

## Extension of *Moringa oleifera* seed aqueous extract shelf life using trona as a preservative

S. B. Saulawa, C. A. Okuofu, A. Ismail, D. B. Adie, J. A. Otun and I. A. Yakasai

### ABSTRACT

The effects of *Moringa oleifera* seed extracts' deterioration and preservation on turbidity removal efficiency were studied. Trona was used as a preservative in the enhancement of the extract's shelf life. An average value of 92.30% was observed at the *Moringa oleifera* seed extracts' dosage of 800 mg/L as the highest average turbidity removal for all the extracts, while the least value 82.16% was observed at a dosage of 320 mg/L. The fresh preserved *Moringa oleifera* seed extract had a value of 95.18% as the highest average percentage turbidity removal against all the extracts for all dosages, while the plain extract had the lowest average percentage turbidity removal value of 74.93%. It was observed also that even though the preserved extract was the most consistent in turbidity removal (overall variance of 1.25), the fresh preserved extract had the overall highest performance. However, the difference in overall performance between the two was only marginal, while substantial difference was observed between the plain extract and the fresh plain extract.

**Key words** | deterioration, *Moringa oleifera* seed extract, preservative, stability, trona

### INTRODUCTION

The treatment of drinking water involves the adoption of several individual processes arranged in a treatment train (a series of processes applied in sequence) to reduce, remove or alter the character of objectionable contaminants. Turbidity removal is an important requirement in water treatment because in addition to aesthetic concerns, high turbidity is sometimes associated with contamination by pathogens. Also, the suspended particles help the attachment of heavy metals, other toxic organic compounds, pesticides and pathogens (Lenntech 2008). In this manner suspended particles act as shields to pathogens against disinfectants.

Flocculation is normally achieved by the addition of coagulants, alum in particular is a coagulant of choice and its use dates back to as early as 1500 BCE (United State Environmental Protection Agency [USEPA] 2000). Unfortunately, some studies have in the past raised some environmental and health concerns over the use of alum

(Crapper *et al.* 1973; Miller *et al.* 1984). Natural coagulants have been used for centuries in traditional water treatment practices throughout certain areas of the developing world. A good example of such coagulants can be found in the seeds of the tree, *Moringa oleifera* commonly referred to as 'Moringa' which is the most widely cultivated species of the genus *Moringa*, which is the only genus in the family Moringaceae. When mixed with water, the powdered seed releases positively charged proteins (molecular weight 13 kDa and isoelectric pH 10–11) that act as an effective coagulant (Folkard & Sutherland 2002). Recent studies have confirmed the status of *Moringa oleifera* as a coagulant, yet its usage in water treatment is still faced with some challenges. One of these is the short shelf life of its extract when stored under room conditions.

Storage was also found to influence the coagulation properties of the extract and to decrease treatment efficiency with increased duration (Katayon *et al.* 2006). The study

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does not discuss the reason for this but it could be assumed that it is due to microbial degradation of the proteins (Emelie *et al.* 2008). A means of arresting this deterioration has to be sought in order to overcome this shortcoming. Being a plant extract, one possible way this may be achieved is by preservation as has been traditionally used on some food items. This study intends to investigate the feasibility of using a local preservative under ambient environmental conditions to extend the shelf life of *Moringa oleifera* seed extract in terms of its coagulation properties.

## MATERIALS AND METHODS

### *Moringa oleifera* seeds

Dry *Moringa oleifera* seeds of good quality were obtained from house backyard gardens within Samaru and Tudunmuntsira villages around Zaria, Nigeria. For water treatment application, the pods were allowed to dry naturally on the tree prior to harvesting (Folkard & Sutherland 2002).

### Water samples

High turbid water samples of initial turbidity ranging from 285 to 341 nephelometric turbidity units (NTU) were collected from a free water surface at the upstream side of the embankment of Kubanni Reservoir, Ahmadu Bello University (ABU) Zaria, Nigeria. The water samples were collected using a 25 L plastic jerry can which was properly rinsed with the sample.

### Preservative

For simplicity of technology and availability of materials, the local substance trona, was chosen to be used as the preservative in this study. Trona is a naturally occurring greyish-white solid mineral. In Nigeria it is sourced from Lake Chad and it is locally known as 'kanwa' in the Hausa and in several other Nigerian languages but commonly called 'potash', which is a misnomer, since its chemical formula is given as  $\text{Na}_2\text{CO}_3 \cdot \text{NaHCO}_3 \cdot 2(\text{H}_2\text{O})$  and is known chemically as sodium sesquicarbonate (Okere & Obimah 1998; SOLVAY Chemicals 2005). It is water soluble and 0.5, 1.0, 1.5 and 2.0% solutions

had an average pH value of  $9.34 \pm 0.02$  with negligible declination observed within 19 days (Saulawa 2010) as can be seen in Figure 1.

