to free fatty acids present in the undefatted Moringa seed powder. Moreover, the active proteins of Moringa seed-derived coagulants are water-soluble cationic proteins with a net positive charge (Ndabigengesere *et al.* 1995).

Table 2 | Comparison of physicochemical parameters of water treated with *Moringa oleifera* crushed seed powder and *Moringa oleifera* seed extract

Parameters	Treatments	Moringa concentrations (g or mL extract)	Moringa crushed seed powder (CS)	Moringa seed extract (CE)
pH	Control	0	8.14 ± 0.006^{a}	8.14 ± 0.006^{a}
	1	10	6.86 ± 0.010^{f}	8.10 ± 0.006^{b}
	2	20	6.62 ± 0.010^{g}	7.93 ± 0.006^{c}
	3	30	6.54 ± 0.021^{h}	7.87 ± 0.010^{d}
	4	40	6.48 ± 0.015^{i}	7.74 ± 0.010^{e}
TDS (mg/L)	Control	0	138 ± 5.196^{e}	138 ± 5.196^{e}
	1	10	256.0 ± 3.000^{d}	128.0 ± 3.606^{f}
	2	20	412.67 ± 2.082^{c}	117.0 ± 5.508^{g}
	3	30	424.67 ± 11.150^{b}	112.67 ± 5.686^{gh}
	4	40	455.33 ± 3.512^{a}	106.1 ± 6.851^{h}
Conductivity (μs/cm)	Control	0	262 ± 3.606^{e}	262 ± 3.606^{e}
	1	10	474.67 ± 5.686^{d}	250.0 ± 3.606^{e}
	2	20	783.33 ± 16.289^{c}	230.0 ± 7.506^{f}
	3	30	800.33 ± 13.650^{a}	227.33 ± 7.506^{f}
	4	40	817.00 ± 3.606^{a}	212.67 ± 8.622^{g}
TSS (mg/L)	Control	0	733.0 ± 107^{a}	733.0 ± 107^{a}
	1	10	515.67 ± 82.124^{bc}	647.33 ± 85.249^{ab}
	2	20	387.0 ± 1.000^{cde}	530.67 ± 127.7^{bc}
	3	30	350.33 ± 27.006^{de}	504.33 ± 114.33^{bcd}
	4	40	277.33 ± 76.644^{e}	490.67 ± 101.86^{bc}

Means with different superscript letters within a column and within a row are significantly different ($p < 0.05$). TDS, total dissolved solids; TSS, total suspended solids.

Adsorption and charge-neutralization have been suggested as the possible mechanisms of Moringa seed-based coagulants where the positive charge in the Moringa seeds neutralizes the negative charge of colloids in water (Chales *et al.* 2022). Thus, the decrease in pH of water observed with increasing dose of Moringa seed powder/extract could also be attributed to possible addition of excess positively charged proton (H^+) into water from the increased concentration of the Moringa seed. When crushed Moringa seed powder is mixed with water, it yields water-soluble proteins that possess a net positive charge (Sutherland *et al.* 1994).

A significant difference ($p < 0.05$) was observed in TDS between the treatment groups and the control and there was an increase in TDS with increasing levels of crushed *Moringa* seed powder (Table 2). This observation is in agreement with the findings of Shan *et al.* (2017) who reported an increase in TDS content of water with increasing concentration of Moringa seed. Kitheka *et al.* (2022) reported a significant increase in TDS of water with increased concentration of Moringa seed powder and showed the existence of a strong positive relationship between Moringa seed coagulant concentration and TDS. Chales *et al.* (2022) reported that dissolved organic carbon (DOC) in well water treated with Moringa seed-derived coagulants increased with increasing dosage of these coagulants. They attributed this to the fact that *M. oleifera* seeds contain a variety of organic compounds, including coagulation-active and coagulation-inactive components, and at least 78% of the DOC of *M. oleifera* whole-seed extract is kept in treated water. Ndabigengesere & Narasiah (1998) also reported that concentration of DOC in treated water increased considerably with the dose of Moringa seed. Thus, these could explain the increase in TDS observed in the present study with an increased dose of Moringa seed powder since TDS is related to DOC. DOC is defined as organic matter that is able to pass a filter which removes material between 0.70 and 0.22 mm in size (Zhuiykov 2014). TDS in wastewater refers to dissolved organic matter and inorganic salts (Mokhatab *et al.* 2019).

