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STUDY ON THE UTILIZATION OF ALUM AND *MORINGA OLEIFERA* IN WASTE WATER TREATMENT

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ABSTRACT
The utilization of Alum and *Moringa oleifera* in the treatment of waste water was study comparing between the *Moringa oleifera* (MO) and Alum was conducted to obtain an insight into the various bacteria associated with wastewater obtained from two different beverages (coca cola and 33) companies in Owerri, Imo State thereby utilizing *Moringa oleifera* and Alum as natural coagulant for the treatment of waste water samples collected from beverage companies. The predominant organisms in the wastewater were *Pseudomonas sp. Staphylococcus aureus, Escherichia coli, Klebsiella sp.* and *Bacillus sp.* processing the waste water by coagulation using *M. oleifera* and Alum as natural coagulant showed that the treatment with *M. oleifera* provided additional advantage of reduced total microbes but in both cases the treated water met WHO water guidelines. MO is found to be a sustainable solution for coagulation and reduction in the microbial load of waste water before its disposal.

KEYWORDS: *Moringa oleifera*, Alum, Coagulation, Microbes.

1. INTRODUCTION
Water is very important to life because the entire living things on earth need water to survive. Today, the quality of water has become a major problem that needs serious attention (Tilley *et al*., 2013) due to adequate treatment. Good quality water has become an expensive item, because many water sources has been polluted by waste coming from the various human activities. This leads to declining quantity of water sources that could not meet the ever growing need (Tilley *et al*., 2013). River water drawn for human consumption and general household use can be highly turbid particularly in the rainy season. River silt is churned into suspension and run off from fields and other surfaces carries solid material, bacteria and other microorganisms into the river (Davis, 2006). It is of paramount importance to remove as much of this suspended matter as possible prior to a disinfection stage and subsequent consumption. This can generally only be achieved by the addition of coagulants to the raw water, within a controlled treatment sequence. In many developing countries, proprietary chemical coagulants, such as aluminium sulphate and synthetic polyelectrolytes are either not available locally or are imported using foreign exchange (Davis, 2006). A viable alternative is the use of crushed seed of *M. oleifera* as a natural coagulant. The seed pods are allowed to dry naturally on the tree prior to harvesting. The mature seeds are readily removed from the pods, easily shelled and then may be crushed and sieved using traditional techniques such as those employed for the production of maize flour (Davis, 2006). Coagulation is one of the most common ways to reduce the pollutant contents in the water body that are present as turbidity, color and organic matters. Coagulation is also used to reduce the metal ion content in water. Separation of these colloids can be done by the addition of synthetic coagulant or biocoagulant followed by slow agitation (flocculation) that causes coagulation of colloidal particles so they can be separated by sedimentation. The common methods of water purification using synthetic materials such as aluminum sulfate (alum) and calcium hypochlorite are not efficient; because these materials are imported and thus make the water cost becomes relatively expensive in most economically developed countries and is not affordable for most rural population. Therefore, some people try to get the water source from dams, mining, small streams, rivers and lakes. Water from these sources is usually turbid and contaminated with microorganisms that may cause various diseases. Several findings from previous research in Postnote (2002) demonstrated the use of synthetic materials for water purification can be severely hazardous to health if something goes wrong in their treatment during processing. The report considered the high level of aluminum in the brain is a risk factor causing Alzheimer's disease. Other studies have raised doubt about the feasibility of inserting aluminum into the environment by the use of aluminum sulfate as a coagulant continuously in the water treatment process.

However, Davis (2006) found no conclusive evidence about the correlation between aluminum and Alzheimer's disease (Aarestrup *et al*., 1996).

