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Moringa Oleifera and Ceramic Filters for *Escherichia Coli* and Turbidity Removal From Drinking Water.

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Abstract: *Porous ceramic filters were prepared by incorporating burnout material into the clay and their efficiency in removal of turbidity and Escherichia coli tested. Moringa oleifera seeds were also tested for their biocoagulant and phytodisinfectant ability in purifying water. The filters reduced E. coli from 390±10 CFU/ml to 0.00 - 0.03 CFU/ml corresponding to an efficiency range of 99.1 to 100%. Turbidity reduced from 64.13±0.75 NTU to 0.92-1.77 NTU equivalent to 97.2 - 98.6% reduction. M. oleifera seeds reduced turbidity of artificially turbid at 100 NTU to 0.83 NTU corresponding to a 99.2 %. With naturally occurring turbid water at initial turbidity of 22.2 NTU, the residue turbidity was 4.02 NTU indicating lower removal efficacy. 1g/l of the seeds reduced E. coli load from 260 CFU/ml to 14.5 CFU/ml equivalent to 94.4% removal. De-oiling the seeds and using the seed cake residue showed similar efficiency in turbidity removal.*

Keywords: *Ceramic filters, M. oleifera, drinking water, E. coli, turbidity*

I. Introduction

Access to safe drinking-water and basic sanitation is essential to human health and survival; however, for many people living in low-resource settings, these vital services remain out of reach [1]. According to WHO and UNICEF recent reports, an estimated 768 million people did not use an improved source for drinking-water in 2011, including 185 million who relied on surface water to meet their daily drinking-water needs. In Kenya, about 39% of the rural population use unimproved sources of drinking water. Of this percent, 30 % use surface water without any treatment on the water [2]. Globally, demand for freshwater and energy will continue to increase significantly over the coming decades to meet the needs of increasing populations, growing economies, changing lifestyles and evolving consumption patterns. This will greatly amplify pressures on limited natural resources and ecosystems [3]. The unimproved sources of drinking water pose a great challenge to a nation by causing water borne diseases that claim the lives of 700,000 children under five each year [4].

The lack of access to piped water has sparked interventions into alternative Point of Use (POU) technologies [5]. One of the most common point-of-use (POU) water purification system is the ceramic water filter (CWF) which has gained widespread use around the world as an inexpensive method to treat microbial contaminated water for potable use [6]. Ceramic filtration involves the use of porous ceramic (fired clay) to filter microbes or other contaminants from drinking water. The ceramic filters are in the range of microfilters and are therefore able to trap particles that are in the micro range as it is the case with the suspended particles and the particles responsible for turbidity, and some bacteria, [7]. The filters have been reported to have a reduction efficiency of 88-100 % and 3-6.8 LRV against *E .coli*. [8]. Since purification is by size exclusion, very turbid water which is the case with most surface water would clog the filters pores resulting in undesirable flow rates. As a result, a coagulative procedure should precede the filtration.

Native plants have also been traditionally used to improve quality of water in many countries in Africa and Latin America. The most common is the *Moringa oleifera*. Its seeds contain proteins that have active coagulation properties and are being used for turbidity and microbial removal in many countries [9]. The protein has been extracted using methanol and tested against *E. coli.* 100 mg/ml of this extract demonstrated a marked inhibition of 15mm as opposed to an inhibition of 1mm and 17 mm for alum and chlorine respectively [10]. Studies on the microscopic structure of aggregates formed with the proteins show that the clusters of material (flocs) that are produced with the protein are much more tightly packed than those formed with conventional flocculating agents. This implies better purification capacity since such flocs are easily separated [11].

II. Experimental Section

2.1 Material collection

Clay was obtained from the department of fine Arts at Kenyatta University, Kenya while, *M. oleifera* seeds were obtained from International Centre for Research in Agroforestry (ICRAF). The local sawdust was purchased from local vendors in the city. These materials were dried under shade for ten days, then ground separately to obtain powder. The powders were sieved with a 425 µM sieve to obtain fine powder and stored in brown paper bags ready for use. The clay was characterised using Philips Minipal2 XRF from Shimadzu.Water

samples for microbial and physicochemical parameters experiments were collected by grab method into 2.5 litres amber glass bottles that were previously washed with detergent and rinsed thoroughly with distilled water. Sampling was done at Nairobi River at the Chiromo bridge (coordinates 1º 16.27' S, 36º 48.436' E). The samples were immediately analyzed on arrival at the laboratory.
All the chemicals used in this work were of analytical grade while the water used was doubly distilled.