On the other hand, treatment of borehole water with *Moringa* seed extract showed an opposite trend with regard to TDS. That is, a decrease in TDS was observed with increasing levels of *Moringa* seed extract (Table 2). The uses of *Moringa* seed extract at the highest concentration (40 mL extract) significantly reduced TDS to 106.1 ± 6.851 (Table 2). The decrease in TDS observed with increasing concentration of Moringa seed extract as compared with Moringa seed powder could be attributed to the strong coagulation property of the Moringa seed extract and its ability to remove TDS as compared with Moringa seed powder. This is expected as Moringa seed extract is more concentrated than the crushed Moringa seed powder and thus has strong effect. TDS is the total amount of mobile charged ions, including minerals, salts, or metals dissolved in a given volume of water, expressed in units of mg per unit volume of water (mg/L) (Thakur & Choubey 2014). TDS is directly related to the purity of water and the quality of water purification systems (Thakur & Choubey 2014).

An increase in conductivity of water was observed with increased levels of addition of crushed *Moringa* seed powder (Table 2). Increasing concentrations of *Moringa* crushed seed powder led to an increase in conductivity, this may be attributed to the increase in cationic polyelectrolyte in *Moringa* seeds thereby producing high dissolved solids that increased the conductivity (Okuda *et al.* 2000). This observation is in agreement with the findings of Shan *et al.* (2017) who reported an increase in conductivity of wastewater with increasing concentration of Moringa seed. Kitheka *et al.* (2022) reported a significant increase in the electrical conductivity of water with the increased concentration of Moringa seed powder and showed a strong positive relationship between Moringa seed coagulant concentration and electrical conductivity. The high conductivity of water observed in the present study could also be attributed to the high TDS content of the water resulting from Moringa seed powder addition which could have increased the ability of the water to conduct an electrical current because of the ions from the dissolved solids in the water samples. However, the present result is not in agreement with the report by Amagloh & Benang (2009) who demonstrated that increasing concentrations of *Moringa* coagulants led to decrease in conductivity values. Water treated with *Moringa* seed powder concentration levels CS3 (30 g) and CS4 (40 g) had significantly higher ($p < 0.05$) conductivity as compared with the other treatments and the control (Table 2). However, there was no significant difference ($p > 0.05$) in conductivity between CS3 and CS4. On the other hand, a decrease in conductivity was observed with an increase in concentration for water samples treated with *Moringa* seed extract (Table 2). The conductivity of water treated with *Moringa* seed extract observed in the present study is much lower than the acceptable conductivity level (1,500 μs/cm) of drinking water according to the Botswana standard (BOBS 2009). CE4 (40 mL extract) resulted in a significantly lower conductivity ($p < 0.05$) as compared with the other treatments (Table 2). *Moringa* seed extract showed a significant decrease in conductivity compared with *Moringa* crushed seed powder which showed an increase in conductivity with an increase in the concentration of the powder (Table 2).

The addition of *Moringa* crushed seed powder to borehole water led to a decrease in TSS in water. An increase in *Moringa* seed powder amount resulted in a significant reduction in TSS as compared with the control (Table 2). CS4 (40 g) is the most effective in reducing TSS with mean value of 277.33 ± 76.644 compared with the rest of the treatments. Treating water with

Moringa seed extract also showed a decrease in TSS but a slight decrease was observed as compared with that of the crushed *Moringa* seed powder (Table 2). Comparison between the two *Moringa* treatment types (*Moringa* crushed seed powder and *Moringa* seed extract) indicated that *Moringa* seed powder at 40 g is the most effective level in reducing TSS in borehole water (Table 2). The decrease in TSS observed in the present study with increasing concentration of Moringa seed powder/extract agrees with the findings of Oluduro & Aderiye (2007) who reported a decrease in TSS with increase in the concentration of Moringa seed extract. They showed that the Moringa seed achieved 90 and 95% sedimentation of suspended particles in groundwater and surface water samples, respectively. A recent study by Kitheka *et al.* (2022) showed that *M. oleifera* seed powder can be used for water clarification as it leads to rapid flocculation of suspended solids in water leading to improved clarity of water. Adsorption and charge-neutralization have been suggested as the possible mechanisms behind turbidity removal of water using Moringa seed-derived coagulants (Chales *et al.* 2022). The turbidity of water is mainly caused by suspended solids and colloidal particles present in water. Coagulation using Moringa seed is caused by the destablization of negatively charged colloids (suspended solids) in water by the positively charged cationic polyelectrolytes in the Moringa powder (Ndabigengesere *et al.* 1995).

These results were within the acceptable TSS value of 500 mg/L for safe drinking water (Olanrewaju *et al.* 2018). The results of the present study are also in agreement with the findings of Olanrewaju *et al.* (2018) who reported that treatment of wastewater with 40 mL of Moringa seed extract reduced the TSS of the wastewater from an initial of 1,240 to 400 mg/L after 24 h settling interval, which means Moringa had a TSS removal efficiency of 67.7%. High level of TSS in water interferes with effective drinking water treatment, that is, high TSS interfere with coagulation, filtration, and disinfection of water and is mostly associated with the possibility of microbial pollution (McAlister & Ormsbee 2005).

