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BIO-FLOCCULATION AND ANTIMICROBIAL ACTIVITIES OF POWDERED *MORINGA OLEIFERA* (LAM) SEEDS AND ALUM ON DOMESTIC WASTEWATER MICROBIAL CONSORTIA

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ABSTRACT

Introduction: *Moringa oleifera* seed is a bio-flocculant liable to purify water and verified to be one of the generally efficient prime coagulants for water treatment. *M. oleifera* seeds also have the potentials to eliminate a broad variety of bacteria, including *Escherichia coli*, *Bacillus subtilis*, *B. cereus*, *Pseudomonas aeruginosa* and *Enterobacter ludwigii*, from domestic wastewater.
Objective: The comparative bio-flocculating ability and antimicrobial activities of powdered *Moringa oleifera* seeds and alum for the treatment of domestic wastewater from a university student' hostels were explored.
Methods: Collection of wastewater samples, physicochemical analysis of wastewater samples and treatment of the wastewater samples with powdered *M. oleifera* seeds and alum were conducted using standard techniques. Enumeration and identification of bacteria using biochemical depiction and (16S RNA) with fungi after treatment were employed via standard protocols.
Results: The optimum pH obtained using powdered *M. oleifera* seeds was 6.00 – 7.38 and in line with the recommended WHO standard. This study revealed that the bacterial count in wastewater samples of Jibowu and Abiola hostels after treatment with 2g of powdered *M. oleifera* seeds and 6g of alum was high (199.67±0.89 CFU/ml); low (26.00±0.57 CFU/ml) for powdered *M. oleifera* seeds and high (87.00±0.57 CFU/ml); low (6.33±0.57 CFU/ml) for alum respectively. The fungal count of the wastewater samples for Akindeko and Jibowu hostels after treatment with 2g of powdered *M. oleifera* seeds and 6g of alum was high (26.00±0.57 Sfu/ml); low (5.00±0.57 Sfu/ml) for powdered *M. oleifera* seeds and high (19.00±0.58 Sfu/ml); low (2.00±0.57 Sfu/ml) for alum respectively. *Escherichia coli*, *Bacillus subtilis* with NCBI-certified *B. cereus* mkbk1, *Pseudomonas aeruginosa* mkbk 2 and *Enterobacter ludwigii* mkbk 3 were isolated from the wastewater samples.
Conclusion: The findings of this study suggest that the bio-flocculating ability of powdered *M. oleifera* seeds accentuated better antimicrobial efficacy of *M. oleifera* over alum as a proviso to the blend of powdered *M. oleifera* seeds and alum for the treatment of domestic wastewaters.

Keywords: Alum; Antimicrobial; Bio-flocculation; *Moringa oleifera*; Physicochemical; Wastewater, 16S RNA

INTRODUCTION

Wastewater is universally labeled as domestic wastewater or industrial wastewater. Domestic wastewater refers to wastewater spawn from non-manufacturing activities routine in inhabited homes which include sewage (from toilets) and grey water (from bathrooms and kitchens) **(Mohamed and Mohamed, 2016).** It comprises of human waste (enclosing pathogens), paper, soap, detergent filtrate and food oddments **(MEIDW, 2018).** Domestic wastewater is the water that has been used by the public and which include all the resources added to the water during its use. It is thus making up of human body wastes (faeces and urine) jointly with the water used for flushing toilets, coupled with the resultant wastewater from delicate washing, laundry, food-making and the cleaning of kitchen paraphernalia **(Duncan, 2003).**
Boasted by the outcome of innate coagulant reports in *Moringa oleifera* seeds, several emergent nations have embraced the usage of the plant seed as a pragmatic coagulant in domestic wastewater management on a small scale **(Thakur and Choubey, 2014). Katayon *et al.* (2006)** have given an account of the utility of *Moringa oleifera* seed powder for plummeting low and high turbidity values in surface water.
The coagulation efficiency of *M. oleifera* in relation to the pH should either be acidic (<6) or should be alkaline (>11), their coagulation efficiency is particularly good due to the domination of positive charges on the amino acids that build the protein molecule as stated by **Sasikala and Muthuraman, (2016)**. *Moringa* seeds have displayed antimicrobial properties on bacteria in precedence attributing to recombinant protein presence in the seeds with inherent potential to flocculate Gram-positive and Gram-negative bacterial cells. In this case, microbes can be detached by resolving in analogous mode as the elimination of colloids in suitably coagulated and flocculated water. Nonetheless, the seeds may also act unswervingly upon microbes consequently leading to growth reticence. According to **Amagloh and Benang, (2009),** domestic wastewater treatment tends to depend on the unstable loading dosage concentrations of the pH. Employing intrinsic resources of plant source to elucidate turbid water like domestic aqueous waste is not a novel scheme as opined by **Bina, (1991); Folkard and Sutherland, (2001**). Amid all the plant materials that have been employed over the years, powder extracted from *Moringa oleifera* seeds has been demonstrated to be one of the chiefly effectual prime coagulants for domestic wastewater management and thereby compared favourably to alum which is a traditional chemical coagulant for domestic aqueous waste management as stated by **Mangale *et al.* (2012).**

