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Synergistic effect of *moringa oleifera* seed and potassium aluminium sulphate (alum) in the remediation of effluent from Nigerian brewery, Kaduna

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Abstract
The purpose of the study was to evaluate the synergistic effects of *Moringa oleifera* seed and Potassium aluminium sulphate in the remediation of effluent from Nigerian Brewery, Kaduna. When compared to the control group, the pH values for the coagulants significantly decreased in the treatment groups. According to the physicochemical analyses, there was no appreciable variation in temperature between the treatment groups and the control group ($P > 0.05$). In comparison to the control group, the treatment groups' electrical conductivity and total dissolved solids both significantly increased due to the coagulants. The values of all parameters in the treatment groups were significantly lower than those in the control group ($P \leq 0.05$), and the effectiveness of the reduction was dose dependent, meaning that greater dosages were more effective. In other words, performance improves when doses are increased. Results from bacteriological studies after treatments with graded dosages of the combined coagulants of 1g, 3g, 5g, and 7g indicated 6 x 10^3 cfu/ml, 4 x 10^3 cfu/ml, 0 cfu/ml, and 0 cfu/ml, respectively, and <2MPN, <2MPN, <2MPN, and 2MPN, respectively, from enumeration of total/feacal coliform count. The average bacterial count revealed a difference between the coagulant doses in terms of bacterial recovery that was statistically significant ($P \leq 0.05$). *Proteus mirabilis* was the microbe that was discovered in the wastewater sample. According to the aforementioned analysis, the combined coagulants demonstrated outstanding physicochemical characteristics and bacteriological quality, which are in compliance with NIS and WHO criteria and are therefore advised for water remediation.

Keyword: *Moringa oleifera* seed, potassium aluminium sulphate, synergistic effect, brewery effluent

Introduction

Conventional methods of water treatment are used throughout the world to guarantee good water quality and safe drinking water. In addition to countering their impacts, this lessens the prevalence of waterborne infections. Processes used in the treatment include coagulation, flocculation, sedimentation, filtering, and disinfection. According to Ida *et al*. (2013) [10], the high price and lack of chemical coagulants and disinfectants make it difficult for underdeveloped nations to implement traditional water treatment technology. It is challenging to have a water treatment system that depends on electricity in sub-Saharan African communities because most don't have access to it (Yongabi *et al*., 2011) [22]. A great deal of safety concerns are also raised by the use of chemicals in the treatment of water. The commonly used water coagulant, aluminum sulphate (alum), produces acidic water that is dangerous for expectant mothers and causes pre-dementia in some persons, among other problems (Yongabi *et al*., 2012) [23].

Traditional natural coagulants of plant origin are a straightforward, trustworthy, and economical technique of water purification that have been used for years in impoverished nations (Ida, 2013) [10]. There is proof that using extracts from plant species with both coagulating and antibacterial characteristics is safe for human health (Dalen *et al*., 2009) [6]. The seeds of the drumstick plant, *Moringa oleifera*, have been discovered to be among the most effective plants for purifying water out of all those that have been employed throughout history. It is also known as the drumstick plant (because to the shape of the seedpods), the horseradish tree (due to the flavor of a condiment made from the roots), the mother's best friend, the miracle tree, and the ben oil tree. According to Olayemi *et al*. (1994) [16], it is known locally in Nigeria as Zogale-gandi or zogale (Hausa), Eweile (Yoruba), and Okwe oyibo (Ibo).

According to Shittu *et al*. (2004) [19], *Moringa oleifera* is a "multipurpose tree" with nutritional, therapeutic, and water purification properties. Almost all plant parts, including the leaves, flowers, seeds, roots, barn, and young pods, are edible and can be utilized for therapeutic and medicinal purposes (Alo *et al*., 2012; Singh *et al*., 2012) [1, 20]. The leaves of *Moringa oleifera* are used as vegetables in soups, the roots are used as spices (condiments) and culinary garnishes, and the oil collected from the seeds is used in cooking (Onuoha *et al*., 2003) [17]. According to studies (Muyibi *et al*., 1994; Bichi *et al*., 2012; Mangale *et al*., 2012) [14, 4, 13], the coagulating and antibacterial properties of *Moringa oleifera* makes it a potent water purification agent.

