Research Journal of Engineering and Environmental Sciences 1(1) 2016 pp. 190-201

Research Journal of Engineering and Environmental Sciences

Journal homepage: www.rjees.com

RJEES

Original Research Article

SYNERGISTIC EFFECTS OF *MORINGA OLEIFERA* SEED CAKE EXTRACT AND CACTUS MUCILAGE IN SURFACE WATER TREATMENT

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ARTICLE INFORMATION

Article history:
Received 10 October 2016
Revised 21 November 2016
Accepted 05 December 2016
Available online 20 February 2017

Keywords:
Moringa oleifera
Cactus mucilage
Bacteria
Turbidity
Shelf life

ABSTRACT

This research investigated the synergistic effects of Moringa oleifera seed cake extract and Opuntia stricta (cactus) mucilage in surface water treatment. The flocculation/coagulation experiments were conducted using a standard jar test apparatus and the properties of the raw and treated waters i.e. turbidity, pH, conductivity, total dissolved solids (TDS) and bacteria count were monitored using appropriate portable meters according to standard methods. For a raw surface water with turbidity of 44 NTU, the optimum doses of Moringa oleifera and Opuntia stricta mucilage were found to be 3 mg/L and 2 mg/L corresponding to 88.6%, and 42.7% reduction in the surface water turbidity respectively when used individually, while a blend of cactus mucilage and Moringa oleifera extracts in the ratio of 40:60 gave turbidity reduction of 86.1%. The blend showed superior ability in terms of bacterial count reduction (82 cells to 2 cells per 100 ml of treated water) when compared to the individual capabilities of 7 cells and 4 cells for Moringa oleifera and cactus mucilage respectively. The shelf life of the water treated with Moringa oleifera, Opuntia stricta mucillage and the blend were 3 days, 3 days and 5 days respectively. The physiochemical properties of the treated water such as conductivity, pH and TDS were found to depend on the chemical composition of the biocoagulants i.e. Moringa oleifera seed cake extract contained 0.700% of K, 0.020% of Na, 0.114% of Ca and 0.074% of Mg while Opuntia stricta (cactus) mucilage contained 0.350% of K, 0.180% of Na, 1.522% of Ca and 0.083% of Mg.

1. INTRODUCTION

Naturally, water sources such as ground water and surface water receive pollutants from surface runoff, sewage and industrial wastes amongst others. An estimated 801,000 children younger than 5 years of age perish from diarrhea each year, mostly in developing countries (Liu et al., 2012). This amounts to 11% of the 7.6 million deaths of children under the age of five and means that about 2,200 children are dying every day as a result of diarrheal diseases (Liu et al., 2012). Worldwide, millions of people are infected with neglected tropical diseases (NTDs), many of which are water and/or hygiene-related, such as Guinea worm disease, Buruli ulcer, trachoma, and schistosomiasis. These diseases are most often found in places with unsafe drinking water, poor sanitation, and insufficient hygiene practices (Global water, sanitation and hygiene, 2016). Therefore, water from all sources must have some form of purification or treatment before consumption.

Various methods are used to make water safe and attractive to the consumer. The method employed depends on the character of the raw water. The common methods of household water treatment require coagulation / flocculation followed by sedimentation, filtration and disinfection (Adejumo et al*.,* 2013,). Several coagulants have been used in conventional water treatment processes for portable water production. These coagulants can be classified into inorganic, synthetic organic polymer and naturally occurring coagulants (Okuda et al*.,* 2000). Synthetic polyelectrolytes are used as primary coagulant as well as coagulant aid to improve the strength of particles aggregates, enhance coagulation and deposition (filtration).

There is evidence that the use of extracts from plant species possessing both coagulating and antimicrobial properties are safe for human health (Muyibi and Okuofu, 1995; Muyibi and Evison 1995; Okuda et al*.,* 2000; Ali et al*.,* 2004; Akinnibosun et al*.,* 2008). It is therefore, desirable to replace the conventional chemical coagulants with plant-based coagulants. The main advantages of using natural plant-based coagulants as point- of- use (POU) water treatment material are apparent; they are cost effective, unlikely to produce treated water with extreme pH and are highly biodegradable (Chun-yang, 2010). Of the large number of plant materials that have been used over the years, the seeds from *Moringa oleifera* have been shown to be one of the most effective primary coagulants for water treatment especially in rural communities (Folkard et al*.,* 2000; Ghebremichael and Kebreab, 2004; Doer, 2005; Onwuliri and Dawang, 2006). Equally well studied is the antibacterial activity of *Opuntia stricta* (cactus) (Ndabigengesere et al., 1995; Goycoolea and Cardenas, 2003).