In general, the results of the analysis of the physicochemical parameters showed that treatment of borehole water with 40 g crushed Moringa seed powder or 40 mL Moringa seed extract was the most effective concentration level in improving the quality of the water. This observation agrees with the findings of Olanrewaju *et al.* (2018) who reported that the optimal concentration level of *Moringa* coagulant for treating wastewater was 40 mL.

Microbiological analysis

The results of the microbial analysis of untreated borehole water (Figures 1 and 2) showed that the total coliforms and *E. coli* counts were as high as 1.37 and 1.04 $\log_{10}$ cfu/mL, respectively. *Salmonella* and pathogenic *E. coli* were not isolated from the untreated borehole water or control.

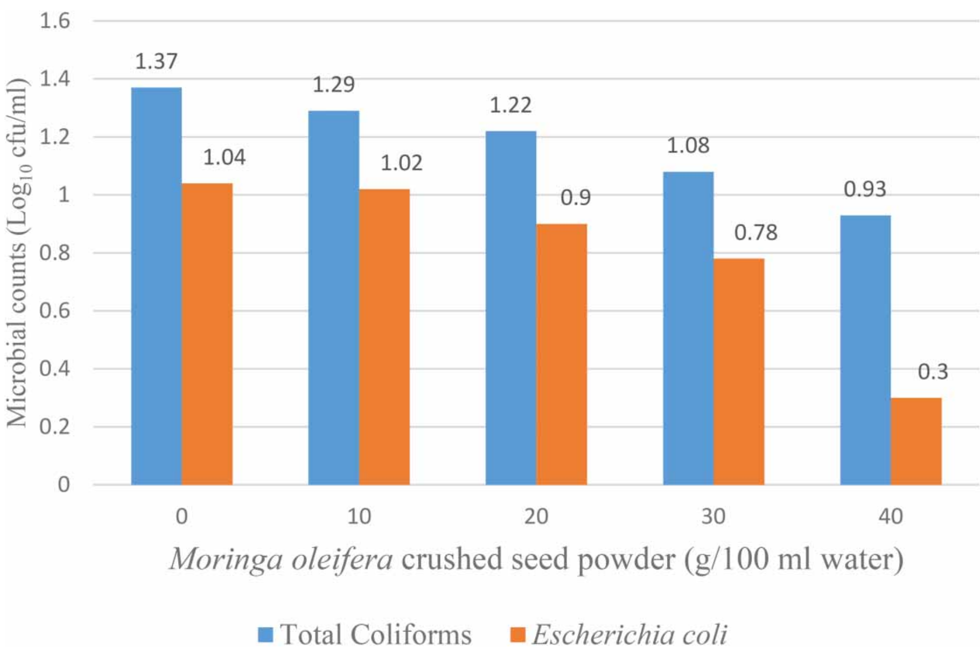


Figure 1 | Effects of *Moringa oleifera* crushed seed powder on microbial quality of borehole water after 2 h of treatment. Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/washdev.2022.100.

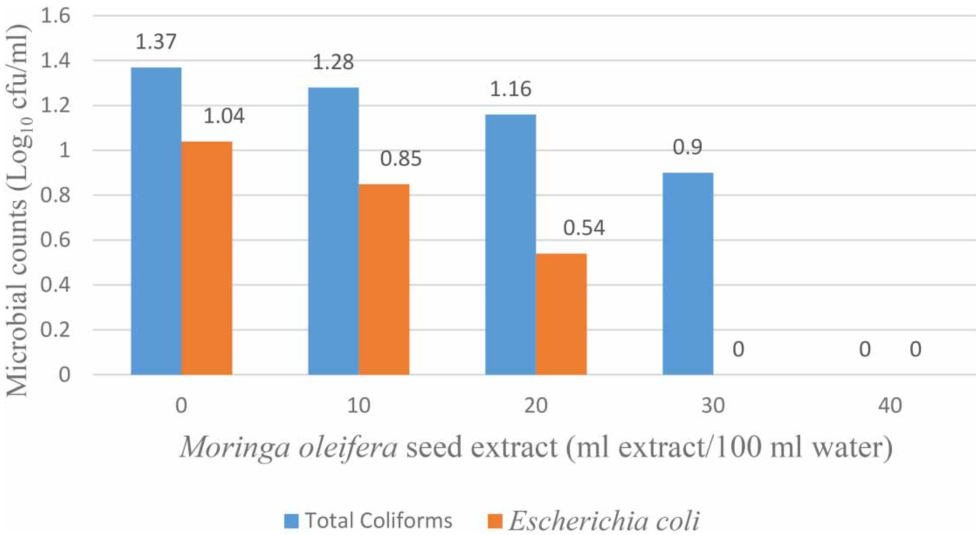


Figure 2 | Effects of *Moringa oleifera* seed extract on microbial quality of borehole water after 2 h of treatment. Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/washdev.2022.100.