According to science, Samia Alazharia Jahn of Germany was the first to prove that *Moringa oleifera* seeds have coagulation characteristics (Schwartz, 2000) [18]. Though the precise type of the protein thought to be the active ingredient is still unknown. There are proteins with coagulating properties that range in size from 3 to 60 kDa, according to research. The proteins function as cationic polyelectrolytes that bind to the soluble particles and form connections between them, forming substantial flocs in the water. The electrostatic flocculation is accelerated by stirring and mixing, and pollutants are condensed in the flocs (Gottsch, 1992) [9].

Materials and procedures

Sample location

The water sample was taken from the Nigerian Brewery Kakuri in Kaduna. It was gathered from a location close to the outflow of the discharging premises where the effluent had been extensively mixed. The Brewery is situated between latitudes 10°2732.13 and 10°2733.39 and longitudes 7°2446.39 and 7°2443.83 (KEPA, 1998) [12].

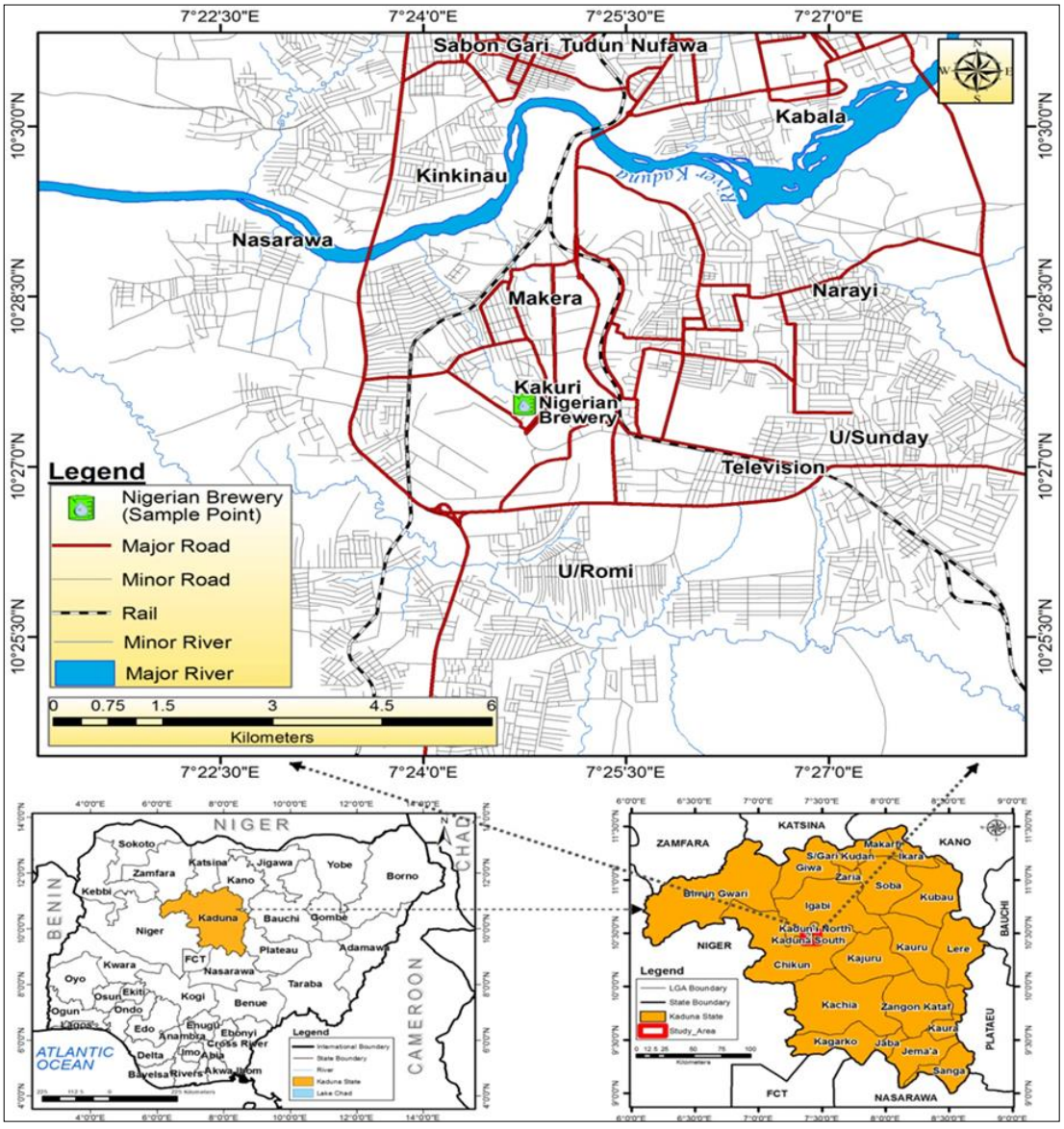

Fig 1: Map of Kaduna showing Sampling Point

Plant materials collection and identification

Moringa oleifera dried seeds weighing 50 grams were taken from a single tree in the Ahmadu Bello University's botanical garden in Zaria. Identification was established in the Herbarium Unit of the Faculty of Life Sciences, Ahmadu Bello University, Zaria, using treatise and the local flora (Dutta, 1979) [7], as well as by comparing the specimens to herbarium sheets of the original species.

Making seed powder from *Moringa oleifera*

To extract the kernels, the gathered seeds were manually shelled. For a further five days, the seed kernels were dried at room temperature (between 23ºC and 25ºC). To avoid photodegradation of some of the plant's phytochemical components, direct sunlight was avoided. A laboratory mortar and pestle was used to crush the dry kernels into powder. After that, a plastic strainer with a 2.5 mm^2 size was used to sift the powder to create a fine powder. To avoid photo-oxidation and prepare it for additional tests, the resulting fine powder was kept in a clean, airtight container in a dark location. (Alo, *et al*., 2012) [1].

Effluent sample collection

Following the standard procedure outlined by APHA (2005) [2], effluent samples were taken aseptically. The effluent sample was obtained by inserting a sterile container with a nominal capacity of 25 liters into the water body at a depth of about 30 cm, allowing it to overflow, then capping the container and removing it from the water. Next, the container was taken to a laboratory for additional analysis.