Numerous reports have demonstrated that the usage of natural coagulants as an alternative for metal salts like ammonium sulphate in alleviating the hazards allied with chemical coagulants. Precedent studies have found *Moringa oleifera* seeds to be safe and suggested as an supplementary plus over the chemical treatment of domestic aqueous waste since it is biological and edible **(Adeniran *et al.*, 2017).**
M. oleifera seeds also have the potentials to eliminate a broad variety of gram-positive and gram-negative bacteria, algae, from domestic wastewater samples **(Akhtar *et al.*, 2007; Suarez *et al.*, 2003).** Another antimicrobial property of *M. oleifera* seeds includes total removal of coliform bacteria in contaminated waters as noted by **Muyibi and Evison, (1995).** A study conducted by **Jabeen *et al.* (2008)** has reported aqueous seed extracts of *M. oleifera* to possess antifungal effects against *Fusarium solani* and *Aspergillus* spp. Alum has been reported to comprise antibacterial activity against Gram Positive and Gram negative bacterial consortia including *Enterococcus faecalis*, *E. faecium*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* **(Putt and Kleber, 2008; Photos-Jones, 2018)** from contaminated domestic waters. Antifungal activity of alum has also been detailed against *Aspergillus, niger, A. fumigatus, Penicillium* spp, *Fusarium* spp and the likes.
However, quite a few drawbacks such as soaring cost and pH variations have been evident by using alum **(Meneghel *et al.* 2013).** Multifarious reports have accomplished the fact that *M. oleifera* seeds do not elicit any noxious outcome when employed for domestic wastewater treatment **(Vikashni *et al.* 2012; Mangale *et al.* 2012).** *Moringa oleifera* seed powder filtrate can be utilized straightforwardly in domestic wastewater management **(Arnoldsson *et al.*, 2008).** The extracted part of *M. oleifera* seed thwarts the growth of coliforms and pathogens **(Arnoldsson *et al.* 2008; Bina *et al.* 2010; Eman *et al.* 2014).** This entails the diminution of the disinfection criteria in domestic aqueous waste management **(Srivastava, 2014; Jabeen *et al.* 2008).**
Domestic wastewater contains an assortment of wastes; which makes the microbial consortia present to proliferate by employing the organic and inorganic substrates therein. Consequently, these types of microbes will have an exact form of features in contrast to bacteria growing in other habitats as stated by **Ishak *et al.* (2008).** Domestic wastewater commonly includes the bacteria consortium of coliforms, *clostridia*, *enterococcus*, lacto bacilli, Micrococci, *Proteus, Pseudomonas, Streptococcus, Staphylococcus* species **(Zaved *et al.*, 2008).**
Aluminium sulphate (alum) is an inert salt and the extensively used coagulant in domestic wastewater treatment. Nevertheless, the utilization of aluminium salt as coagulant has come under inquiry. Moreover, elevated residues of aluminium

remnants in the treated aqueous waste have raised apprehension on human wellbeing. Prior investigations have been critical on the intake of huge total of aluminium salt eliciting an up-rise of neurodegenerative maladies **(Ahmad *et al.*, 2018). Bala *et al.* (2011)** reported aluminium sulphate had an appreciable antimicrobial activity against *E. coli* DH5α and *Staphylococcus epidermidis* NCIMB 12721 which confirms the potential of alum for water disinfection application.

Pathogenic fungi also have a considerable effect on the biology of the domestic wastewater environment as stated by **Brown *et al.* (2012).** Fungal members such as *Acremonium, Rhodotorula, Candida, Cladosporium, Sporothrix, Geotrichum candidum, Aspergillus, Penicillium, Trichophyton* and *Scopulariopsis* are reported as often occurring fungal genera implicated in domestic wastewater milieu detected using culture-dependent methods as noted by **Liu *et al.* (2017).**

Hence the purpose of this study is to independently evaluate the bio-flocculation and antimicrobial activities of *Moringa oleifera* (lam) seeds and alum on domestic wastewater samples collected from Federal University of Technology, Akure male and female students' hostels. This is to prove the bio-flocculating ability of powdered *M. oleifera* seeds and to accentuate the comparative antimicrobial efficacy of powdered *M. oleifera* seeds and alum. The effects of treating the domestic wastewater with *M. oleifera* seed powder and alum with respect to the physicochemical profile of the domestic wastewater samples were also evaluated.

EXPERIMENTAL PROTOCOLS

Depiction of catchment area

The study was carried out at male hostels (Akindeko, Abiola, Adeniyi) and female hostels (Jibowu and Jadesola) situated within the vicinity of the Federal University of Technology, Akure, Ondo State, Nigeria (Figure 1). The University is located between latitudes 7˚16 and 7˚18 N and longitudes 5˚ 009 and 5˚ 011 E. It is situated along Akure–Ilesha expressway, with Awule, Ibule and Ilara as the bordering rural communities.

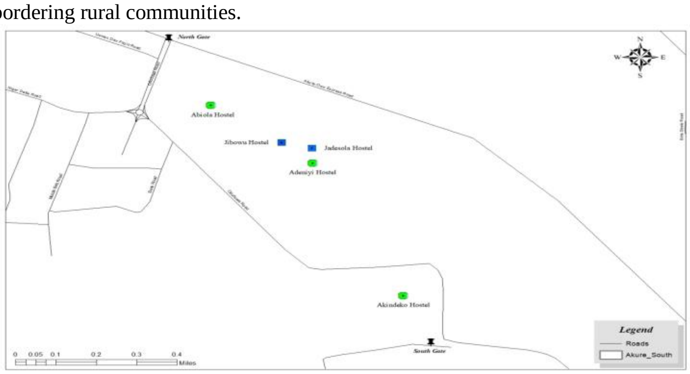

Figure 1 Locality map showing sampling points of male and female hostels in the tertiary study setting

Preparation of *M. oleifera* seeds powder

The seeds were obtained from Bodija market in Ibadan, Oyo state. The seeds were dried, manually de-shelled and the seed kernels were homogenized using an electric high power blender (Marlex Electrolyne IS: 250). The powder was stored in a sterile plastic bag and stored in room condition.

Collection and treatment of the wastewater samples

Domestic wastewater was collected from discharge outlets in students' residential hostels (Jibowu, Jadesola, Akindeko, Adeniyi, Abiola) in Federal University of Technology, Akure which is the area of study. The samples were collected into clean, transparent 5 liter kegs. The samples were collected at the run-off point, where it is released into the environment. The wastewater samples were gently homogenized in 500 ml keg before pouring into sterilized plastic containers, 500 ml of the collected wastewater specimens was poured into each bowl, powdered *M. oleifera* seeds and crushed (weighted) alum was added to each plastic bowl using varying concentrations (2 g, 4 g and 6 g). Each bowl was stirred using a stirrer continuously for 5 min and then allowed to stand for 60 min**.** Standard pour plate method was used in bacterial characterization with 0.5 ml of the supernatant. The plates were incubated at appropriate temperature and observed after 24 hr for bacteria and 72 hr for fungi enumeration.