Besides synthetic chemicals, there are natural ingredients that can be derived from tropical plants which can be used as coagulants, including moringa seeds (*Moringa oleifera*). The use of natural ingredients from local indigenous plants to clear muddy water is not a new idea. From existing reports, there were allegations that the powder of Moringa seeds has antimicrobial properties. Previous research found that Moringa is not toxic (Aarestrup *et al*., 1996) and recommended for use as a coagulant in developing countries. Various studies have been conducted and showed that moringa seeds are effective as biocoagulant to improve physico-chemical properties of contaminated water. *M. oleifera* functions as coagulant trough adsorption and neutralization mechanisms. *Moringa oleifera* is potential as organic pollutant absorber in simulation solution. *M. oleifera* is reported able to eliminate the turbidity and dissolved organic matters of river water. Damayanti *et al.* (2011) made a membrane consisted of *M. oleifera*, PAC and zeolite for palm oil effluent treatment. Nigeria is rich in biodiversity, and moringa tree can grow well, easy to find and easy to cultivate in various regions. Therefore it is not difficult to use Moringa seeds as a natural coagulant or biocoagulant for water clarifying process. The use of natural coagulants in water treatment process is expected to provide more advantages than the use of synthetic materials because they are natural and reported as safe to be consumed. The cost of using natural coagulants was less expensive than that of alum (Aarestrup *et al*., 1996). Effectiveness of natural coagulant for water purification was tested also in the wastewater treatment process (Aarestrup *et al*., 1996). This work is aimed at utilizing Alum and *Moringa oleifera* (MO) in treatment waste water.

2. MATERIALS AND METHODS

2.1 Waste Water Collection

The wastewater samples for this present study were collected from two different beverage industries wastewater Treatment Plants. Each water sample was collected in screw capped sterile glass bottles. The bottles were labeled with the details of source, date and time of collection. The bottles were carried in icebox and brought to the Microbiology Laboratory and stored in refrigerator until analysis. The waste water was collected from 3 different point of the treatment plant. In general 6 different samples were collected; 3 from each beverage company labeled A, B, C, D, E and F.

2.2 Measurement of Turbidity

The wastewater turbidity was determined using the spectrophotometric method for absorbance. Using a cuvate; 2 ml of distilled water was used as blank to zero the ultraviolet-visible spectrophotometer. 2 ml of the wastewater samples each were measured for turbidity before and after introduction of the extracts. All the samples were measures at a wavelength of 500 nm.

2.3 Collection of *Moringa oleifera* (Mo) Extracts

Moringa oleifera (MO) seeds were collected from Uratta Village in Owerri. It were completely dried, shelled then crushed to a fine sieved powder in order to be effective as Ndabigengesere *et al*. (1995) similarly did it. The shells were ground to a fine powder using a mortar. The powder was then weighed and dissolved in distilled water to make a 50 g/l solution. The solution was stirred for 30 minutes using a centrifuge, and finally filtrated through a Whatman filter no. 40. A fresh solution was prepared every day in order to avoid ageing effects (Ukanwa, 2010).

2.4 Collection of Alum Extract and Test Water

Alum was bought from vendors in Ekonuwa market in Owerri. It was completely crushed to a fine sieved powder in order to be effective as Gideon *et al*. (2005) similarly did it. The shells were grind to a fine powder using a mortar. The powder was then weighed and dissolved in distilled water to make a 100 g/l solution. The solution was stirred for 30 minutes and filtrated through a Whatman filter no. 40. A fresh solution was prepared every day in order to avoid ageing effects (Ukanwa, 2010).

2.5 Assessment of Coagulation Potentials of the Alum and *Moringa oleifera*

Method of James (2002) was adopted were 1.0 L of each wastewater samples was put in a sterile beaker, respectively. The extracts each (*Moringa oleifera* and Alum) were introduced into each of the beaker containing the waste water in different concentration and mixed for 30 minutes. The samples were then allowed to settle for 2-3 hours until all of the floc had fully settled. Before and after treatment samples were measured for turbidity and microbial load. The goal of the testing was to identify the dose of *the M. oleifera* and Alum required achieving a turbidity of less than 1.0. The tests were performed at doses of 0 (control, before), 10, 25, 50, 75, and 100 mg/L of Alum and *M. oleifera*.