Experimental design

On a work station, six (6) 1000ml sterile plastic containers with labels were set out. An effluent sample was placed in a plastic container as a negative control, and distilled water was placed in a different container as a positive control. Graded doses of the mixed coagulants were applied to effluent samples in four (4) plastic containers.

The following steps were taken:
The positive and negative controls' physicochemical characteristics and bacteriological quality were identified.

- Powdered *Moringa oleifera* seed and potassium aluminium sulphate were introduced in amounts of 1g, 3g, 5g, and 7g to four plastic containers that each held 1000 ml of effluent samples at a 1:1 ratio.
- The four plastic containers containing effluent samples filled with various concentrations of the coagulants, were then agitated for 60 seconds with a glass rod before being slowly and gently mixed for five minutes.
- Larger particles (flocs) were able to "condense the contaminants" as a result of the gradual, gentle mixing, which also sped up electrostatic flocculation (Bergman and Arnoldsson, 2008) [3].
- For six hours, the contents were allowed to settle. The supernatants were properly decanted after sedimentation, and the physicochemical parameters and bacteriological status of the treated samples and the negative control were then measured and compared to the values of the Nigerian Industrial Standard (APHA, 2005) [2].

Measuring the physicochemical characteristics of the effluent sample
The raw water's physicochemical characteristics were assessed both before and after treatments. A Combo Hanna multiparametric meter was used to detect pH, temperature, Total suspended solids (TSS), and electrical conductivity (EC) in situ. The procedures outlined in full by APHA (2005) [2] were used to determine other physical and chemical parameters.

Bacterial analysis of the effluent sample
Before and after treatment with the graded doses of the combined coagulants, the bacteriological investigation of the effluent sample was carried out. The total number of bacteria were counted in order to complete the test.

Preparation of media
The nutrient agar (NA) media, which was used for the bacteriological examination, was made by combining 28g of the NA powder with 1 liter of distilled water, autoclaving it, and then letting it cool. Using Petri dishes with labels ranging from 1 to 25, the NA media was added and incubated for 24 hours in an electro-thermal incubator. The growth of the microorganism was then observed and counted per ml (number of microorganisms per ml of water samples).