## Procedures

### Preparation of trona solution

Blocks of trona were ground to powder using a pestle and mortar. A measure of 0.5 g of the powder was added to 100 mL of distilled water and the setup was stirred on a magnetic stirrer at high speed. After 10 min stirring, the resulting solution was filtered through a Whatman No 1 filter paper.

### Extract preparation

The winged seed cover was removed by gentle pounding with a pestle to obtain shelled kernel. The kernel was then ground to a fine powder using a kitchen blender (NAKAI Japan Model No: NJ1731). The powder was then sieved through a domestic sieve. A weight of 2.0 g of the powder was added to 100 mL of distilled water and the setup was stirred on a magnetic stirrer at high speed for 15 min to obtain a suspension. The suspension was first filtered through a four-layer muslin cloth, then through a Whatman No 1 filter paper. The filtrate will be assumed to contain 2% (w/v) or 20,000 mg/L concentration of the extract (dosages are given as equivalent weight of seed powder or presscake material required to make up the dosing solution, Dishna 2000) and shall henceforth be referred to as the 'fresh

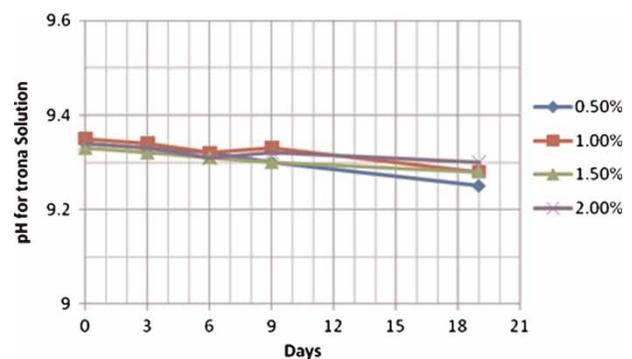


Figure 1 | Timely pH variations of different trona solutions.

plain extract'. When this extract is kept under room conditions for more than 24 hours (i.e. since preparation) it is referred to as 'plain extract'.

### Extract preservation

In order to arrest the extracts' deterioration, 0.5% (w/v) trona solution was used instead of distilled water in the preparation (as in 'Extract preparation' above) of the extract and the extract is referred to as the 'fresh preserved extract'. It does not have a definite appearance just after filtration as it is sometimes colourless or at times cloudy. When this extract is kept under room conditions for more than 24 hours it is referred to as 'preserved extract'.

### Coagulation analysis

Using a 500 mL graduated cylinder, 500 mL of the raw water to be treated was added to each of the jar test beakers. Each beaker was dosed with increasing amounts of the prepared coagulant (fresh plain, plain, preserved or fresh preserved *Moringa oleifera* seed aqueous extract) stock solution. The stirrer was operated at 100 rpm for approximately 1 min immediately after adding the coagulant. The speed of the stirrer was then reduced to 10 rpm, and it was allowed to stir for 15 min at the end of which the stirrer was then turned off and a settling time of 1 hour was given to allow the flocks to settle. To determine which sample is the clearest, a portion from the top of each beaker was carefully decanted into a clean flask and then turbidity measurements were made.

### Assessment of the effect of deterioration and preservation of *Moringa oleifera* seed extract on turbidity removal efficiency

The performance of four different extracts (fresh plain, plain, preserved and fresh preserved) having the same strength (2%) *Moringa oleifera* seed powder was compared by conducting periodic jar tests over a period of 12 days at four-day intervals using the water samples. The plain and preserved extracts were prepared on day zero (0) and kept under room conditions throughout the 12-day period, while the fresh plain and fresh preserved extracts were prepared on

days jar tests were conducted. Individual performance was measured in terms of percentage turbidity removal.

### Assessment of the effect of *Moringa oleifera* seed extracts' deterioration and preservative on water quality

Four preserved extracts (labelled B, C, D and E) having the same concentration (2% w/v) of *Moringa oleifera* seed powder but with incremental dosage of trona (0.5–2.0 g) were prepared. A plain extract (labelled A) having the same concentration as the other preserved extracts was prepared as a control. Each of the five extracts was used in a jar test in order to determine a dosage range within which it effectively removes the turbidity of a turbid water sample. Jar tests were conducted at three-day intervals by adding the effective dosages established earlier for each extract. The beaker with the best result was chosen and the clear water obtained therein was then subjected to quality examinations.

