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Assessment of the Antimicrobial Efficiency of *Moringa oleifera* Seed Extracts in the Treatment of Grey Water

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Authors' contributions

Authors MIA, SBI, SAA and BOO designed the study, wrote the protocol, performed part of the microbial analysis and wrote the first draft of the manuscript. Author SOD performed the physico-chemical and statistical analysis, and managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

The antimicrobial efficiency of *Moringa oleifera* seed extracts in the treatment of grey water was investigated in this study. The *Moringa oleifera* seeds were collected, prepared and used to treat both filtered and unfiltered grey water at different concentrations. Physical parameters like Biochemical Oxygen Demand (BOD), Turbidity, Electrical conductivity (EC) and Total dissolved solids (TDS) of the raw, filtered and treated grey water samples were conducted. The result revealed 65.7%, 67.1% and 80.1% BOD removal for the 50mg/l, 100mg/l and 150mg/l *Moringa* treatment respectively. An average of 97.4% turbidity removal was achieved for the filtered grew water sample after treatment with 150mg/l of *Moringa* seed extract. For the Electrical conductivity, a 27.3% removal was achieved for the unfiltered sample while for the Total dissolved solids, a 91.52% removal was recorded for the filtered grew water sample using 150mg/l of seed extract. The analysis of the total coliforms and pH conducted showed that 50mg/l, 100mg/l and 150mg/l of *Moringa oleifera* seed extracts solution respectively achieved 68.33%, 85.00% and 97.50% removal for the filtered grey water with pH values of 6.37,

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5.88 and 5.83 respectively. It was calculated that the optimum dosage of *Moringa* seed powder required to maintain minimum crop pH requirement of (6.5) was 32.89mg/l (44.95%). It was concluded that the seed extract of *Moringa oleifera* acts as a natural coagulant, flocculent and absorbent for the treatment of grew water. The presence of indicator organisms shows that the water samples are faecally contaminated and is only safe for irrigation purposes after the treatment.

Keywords: Grey water; antimicrobial efficiency; Moringa oleifera; dosage; total coliform; pH.

1. INTRODUCTION

In many parts of the world, water scarcity is one of the most significant challenges to human health and environmental integrity. As the world's population grows and prosperity spreads, water demand increases without the possibility for an increase in supply [1]. As pressures on freshwater resources grow around the world and new sources of supply become increasingly scarce, expensive, or politically controversial, efforts are underway to identify new ways of meeting water needs. Of special note are efforts to reduce water demand by increasing the efficiency of water use and to expand the usefulness of alternative sources of water previously considered unfit for use [2]. Grey water reuse is one of such strategy, and its usefulness to fulfill non-potable water needs is being thoroughly investigated by researchers. Grey water is wash water coming from showers, bathtubs, washing machines and bathroom sinks [3].

Globally, over 70% of freshwater consumption is devoted to agricultural activities [4]. Recently, declining productivity of commercial farms has led international policy networks to recommend the promotion of urban and peri-urban agriculture as an escape from food crisis situations [5]. However, many households in poorer areas lack access to fertilizers and have a limited supply of fresh water. Wastewater treatment and reuse at the individual level can provide a combined solution to these problems by supplying the water and nutrients needed for household food production. Indeed, this strategy is already in use by millions of farmers worldwide and it is estimated that 10% of the world's population consumes foods irrigated with wastewater [6]. Wastewater treatment and reuse for irrigation may well hold the key to easing demand on limited freshwater reserves while improving the food production capacity of households and farms.

However, there are significant concerns about the safety of wastewater reuse for irrigation purposes. The key issue involved is the potential for damaging effects of poor-quality water on soil, plants and humans. Water quality requirements for agricultural irrigation are a subject of much interest to researchers. In theory, agricultural water need not be of potable quality, opening the door to wastewater and surface water irrigation [6]. However, the microbial population of untreated water is very diverse, and dangerous organisms can be present. Microorganisms that can cause illness or disease, collectively known as pathogens, are usually associated with human or animal fecal matter present in wastewater and surface water sources.

Irrigation water contaminated with pathogens has often been blamed for outbreaks of foodborne illness. It is important to carefully manage this risk when promoting the reuse of non-potable water sources to fulfill the water demand of agricultural irrigation activities.

The pathogens content of grey water is generally low, because pathogens are primarily added to waste water through faeces. However, grey water can be seen as a health hazard mainly because of the presence of indicator bacteria that are found in it. It has been shown that grey water especially bath/shower and laundry water can contain pathogens. They can have low content of pathogens, bacteria and virus. Particularly laundry and bath/shower water from infants and sick older people [7].