2.2 Filters

Moulds of clay were prepared on a clay- sawdust powder (C-S) volume ratio basis by mixing clay and appropriate amount of sawdust powder with water. Four ration that is, 50:50, 55:45, 60:40 and 65:35 of clay to sawdust ration were selected and frustum shaped filters prepared from them in triplicates. The filters were dried under shade for five days and then fired by first preheating at 100 °C for two hours followed by sintering at 850°C for 8 hours. Another set of filters was prepared in the same way but fired at 650°C.
Water of known levels of contamination was passed through the filters and the filtrate collected for analysis. For the determination of flow rates, distilled water was passed through.

2.3 Coagulation and disinfection

200 ml of the contaminated water was put in a 250 ml conical flask. Various amount of ground *M. oleifera* seeds powder ranging from 5mg to 200 mg were added to the conical flasks. The flasks were shaken in an orbital shaker at 150 rpm for 4 minutes followed by slow mixing at 50 rpm for ten minutes. A blank sample was treated in a similar way to act as a control. The conical flasks were then removed from the orbital shaker and left to stand on the bench for one hour. At this point the measurements were taken for the various target contaminants to determine the coagulative and disinfection effects.

2.4 Extraction of hexane soluble oil from *M. oleifera* Seeds

Hexane soluble oil was extracted from the seeds by soaking around 5 g of dry *M.oleifera* seeds powder in 50 ml HPLC grade hexane in 100ml beaker. The contents of the beaker were shaken for 15 minutes in an orbital shaker before being allowed to stand overnight. Whatman filter paper was used to filter out the seed cake residue. This seed cake residue was dried in the air for one hour before drying in an oven at 105°C for another two hours and cooling it to room temperature. The weight difference of the seed cake was used to compute the percentage composition of the oil.

2.5 Turbidity and *E. coli* analyses

Turbidity was determined using a turbidity meter model LaMotte TC-3000e Tri-Meters. The spectrophotometric turbidity meter was calibrated using standard solutions of turbidity 1 and 10 NTU with zeroing of the meter done with distilled water. Water samples were placed in the glass bottles provided and the bottles placed into the sample holders. The turbidity readings were taken and recorded. *E. coli* count was determined using the 3M *E.coli* Petrifilm plates. The count plate was placed on a flat surface, top film lifted and 1 ml of sample dispensed onto the centre of the bottom film. Slowly, the top film was rolled down onto the sample to prevent the entrapment of air bubbles. The plate was then left undisturbed for one minute to permit even distribution of the sample and solidification of the gel.
The plates were then incubated in a horizontal position with the clear side up at 37.5°C for 24 ±2 hours. After the incubation, the plates were removed and enumeration of *E. coli* carried out. Blue colonies associated with entrapped gas were counted as *E. coli*.

2.6 pH adjustments

pH of the samples was adjusted to 5, 7, and 9 using 0.1 M HCl and 0.1 M NaOH.

III. Results and Discussion

3.1 Filters

3.1.1 Clay composition

The XRF analysis of the clay used in making the filters revealed that it contained the following oxides.

Table 1. Chemical composition and loss on ignition (LOI) of the clay used.

Oxides	SiO_2	Al_2O_3	CaO	MgO	Na_2O	K_2O	TiO_2	MnO	Fe_2O_3	LOI
%Composition	57.8	15.37	1.11	1.90	2.34	2.10	0.49	0.10	7.62	10.75

The soil was rich in Silicates (57.8%) and Alumina (15.37%) and had low concentration of titanium oxide (0.49%) and manganese oxide (0.10%) as indicated in Table 1. This is in agreement with chemical characterisation of clays as aluminosilicate minerals [12].

3.1.2 Determination of flow rates

The filters flow rates ranged between 20ml/hr and 104.5 ml/hr. The rates were dependent on the ratio of clay to sawdust (C:S) and the thickness of the filters wall. They increased with increase in the amount of sawdust for the first two filters which could be attributed to the fact that as the sawdust increased, more pores were available for the passage of water. However, a decrease is observed with an addition of more sawdust to the clay. With addition of sawdust, the elasticity of the clay is lost. For this reason , the 55:45 and 50:50 filters were made from inelastic mould of clay that implied making filters with thicker walls to compensate for the inelasticity. As a result, their flow rates decreased with the 50:50 filter having the lowest flow rate. The flow rates can be enhanced without compromising the efficiency of the filters by reducing the wall thickness of the filters and making larger filters that can accommodate more water. The flow rates are graphically presented in Fig. 1.