It is therefore, the goal of the present paper to investigate the potential of combining both plants in surface water treatment to enhance clarity, bacteria load reduction and shelf life elongation of the treated water.

2. MATERIALS AND METHODS

2.1. Materials

Opuntia stricta (cactus) pads were obtained from Jos, Plateau State, while *Moringa oleifera* seeds were obtained from Zaria, Kaduna State. The surface water sample was obtained from

Ahmadu Bello University's water dam. The reagents used in this study included distilled water, deionized water, sodium hexametaphosphate, ethanol, sodium chloride, n-hexane, hydrochloric acid, buffer solution, sodium hydroxide, nitric acid, sulphuric acid etc. Other materials used include distiller, jar test apparatus (Stuart flocculator equipment), centrifuge, soxhlet apparatus, turbidimeter (HACH 2100P Model), pH meter (KENT EIL 7055), conductimeter (Jenway 4010), magnetic stirrer, oven, retort stand, test tubes, Berzelius beakers, pipette, Erlenmeyer flasks etc.

2.4. Preparation and Characterization of Cactus Mucilage and Moringa Coagulant

2.4.1. Extraction of mucilage from cactus

Mucilage was extracted from *Opuntia stricta* pads according to the methods described by Goycoolea and Cardenas, (2003), Turquois et al. (1999) and Medina-Torres et al. (2000). The mucilage was extracted at a temperature of 85^oC.

2.4.2. Preparation of *Moringa oleifera* seed cake extract

The seed wings and coat were removed from the selected *Moringa oleifera* seeds and the nuts ground to a fine powder with the domestic food blender and then it was extracted using n-hexane as solvent in a soxhlet apparatus. The temperature for the extraction was about 40-60^oC

2.4.3. Characterization of the cactus mucilage and *Moringa oleifera* extract

Elemental analysis of the cactus mucilage and *Moringa oleifera* (Ca, Na, K and Mg) was performed using flame photometer and atomic absorption spectrophotometer (Perkin-Elmer Corporation).

2.5. Jar Test

Standard Jar test procedures were employed for this purpose (American Public Health Association/ American Water Works Association/ Water Pollution Control Federation (APHA/AWWA/WPCF)) (Muyibi, 1995). The optimizations for cactus and *Moringa oleifera* were conducted using the jar test apparatus. Varying concentrations of *Moringa oleifera* (1 – 35 mg/l) and cactus mucilage (1 – 30 mg/l) were tested in separate parallel runs. The combined effects of cactus extract and *Moringa* extract were studied using varying ratios (100:0 to 0:100)

2.6. Analysis

Water samples were analyzed for bacteria load (using the tube method) and *E. coli* count using MacConkey agar (Tadesse, 2006). The pH of the sample was read using a calibrated Jenway 3505 pH meter. Total dissolved solid (TDS) and electric conductivity (EC) were determined using the TDS meter (Jenway 4510). The turbidity of the sample water was determined using a direct reading potable turbidimeter (HACH 2100P).

2.7. Confirmation Test

A loopful of a positive lactose broth culture was transferred from a tube inoculated with the smallest inoculum giving a positive presumptive test (0.1 ml in above test) into 2% brilliant green bile broth. It was incubated between 35-37 ^oC overnight. It was then looked for cloudiness and gas production for a positive result (Tadesse, 2006).

2.8. Complete Test

A loopful of a positive brilliant green bile broth, 2%, culture was streak onto MacConkey agar plates. It was then incubated between 35-37 ^oC overnight. Coliform bacteria was detected which produce brick-red colonies with a surrounding zone of precipitate bile on MacConkey. Stain representative colonies by gram method was used to detect gram negative, non-sporulation rods microscopically (Tadesse, 2006).

2.8. Data Analysis

Data were analysed using the SPSS statistical package. A two sided *t*-test was used at 5% level of significance to determine if there were significant variations among the *Moringa oleifera* and cactus mucilage treatments used in the study.