Various treatment technologies have been employed to store grey water for irrigation purposes. The common treatment technologies are physical treatment (sand filtration, membrane filtration) and biological process followed by chemical treatment (disinfection). Different disinfectants have been employed to store the grey water for a long time and they are chloramine, chlorine, hydrogen peroxide, Ultra Violet light and oils. The chemical and physical quality of the grey water will heavily influence what type of disinfection method is most suitable [8]. For example, presence of organic matter and suspended solids in grey water can affect efficiency of disinfection and disinfectant demand. Organic material generally reacts with disinfectant and therefore a greater initial dose is needed to achieve total inactivation of bacteria [9]. Asano et al., [10] also found that a greater initial dose of chlorine is needed to overcome its interaction with organic matter. It was also found that larger particles can help shield bacteria from disinfection [11].

Chlorine has been the major chemical widely used for water disinfection since the early 1900's, although it was discovered as far back as 1777 [12]. Chlorine has been identified to form Disinfection Bye-products (DBPs) which have tendencies of being carcinogenic over long exposure [13]. Although treated grey water is not expected to be used for potable application, the avoidance of the risk of using chlorine will go a long way to protect the health of the end users. Seeds of *Moringa oleifera* have been found to be the most effective natural materials that have been used to clarify water [14]. Apart from the coagulation ability of *Moringa oleifera*, it is also a natural antihelmintic, antibiotic, detoxifier, outstanding immune builder and is used in many countries to treat malnutrition and malaria. This properties qualify it for use in water purification, thus helps in reducing the incidence of water borne diseases [15]. It is for this reason that research such as this one was carried out to explore the effectiveness of *Moringa oleifera* seed extracts as a natural disinfectant and antimicrobial that would combat the disadvantages of the chemical disinfectants.

2. MATERIALS AND METHODS

2.1 Grey Water Sampling

The sample was collected from drainage in the residential area in Ahmadu Bello University main campus, Zaria. The sample had been collected in a 10L High Density Poly-ethylene container after which it was transported as soon as possible under dark conditions to the Environmental Health Engineering Laboratory of the Department of Water Resources and Environmental Engineering, Ahmadu Bello University, Zaria for analysis to reduce storage time before testing.

2.2 Preparation of *Moringa oleifera* Seed Extracts

Seeds of *Moringa oleifera* were collected from new extension area of Samaru, Zaria, Kaduna State, dried and manually grounded to powder form. The crushed kernels were sieved using a sieve size of <300mm and divided by weight into three categories, (that is, 2 each of 5g, 10g, and 15g). The weighed *M. oleifera* seed powder were dissolved each in 100ml of distilled water to vary the concentration (Dosage) after they were in turn used to treat both the filtered and unfiltered grey water samples.

Fig. 1. (a) *Moringa oleifera* seeds with seed coating (b) seeds without seed coating

2.3 Experimental Design

The experiment was carried in two sets. The collected grey water sample was divided into two equal parts and used for each set of the experiments. The first part was left unfiltered and labeled as 'Raw sample' while the second part was labeled 'filtered sample' after passing through filtration to evaluate the effect of big suspended solids on treatment. The raw and the filtered samples were respectively treated with varying doses of *M. oleifera* seed extracts (i.e 50 mg/L, 100 mg/L and 150 mg/L). The powder was manually added to the specified quantity of water in 100liters PVC black drum in each case, the solution was mixed in thorough agitation with the aid of a stirrer for about 30 minutes before leaving to settle for 7 days under dark condition with the drums tightly covered to avoid further microbial and other contaminants. After the experimental period, the solution was decanted thereby removing the purified water at the top portion of the containers while the sediments were gotten rid of. Consequently, physical parameters pH, Biochemical Oxygen Demand (BOD), Electrical conductivity (EC), Total Dissolved Solid (TDS) and Turbidity as well as and Total Coliforms were monitored to assess the grey water quality changes before and after filtration and after treatment with varying doses of *M. oleifera* seed extracts.

2.4 Physical Parameters Analysis

All the physical analyses were carried out according to standard methods for analysis of water and waste water described by [16]. The pH, Electrical conductivity (EC), Total dissolved solids (TDS) and Turbidity were determined using Hodel Carbo manufactured by Hanna Instruments (P) Ltd. The BOD test was performed using a dissolved oxygen test kit. It was determined by comparing the DO level of the sample taken immediately with the DO level of sample that was incubated in complete darkness at 20^{o}C for 5 days.