Physiochemical analysis of the domestic wastewater samples before and after treatment with *M. oleifera* seed powder and alum

The wastewater samples were analyzed for physiochemical properties including electrical conductivity, pH and turbidity before (initial) and after treatment with both *M. oleifera* seeds and alum. Also after treatment, the supernatant gotten from the treated wastewater were also analyzed physico-chemically via the above listed parameters.

A calibrated Crison Conductimeter Basic C30 was used. The conductimeter was switched on and allowed to warm for about 5 minutes. The probe was calibrated using distilled water. The conductivity meter probe was then inserted making sure it did not touch the beaker. The reading was recorded from the LCD display after it had stabilized.

The pH of the raw wastewater and the supernatants from the treated sample was determined using pH meter as described by **AOAC, (2012).** This was done by first standardizing the pH meter (Hanna multi–parameter –H1-9828) fitted with glass electrodes using buffer solutions of pH 4.0 and pH 7.0. The electrode was then rinsed with distilled water and immersed into the sample and making sure it did not touch the beaker. The reading was recorded from the LCD display after it had stabilized.

Spectrophotometer (752 p B&D England) was used to check the turbidity and distilled water was used as reference (to blank the spectrophotometer). The level of difference between the distilled water and the filtrate was used to indicate turbidity levels of the wastewater samples. The spectrophotometer was blanked (or zeroed) using distilled water, about 25 ml of the filtrate was poured into the curvette and the side was clean with a tissue paper before inserting into the spectrophotometer. The absorbance was read at a wavelength of 750 nm. The turbidity of the wastewater samples was displayed on the LCD panel of the instrument in Nephelometric Turbidity Units (NTU). After each reading, the spectrophotometer was calibrated again with the distilled water before being used on the next sample.

Isolation and identification of bacteria from the wastewater samples

Serial dilution and standard pour plate method using one (1) ml of 10^{-4} diluent of domestic wastewater samples was employed as bacterial organisms were enumerated on nutrient agar (Hi-Media, India) and MacConkey agar (Hi-Media, India) plates as demonstrated by **Fawole and Osho, (2001).** The bacteria petri-plates were incubated for duration of 18-24 hours at 37 ˚C. The bacterial colonies were then sub-cultured for identification through molecular analysis. Biochemical confirmatory tests were conducted on the isolated bacteria including; gram reaction, catalase, coagulase, motility, urease, indole, oxidase, citrate and sugar fermentation tests for their identification via comparism with bergey's manual of systematic bacteriology (Don *et al.*, 2006).

Isolation and identification of fungi from the wastewater samples

Serial dilution and standard pour plate method using one (1) ml of 10^{-4} diluent of domestic wastewater samples was employed as fungal organisms were enumerated on potato dextrose agar (Hi-Media, India) plates as described by **Fawole and Osho, (2008).** The fungal petri-plates were incubated for duration of 72 hours at 27 ˚C. The fungal isolates were identified by transferring a mycelia growth on the plates with the aid of sterile needle from a pure culture onto a grease-free glass slide containing a drop of lactophenol cotton blue, the mycelium was placed on the slide, covered with a cover slip and then viewed under the microscope at low power X40 magnification objective.

16S gene amplification of bacterial isolates from the wastewater samples

Polymerase chain reaction (PCR)-based identification and characterization of bacterial isolates were carried out with DNA extraction of bacteria isolates according to the technique illustrated by **Gurakan *et al.* (2008).** The 16SrRNA gene of the bacteria was amplified using the primer pair 27F-5′-AGAGTTTGATCCTGGCTCAG-3', and 1492R 5'GGTTACCTTGTTACGACTT-3'. PCR extension was carried as demonstrated by **Sambrook *et al.* (2001);** the PCR profile used was initial denaturation temperature of 94 ˚C for 3 mins, followed by 30 cycles of 94 ˚C for 60 sec, 56 ˚C for 60 sec, 72 ˚C for 120 seconds and the final extension temperature of 72 ˚C for 5 minutes followed by sequencing and genetic make-up blasting via the National centre for biotechnological information (NCBI) server. PCR-sequencing was conducted via DNA Sanger sequencing and data was analyzed by ABI Sequencing Analysis software (version 5.2).

Data analysis

Analysis of study statistics was carried out using analysis of variance (ANOVA) to determine if significant differences (standard error of mean) exist between the initial, after treatment with *M. oleifera* seeds and after treatment with alum, for each physiochemical parameter ($p \leq 0.05$) via SPSS (version '22).

RESULTS

Effects of powdered *Moringa oleifera* seeds and alum on electrical conductivity of domestic wastewater samples

The electrical conductivity (µs/cm) at the point of collection of the wastewater samples from the students' hall of residences was high at 1910.67 µs/cm for Abiola hostel and low at 420.00 µs/cm for Jadesola. The electrical conductivity of 2 g of powdered *M. oleifera* seeds was high at 668.33 µs/cm for Abiola hostel and low at 199.00 µs/cm for Jadesola hostel. The electrical conductivity of 4g of powdered *M. oleifera* seeds was high at 701 µs/cm for Abiola hostel and low at 248.33 µs/cm. The electrical conductivity of 6 g of powdered *M. oleifera* seeds was high at 757.33 µs/cm for Abiola hostel and low at 269 µs/cm. The electrical conductivity of 2 g of alum was high at 911.61 µs/cm for Abioa hostel and low at 302.33 µs/cm for Jadesola hostel. The electrical conductivity of 4 g of alum was high at 1132 µs/cm for Abiola hostel and low conductivity was observed at 347.66 µs/cm for Jadesola hostel. The electrical conductivity of 6 g of alum was high at 1188.67 µs/cm for Abiola hostel and the least conductivity at 381.67 µs/cm for Jadesola hostel as observed as illustrated in Table 1.