3. RESULTS AND DISCUSSION

The effect of *Moringa* and cactus dosage on percent turbidity reduction of surface water is as presented in Figure 1. At *Moringa* dosages of 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, and 35 mg/l respectively, the % turbidity reduction of the water sample were 55.5, 72.5, 88.6, 83.4, 81.8, 81.7, 76.6, 73.6, 72.3, 70, and 69.5% respectively. Hence, an optimum *Moringa* dosage 3 mg/l can be deduced from the figure, similarly the cactus mucilage's optimum dosage of 2 mg/l was obtained. The settling rate of coagulated floc from water with *Moringa oleifera* seed extract was faster and apparently higher than that of *Opuntia stricta* cactus given the same initial turbidity. In polluted surface water, impurities are dominated by negatively charged ions. The predominant coagulating agent in *Moringa oleifera* was reported by Gassenschmidt et al., (1995) as cationic polyelectrolytes, while the coagulating agent in cactus mucilage was suggested by Seanz et al., (2004) to consist of viscous and complex carbohydrate stored in the cactus inner pad especially the galacturonic acid. Therefore, from the physicochemical properties of *Moringa oleifera* and *Opuntia stricta* are shown in Table 1, the high concentration of Ca^{2+} in the cactus may be responsible for its colloidal nature, unlike Moringa with K+ being highly soluble in water. (Buttice et al., 2010). The turbidity of *Moringa oleifera* treatment was statistically significant as compared to that of cactus mucilage treatment ($p=0.000$ and $p<0.05$ respectively).

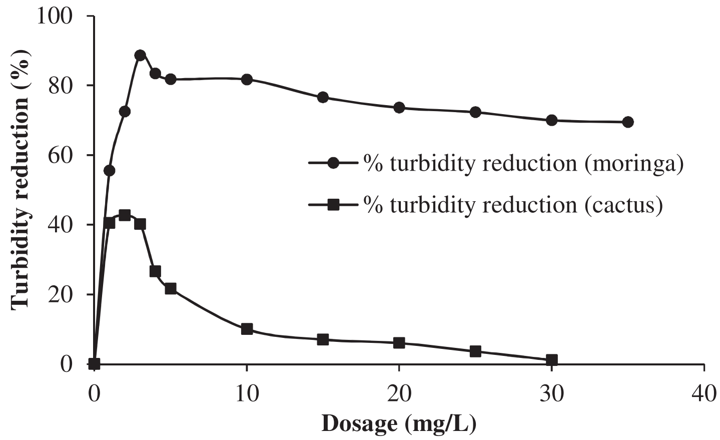

Figure 1: Effect of Moringa and cactus dosage on % turbidity reduction of surface water

Table 1: Physicochemical properties of *Moringa oleifera* and *Opuntia stricta* cactus

Parameters	Moringa	Cactus
Potassium (K) %	0.700	0.350
Sodium (Na) %	0.020	0.180
Calcium (Ca) %	0.114	1.522
Magnesium (Mg) %	0.074	0.083

The effect of *Moringa* and cactus dosage on conductivity and total dissolved solid of surface water are shown in Figures 2 and 3. Increase in both *Moringa oleifera* and cactus mucilage coagulants dosage increased the conductivity and TDS of the treated water. This is in consonance with the findings by Muhammad (2011). For instance, 3 mg/l dosage of *Moringa oleifera* coagulant gave the lowest turbidity (5.03NTU) indicating the optimum values at conductivity and TDS of 117.8 (µS/cm) and 72.1 mg/l respectively. In cactus mucilage treatment, 2 mg/l gave the lowest turbidity of 25.2NTU at conductivity and TDS of 122.8 (µS/cm) and 74.8 mg/l respectively.

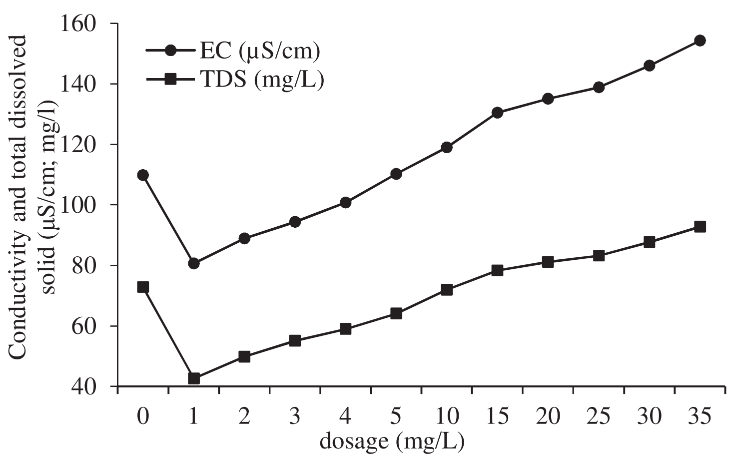

Figure 2: Effect of *Moringa* dosage on conductivity and total dissolved solid of surface water