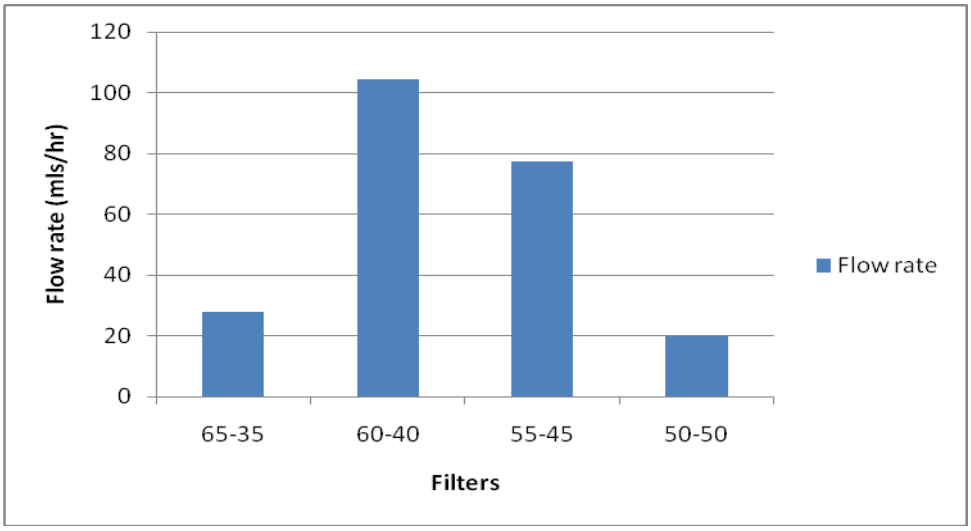

Figure 1. Illustration of the various filters flow rates

By varying the grain size of the sawdust (300, 600 and 900 µm) and using a clay: sawdust ratio 1: 2, Varkey and Dlamini [13] obtained flow rates ranging between 50- 140 ml/h which compares to flowrates recorded for some of the filters prepared in this work.

3.1.3 Filters efficiency in turbidity removal

Sample water at different initial turbidity was used in testing the filters efficiency in turbidity removal. For all the water samples, the residue turbidity was below 3 NTU. It was observed that the higher the turbidity of the raw water, the lower the turbidity of the treated water recorded as shown in Table 2. Naturally occurring turbid water obtained from Nairobi River at the Chiromo Bridge was used in the experiment and therefore turbidity's higher than 65 NTU could not be achieved.

Table 2. Efficacy of the filters in turbidity removal for water of different initial turbidity.

Initial Turbidity (NTU)	Residue turbidity (NTU)
5	2.37±0.06
10	2.06±0.05
20	1.45±0.05
50	1.21±0.03
65	0.90±0.03

The improved efficiency with an increase in initial turbidity has also been reported by Sagara [14]. He suggested that as the particles in water get filtered, the effective pore size of the filter gets smaller due to the clogging of the pores by the filtered particles. This therefore implies that the highly turbid water would reduce the pore size more and thus the higher efficiency. However, very turbid water would result into total clogging thus preventing any filtration to take place.

3.1.4 Microbial contaminants

The sample water obtained from Nairobi River at the Chiromo Bridge contained 390 CFU/ml *E. coli*. Nairobi River has generally been reported to contain high levels of the indicator organism, *E. coli*. Musyoki *et al.*, [15] recorded an average of 980 ± 130 CFU/ml for the river before the Dandora Sewage Treatment Plant

(DSTP) and an average of 1000 ± 110 CFU/ml after the DSTP point. This high levels of the feacal coliforms contamination could be attributed to the surface runoffs from the city as well as wastewater pollution from the informal settlements located alongside its course. Fig. 2 is a pictorial representation of the results.

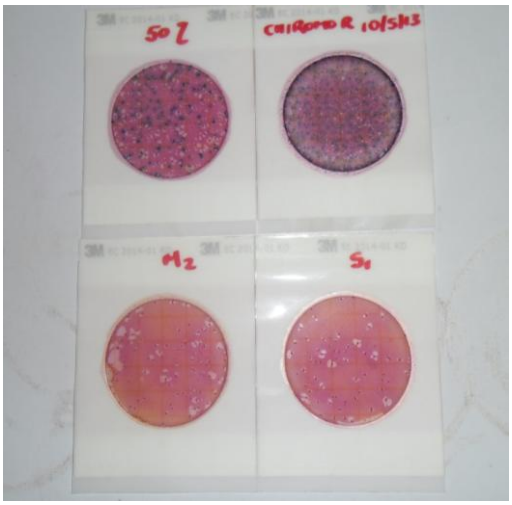

Figure 2. Pictorial presentation of the E. coli results after filtration.