2.6 Microbial Evaluation of the Wastewater Sample

Microbial investigations were carried out on the wastewater samples to determine effect of the two extracts (*M. oleifera* and Alum) on the microbial load of the samples. The 6 different samples collected from the two different beverage industries in Owerri were subjected to serial dilution according to Uwaezuoke, (2006). After the serial dilution, 0.1 ml of the serially diluted samples each was inoculated into freshly prepared Nutrient agar (for Total Heterotrophic Plate Count), MacConkey agar (for Coloform count) and Manitol salt Agar (for staphylococcus count) respectively using a sterile pipette and spread plate method was adopted. Nutrient Agar, MacConkey agar and Manitol salt Agar, culture plates was incubated at 37°C for 24 hours. The Nutrient Agar, MacConkey and

Manitol salt Agar plate were checked for growth after 24 hours of incubation and were counted. Only plates that showed not less than 30 colonies and not more than 300 colonies were counted and Colony forming unit (CFU) was used as the standard unit. The colonies from the Nutrient agar, MacConkey agar and Manitol salt Agar after counting were purified by sub culturing them in fresh nutrient agar plates. After purification, the isolates were maintained using nutrient agar slant and were kept in the refrigerator at 4^0C for identification.

2.7 Identification of Bacterial Isolates

The isolates were identified using colonial and cellular characteristics, then biochemical properties. Biochemical tests to be carried out shall include; Urease test, Citrate utilization test, Indole test, voges-proskauer test, Motility test, Methyl-red test, Coagulase test, Sugar fermentation test and Catalase test.

2.8 Statistical Analysis

All experimental set ups were carried out in triplicates. Results were presented as mean and standard deviations of triplicate determinations.

3. RESULT

3.1 Bacteria Associated with the Waste Water

In the study, two beverage industries waste water was analyzed for the presence of bacteria isolates before its treatment with Alum and MO. After the analysis it was, five (two gram positive and three gram negative) bacteria genera were associated with the samples collected in the wastewater of the two beverage Companies. The bacteria include; *Pseudomonas* spp. *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* spp. and *Bacillus* spp. The morphology and biochemical characterization is shown in Table 3.1 and Table 3.2, respectively.

Table 1: Morphological characteristic of the bacteria isolates.

GROWTH MORPHOLOGY (ON NUTRIENT)	POSSIBLE ORGANISM
Large opaque purple raised round colonies with smooth edge on Nutrient Agar	***Pseudomonas* spp.**
Small round milky colonies with smooth edges, raised, pinkish on Nutrient agar	***Staphylococcus aureus***
Small round milky colonies with smooth edges, raised, pinkish on MacConkey	***Escherichia coli***
Blackish blue colonies with rough edge on E.M.B	***Klebsiella* spp.**
Small round milky colonies with smooth edges, raised, pinkish on MacConkey	***Bacillus* spp.**

Table 2: The Biochemical characterization of the bacteria.

BIOCHEMICAL IDENTIFICATION TEST															POSSIBLE ORGANISM
Gram Stain	Shape	Motility	Catalyst	Coagulasee	Oxidase	Citrate	Indole	Urease	M-r	V-p	H_2s	Glucose	Sucrose	Lactose	
-	R	+	+	-	-	+	-	+	+	-	+	A	A	A	*Pseudomonas* sp.
+	C	-	+	+	+	+	-	+	+	-	-	A	A	-	*Staphylococcus aureus*
-	R	+	+	-	-	-	+	-	+	-	-	A/G	A/G	A/G	*Escherichia Coli*
-	R	+	+	-	-	+	-	+	-	+	-	A/G	A	A	*Klebsiella* sp.
-	R	+	+	-	-	-	+	-	+	-	-	A/G	A/G	A/G	*Bacillus* sp.

KEY

+ = positive, - = negative, A =acidic, B =basic, G = gas, R = Rod, C = Cocci.