The clear water obtained was examined for the following water quality parameters: alkalinity, pH, turbidity, electrical conductivity and total dissolved solids (TDS). Suitability of the resulting treated water was appraised in accordance with the set standards shown in Table 1.

## RESULTS AND DISCUSSIONS

### Effect of deterioration and preservation of *Moringa oleifera* seed extract on turbidity removal efficiency

The effects of *Moringa oleifera* seed extracts' deterioration and preservation on turbidity removal efficiency were studied and analysed in three ways, namely: performance of each individual dosage administered for all the extracts, performance of each individual extract for all dosages administered

**Table 1** | Maximum permissible limits for some water quality parameters

Parameter	Unit	Maximum permissible limit
Electrical conductivity	µS/cm	1,000
pH	–	6.5–8.5
Total dissolved solids	mg/L	500

Source: Nigerian Industrial Standard (NIS 2007).

and finally the performance of all the extracts for all dosages administered for a given day. These were analysed in terms of percentage turbidity removal which so far seems to be a significant parameter that has been applied in several other studies (Muyibi & Okuofu 1995; Folkard & Sutherland 2002; Katayon *et al.* 2004; Dorea 2006; Emelie *et al.* 2008).

The result for the entire experiment is presented in Tables 2 and 3, which summarise the average percentage turbidity removal and variance in percentage turbidity removal of all extracts for given dosages and days. It should be noted however that dosages below 320 mg/L were not included in the study because after running trial jar test protocols, they were generally found to be inadequate in producing a significant reduction in the turbidity.

### Performance of each individual dosage for all the extracts

Considering individual dosages for the various *Moringa oleifera* seed extracts in Table 2, higher dosages of the extracts appear to be more consistent in their performance on turbidity removal. An average value of 92.30% was observed at the

*Moringa oleifera* seed extracts' dosage of 800 mg/L as the highest average turbidity removal, while the least value 82.16% was observed at a dosage of 320 mg/L. The 320 mg/L dosage of the plain extract had the least performance with an average turbidity removal of 58.99% against 95.50% produced by the preserved extract. The highest performance was observed at a dosage of 800 mg/L when the preserved extract had an average turbidity removal of 95.65% against 84.00% produced by the plain extract.

The dosage requirements (especially the plain extracts) in this study have been observed to be higher than what has been reported by previous studies (Folkard & Sutherland 2002; Emelie *et al.* 2008) but similar to what was observed by Katayon *et al.* (2004) for water of initial turbidity above 200 NTU. This difference according to Katayon *et al.* (2004) may be attributed to difference in the *Moringa oleifera* species. According to Jahn (1988) about 14 species of *Moringa oleifera* have so far been identified and although all *Moringa* suspensions acted as primary coagulants, the different species did not have the same coagulation efficiency.

In terms of consistency in turbidity removal, Table 2 shows that the 800 mg/L dosage of *Moringa oleifera* seed extract appears to be the most stable for it had the least

**Table 2** | Performance of *Moringa oleifera* seed extracts' dosages

Dosage (mg/L)	Plain		Fresh plain		Preserved		Fresh preserved		Average	
	$\lambda^*$	$\gamma^*$	$\lambda^*$	$\gamma^*$	$\lambda^*$	$\gamma^*$	$\lambda^*$	$\gamma^*$	$\lambda^*$	$\gamma^*$
320	58.99	1287.03	78.94	22.83	95.00	1.36	95.71	1.68	82.16	328.23
480	74.80	1487.07	95.41	1.50	95.02	0.38	94.94	0.21	90.04	372.29
640	81.94	474.13	96.11	2.12	94.47	2.49	95.51	1.30	92.01	120.01
800	84.00	299.10	94.97	3.10	95.65	0.77	94.57	7.48	92.30	77.61
Average	74.93	886.83	91.36	7.39	95.03	1.25	95.18	2.67		

$\lambda^*$  = Average turbidity removal (%),  $\gamma^*$  = Variance of percentage turbidity removal.