Table 1 Effects of powdered *Moringa oleifera* seeds and alum on electrical conductivity of domestic wastewater samples

School hostels	**Initial (µs/cm)**	**After treatment with powdered *M. oleifera* seeds (µs/cm)**			**After treatment with alum (µs/cm)**		
		2g	4g	6g	2g	4g	6g
Jibowu	1007.67±8.88^{g}	432.33±1.86^{a}	493.67±0.88^{b}	535.33±0.64^{c}	692.33±1.45^{d}	756.33±0.33^{e}	822.00±1.16^{f}
Jadesola	420.00±1.16^{g}	199.00±0.58^{a}	248.33±0.33^{b}	269.00±0.58^{c}	302.33±1.45^{d}	347.66±1.45^{e}	381.67±1.67^{f}
Akindeko	649.67±0.88^{g}	271.67±2.03^{a}	356.00±0.58^{b}	376.67±0.88^{c}	445.00±2.52^{d}	547.67±1.45^{e}	573.00±2.08^{f}
Adeniyi	1464.00±0.58^{g}	565.00±1.53^{a}	630.33±0.88^{b}	701.00±2.08^{c}	852.00±1.16^{d}	948.33±0.88^{e}	1100.33±1.45^{f}
Abiola	1910.67±1.76^{g}	668.33±0.88^{a}	701.00±0.58^{b}	757.33±0.88^{c}	911.67±0.88^{d}	1132.00±1.16	1188.67±0.88^{f}

Figures are means ± standard error. Means with the parallel superscript have no noteworthy variation at $p \leq 0.05$ level of significance. µs/cm=microsecond/centimeter

Effects of powdered *M. oleifera* seeds and alum on the pH of the domestic wastewater samples

The pH of the domestic wastewater (initial pH) at the point of collection was high at 8.5 for Jibowu hostel and the lowest pH was recorded at 3.06 for Adeniyi hostel. The pH of the wastewater with 2 g of powdered *M. oleifera* seeds was high at 7.36 while the lowest pH was observed to be 6.34 for Adeniyi hostel. The pH of the wastewater dropped with 4 g of powdered *M. oleifera* seeds introduced at 7.02 for both Abiola and Akindeko hostels while the lowest pH was recorded at 6.23 for Adeniyi hostel. The pH of the wastewater dropped further with 6 g of powdered *M. oleifera* seeds introduced at 6.67 for Abiola hostel while the lowest pH was recorded at 6.00 for Jibowu hostel. The pH of the wastewater dropped even further with 2 g of alum introduced at 4.40 for Jibowu hostel while the lowest pH was recorded at 2.67 for Adeniyi hostel. The pH of the wastewater with 4 g of alum introduced at 4.30 for Jibowu hostel while the lowest pH was recorded at 2.16 for Adeniyi hostel. The pH of the wastewater with 6 g of alum introduced at 4.20 for Jibowu hostel while the lowest pH was recorded at 1.90 for Adeniyi hostel as demonstrated in Table 2.

Table 2 Effects of powdered *M. oleifera* seeds and alum on the pH of the domestic wastewater samples

School hostels	**Initial pH**	**After treatment with powdered *M. oleifera* seeds**			**After treatment with alum**		
		2g	4g	6g	2g	4g	6g
Jibowu	8.53±0.09^{d}	7.00±0.12^{c}	6.40±0.88^{b}	6.00±0.18^{b}	4.40±0.12^{a}	4.30±0.06^{a}	4.20±0.07^{a}
Jadesola	6.67±0.09^{e}	7.36±0.12^{f}	6.25±0.15^{d}	6.20±0.67^{c}	3.19±0.00^{b}	3.03±0.01^{a}	2.92±0.03^{a}
Akindeko	4.78±0.01^{c}	7.24±0.01^{e}	7.02±0.02^{e}	6.50±0.08^{d}	2.82±0.01^{ab}	2.70±0.07^{b}	2.55±0.07a
Adeniyi	3.06±0.01^{d}	6.34±0.01^{f}	6.23±0.02^{f}	6.05±0.03^{e}	2.67±0.01^{c}	2.16±0.01^{b}	1.90±0.60^{a}
Abiola	5.05±0.01^{d}	7.05±0.01^{f}	7.02±0.01^{f}	6.67±0.10^{e}	3.75±0.02^{c}	3.27±0.01^{b}	3.01±0.02^{a}

Figures are means ± standard error. Means with the identical superscript have no major disparity at $p \leq 0.05$ level of significance

Effects of powdered *M. oleifera* seeds and alum on the turbidity of domestic wastewater samples

The turbidity at the point of collection of the wastewater was high at 0.958 NTU for Jibowu hostel and low at 0.128 NTU. The turbidity level of the wastewater with 2 g of powdered *M. oleifera* seeds was high at 0.101 NTU for Adeniyi and the lowest turbidity was recorded at 0.066 NTU for Jadesola hostel. The turbidity level of the wastewater with 4 g of powdered *M. oleifera* seeds reduced at 0.096 NTU for Adeniyi hostel while the lowest turbidity was recorded at 0.057 NTU for Jadesola hostel. The turbidity level of the wastewater with 6 g of powdered *M. oleifera* seeds introduced also reduced at 0.088 NTU for Adeniyi hostel while the lowest turbidity was recorded at 0.029 NTU for Jadesola hostel. The turbidity level of the wastewater with 2 g of alum introduced was high at 0.212 NTU for Jibowu hostel while the lowest turbidity was recorded at 0.049 NTU for Jadesola hostel. The turbidity level of the wastewater with 4 g of alum introduced reduced at 0.918 NTU for Jibowu hostel while the lowest turbidity was recorded at 0.047 NTU for Jadesola and Akindeko hostels. The turbidity level of the wastewater with 6 g of alum introduced reduced further at 0.184 NTU for Jibowu hostel while the lowest turbidity was recorded at 0.039 NTU for Akindeko hostel which is lower than the WHO standard of 5 NTU as shown in Table 3.