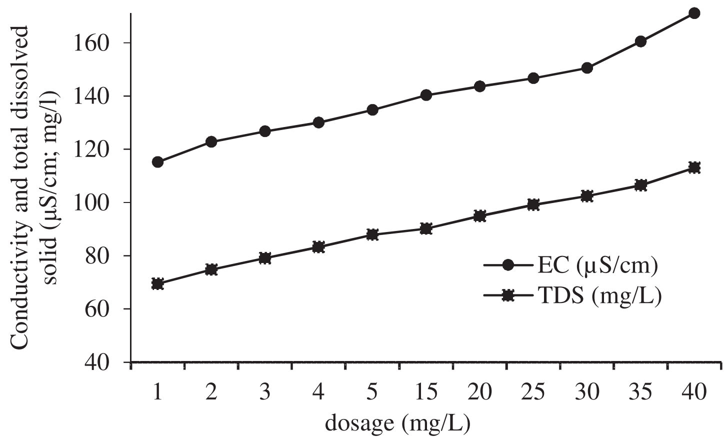

Figure 3: Effect of cactus dosage on conductivity and TDS of surface water

All the treated water samples gave conductivity and TDS values below the world health organization (WHO) maximum limits of 1000 µS/cm and 500 mg/l respectively (WHO 2006; WHO 2011). The control sample had conductivity and TDS values of 109.8 (µS/cm) and 67.5 mg/l which was also lower than the WHO limits (WHO 2006; WHO 2011). This is also in consonance with the finding of Muhammad (2011), who reported that the TDS of river water is less than 200 mg/l. The conductivity of *Moringa oleifera* treatment is statistically not significant as compared to that of cactus mucilage treatment ($p=0.08$ and $p>0.05$ respectively). The TDS of *Moringa oleifera* treatment is not statistically significant as compared to that of cactus mucilage treatment ($p=0.055$ and $p>0.05$ respectively).

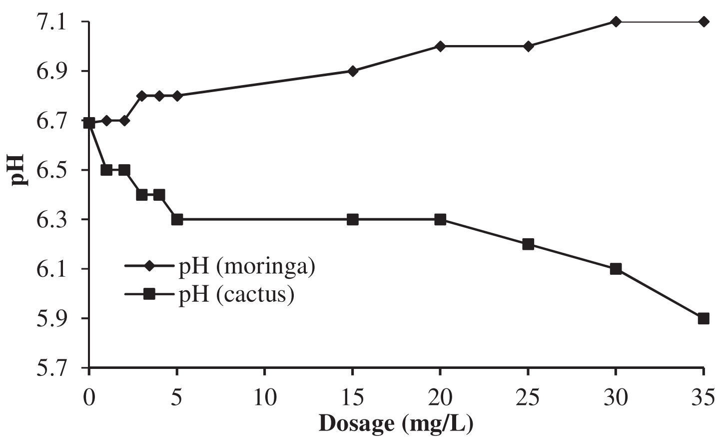

Figure 4: Effect of pH on *Moringa* and cactus dosage of surface water (mg/l)

The recommended acceptable range of pH for drinking water as specified by the WHO is between 6.5 and 8.5 (WHO 2006; WHO 2011). The pH increase slightly from 6.69 to 7.1 after *Moringa oleifera* extract sedimentation as shown in Figure 4. Ndabigengesere et al. (1995), reported that the action of *Moringa oleifera* as a coagulant lies in the presence of water soluble cationic proteins in its seeds. This suggests that in water, the basic amino acids present in the protein of *Moringa* would accept a proton from water resulting in the release of a hydroxyl group making the solution basic. This accounted for the basic pH values observed for *Moringa* treatments compared with cactus mucilage treatments. Cactus mucilage gave a pH range of 5.9 to 6.69. The pH values were decreasing as the concentration of the cactus mucilage increased as shown in Figure 4. This was attributed to the fact that cactus mucilage contains galactunic acid which lowered the pH levels as reported by Buttice et al. (2010). There was no significant effect in pH when cactus and *Moringa* were combined as shown in Table 2. The pH of *Moringa oleifera* treatment was statistically significant as compared to that of cactus mucilage treatment (p=0.000 and p<0.05 respectively).