**Table 3** | Performance of *Moringa oleifera* seed extracts for given days

Days	Plain		Fresh plain		Preserved		Fresh preserved		Average	
	$\lambda$	$\gamma$	$\lambda$	$\gamma$	$\lambda$	$\gamma$	$\lambda$	$\gamma$	$\lambda$	$\gamma$
0	92.97	25.06	92.97	25.06	95.56	0.29	95.56	0.29	94.27	12.67
4	90.28	36.36	91.30	79.62	94.75	1.99	95.51	0.53	92.96	29.63
8	83.22	250.01	90.55	71.58	95.52	1.94	95.36	1.79	91.16	81.33
12	33.25	593.28	94.30	123.23	94.30	0.19	94.30	7.75	78.11	181.11
Average	74.93	226.18	91.36	74.87	95.03	1.10	95.18	2.59		

average variance of 77.61 for all the extracts. This can be seen from Figure 2(d) which shows the least fluctuations in percentage turbidity removal. On the other hand, the 480 mg/L dosage appeared to be the least stable with the highest average variance of 372.29 and Figure 2(a) shows the widest fluctuations in percentage turbidity removal. A general decline in performance can be observed from the 8th day in Figures 2(a–d). It can consequently be deduced from this that it is uneconomical to store plain extracts beyond 8 days because more dosage than ordinarily required would be applied to treat highly turbid waters.

### Performance of each individual *Moringa oleifera* seed extract for all dosages administered

From Table 2, it can be seen that the fresh preserved *Moringa oleifera* seed extract had a value of 95.18% as the highest average percentage turbidity removal against all the extracts for all dosages, while the plain extract had the lowest average

percentage turbidity removal value of 74.93%. The preserved *Moringa oleifera* seed extract however showed the most consistency in turbidity removal against all the extracts, it had negligible fluctuations (Figure 3(c)) for all dosages compared to the other extracts and it had a value of 1.25 as its overall average variance which is the least when compared to values of 886.83, 7.39 and 2.67 for the plain, fresh plain and fresh preserved extracts respectively. The plain extract on the other hand is the most inconsistent with the widest fluctuation (Figure 3(a)) and the highest average variance.

This can be attributed to loss of coagulation property with time as reported by Katayon et al. (2004) which in turn could be caused as a result of microbial degradation of the proteins content (Emelie et al. 2008) which has been reported to be the active ingredient of the extract (Gassenschmidt et al. 1995; Ndabigengesere et al. 1995; Okuda et al. 1999; Folkard & Sutherland 2002). It has also been reported that *Moringa oleifera* seed powder contains 31.65–37.8% protein (Anwar & Rashid 2007; Oluduro & Aderiyi 2007).