Table 3 Effects of powdered *M. oleifera* seeds and alum on the turbidity (NTU) of domestic wastewater samples

School hostels	Initial turbidity	After treatment with powdered *M. oleifera* seeds (NTU)			After treatment with alum (NTU)		
		2g	4g	6g	2g	4g	6g
Jibowu	0.958±0.001^{d}	0.095±0.000^{b}	0.089±0.000^{ab}	0.029±0.000^{a}	0.212±0.000^{c}	0.198±0.000^{bc}	0.184±0.00^{bc}
Jadesola	0.102±0.000^{c}	0.066±0.000^{bc}	0.057±0.000^{b}	0.043±0.000^{a}	0.049±0.000^{a}	0.047±0.000^{a}	0.046±0.000^{a}
Akindeko	0.649±0.000^{c}	0.072±0.000^{b}	0.063±0.000^{b}	0.055±0.000^{ab}	0.059±0.000^{ab}	0.047±0.000^{ab}	0.039±0.000^{a}
Adeniyi	0.888±0.001^{e}	0.101±0.000^{d}	0.096±0.000^{cd}	0.088±0.000^{c}	0.089±0.000^{c}	0.077±0.000^{b}	0.070±0.000^{a}
Abiola	0.765±0.001^{f}	0.089±0.000^{bc}	0.081±0.000^{b}	0.071±0.000^{a}	0.121±0.000^{e}	0.101±0.000^{d}	0.090±0.000^{bc}

Values are means ± standard error. Means with the identical superscript have no considerable dissimilarity at $p \leq 0.05$ level of significance. Abs=Absorbance

Effects of powdered *M. oleifera* seeds and alum on bacterial count of domestic wastewater samples using nutrient agar

There was an increase in the initial bacterial count with 311 Cfu/ml recorded for Jibowu hostel while Abiola hostel had the lowest bacterial count of 176 Cfu/ml in the domestic wastewater on the point of collection. There was a considerate diminution of bacterial counts from the 2 g to 6 g of *M. oleifera* seed powder in subsequent treatment of the wastewater. The bacterial count of the wastewater reduced after the initial count with 2 g of *M. oleifera* seed powder at 199 Cfu/ml for Jibowu hostel while Abiola hostel had the lowest bacterial count of 56 Cfu/ml. Four (4 g) of the *M. oleifera* seed powder introduced into the wastewater elicited 124 Cfu/ml for Jibowu hostel while Abiola hostel had the lowest count of 37 Cfu/ml. Six (6 g) of the *M. oleifera* seed powder elicited 100 Cfu/ml for Jibowu hostel while Abiola hostel had the lowest count at 26 Cfu/ml. Two (2 g) alum treatment of the wastewater elicited reduced bacterial count at 87 Cfu/ml for Adeniyi hostel and 29.33 cfu/ml for Abiola hostel. The bacterial count of the wastewater further reduced using 4 g of alum thereby eliciting 80 Cfu/ml for Adeniyi hostel while 16 Cfu/ml was recorded for Abiola hostel. Six (6 g) of the alum introduced for treatment brought about 62 Cfu/ml for Jibowu hostel and 6.33 Cfu/ ml for Adeniyi hostel as demonstrated in Table 4.

Table 4 Effects of powdered *M. oleifera* seeds and alum on bacterial count of domestic wastewater samples using nutrient agar

School hostels		After treatment with powdered *M. oleifera* seeds (cfu/ml)			After treatment with alum (cfu/ml)		
	Before	2g	4g	6g	2g	4g	6g
Jibowu	311.00±0.58^{g}	199.67±0.89^{f}	124.33±1.20^{e}	100.00±0.57^{d}	81.00±0.57^{c}	73.33±0.89^{b}	62.00±0.57^{a}
Jadesola	219.00±0.57^{f}	86.00±0.57^{e}	54.00±0.57^{d}	41.00±0.57^{c}	55.00±0.57^{d}	41.00±0.57^{b}	21.00±0.57^{a}
Akindeko	278.33±1.20^{e}	139.00±0.57^{d}	75.00±1.15^{c}	41.00±0.57^{b}	76.00±0.57^{c}	42.00±0.57^{b}	21.00±0.57^{a}
Adeniyi	202.00±1.15^{g}	102.00±0.57^{f}	90.33±0.88^{e}	75.00±0.57^{b}	87.00±0.57^{d}	80.00±0.57^{c}	61.00±0.88^{a}
Abiola	176.00±0.57^{g}	56.00±0.57^{f}	37.00±0.33^{e}	26.00±0.57^{c}	29.33±0.33^{d}	16.00±0.57^{b}	6.33±0.57^{a}

Values are means ± standard error. Means with the parallel superscript have no noteworthy variation at $p \leq 0.05$ level of significance. Cfu/ml=Colony forming unit per millimetre

Effects of powdered *M. oleifera* seeds and alum on lactose fermenters isolated from domestic wastewater samples using MacConkey agar

There was an increase in the initial lactose fermenter count before the addition of *M. oleifera* seed powder and alum with 177 Cfu/ml recorded for Adeniyi hostel while both Abiola and Jibowu hostels had the lowest lactose fermenter count of 76 Cfu/ml in the domestic wastewater on the point of collection. There was a considerate diminution of lactose fermenter count from the 2 g to 6 g of *M. oleifera* seed powder in subsequent treatment of the wastewater. The lactose fermenter count of the wastewater reduced after the initial count with 2 g of *M. oleifera* seed powder at 72 Cfu/ml for Adeniyi hostel while Abiola hostel had the lowest lactose fermenter count of 20 Cfu/ml. Four (4 g) of the *M. oleifera* seed powder introduced into the wastewater elicited 55 Cfu/ml for Adeniyi and Jadesola hostels while Abiola hostel had the lowest count of 17 Cfu/ml. Six (6 g) of the *M. oleifera* seed powder elicited 41 Cfu/ml for Jadesola hostel while Abiola hostel had the lowest count at 11 Cfu/ml. Two (2 g) alum treatment of the wastewater elicited reduced lactose fermenter count at 37 Cfu/ml for Jadesola hostel and 11 cfu/ml for Jibowu hostel. The lactose fermenter count of the wastewater further reduced using 4 g of alum thereby eliciting 18 Cfu/ml for Jadesola hostel while 7 Cfu/ml was recorded for Jibowu hostel. Six (6 g) of the alum introduced for the wastewater treatment brought about 15 Cfu/ml for Adeniyi hostel and 3.67 Cfu/ ml for Jibowu hostel as displayed in Table 5.