The M2 and S1 are plates incubated with water filtrate. The absence of the blue colonies in these plates indicates that all the *E. coli* have been filtered out.
Enumeration of the *E. coli* after filtration and incubation was done and the results presented in a tabular form in Table 3. The results indicate that three of the four sets of filters prepared eliminated all the *E. coli.*

Table 3. Residue *E. coli* count after filtration

	E. coli (CFU/ml)
Raw water	390.00±10.00
Filter 1	0.00±0.00
Filter 2	0.00±0.00
Filter 3	0.33±0.58
Filter 4	0.00±0.00

The filters recorded an average efficiency of 99.98 % *E. coli* reduction. From literature, ceramic filters have been reported to remove *E. coli* with an efficiency ranging between 98%-100%. By using water spiked with 6.0 * $l0^6$ CFU/ml *E. coli*, Simonis and Basson [8] obtained an average reduction efficiency of 99.999% after filtration through ceramic filters.

3.1.5 Effect of firing temperature

To investigate the effect of firing temperatures, one set of the filters was fired at 650° C while the other one was fired at 850° C. Both set of filters were similar in appearance after the firing and were equally effective in removing Feacal Coliforms and turbidity as shown graphically in Fig. 3.

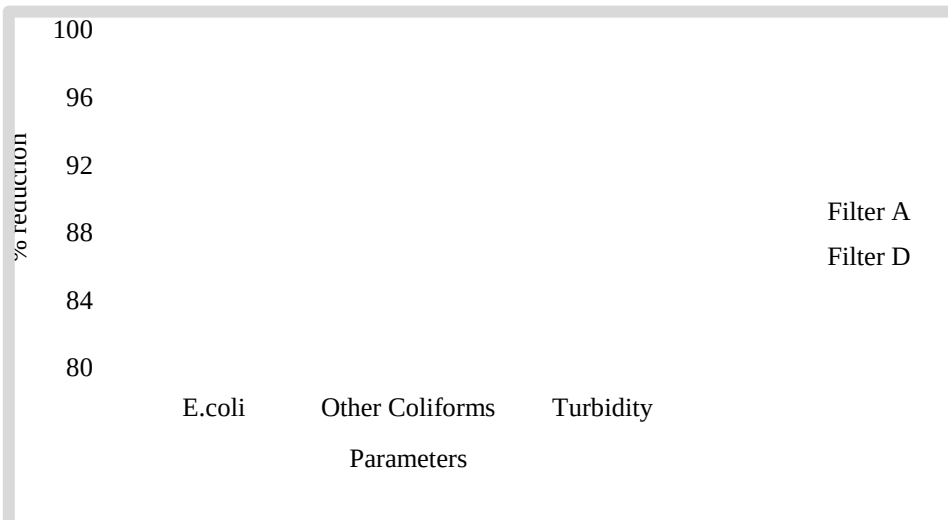

Figure 3. Efficiency of two filters: Filter A fired at 850° C and Filter D° fired at 650 C

Since firing at 650° C was equally as effective as firing at 850° C,firing for this type of clay used should be done at the lower temperature to save energy. The temperature should be high enough to vitrify clay and make it hard and resistant to stress. This ensures that it does not change shape when water is added to it and neither will it contaminate the water filtered through.

3.2 Coagulation and disinfection using *M. oleifera* seeds

3.2.1 Effect on turbidity

Both synthetic and naturally turbid water was used in testing the effects of the seeds and the optimal dose on turbidity.

Synthetic turbid water at 100 NTU

Various amount of the seeds were applied to water at this turbidity and the residue turbidity's recorded after 0, 4 and 18 hrs. The results are presented in Fig. 4. The optimal dosage was found to be 0.2 g/l corresponding to a reduction from 100 to below 5 NTU after one hour. This dose further reduced the turbidity to 2.74 and 0.83 NTU after 4 and 18 hours respectively. Optimal dosage as low as 0.1g/l have also been reported initially. Yongabi, [10] in his findings reported that 2 grams of crushed *M. oleifera* seeds was used to treat 20 litres of water in Malawi resulting in residue turbidity below 5 NTU.