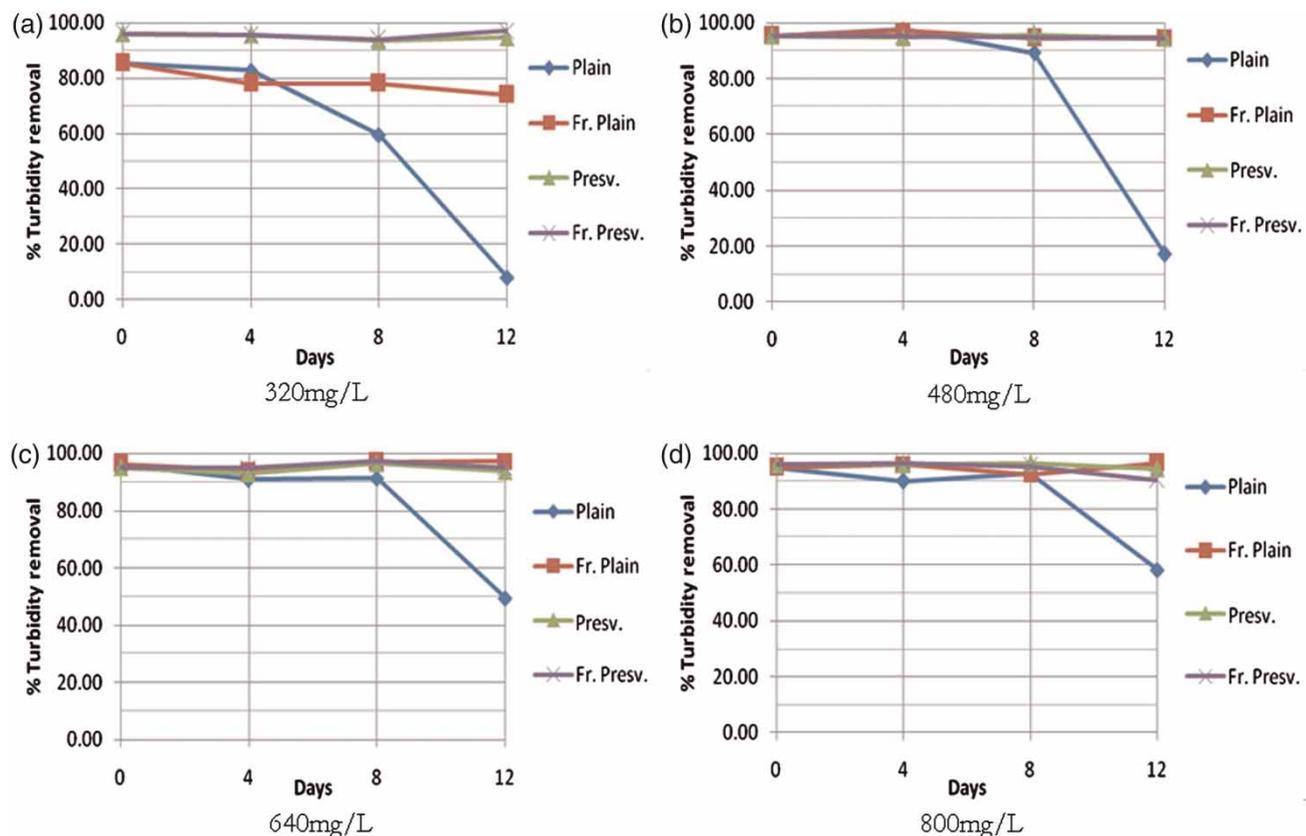
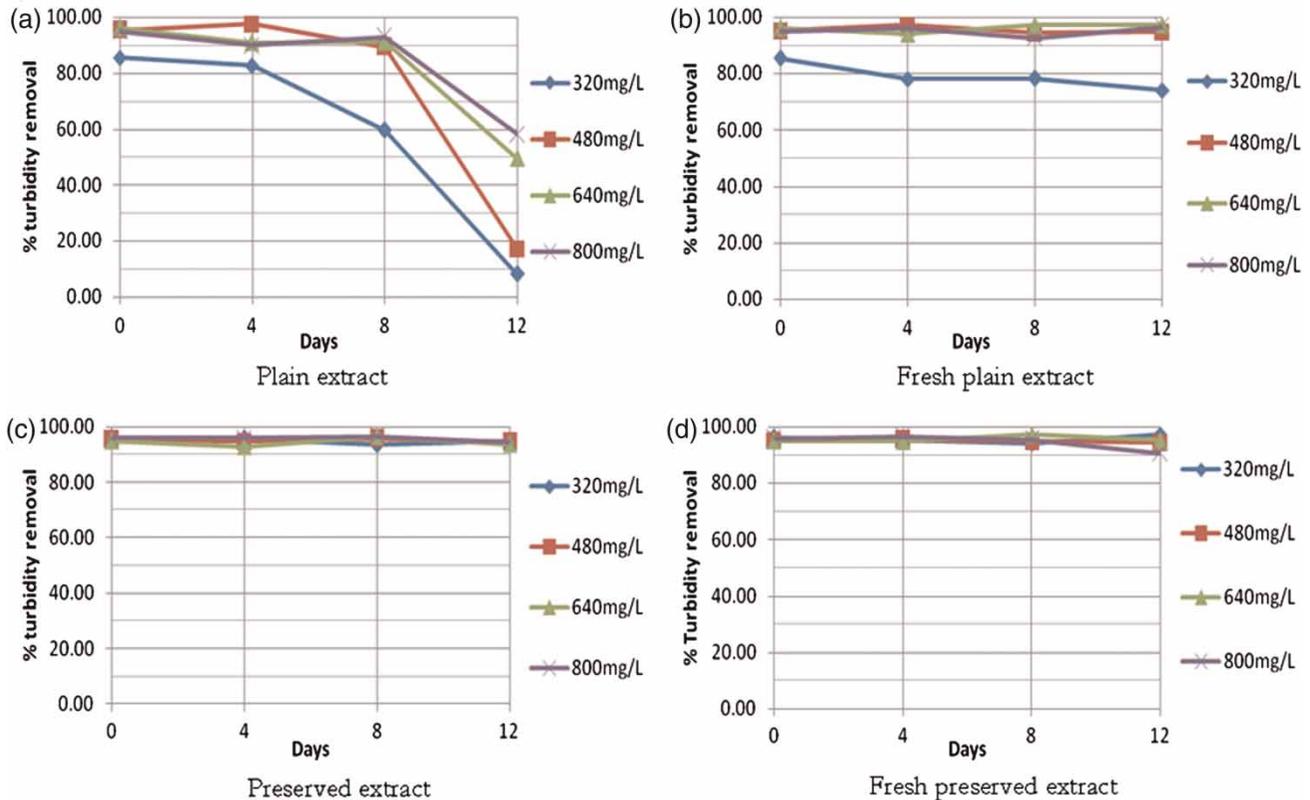


Figure 2 | Turbidity removal performances of varying dosages of different *Moringa oleifera* seed extracts having the same strength.