3.2 Total Heterotrophic Bacteria Count of the Isolates

Table 3.3 shows the total heterotrophic bacteria count of the wastewater (A, B, C, D, E, and F) samples, respectively. In the study heterotrophic bacteria counts showed that sample D had the highest heterotrophic count of 1.6×10^8 Cfu/ml before the treatment while sample A had the least heterotrophic bacteria count of 1.3×10^6 Cfu/ml before the treatment. After the treatment of the waste water with Alum and MO, it was observed that there was a reduction in the aerobic count; were sample E had the least viable count of 2.7×10^4 Cfu/ml after treatment with Alum while sample A had the least THBC of 1.3×10^3 Cfu/mL after treatment with MO.

Table 3: Total Heterotrophic Count Bacteria (THBC).

Sample	Before treatment (Cfu/mL)	After treatment with ALUM (Cfu/mL)	After treatment with MO (Cfu/mL)
A	1.3×10^6	3.3×10^4	1.3×10^3
B	1.5×10^7	5.5×10^5	3.5×10^5
C	1.9×10^6	9.2×10^4	2.7×10^5
D	1.6×10^8	4.7×10^6	5.6×10^5
E	3.5×10^6	2.7×10^4	5.4×10^3
F	1.9×10^6	6.5×10^4	3.7×10^4

3.3 TOTAL COLIFORM COUNT

The result for Total coliform count (TCC) of the wastewater samples before the treatment showed that sample C had the highest coliform count of 4.5×10^6 Cfu/ml while sample F had the least coliform count of 1.2×10^3 Cfu/ml. After the treatment with Alum and MO, it was observed that there was also a reduction in the coliform count were sample E had the least coliform count of 3.2×10^2 Cfu/ml after treatment with Alum while sample B had the least coliform count of 1.3×10^2 Cfu/mL after treatment with MO.

Table 4: Total Coliform Count.

SAMPLE	Before treatment (Cfu/mL)	After treatment with ALUM (Cfu/mL)	After treatment with MO (Cfu/mL)
A	3.0×10^6	6.2×10^3	1.0×10^3
B	1.3×10^5	2.4×10^3	1.3×10^2
C	4.5×10^6	7.8×10^3	1.5×10^3
D	3.2×10^3	5.5×10^3	3.3×10^2
E	2.4×10^3	3.2×10^2	2.4×10^2
F	1.2×10^3	5.2×10^2	2.0×10^2

3.4 Total *Staphylococcus* Count

Result for *Staphylococcus* count showed that sample C had the least *Staphylococcus* count of 1.5×10^3 Cfu/ml while sample D had the highest Staphylococcus count of 2.8×10^3 Cfu/ml, respectively before treatment with Alum, and MO. After the treatment of the waste water with Alum and MO, it was observed that there was no *Staphylococcus* count in Sample C and F after treatment with Alum. After the treatment of the waste water samples with *Moringa oleifera* it was observed that there was no *Staphylococcus* count in sample A, C, E and F after the treatment of the waste water with MO. The result is shown in Table 3.5 below.

Table 3.5: Total *Staphylococcus* Count.

Samples	Before treatment (Cfu/mL)	After treatment with ALUM (Cfu/mL)	After treatment with MO (Cfu/mL)
A	2.5×10^3	1.5×10^1	-
B	2.0×10^3	1.6×10^1	2.0×10^1
C	1.5×10^3	-	-
D	2.8×10^3	1.5×10^1	2.8×10^1
E	2.2×10^3	1.9×10^1	-
F	-	-	-

3.5 Effect of Coagulant Addition (Alum and Mo) to the Wastewater Samples

According to the turbidity analysis as indicated in Table 3.5; it was observed that the use of MO and Alum was effective in turbidity reduction from 10 to 75 mg/L but at 100 mg/L there was a slight increase. Although initial turbidity for the waste water sample was 7.84 NTU that is beyond WHO recommended limit of 5 NTU but after treatment turbidity reduced to the recommended limit.

Table 5: Effect of Coagulant Addition (Alum and Mo) to.