Table 2: Physicochemical properties of *Moringa (M)* and cactus (C) treated surface water

Dose C+M (mg/l)	Turb. (NTU)	% reduction Turb.	pH	Cond. (µS/cm)	TDS (mg/l)	T (°C)
Untreated	44	0	6.70	109.8	67.5	24.5
80:20	13.1	70.2	6.57	112.5	68.1	25.5
60:40	12.4	71.8	6.58	112.2	67.7	25.5
40:60	5.1	86.1	6.61	110.5	67.0	25.0
20:80	8.9	79.7	6.65	110.3	66.7	25.1
WHO Limit	≤ 5		6.5-8.5	≤ 1000	≤ 500	ambient

As presented in Table 2, 2 mg/l dosage was used to set the ratio of cactus mucilage to *Moringa oleifera* at 80:20, 60:40, 40:60, and 20:80, resulting in turbidity of 13.1, 12.4, 5.1 and 8.9 NTU respectively. It could be noticed that the total dissolved solids, conductivity and pH were not significantly affected by the combination ratio of cactus to *Moringa oleifera*. The TDS, electric conductivity and pH levels were all very close to that of the control sample.

Table 3: Bacteria analysis using *Moringa oleifera* extract for the treated water sample

Dosage (mg/l)	No. of Coliform	Confirmed test	Complete test	% reduction in coliform
Untreated	82	+	+	
1	46	+	+	43.9
2	32	+	+	60.9
3	7	+	-	91.4
4	14	+	-	82.9
5	20	+	+	75.6

Key: + means presence of *E. coli*; - means absence of *E. coli*.

At *Moringa oleifera* dosages of 1, 2, 3, 4, and 5 mg/l, the % reduction in coliform was 43.9, 60.9, 91.4, 82.9 and 75.6% respectively as shown in Table 3. This shows that the optimum dosage is at 3 mg/l. This is in agreement with Madsen et al. (1987) who reported a 90% reduction in the bacterial load of water treated with *Moringa* seed paste. The active ingredients in *Moringa* seeds are water soluble cationic peptide as seen from their coagulation and antimicrobial activities (Ndabigengesere et al., 1995). The mode of *Moringa oleifera* seed extract action on *E. coli* cells was explained as rupturing of cells and damaging the intercellular components, when water dips into the cell which causes it to swell more and burst leading to death. The active agents in *Moringa oleifera* that possesses antimicrobial activity have been reported to include: 4-(4'-O-acetyl- -L rhamnopyranosyloxy) benzyl isothiocy-anate (Abrams et al., 1993), 4-(-L rhamnopyranosyloxy) benzyl isothiocy-anate (Abuye et al., 1999), niazimicin (Akhtar *et al.*, 1995), pterygospermin (Anderson et al., 1986), benzyl isothiocyanate (Anwar and Bhanger 2003) and 4-(-L-rhamnopyranosyloxy) benzyl glucosinolate (Asres, 1995).

Table 4: Bacteria analysis using Cactus mucilage for the treated water sample

Dosage (mg/l)	No. of Coliform	Confirmed test	Complete test	% reduction in coliform
Untreated	82	+	+	
1	6	+	-	92.7
2	4	+	-	95.1
3	8	+	-	90.2
4	20	+	+	75.6
5	30	+	+	63.4

Key: + means presence of *E. coli*; - means absence of E. coli.

At cactus mucilage dosage of 1, 2, 3, 4 and 5 mg/l, the % reduction in coliform were 92.7, 95.1, 90.2, 75.6 and 63.4% respectively as presented in Table 4. This shows that the optimum dosage was at 2 mg/l, which was used to set the dosage of the ratio of cactus to *Moringa* at 80:20, 60:40, 40:60 and 20:80. Previous studies have shown cactus mucilage to contain carbohydrates such as arabinose, xylose, galactose, rhamnose and galacturonic acids (Buttice et al., 2010). Galacturonic acid could possibly be the active ingredient that affords the coagulation capability of *Opuntia* specie whereas the combination of arabinose, xylose, galactose, rhamnose and galacturonic acids is responsible for the antibacterial activity. This is in consonance with the findings of Alcantar (2007) who observed 98% reduction of bacteria from water when treated with cactus mucilage. Alcantar (2007) recorded that cactus mucilage engulf around the bacteria in the water, depriving them of their food and starving them to death. The cactus mucilage also inhibit the growth of the bacteria. The bacteria analysis (*E. coli*) of *Moringa oleifera* treatment was statistically significant as compare to that of cactus mucilage treatment ($p=0.02$ and $p<0.05$ respectively).