Table 5 Effects of powdered *M. oleifera* seeds and alum on lactose fermenters isolated from domestic wastewater samples using MacConkey agar

School hostels		After treatment with powdered *M. oleifera* seeds (cfu/ml)			After treatment with alum (cfu/ml)		
	Before	2g	4g	6g	2g	4g	6g
Jibowu	76.00±0.57^{g}	25.33±0.88^{f}	20.00±0.57^{e}	14.00±0.57^{d}	11.00±0.57^{c}	7.00±0.57^{b}	3.67±0.33^{a}
Jadesola	149.67±0.88^{g}	60.00±0.57^{f}	55.00±0.57^{e}	41.00±0.57^{d}	37.00±0.57^{c}	18.00±0.57^{b}	11.67±0.33^{a}
Akindeko	169.67±0.88^{f}	64.00±0.88^{e}	41.00±0.88^{d}	26.00±0.57^{c}	26.00±0.57^{c}	16.00±0.57^{b}	7.33±0.33^{a}
Adeniyi	177.00±0.57^{g}	72.00±0.57^{f}	55.00±0.57^{e}	40.33±0.33^{d}	26.63±0.88^{c}	11.00±0.57^{a}	15.00±0.57^{b}
Abiola	76.00±0.57^{e}	20.00±0.57^{d}	17.00±0.57^{c}	11.00±0.57^{b}	16.00±0.57^{c}	11.00±0.57^{b}	6.00±0.57^{a}

Figures are means ± standard error. Means with equivalent superscript have no major disparity at $p \leq 0.05$ level of significance. Cfu/ml= Colony forming unit per millimeter

Effects of powdered *M. oleifera* seeds and alum on fungal count in domestic wastewater samples

There was an initial increase in the fungal count before the addition of *M. oleifera* seed powder and alum with the highest fungal count of 45 Sfu/ml recorded for Akindeko hostel while Jibowu hostel had the least fungal count of 22 Sfu/ml in the domestic wastewater on the point of collection. There was subsequent reduction of fungal counts from the 2 g to 6 g of *M. oleifera* seed powder introduced for the wastewater treatment. The lactose fermenter count of the wastewater reduced after the initial count with 2 g of *M. oleifera* seed powder at 26 Sfu/ml for Akindeko hostel while Abiola hostel had the lowest fungal count of 13 Sfu/ml. Four (4 g) of the *M. oleifera* seed powder introduced into the wastewater elicited the highest fungal count of 18 Sfu/ml for Akindeko hostel while Jibowu hostel had the lowest fungal count of 7 Cfu/ml. Six (6 g) of the *M. oleifera* seed powder elicited a further reduced fungal count of 11 Sfu/ml for Akindeko hostel and 5 Sfu/ml for Jibowu hostel. Two (2 g) alum treatment of the wastewater elicited reduced fungal count at 19 Sfu/ml for Akindeko hostel and 7 Sfu/ml for Jibowu hostel. The fungal count of the wastewater further reduced using 4 g of alum thereby eliciting 15 Sfu/ml for Akindeko hostel while 5 Sfu/ml was recorded for Jibowu hostel. Six (6 g) of the alum introduced for the wastewater treatment brought about 10 Sfu/ml for Akindeko hostel and 2.0 Sfu/ml for Jibowu hostel as illustrated in Table 6.

Table 6 Effects of powdered *M. oleifera* seeds and alum on fungal count in domestic wastewater samples

School hostels		After treatment with powdered *M. oleifera* seeds (sfu/ml)			After treatment with alum (sfu/ml)		
	Before	2g	4g	6g	2g	4g	6g
Jibowu	22.67 ± 0.88^{d}	15.00 ± 0.57^{c}	7.00 ± 0.57^{b}	5.00 ± 0.57^{b}	7.00 ± 0.57^{b}	5.00 ± 0.57^{b}	2.00 ± 0.57^{a}
Jadesola	31.00 ± 0.57^{f}	21.00 ± 0.57^{e}	12.00 ± 0.57^{d}	9.00 ± 0.57^{bc}	10.33 ± 1.20^{cd}	8.00 ± 0.57^{ab}	6.00 ± 0.57^{a}
Akindeko	45.33 ± 0.88^{e}	26.00 ± 0.57^{d}	18.00 ± 0.57^{c}	11.00 ± 0.57^{a}	19.00 ± 0.88^{c}	15.00 ± 0.57^{b}	10.00 ± 1.15^{a}
Adeniyi	28.00 ± 0.57^{e}	17.00 ± 0.57^{d}	16.00 ± 0.57^{d}	9.00 ± 0.88^{c}	8.00 ± 0.57^{c}	6.00 ± 0.57^{b}	3.00 ± 0.57^{a}
Abiola	24.00 ± 0.57^{e}	13.00 ± 0.57^{d}	7.67 ± 0.33^{b}	6.00 ± 0.57^{a}	11.00 ± 0.57^{c}	9.00 ± 0.57^{b}	5.00 ± 0.57^{a}

Figures are means ± standard error. Means with the same superscript have no significant difference at $p\leq0.05$ level of significance. Sfu/ml=Spore-forming unit per millimeter

Bacterial organisms isolated from the domestic wastewater samples

Three of the bacteria isolated after treatment of the wastewater samples with *M. oleifera* seeds were biotechnologically-identified after the determination of their biochemistry and were successfully submitted into the NCBI gene bank including; *Bacillus cereus* strain mkbk 1 with accession number MT199666, *Pseudomonas aeruginosa* strain mkbk 2 with accession number MT199667 and *Enterobacter ludwigii* strain mkbk 3 with accession number MT199668 as displayed in Table 7 excluding *Escherichia coli*, and *Bacillus subtilis* which were identified via the nominal biochemical characterization with results compared with Bergey's manual of systematic bacteriology **(Don *et al.*, 2006)** for presumptive identification as juxtaposed in Table 8.