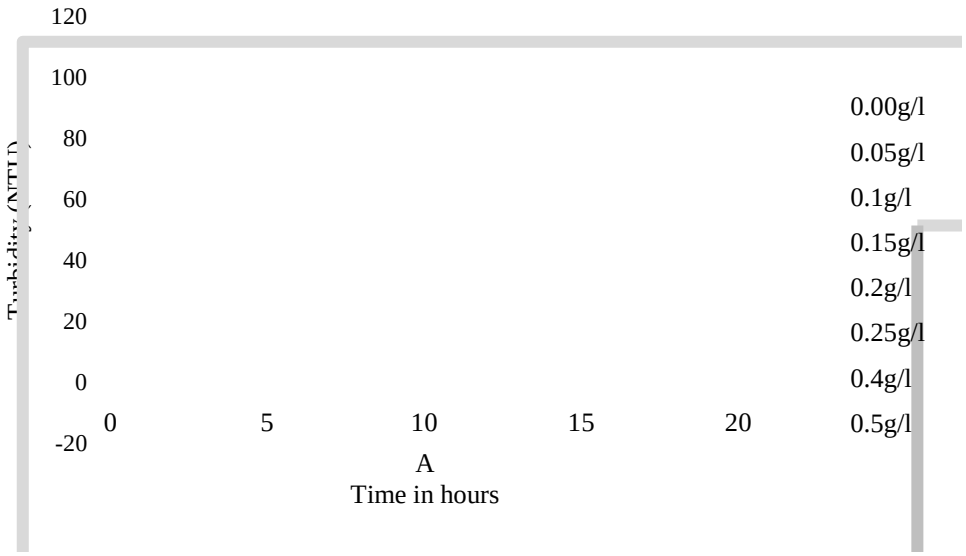

Figure 4. *M. oleifera* optimal dosage for synthetic turbid water at 100 NTU initial turbidity

Synthetic turbid water at 16.3 NTU

With water at this initial turbidity, lesser amount of the seeds would be required as indicated in Fig 5. The optimal dosage for water with an initial turbidity of 16.3 NTU was 0.025 g/l. This dose reduced the turbidity to 2.88 NTU and 1.44 NTU after 1 and 4 hours respectively corresponding to a reduction efficiency of over 91.2 %.

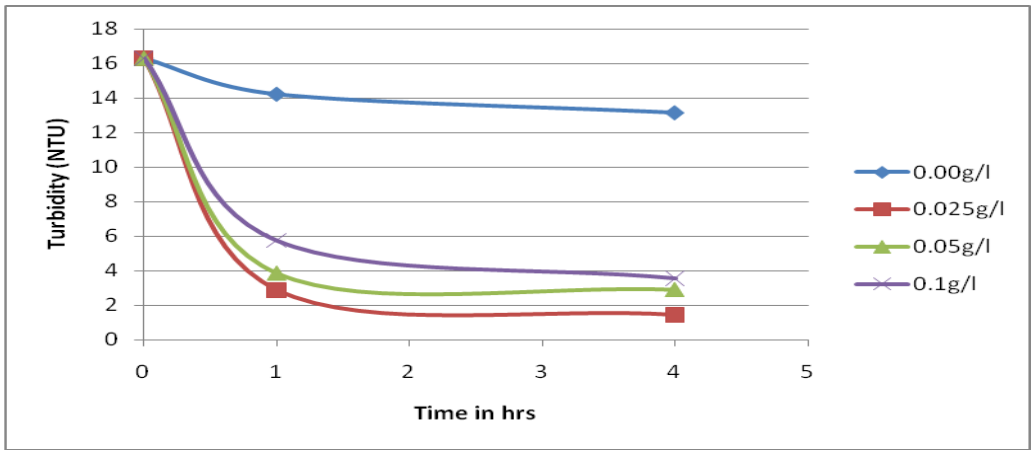

Figure 5. Optimal *M. oleifera* dosage for synthetic turbid water at 16.3 NTU initial turbidity

The lowest turbidity value achieved for this water sample with an initial turbidity of 16.3 NTU was 1.44 NTU which is high compared to 0.82 NTU value achieved by using water at initial turbidity of 100 NTU. The behaviour has been reported by other scholars and could be attributed to the fact that low turbidity waters contain limited colloidal matter; hence, a very limited inter-particle contact system for the polyectrolyte [17, 18].

Naturally turbid water

Naturally turbid water obtained from Chiromo River and at an initial turbidity of 22.5 NTU was used in this test.