Table 5: Cactus mucilage (C) and *Moringa oleifera* (M) bacteria analysis for the water sample

Dosage (mg/l) C+M	No. of Coliform	Confirmed test	Complete test	% reduction in coliform
Untreated	82	+	+	
80:20	6	+	-	92.7
60:40	5	+	-	94.0
40:60	2	+	-	97.5
20:80	3	+	-	96.3

Key: + means presence of *E. coli*; - means absence of *E. coli*

At cactus to *Moringa* ratio of 80:20, 60:40, 40:60 and 20:80, the % reduction in coliform were 92.7, 94, 97.5 and 96.3% respectively as shown in Table 5. This means that the synergistic effect of cactus mucilage and *Moringa oleifera* coagulant could be effectively used as natural disinfectant. The active agents *Moringa oleifera* seeds cake powder extract are water soluble materials as seen from their coagulation and antimicrobial activities of the raw turbid water.

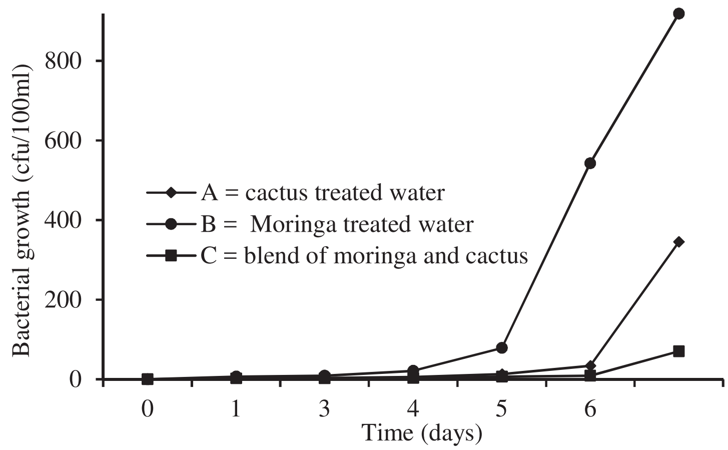

Figure 5: Determination of shelf life of treated water

The samples were analysed for coliform count at an interval of 24 hours each for six days. Samples were found to be contaminated above WHO standards at day 3 for *Moringa oleifera* and cactus mucilage coagulant alone while the blend of the coagulants lasted for 5 days as shown in figure 5. The bacteria re-growth rate was faster in *Moringa oleifera* than in cactus while cactus and *Moringa oleifera* coagulant blend gave the least bacteria re-growth rate. The bacteria re-growth rate increased in geometric progression as the water stayed longer. This confirms earlier works by Eilert et al. (1981), Jahn (1986), Madsen et al. (1987), Bina (1991), Schwartz et al. (2000) and Doerr (2005) who showed that the water treated is not safe after 48 hours as a result of bacterial re-growth. This bacteria regrowth is ascribed to two causes: the increase in the nutrients in the settled solids/sludge after coagulation from which the bacteria feed and multiply rapidly, and the mechanism of bacteria removal involves that some of the bacteria being carried down with the flocs and settling with the solids/sludge during the coagulation and settling process. The dissolved *Moringa oleifera* seed cake extract has high solubility rate of the nutrients (Ca, Na, K and Mg) (Oluduro *et al*., 2007) whereas the dissolve nutrients (Ca, Na, K and Mg) in *Opuntia stricta* has low solubility rate. This is why the bacteria re-growth rate is faster in *Moringa* than *Opuntia stricta*. The nutrients dissolved more in the water as the water stay longer. This condition provides a good environment for bacteria to feed and multiply rapidly.

4. CONCLUSION

Based on the results obtained in this work, the following conclusions were drawn:

1. The blend of cactus and *Moringa oleifera* seed cake extract showed characteristic synergy in water treatment than using cactus alone or *Moringa* alone.
2. The blend of cactus and *Moringa oleifera* seed cake powder in water treatment can effectively improve water sanitation in third world countries at low cost because the plant is cultivated locally.
3. The *Moringa oleifera* protocol can produce potable water of higher quality than that of the original source.
4. Finally, it could be concluded that treated water with *Moringa oleifera* seed extract at varying concentrations lead to drastic reduction in water microbial counts.

5. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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