2.5 Microbial Isolation and Identification

Total coliform analysis was carried out on the raw, filtered as well as samples treated with varying doses of *M. oleifera* seed extracts using the plate count method described previously in [17,18]. The media used included Eosin methylene blue (EMB) and MacConkey Agar (for *E. coli* and total coliforms isolation), Nutrient Agar (for heterotrophs isolation). For total coliforms and *E. coli,* the plates were incubated at 37°C for 24hrs while for heterotrophs; the plates were incubated at 37°C for 48hrs.

After incubation, number of bacterial colonies was determined using colony counter and expressed as colony forming units (CFU) per 100ml. Individual colonies were purified and identified by morphological and biochemical techniques [19].

3. RESULTS

Table 1 shows the results for the physical tests carried out on the raw, filtered and treated grey water with varying concentration of *Moringa oleifera* seed extracts. The results reveal that 65.7%, 67.1 and 80.1% BOD removal was achieved for 50mg/l, 100mg/l and 150mg/l respectively for the raw sample of grey water while 71.4%, 74.3% and 81.4% BOD removal was recorded for 50mg/l, 100mg/l and 150mg/l respectively for the filtered grew water sample. The BOD removal efficiency of the *Moringa oleifera* seed extracts increased with increased concentration of the seed extracts used. The BOD removal efficiency was also observed to be higher for the filtered grey water sample relative to the raw samples. The difference in the percentage reduction in turbidity with varying concentrations of the seed extracts was marginal. An average of 97.4% turbidity reduction was recorded. The results for the electrical conductivity and total dissolved solids also followed the same trend with the BOD and turbidity.

Table 2 shows that the antimicrobial efficiency of the *Moringa oleifera* seed extracts on the grey water samples increased with increasing dosage of *Moringa oleifera* seed extracts while an attendant decrease in pH was observed. The highest antimicrobial efficiency of 97.50% was recorded with 150mg/l dose of *Moringa oleifera* seed extracts on the filtered grey water sample while 50mg/l and 100mg/l dosage of *Moringa oleifera* seed extracts achieved 68.33% and 85.00% antimicrobial efficiency on the filtered grey water samples respectively. The Results also show that the filtration of the grey water before application of *Moringa oleifera* seed extracts improved the antimicrobial efficiency of the *Moringa oleifera* seed extracts by an average of 9.73%.

The optimum dosage (D_{opt}) of *M. oleifera* seed extracts that would maintain the treated grey water pH at a minimum of 6.5 tolerable to vegetable garden according to [20] was estimated by interpolation between 0mg/l and 50mg/l using the formula below:

$$D_{opt} = \frac{(50-0)(6.5-6.75)}{(6.37-6.75)} + 0 = 32.89\ mg/l \quad (1)$$

Table 1. Mean and Standard deviation of Physical parameters of Raw and Filtered Grey water on treatment with *Moringa oleifera* seed powder

Sample	BOD (mg/l)				EC (µhos/cm)				TDS (mg/l)				Turbidity (NTU)			
	Raw Sample	% Removed	Filtered Sample	% Removed	Raw Sample	% Removed	Filtered Sample	% Removed	Raw Sample	% Removed	Filtered Sample	% Removed	Raw Sample	% Removed	Filtered Sample	% Removed
Raw sample	700.0000±1.1547	0	700.0000±1.1547	0	139.2333±0.2082	0	112.2667±0.2517	0	1300.2670±0.2517	0	1300.2670±0.2517	0	1345.2670±0.2517	0	1300.2670±0.2517	0
Treatment with 50mg/l of *M.oleifera* seed extract	240.0000±1.0000	65.7	200.3000±0.2646	71.4	112.2667±0.2517	19.4	102.3000±0.2646	8.9	601.6000±0.2646	53.7	340.6333±0.0577	73.8	53.3667±0.0577	96.0	48.3333±0.0577	96.3
Treatment with 100mg/l of *M.oleifera* seed extract	230.4667±0.4163	67.1	180.3000±0.2646	74.3	105.2333±0.2080	24.5	100.3000±0.2646	10.7	501.1000±0.1000	61.5	220.1000±0.1000	83.07	41.5333±0.0577	96.9	44.5000±0.1000	96.6
Treatment with 150mg/l of *M.oleifera* seed extract	130.4333±0.4509	80.1	130.4333±0.4509	81.4	101.2000±0.2000	27.3	99.1667±0.1528	11.6	300.8667±0.0577	76.9	110.2067±0.1528	91.52	39.0667±0.0577	97.1	34.3900±0.1000	97.4