**Figure 3** | Turbidity removal performance of given *Moringa oleifera* extract for all doses administered over a period of 12 days.

A study by Saulawa *et al.* (2011) explained the deterioration of the extract in terms of shift in pH over time. A steady decline in the pH of plain extracts was observed with the first two days of storage and from then on extract became progressively more acidic; perhaps due to microbial degradation of organic content of the extract with increasing storage duration. Furthermore, the study reported that for trona preserved extracts however, the pH remained steadily within the alkaline region with slight variation.

#### Performance of all *Moringa oleifera* seed extracts for all dosages administered for a given day

The periodic performances of the extracts were analysed according to the interval of days the jar tests were conducted on as shown in Table 3. It was found that the overall best performance (an overall mean value for average percentage turbidity removal of 94.27%) for all the extracts was recorded on day 0. This value steadily decreased to a value

of 78.11% (the least performance observed) on day 12. It was observed also that the preserved extract was the most consistent in turbidity removal with an overall average variance of 1.10 which is the least (also the least observed fluctuation as can be seen Figure 3(c)) for all the extracts. With an average turbidity removal of 95.18%, the fresh preserved extract had the overall highest performance, which is just marginally greater than that recorded for the preserved extract.

Generally the preserved extracts performed better against the plain extracts both individually (i.e. Presv. Vs Plain and Fr. Presv. Vs Fr. Plain) and when combined (i.e. Presv. and Fr. Presv. Vs Plain and Fr. Plain). The performance of the plain extract deviated widely away from what was obtained with the fresh plain extract, while the preserved extract had an almost identical performance to that of the fresh preserved extract as can be seen in Figures 4(a–d).

Of all the extracts, the preserved extract was the most consistent in turbidity removal over the period of study. It