Dose (Mg/L)	Turbidity (NTU)	
	ALUM	*Moringa oleifera* (MO)
0 (Control)	7.84	7.84
10	4.04	3.56
25	2.70	3.01
50	1.85	0.87
75	1.46	0.65
100	0.53	0.32

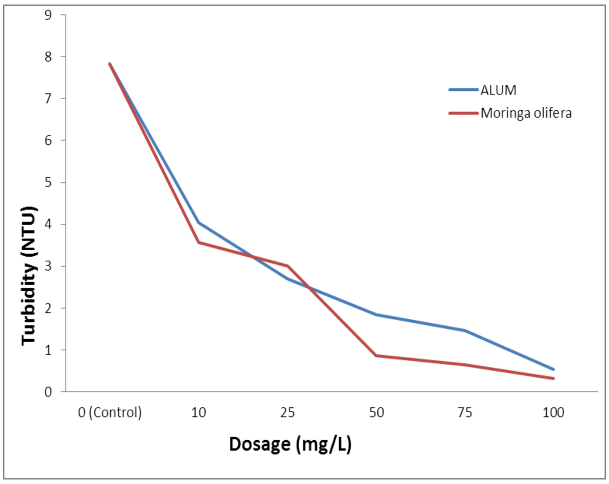

Fig 1: Effect of Coagulant Addition (Alum and MO) to the Wastewater Samples.

4 DISCUSSION

The present study was conducted to obtain an insight into the various bacteria associated with wastewater obtained from two different beverages companies in Owerri, Imo State thereby utilizing *Moringa oleifera* and Alum as natural coagulant for the treatment of waste water samples collected from beverage companies. The predominant organisms in the wastewater were *Pseudomonas sp. Staphylococcus aureus, Escherichia coli, Klebsiella sp.* and *Bacillus sp.* The finding agrees with the report of previous works done by (Norton and Lechuevallier, 2002, Colford *et al.*, 2002). The predominant genera were the *Klebsiella sp, Escherichia coli*, Bacillus sp., *Staphylococcus sp.* and *Pseudomonas aeruginosa*. These are considered ubiquitous and opportunistic pathogens by forming spores and becoming dehydrated. Dehydrated spores can then be packaged and sold as viable cultures. Waste becomes a limited liability in an ecological area, acquiring the spread of ticks, mosquitoes, rodents etc. The populations at greatest potential risk of exposure are infants and toddlers, who may ingest soil through mouthing of hands, toys and other hap ten objects. It has been reported that infants and toddler ingest 10mg to 10g day -1 of soil (Lewis *et al.*, 1994). Other pathogens like the prevalence of *Staphylococcus aureus* and *Pseudomonas sp.* in dried materials during bedding, handling handkerchiefs, sweeping floor account for the entry of microorganisms in human population (Nandalal and Somasheka 2007) and represent a significant microbial disease health risk, epidemics may occur periodically as a result. While the most common bacteria associated with the samples were *Klebsiella*, *Staphylococcus* and *Escherichia coli*, these little variations may be as a result of the difference in the waste water.

In the study heterotrophic bacteria counts showed that sample D had the highest heterotrophic count of 1.6 x 10^8 Cfu/ml before the treatment while sample A had the least heterotrophic bacteria count of 1.3 x 10^6 Cfu/ml before the treatment. After the treatment of the waste water with Alum and MO, it was observed that there was a reduction in the aerobic count were sample E had the least viable count of 2.7 x 10^4 Cfu/ml after treatment with Alum while sample A had the least THBC of 1.3 x 10^3 Cfu/mL after treatment with MO (Table 3.3). The result for Total coliform count (TCC) of the wastewater samples before the treatment with Alum and *Moringa oleifera* showed that sample C had the highest coliform count of 4.5 × 10^6 Cfu/ml while sample sample F had the least coliform count of 1.2 x 10^3 Cfu/ml. After the treatment with Alum and MO, it was observed that there was also a reduction in the coliform count were sample E had the least coliform count of 3.2 × 10^2 Cfu/ml after treatment with Alum while sample B had the least coliform count of 1.3 x 10^2 Cfu/mL after treatment with MO (Table 3.4). Result for Staphylococcus count showed that sample C had the least Staphylococcus count of 1.5×10^3 Cfu/ml while sample D had the highest Staphylococcus count of 2.8×10^3 Cfu/ml, respectively before treatment with Alum, and MO. After the treatment of the waste water with Alum and MO, it was observed that there was no *Staphylococcus* count in Sample C and F after treatment with Alum. After the treatment of the waste water samples with *Moringa oleifera* it was observed that there was no *Staphylococcus* count in sample A, C, E and F after the treatment of the waste water with MO (Table 3.5). Processing the waste water by coagulation using *M. oleifera* and Alum as natural coagulant showed that the treatment with *M. oleifera* provided additional advantage of reduced total microbes.