Table 2. Mean and Standard deviation of pH and Total Coliforms Count for Raw and Filtered Grey water on treatment with *Moringa oleifera* seed powder

Sample	**Total Coliforms Count (x 10^5 CFU/100ml)**				**pH**
	Unfiltered Grey Water Sample	**Percentage Removed (%)**	**Filtered Grey water Sample**	**Percentage Removed (%)**	
Raw sample	120.6000±0.5292	0	120.6000±0.5292	0	6.75
Treatment with 50mg/l of *M. oleifera* seed extract	60.1667±0.2887	50	38.1667±0.3786	68.33	6.37
Treatment with 100mg/l of *M. oleifera* seed extract	23.3000±0.5196	80.83	18.1667±0.3786	85.00	5.88
Treatment with 150mg/l of *M. oleifera* seed extract	11.1667±0.1528	90.80	3.1000±0.1000	97.50	5.83

Each value is an average of three (3) counts

4. DISCUSSION

In the present study, different doses of *Moringa oleifera* seed powder was used for the treatment of grey water samples. After treatment with *Moringa,* the range of pH was found to be between 5.83-6.37 which is weakly acidic falling within the World Health Organization (WHO) limit of 6.5 for garden crops. This is in contrast to earlier report that the action of *M. oleifera* as a coagulant lies in the presence of water soluble cationic proteins in the seeds suggesting 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 [21].

This result is also supported by the report of [22] on the treatment of river water with *Moringa oleifera* seed powder. It was observed that after treatment with *Moringa s*eed powder; pH was decreased at 50 and 100 mg/l dose, but at 150 mg/l dose, it was partially increased. After treatment the range of pH was 7-7.5 and it is within the limit specified by [23].

It may be inferred from the investigations that 97.5% antimicrobial efficiency was achieved in the treatment of grey water using 150mg/l of *Moringa oleifera* seed extracts tolerable only to few acid-loving garden plants like *Capsicum species*. An optimum dosage of 32.89mg/l was estimated to be a safe limit to maintain the treated water pH at 6.5 for vegetable gardens.

Total dissolved solid (TDS) was found in this study to decrease with increasing dose of *Moringa* seed powder. This is supported by the finding of [24] who reported maximum reduction for both total solids and total dissolved solids in ground water after treatment with *Moringa oleifera*. Also, [25] substituted *Moringa* for alum in the removal of solids from water for drinking purposes w decision that was informed by its availability and effectiveness. This reducing ability of *Moringa oleifera* may be due to the fact that it is known to be a natural cationic polyelectrolyte and flocculant in agreement with [26].

The decreasing trend observed for turbidity values in this research corroborates the report of [24] who reported that the use of *Moringa oleifera* seed powder showed decreased turbidity in river water with increased dose from 50, 100 and 150 mg/l respectively. In the same vein, [27] reported that the use of *Moringa* seed powder achieved highest turbidity removal over the use of alum and alum with *Moringa* respectively. In an earlier study, [28] recorded 90-99% removal of turbidity in water from treatment plant using *Moringa.*

Moringa acts as antimicrobial agents against microorganisms as seen in this study and this agrees with [29]. There was an observed reduction in the coliform bacteria as the dosage increased and the highest reduction was found in the filtered sample treated with 150mg/L of seed powder. The presence of coli forms after treatment indicates that the water is faecally contaminated and is only safe for irrigation purpose. Mangale *et al.,* [24] observed that the initial MPN was present beyond the limits of WHO standards. After the treatment, MPN/100ml coli form was decreased from low dose to high level dose of *M. oleifera* seed powder**.** The result obtained here further corroborates the findings of [30] that the use of *Moringa* seed extract achieved 90.00-99.99% bacterial removal in previously untreated water.

5. CONCLUSIONS

It can be concluded from this investigation that *Moringa oleifera* seeds acts as a natural coagulant, flocculent and absorbent for the treatment of grew water. It reduced the BOD, total hardness and turbidity after the treatment. It also acts as a natural antimicrobial active agent against the micro-organisms which are present in the grey water and decrease the bacteria count. The Coliform count was equally reduced after treatment with higher dose (150 mg/l) of *Moringa* seed powder. The presence of coliform in the treated water even at reduced rate is an indication of faecal contamination and as such, the treated grey water is only safe for irrigation purposes after the treatment.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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