Total number of bacteria
A 10 fold serial dilution of water sample was carried out. 9ml of sterile (the diluents) were placed into 5 different test-tubes each arranged in a rack. The water sample was shaken to mix and 1ml was taken using sterile 2ml syringe and then added into the first test tube in the rack and shaken properly to mix. 1ml of the water was taken from the first test tube and mixed. This process was repeated for 5-test tubes. 0.1ml aliquot of the 1 and 3rd dilution were plated in an already solidified nutrient agar. The effluent sample was spread evenly on the surface of the agar using a sterile hockey stick. The inoculated media were allowed to solidify and then incubated at 37ºC for 24 hours. After the incubation period, the number of colony growths on the agar were counted and recorded as colony forming unit per ml

$$(\text{cfu/ml}) = \frac{\text{No of colonies}}{\text{Volume plated}} \times \text{Dilution factor}$$

Examination of total and faecal coliform
Presumptive test: According to APHA's 2005 description, multiple tube fermentation tests were used to count the total and feacal coliforms. The Most Probable Number (MPN) three tube assay was used to calculate a coliform count. MacConkey broth (Oxoid) was used for the presumptive test. Sterilized 10 ml double-strength broth was administered to the first set of the five tubes, while 5 ml single-strength broth was administered to the second and third sets. Before sterilizing, Durham tubes were inserted into each tube with their ends inverted. Using sterile pipettes, the three sets of tubes were given effluent sample amounts of 10 ml, 1 ml, and 0.1 ml. Tubes were carefully labeled and incubated at 37ºC for 24 hours for estimation of total coliforms and at 44.5ºC for 24 hours for estimation of feacal coliforms and examined for acid and gas production. Acid production was determined by the color change in the broth from reddish purple to yellow, while gas production was checked for by the entrapment of gas in the Durham inverted tubes. The MPN for the three sets of tubes was determined from the MPN table.

Confirmed test: A confirmed test was carried out on all primary fermentation tubes that showed gas formation after 24 and 48 hours of incubation. The tubes were carefully labeled, incubated for 24 hours at 37ºC for the estimation of total coliforms and at 44.5ºC for the measurement of fecal coliforms. Acid and gas production was also observed. Acid production was identified by the reddish-purple broth turning yellow, and gas production was detected by the presence of trapped gas in the Durham inverted tubes. From the MPN table, the MPN for each of the three sets of tubes was calculated.

Confirmed test: After 24 and 48 hours of incubation, all primary fermentation tubes that had gas production were subjected to a confirmed test. It was done by transferring a loopful of culture from a tube that tested positive for the presumptive test into a tube that contained Durham tubes and Brilliant Green Lactose Bile (BGLB) broth (oxoid). For the purpose of observing gas production, the tubes were incubated for 24 hours at 37ºC for total coliforms and for 44.5ºC for fecal coliforms.

Completed test: A loopful of broth from a tube that tested positively in the confirmed test was streaked onto Eosine Methylene Blue (EMB) agar plates to check for pure colonies. The plates were incubated for 24 hours at 37°C. The Gram staining method was used to further identify the colonies that emerged on EMB agar.

Identification of bacteria
Gram staining
Gram staining was carried out to differentiate the bacterial species into two large group base based on their cell wall constituents. The Gram stain procedure distinguishes between Gram positive and Gram negative groups by the colour of their cells i.e. red or violet (Gram reaction). A

pure colony of bacteria was removed using a sterile wire loop and smeared on a clean slide then heat fixed. The smear was then flooded with gram crystal violet primary stain to stain for 1 minute, after which it was washed off with cold water. The slide was then flooded with grams iodine mordant and left to seat for 1 minute, and then washed off with safranin counter stain solution. More counter stain solution was added to the slide to stain for 50 seconds after which it was washed off with cold water and the slide blot dried. Using a light microscope, the slide was mounted and observed under oil immersion (APHA, 2005) [2].

Biochemical test

All colonies identified after Gram staining above were further screened biochemically using procedures described in details by (APHA, 2005) [2].

Table 1: Physico-chemical parameters of untreated and combined coagulants treated effluent