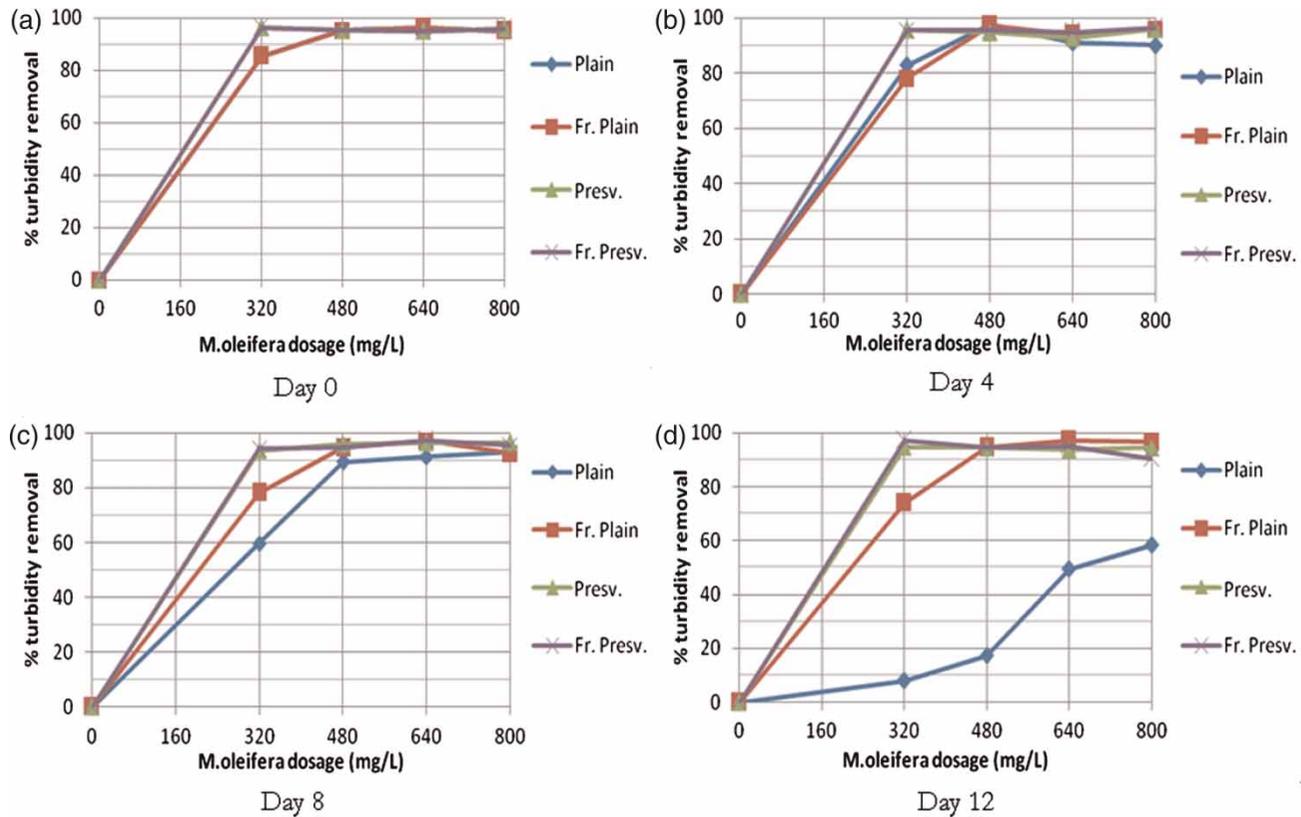


Figure 4 | Turbidity removal performance of all *Moringa oleifera* seed extracts for all dosages administered for a given day.

had the least overall average variance of percentage turbidity removal of 1.10 against similar values of 2.59, 74.87 and 226.18 for fresh preserved, fresh plain and plain extracts respectively, (see Table 3).

During a different session, after 50 days of storage under room conditions since preparation, a plain and preserved extract of the same concentrations were used for coagulation in water with initial turbidity of 341 NTU. A 640 mg/L dosage of the preserved extract was able to affect a turbidity reduction of 97%, while the plain extract was only able to produce a mere 12% turbidity reduction, compared in terms of turbidity removal.

#### Effect of *Moringa oleifera* seed extract's deterioration and effect of added preservative on treated water quality

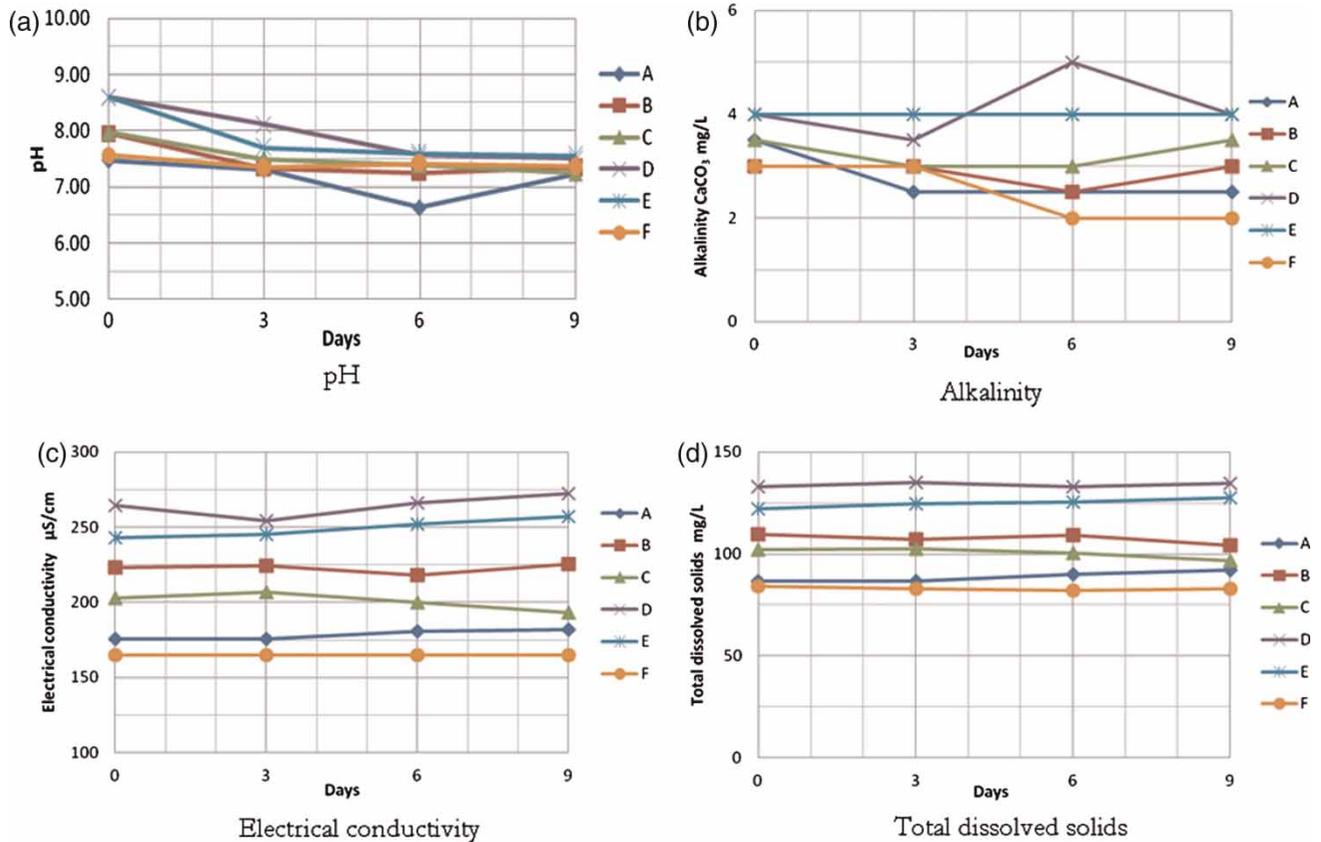
Five extracts (labelled A to E), were prepared and used as explained earlier. The effective dosage for the plain and