Table 7 Molecular identification of the domestic wastewater bacterial isolates

Isolate codes	Biochemical Identity	Molecular identity	Percentage similarity	Strain number	NCBI-assigned Accession number
Ba	*Bacillus cereus*	*Bacillus cereus*	99.31%	Mkbk 1	MT199666
Pa	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	96.93%	Mkbk 2	MT199667
El	*Enterobacter ludwigii*	*Enterobacter ludwigii*	97.43%	Mkbk 3	MT199668

Keys: Ba- *Bacillus cereus*; El- *Enterobacter ludwigii*; Pa- *Pseudomonas aeruginosa*

Table 8 Biochemistry of bacterial isolates from the domestic wastewater samples

Catalase	Coagulase	Motility	H_2S	Urease	Indole	Oxidase	Sugar Fermentation: Glucose	Sucrose	Lactose	Mannitol	Citrate	Gram reaction	Probable Organism
+	-	+	+	-	+	-	+	+	+	+	-	-ve rods	*Escherichia coli*
-	-	-	-	-	-	-	+	+	+	+	-	-ve rods	*Enterobacter ludwigii*
+	-	+	-	-	-	+	-	+	-	+	+	-ve rods	*Pseudomonas aeruginosa*
+	+	+	-	NA	-	-	+	+	-	-	+	+ve rods	*Bacillus cereus*
+	+	+	-	NA	-	-	+	+	-	-	+	+ve rods	*Bacillus subtilis*

Keys: + = positive; - = negative; NA – Not applicable

1500 bp

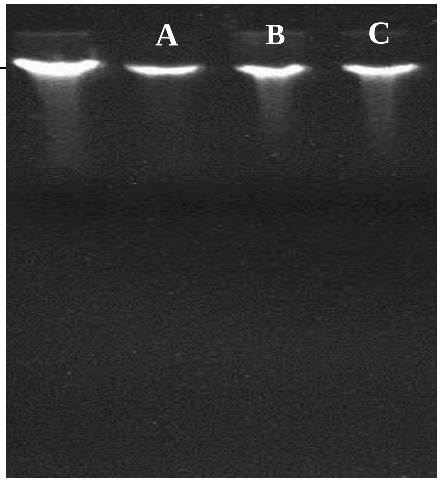

Plate 1 The 16S rRNA gene amplified by polymerase chain reaction of the test bacterial isolates *Bacillus cereus* (A), *Pseudomonas aeruginosa* (B), *Enterobacter ludwigii* (C)

Fungal organisms enumerated from the domestic wastewater samples

The morphological traits of fungal organisms isolated from the wastewater samples are *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *Candida parapsilosis*, *Fusarium oxysporum*, and *Penicillium citrinum* as displayed in Table 9.

Table 9 Macro and micro-morphological features of fungal organisms from the domestic wastewater samples

Macroscopic features	Microscopic features	Probable fungal organisms
Black mycelia growth	Hypha is septate. Possess simple upright conodiophores that terminates in a globosely	*Aspergillus niger*
Greyish blue colonies	Versicular shape with rough uniseptate sporangiosphore	*Aspergillus fumigatus*
Light green and powdery growth	Long, erect septate and condiophores	*Aspergillus flavus*
Yellow and cottony-like growth	Long erect conidiophores with round-shaped conidia	*Penicillium citrinum*
Whitish mycelia growth	Round white-phase and elongated, opaque-phase	*Candida parasilopsis*
Yellow pink creamy colonies	Cylindrical to ovoid conidia with curved septate conidiophores	*Fusarium oxysporum*

DISCUSSION

This study was carried out to evaluate the bio-flocculating and antimicrobial activities of powdered *M. oleifera* seeds and alum for the treatment of domestic wastewater samples obtained from five different school hostels as sampling points at Federal University of Technology, Akure (FUTA). This study revealed conductivity decreased generally as aluminum sulfate (alum) and powdered *M. oleifera* seeds showed similar trend of decreasing conductivity. However, powdered *M. oleifera* seeds showed a lower conductivity than alum which implies that it contains less impurities than alum. There was a decrease in the conductivity in this study carried out which is analogous with observations of the study carried out by **Hendrawati *et al.* (2016)** who also noticeably observed a decrease in the conductivity of water samples used. **Tunggolou and Payus, (2017); Bakare, (2016)** did not observe any significant difference between the initial electrical conductivity and after treatment of the wastewater samples with powdered *M. oleifera;* this might be due to the removal of the epicarp on *M. oleifera* seeds before using it for the treatment of the wastewater samples. This investigation further revealed that powdered *M. oleifera* seeds at 2g were most effectual for the removal of impurities been significantly different from other concentration used. The initial decrease in electrical conductivity of domestic wastewater that was treated with powdered *M. oleifera* seeds could have been as a result of the ions formed during the coagulation process, formation of the ions may also contribute to the overall conductivity. Another reason that might contribute to the increased conductivity would be the dissociation of the inorganic compounds in alum which results in the wastewater's ability to conduct large electric current; this was also observed by **Tunggolou and Payus, (2017).**