In the comparison analysis on the use of Alum and *Moringa oleifera* (MO) as natural coagulant; it was observed that a *Moringa oleifera* (MO) had greater reduction potency in the microbial load and turbidity of the waste water samples. The turbidity analysis showed that the use of MO and Alum was effective in turbidity reduction from 10 to 75 mg/L but at 100 mg/L there was a slight increase. Although initial turbidity for the waste water sample was 7.84 NTU that is beyond WHO recommended limit of 5 NTU but after treatment turbidity reduced to recommended limit (WHO, 2006). *M. oleifera* seeds also have bactericidal activity, which have been proved by Oluduro and Aderiye (2007). In their research; bacterial species *S. faecalis* and *P. aerugenosa* which were cultured in water, stop growing back after *M. oleifera* seeds were added. When the seeds of *M. oleifera* are crushed and dissolved into the water, protein produces a positive charge that acts like a magnet and attracts dominant negatively charged particles such as clay, silk, and other toxic particles. This is in accordance with the invention of Schwarz (2000) that the flocculation process removes about 90-99% of bacteria that are usually attached to solid particles, so the bacteria will be aggregated together to form flocs and can be removed from the water (Schwarz, 2000). This suggests that bacteria in water were not only inactivated in a dormant state, but also were killed. *M. oleifera* removes both gram negative and gram positive bacteria (Madsen *et al*., 2005; Lurling *et al*., 2009).

Several scientific studies suggested that *Moringa* seeds could serve as coagulant because it contains low molecular weight water soluble protein (Fayos *et al*., 2010; Kebreab *et al*., 2005). Protein will be positively charged when dissolved in water (Sahni *et al*., 2008). Protein will act as positively charged synthetic materials (Broin *et al*., 2002) and can be used as synthetic polymer coagulant. Therefore, *Moringa* can be called as a coagulant. Since this coagulant is derived from plants and without any synthetic process, it is also called natural coagulant or biocoagulant. The most likely mechanism that occurs in the coagulation process is the adsorption and neutralization of the voltage or adsorption and bonding between unstable particles. It is difficult to determine which mechanism that occurs because both mechanisms may occur simultaneously. But, the most common mechanisms of coagulation that involves *Moringa* seeds are the adsorption and voltage neutralization (Schwarz, 2000). When *Moringa* seeds that have been processed (powder) were poured into the dirty water, the protein in the seeds will bind the negatively charged particulates that cause turbidity, such as clay, bacteria, dust, and others. Thus, the particulates are collected and agglomerated into larger molecules, which will settle to the bottom, and then it will be easy to separate the water and contaminant.

4.1 CONCLUSION AND RECOMMENDATIONS

Alum sulphate and *Moringa oleifera* was used to treat waste water collected from two different beverage companies in Imo State. The result reviewed that the local materials showed coagulative effect against the microbial and turbidity population of the waste water but the result reviewed a high effect on the use of *Moringa oleifera* and it was proven to be able to be used for wastewater treatment *M. oleifera* can be used in the coagulation process because it has properties as a natural coagulant. The result suggested that *M. oleifera* is effective at the concentration of 80 to 100 mg/L as a coagulant to treat wastewater from the beverage industry. *M. oleifera* optimum coagulant dose is also influenced by the initial state of the sample to be coagulated. The heavier the burden of pollution, the higher the optimum dose that is needed.

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