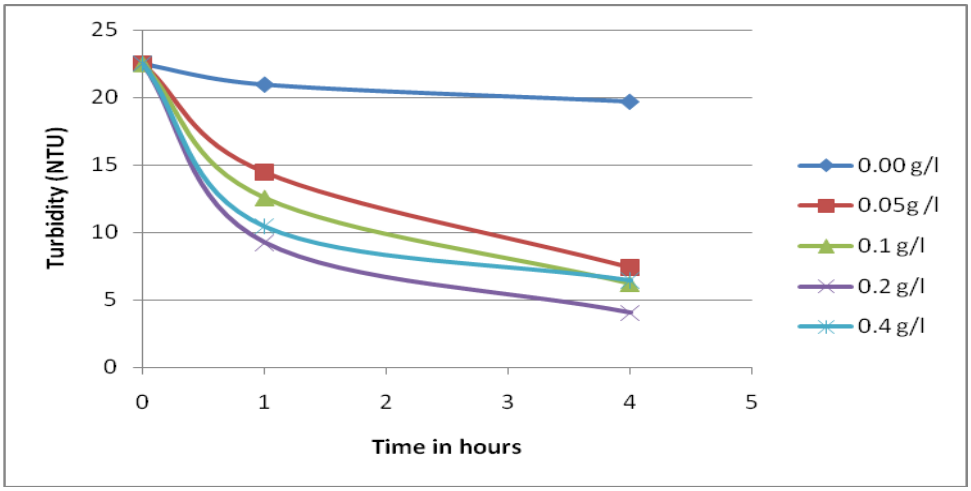

Figure 6. *M. oleifera* optimal dosage for naturally turbid water

The optimal dose was found to be 0.2 g/l lowering the turbidity from 22.5 to 9.23 and 4.02 NTU after 1 hr and 4 hrs respectively. This corresponds to a 59 and 82% reduction. Mathematically, the optimal dosage for this natural water is around 10 times greater than that of the synthetically turbid water of the same initial turbidity range. Moreover, this lowest residue turbidity achieved is higher than that of the synthetic turbid water which gave a residue turbidity of 1.44 NTU.

Pritchard *et al* [18] had previously reported that the seeds were not as effective with the natural turbid water as they were with artificial turbid water. They reported that natural water require far higher doses (x15) of *M. oleifera* seeds than the model turbid water to successfully produce coagulation. It has been proposed that the reduced efficiency with water of lower and natural turbidity could be as a result of formation of small and less dense flocs that could not settle effectively, [19].

For all the cases studied, the optimum dosage that reduced turbidity to acceptable limits for drinking water was found to be equal or less than 0.2g /l. Increasing the dosage beyond the optimal dosage resulted in

slight increase in the residue turbidity. Sutherland *et al*., [20] in their work have mentioned that once the optimal dosage is achieved, the excess polyelectrolyte proteins repel each other due to their charged nature leading to the flocs floating or suspending in the water. Such floating flocs could be filtered to achieve lower turbidity [21].

Effect of de-oiling the seeds on turbidity removal

The *M. oleifera* seeds were de oiled and the hexane soluble oil content found to be 26.40±1.04 % of the total mass of the seeds. From literature, the seeds have been reported to contain over 40% edible oil [22]. The de oiled seeds were as effective as the shelled seeds in turbidity removal as illustrated by the residue turbidity's in Table 4.

Table 4. Comparison of the shelled and de oiled seeds in turbidity removal.

Amt of *M. oleifera* seeds (g/l)	Residue turbidity (NTU)	
	Shelled seed powder	De-oiled seed powder
0.05	4.925±0.078	4.85±0.071
0.1	3.725±0.078	3.66±0.057
0.2	2.45±0.212	2.45±0.106
0.25	3.665±0.078	4.83±0.028
0.5	5.44±0.042	6.62±0.056

3.2.2 Disinfection properties of the seeds.

An experiment with raw water at an initial concentration of 260 CFU/ml and 300 CFU/ml *E. coli* and other Coliforms respectively was used. Fig. 7 is a plot of the residue number of the faecal Coliforms after one hour against the various amounts of the seed powder used.