The recommended pH range by World Health Organization (WHO) for wastewater discharged into water bodies or land is 5.0 – 8.0. The optimum pH obtained using powdered *M. oleifera* seeds was 6.00 – 7.38 and in line with the recommended standard **WHO, (2006).** This further elaborated the fact that the lowest concentration of powdered *M. oleifera* seeds brought the pH to a neutral value and as the concentrations increased, it gradually moved to a slightly acidic value, this was the same trend noticed by **Maduabuchi, (2018).** The change in the pH reading when seeds were used results in the final pH reading being within the permitted range of standard for domestic wastewater. **Sasikala and Muthuraman, (2016)** stated that for the coagulation efficiency using *M. oleifera*, the pH should either be acidic (<6) or should be alkaline (>11), their coagulation efficiency is particularly good due to the domination of positive charges on the amino acids that build the protein molecule as also corroborated in this study. **Hendrawati *et al.* (2016)** also observed similar trend in their study carried out on domestic wastewater treatment and expatiated on the fact that wastewater became slightly alkaline due to the ability of *M. oleifera* seeds to present its cationic water-soluble protein found in its skins and seeds, this causes the acceptance of protons by the alkaline amino acid to release the hydroxyl group which makes the solution slightly acidic. Opposing this trend was **Ewan *et al.* (2009)** who noticed that the pH did not change significantly after treatment with powdered *M. oleifera* seeds; but this might be due to the removal of the seed oil present in the seed and using the seed cake for water treatment. Alum had the lowest pH which was below the recommended standard due to alkalinity consumption during hydrolysis by the coagulant. This is consistent with the results obtained from **Jowa and Mguni, (2005)** where they stated that alum was more acidic and hence its consumption of alkalinity is higher to prevent acidity of water samples in their study. This further buttress the suggestion earlier raised by **Ahmad *et al.* (2018)** who noted elevated residues of aluminium remnants in treated domestic wastewater with a tendency for increased apprehension on human wellbeing due to its acidity.

The initial absorbance obtained showed that the wastewater samples were quite high which implied high turbidity. The mechanism responsible for reduction of turbidity by powdered *M. oleifera* seeds were due to the ability of the powdered seeds to adsorb both positively and negatively-charged colloids and neutralize their charge in the wastewater samples as supported by **Ndagengesere *et al.* (2015).** The result obtained from this study meet the domestic wastewater quality standard recommended by World Health Organization **(WHO), (2006)** with the lowest turbidity obtained at a concentration of 6 g which was significantly different from alum also at 6 g. **Bakare, (2016)** also observed a 90 % decrease in turbidity from the use of powdered *M. oleifera* seeds. The study carried out by **Tunggolou and Payus, (2017)** also showed that *M. oleifera* was more effective at reducing turbidity than alum. The ability of alum to reduce turbidity of the wastewater samples is based on aluminum salts been hydrolyzed to produce cationic species responsible for absorbing negatively-charged colloidal particles and also neutralizing their charges as destabilization of the particles occur as reported by **Vinoth *et al.* (2012).**

Reduced microbial population with powdered *M. oleifera* seeds at 4 g may be due to the presence of some active biochemical agents in *M. oleifera* seeds according to **Aromolaran, (2013).** From the comparism of the microbial population before and after treatment, it was observed that *M. oleifera* reduced the microbial population (bacteria and fungi); although, the microbial population for *M. oleifera* at 6 g was still higher than that noticed in alum at 4 g quantity. This might be due to the compounds contained in the *M. oleifera* seeds from which some may serve as growth factors for microorganisms; so after bio-flocculation, organisms may proliferate using nutrients supplied by *M. oleifera* seeds. Alum was able to reduce the microbial load more significantly; this may suggest it has the ability as a chemical flocculant to alter the water pH, a factor necessary for growth of microorganisms and hence less microbial population after treatment as opined by **Madsen *et al.* (2017).** The high bacterial and fungal count of the domestic wastewater samples in this study indicates a very high presence of different microbial contamination due to the presence of organic residues and partly due to human-related activities inherent in the wastewater samples in this study as supported by **Olalemi *et al.* (2021)** who correlated the faecal bacterial count of hospital wastewater with diarrheagenic faecal samples.

The results of the bacterial consortium enumerated from the domestic wastewater samples in this study are consistent with **Brown *et al.* (2012)** who confirmed the occurrence of coliforms, *Clostridia*, *Enterococcus*, lacto bacilli, Micrococci, *Proteus*, *Pseudomonas*, *Streptococcus*, *Staphylococcus* species while the outcome/findings of fungal organisms isolated from the domestic wastewaters are also analogous to **Liu *et al.* (2017)** who also affirms the presence of *Candida, Geotrichum, Geotrichum candidum, Aspergillus,* and *Penicillium* spp. **Putt and Kleber, (1995); (Photos-Jones, 2018)** also affirms the occurrence of Gram positive and Gram negative bacterial consortia including *Enterococcus faecalis*, *E. faecium*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* respectively from contaminated domestic waters which is as well evident in this study.

CONCLUSION

Wastewater treatment using powdered *M. oleifera* seeds revealed its efficiency in aiding maximum suspended materials removal and reduction of pH from alkalinity to acidity together with alum. The seeds displayed effective coagulation properties which is efficient in reducing the presence of microorganisms in domestic wastewaters as evident in this study. *Moringa oleifera* seed powder was also efficacious in improving the physicochemical profile of the wastewater in this study compared to alum. The powdered *M. oleifera* seeds has been tested and confirmed to be an effective material for the bacteriological and fungal treatment of domestic wastewater. The findings of this study suggest that the bio-flocculating ability of powdered *M. oleifera* seeds accentuated better antimicrobial efficacy of *M. oleifera* over alum as a proviso to the blend of powdered *M. oleifera* seeds and alum for the treatment of domestic wastewaters. To ensure environmental safety, it is hereby recommended domestic wastewater should be treated before releasing into the environment to reduce environmental pollution. *M. oleifera* should be used for the treatment of any type of wastewater before their release to the environment. Appropriate sewer system should be built by water-regulatory bodies to ease the treatment of the domestic wastewaters especially in the developing countries.

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