preserved extracts was determined from jar tests and is presented in Table 4.

The analysis of water quality after treatment with the preserved extract revealed that it had not altered the quality of water away from what was normally observed when fresh extracts are used in water treatment. Although a slight

Table 4 | Effective dosages for *Moringa oleifera* seed extracts with varying preservative concentration

Preservative concentration (g/100 mL)	Effective dosage (mg/L)	Sample label
0	8.00	A
0.5	5.00	B
1.0	2.00	C
1.5	3.00	D
2.0	2.00	E
Raw water	0	F



**Figure 5** | Daily values of measured water quality parameters for raw water and water treated with *Moringa oleifera* seed extracts.

increase in electrical conductivity and TDS was observed, which could be due to soluble minerals, salts, metals and ions that may be present in the preservative, all measured water quality parameters (see Figure 5) were within the acceptable limits as stipulated by Nigerian Industrial Standard NIS 554 (2007) as was shown in Table 1.

In the standard referred to above, no limit was recommended for alkalinity, however some countries suggest a range; Canada for instance recommends a range between 80 and 120 ppm (Safe Drinking Water Foundation [SDWF] 2007). Alkalinity in drinking water is in a sense not a pollutant but just a measure of its capacity to neutralise acid. Alkali substances in water include hydroxides or bases. Strongly alkaline waters have an objectionable 'soda' taste. The USEPA Secondary Drinking Water Regulations limit alkalinity only in terms of TDS (500 ppm) and to some extent by the limitation on pH (Advanced Purification Engineering Core [APEEC] 2008).

## CONCLUSION

The effect of storage duration on the turbidity removal performance of preserved and unpreserved *Moringa oleifera* seed aqueous extract has been studied. Results obtained have affirmed earlier findings which have shown that storage duration has an influence on the coagulation property of unpreserved extract by decreasing the treatment efficiency with increased duration. For the preserved extract however, coagulation activity was found to be maintained appreciably i.e. always above 90% turbidity removal throughout the storage duration.

In his work, Okuda et al. (1999, 2001) extracted *Moringa oleifera* coagulant using 1.0 mol/L NaCl solution and the extracted coagulant showed better coagulation activity with a dosage 7.4 times lower than that of *Moringa oleifera* distilled water extract for similar turbidity removal. In that study, the improvement in coagulation activity was

attributed to the salting-in mechanism of salt on proteins present in the seed powder suspension. As a mineral, trona may act in a similar way to NaCl when used in the extraction of the bio coagulant in *Moringa oleifera*. This could be the reason why the preserved extracts performed better than the plain extracts.

It has also been found that the preserved extract does not affect the pH and alkalinity of water after treatment. However, a slight increase in electrical conductivity and TDS was observed, which could be due to soluble minerals, salts, metals and ions that may be present in the preservative. It is therefore likely that a high dose of trona could cause taste and odour problems in water.

This shows that for conditions similar to those under which this study was performed, trona has the potential to extend the shelf life of *Moringa oleifera* seed aqueous extract in terms of its turbidity removal efficiency, especially when the preserved extract is compared to the plain extract (which is the control).

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