Figure 7. Optimal dose disinfection results

The various dosages ranging from 0.125 to 2 g/l resulted in residue *E. coli* count ranging from 60 to 15 CFU/ml corresponding to reduction efficacy range of 55.67 - 92.33%. 1g/l dosage recorded the best efficiency of 92.33% reduction. The results were within the range reported by other scholars. Yongabi *et al*., [10] reported a 95% efficiency reduction of the total aerobic mesophilic bacterial counts, *E. coli* counts as well as coliforms counts. They reported an optimal dosage of between 4 and 5 g/l. Pritchard *et al*., [18] reported different efficiency at different working conditions, by changing the initial microbial load, and initial turbidity of the test water, they observed efficiency that ranged between 84 and 88% for artificial model water and 77 and 88% for natural water. Their model water was spiked with 100-300 CFU/ml *E. coli* while their river was reported to contain an average of 26.5 CFU/ml. For their work, they reported an optimal dose ranging between 0.75 and 1.25 g/l.

3.2.3 Effect of time on *E. coli* disinfection

The minimum amount of *M. oleifera* seeds that could result in maximum disinfection when the treated water samples were left to stand for upto six hours was sought in this experiment. The jar tests were carried out as previously outlined with the sampling of water for inoculation conducted after 1, 3 and 6 hrs. Fig. 8 is a pictorial presentation of the effect of time on the disinfection properties of the *M. oleifera* seeds.

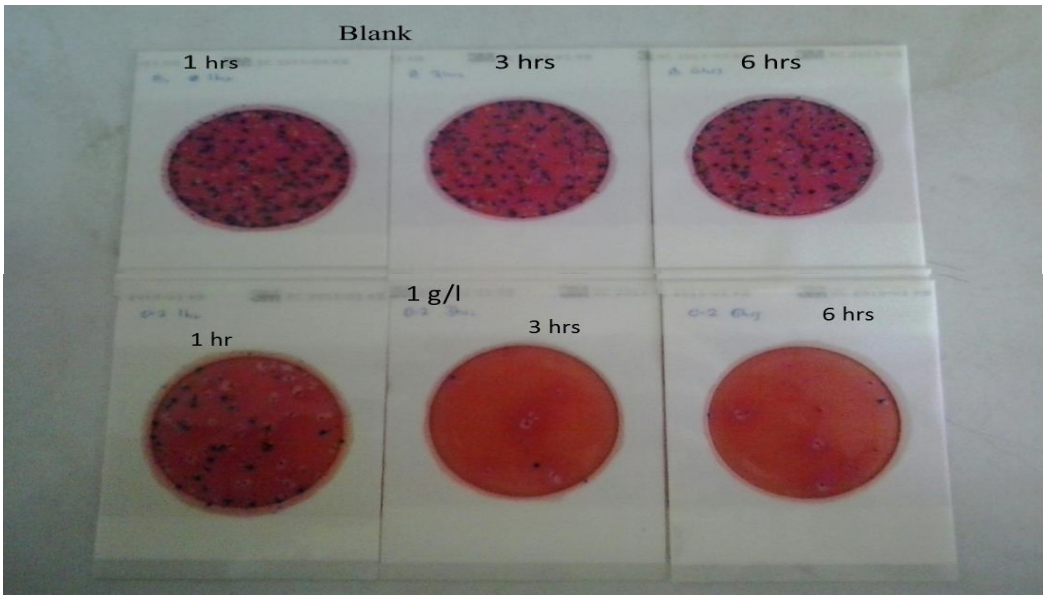

Figure 8. Pictorial illustration of the effect of time for the blank and 1g/l dosages after 1, 3 and 6 hours of coagulation.

The blank sample had a high residue number of Coliforms as evidenced from the blue and red dots even after leaving the waters to stand for six hours. With a dose of 1 g/l, the residue coliforms considerably reduced after one hour with an almost 100 % reduction after 3 and 6 hours. Enumeration of residue Coliforms was carried out and the results presented in Table 5.

Table 5. Effect of time on *E. coli* disinfection

Amount of *M. oleifera* (g/l)	Residue E. coli count with time			
	0 hr	1 hr	3 hrs	6 hrs
0	260±10	125±5.0	92.3±4.2	96.5±7.8
0.1	260±10	115.3±4.7	76.3±4.9	92.3±2.6
0.2	260±10	86.7±2.5	67.3±5.9	69±2.8
0.25	260±10	69±2.6	36±3.6	59.7±1.5
0.5	260±10	43.3±3.8	26±5.3	39.7±8.5
1	260±10	28±2.6	3.7±2.5	3.0±1.0
2	260±10	28.7±4.2	2.0±1.0	2.7±1.5