Treatment of borehole water with *M. oleifera* crushed seed powder at different treatment levels led to decrease in the microbial counts in the water (Figure 1). A decrease in total coliform and *E. coli* counts were observed with increasing concentration level of crushed *Moringa* seed powder (Figure 1). Oluduro & Aderiye (2007) reported that treatment of surface water with *M. oleifera* seed extract reduced total bacteria and coliforms counts by 88 and 97.5%, respectively, after 24 h whereas treatment of underground water with the seed extract reduced total bacteria and coliforms by 88.3 and 93.3%, respectively, after 24 h. A recent report by Chandrashekar *et al.* (2020) indicated that crude extract and coagulant protein from *Moringa* seeds exhibited cell aggregation and growth inhibition of six different pathogenic microorganisms including *E. coli* and *Salmonella* Paratyphi B and they attributed this to the presence of peptides with antibacterial property in the Moringa seeds. The antibacterial effects of the Moringa seed extract observed in the present study could be attributed to its possession of antibacterial proteins or the presence of various phytochemicals with antimicrobial properties in the seeds as reported by Begum *et al.* (2021).

The lowest total coliforms and *E. coli* counts were observed at 40 g concentration level (Figure 1). Treatment of borehole water with 40 g crushed *Moringa* seed powder resulted in a reduction of total coliforms and *E. coli* counts by 0.44 and 0.74 $\log_{10}$ cfu/mL, respectively (Figure 1). The results obtained in this study are in line with observations made by Jabeen *et al.* (2008) who stated that the antimicrobial activity of *M. oleifera* seed powder is dependent on the quantity of the seed powder, as the quantity increases the antibacterial activity also increases. Nkurunziza *et al.* (2009) reported that treatment of turbid water with *M. oleifera* seed powder resulted in 96% removal of *E. coli* from the water.

Figure 2 indicates that *M. oleifera* seed extract is more effective in reducing microbial counts in borehole water as compared with crushed *Moringa* seed powder. As *Moringa* seed extract concentration increases, a significant reduction in total coliform and *E. coli* counts were recorded (Figure 2). No *E. coli* count was observed at *Moringa* seed extract concentrations of 30 and 40 mL. The highest concentration (40 mL) of *Moringa* seed extract resulted in inhibition of growth of both total coliforms and *E. coli* (Figure 2). The results indicate that concentration of 40 mL of *Moringa* seed extract is the most effective level to inhibit the growth of total coliforms and *E. coli* in water. The inhibitory effect of the *Moringa* seed extract observed might be attributed to the fact that the seeds of *M. oleifera* contain a number of benzyl isothiocyanate and benzyl glucosinolates which act as antibiotics (Bichi *et al.* 2012). Bichi *et al.* (2012) reported that the mode of attack of the *M. oleifera* seed extract on the *E. coli* cells is by rupturing the cell and damaging the intracellular components, allowing water to enter into cell which causes it to swell more and burst leading to death. Also, an *in vivo* study by Fuglie (1999) showed that an aqueous extract made from the seeds of *M. oleifera* was effective against *E. coli*.

CONCLUSION

Treatment of borehole water with 40 mL of *Moringa* seed extract significantly reduced the pH, TDS, conductivity, and TSS as compared with lower concentration levels. At higher concentration of 40 g or mL extract, a reduction in *E. coli* and total coliforms was observed in water samples treated with both *Moringa* seed extract and *Moringa* crushed seed powder. Therefore, concentration of 40 g or mL extract is the most effective level in improving the physicochemical properties and microbial quality of borehole water. Treating water using *Moringa* seed extract is the most effective in improving both physicochemical and microbiological parameters compared with *Moringa* crushed seed powder. The results of this study showed that *M. oleifera* seed can be used as an alternative, cheap and locally available method for treatment of borehole water and significantly improve its physicochemical properties and microbial quality. This is particularly important in rural Botswana where people often do not have access to and/or afford to buy potable drinking water.

ACKNOWLEDGEMENTS

The authors thank the National Food Control Laboratory for allowing the researchers to use their facilities and assisting during physicochemical and microbiological tests. We are also very grateful to all households in Gaborone who generously provided the Moringa seed samples used in this study. Mr Joshua Makore's assistance during the statistical analysis of the data is highly appreciated. This study was funded by the Department of Tertiary Education of Botswana.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare that there is no conflict.

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First received 24 April 2022; accepted in revised form 27 August 2022. Available online 7 September 2022