The *E.coli* were found to reduce for the first 3 hours for all the dosages. At 6 hours, the *E. coli* residue has increased for dosages lower than the optimal dose. For instance at the lowest dose of 0.1 g/l, the residue count after 3 hours is 76.3±4.9 CFU/ml. After six hours, the same sample record a residue *E. coli* count of 92.3±2.6 CFU/ml. The increase would be as a result of the residue *E.coli* feeding on the nutrients present in the water and multiplying thus resulting in the increase. At a dose of 1 and 2 g/l the residue number of *E. coli* was 3.7±2.5 and 2.0±1.0 CFU/ml respectively after three hours. After 6 hours, the average residue counts were 3.0±1.0 for the 1 g/l dosage and 2.7±1.5 CFU/ml for the 2 g/l dosage. Since almost all the *E. coli* were eliminated after the first 3 hours for the 1 and 2 g/l dosages, there was no significant increase in the number of Coliforms after leaving the samples to stand for an extra 3 hours. Leaving blank samples on the bench after shaking also resulted in decreased number of colonies due to the natural dying process.

3.2.4 Effect of pH on *E. coli* dsinfection

A dosage of 1g/l *M. oleifera* was chosen and the samples pH adjusted to acidic, neutral and basic conditions. The disinfectant tests were carried out and the enumeration of *E.coli* after 1, 3 and 6 hours done. Residue fraction was calculated using equation (1):

Residue fraction = N/N° **Equation 1**

Where N is the number of coliforms after phytodisinfection

N° is the initial number of Coliforms before disinfection.

Fig. 9 gives the results for the effect of pH on *E. coli* disinfection. For all the pH values chosen, the residue fraction after one hour was between 0.02 and 0.06 with the highest residue figure recorded at pH 5 and the

lowest figure at pH 9. The same trend is observed after the third and sixth hour but with reduced discrepancy between the residue fractions values.

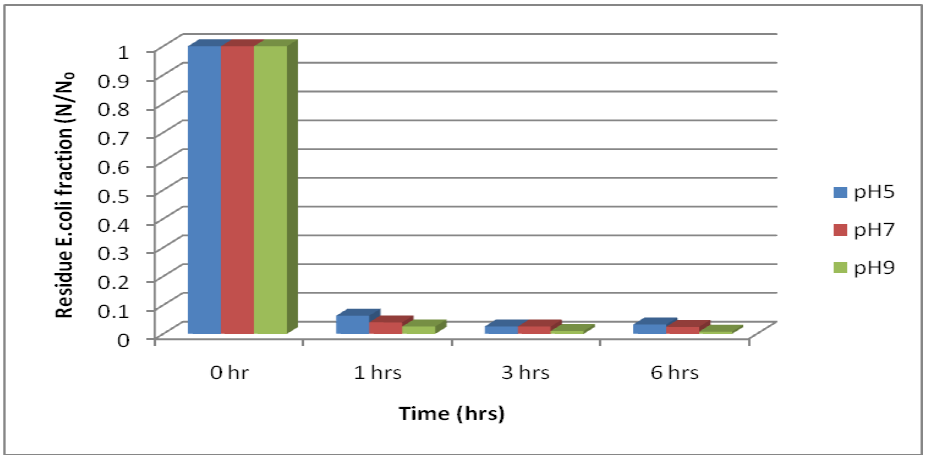

Figure 9. Effect of pH on *E. coli* disinfection

A basic media of pH 9 was found to be a better working pH for the *M. oleifera* seeds while acidic conditions were found to lower the efficiency of the seeds. Since natural water is generally slightly basic, then the seeds would be effective in disinfecting water.

Previous work by Pritchard and co-workers [23] had reported that the seeds were more effective in neutral pH but also observed that alkaline conditions were overall more favourable than acidic conditions.

IV. Conclusion

The study investigated the effectiveness of ceramic filters and *M. oleifera* in the removal of *E. coli* and turbidity from drinking water. The filters efficiency in turbidity removal was above 97.2 % while it ranged between 99.91 and 100% *for E. coli*. However, filtering turbid water clogs the filters and may block the filters completely thus a pretreatment process is required. The *M. oleifera* seeds were effective in lowering turbidity and the feacal Coliform loads of water samples. A turbidity reduction efficiency of 97% was achieved by a dosage of 0.2 g/l, that is, turbidity was lowered from 100 NTU to 2.74 NTU. With *E. coli* 1g/l dosage reduced the total count from 260 CFU/ml to 14.5 CFU/ml. The deoiled seeds were also effective in treating the water implying that the edible oil could be extracted first before the residue seed cake is used in water treatment. Combining the two POU (filtration and use of *M. oleifera* seeds) would produce quality water and also prevent clogging of filters.

Acknowledgement

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