Parameters	Before Treatment	After Treatment (Ratio Of Coagulants = 1 : 1)				NIS/WHO
	CONTROL	1g (Mean ± S.E)	3g (Mean ± S.E)	5g (Mean ± S.E)	7g (Mean ± S.E)	
PH	9.30 ± 0.23^{c}	8.00 ± 0.46^{b}	6.67 ± 0.20^{a}	5.66 ± 0.21^{a}	5.44 ± 0.08^{a}	6.5 – 8.5
TEMP	25.40 ± 0.80	24.95 ± 0.37	24.50 ± 0.29	25.30 ± 0.69	25.36 ± 0.37	40°C
TDS	581.20 ± 0.10^{b}	655.60 ± 0.05^{a}	664.28 ± 0.04^{c}	727.80 ± 0.06^{d}	763.93 ± 0.03^{e}	1500mg/l
EC	1056.50 ± 0.28^{b}	1153.33 ± 0.33^{a}	1220.83 ± 3.17^{c}	1323.67 ± 0.33^{d}	1394.90 ± 0.10^{e}	1000µS/cm
Phosphate	2.50 ± 0.00^{c}	1.55 ± 0.00^{b}	0.53 ± 0.02^{a}	0.50 ± 0.02^{a}	0.52 ± 0.00^{a}	0.3mg/l
Sulphate	385.00 ± 0.00^{e}	300.03 ± 0.03^{d}	260.01 ± 0.01^{c}	210.02 ± 0.02^{b}	100.02 ± 0.01^{a}	100mg/l
Nitrate	27.50 ± 4.33^{c}	25.02 ± 0.01^{a}	25.01 ± 0.01^{a}	30.01 ± 0.01^{b}	30.03 ± 0.03^{b}	50mg/l
Chloride	260.03 ± 0.03^{e}	175.04 ± 0.04^{d}	150.02 ± 0.02^{c}	148.01 ± 0.01^{b}	140.02 ± 0.01^{a}	250mg/l
Calcium	80.17 ± 0.01^{d}	28.04 ± 0.01^{c}	24.05 ± 0.00^{b}	23.25 ± 0.00^{a}	23.24 ± 0.00^{a}	75mg/l
Hardness	280.05 ± 0.03^{a}	520.07 ± 0.07^{b}	648.01 ± 0.02^{c}	760.04 ± 0.27^{d}	1000.06 ± 0.03^{e}	150mg/l
Alkalinity	188.01 ± 0.01^{e}	143.01 ± 0.01^{d}	120.03 ± 0.017^{c}	105.01 ± 0.01^{b}	80.02 ± 0.01^{a}	120mg/l
Turbidity	125.01 ± 0.01^{e}	77.02 ± 0.01^{d}	75.02 ± 0.03^{c}	71.01 ± 0.01^{b}	68.01 ± 0.01^{a}	5NTU
TSS	3.04 ± 0.03^{d}	2.00 ± 0.01^{c}	1.92 ± 0.01^{b}	1.00 ± 0.00^{a}	1.01 ± 0.01^{a}	
DO	4.02 ± 0.02^{b}	2.95 ± 0.54^{ab}	2.75 ± 0.02^{a}	2.55 ± 0.03^{a}	2.50 ± 0.05^{a}	
BOD	2.52 ± 0.01^{b}	1.45 ± 0.26^{a}	1.37 ± 0.01^{a}	1.27 ± 0.01^{a}	1.25 ± 0.02^{a}	300mg/l
COD	8.52 ± 0.01^{d}	5.18 ± 0.03^{c}	4.52 ± 0.02^{b}	4.02 ± 0.02^{a}	4.02 ± 0.01^{a}	4mg/l

Values along the same row with different superscripts a, b, c, d, and e are significantly different ($p \leq 0.05$).

Table 2: Bacteriological characteristics of untreated and treated effluent with graded doses of the coagulants

	Control	1g	3g	5g	7g	NIS/WHO
TBC of MOS + PAS	26000.100 ± 0.10^{d}	6000.06 ± 0.7^{c}	4000.17 ± 0.17^{b}	0.00 ± 00^{a}	0.00 ± 0.00^{a}	10
MPN	>1800.00	<2	<2	<2	<2	0

Values along the same row with different superscripts a, b, c, d, and e are significantly different ($p \leq 0.05$).

Keys

TBC = Total Bacterial Count,
MOS = *Moringa oleifera* seed,
PAS = Potassium aluminium sulphate,
MPN = Most Probable Number

Results and discussion

Physico-chemical characteristics of combined coagulant-treated effluent and untreated effluent

The pH values in the treatment groups were significantly lower than those in the control group, as shown in Table 1. The pH level of the effluent sample decreased with different doses of the mixed coagulant. Treatments with 1g/l and 3g/l resulted in pH reductions within the NIS/WHO allowed range of 6.5 to 8.5, however 5g/l and 7g/l treatments resulted in pH reductions below the approved level. This might be because throughout the treatment procedure, a number of hydrolytic processes result in hydrogen ions. Practically speaking, this means that the ideal combination of *Moringa oleifera* and alum for water purification should not require additional chemical addition for pH adjustment.

In terms of temperature, there was no discernible difference between the treatment groups and the control group ($P > 0.05$). Due to the combined coagulants' high levels of organic and inorganic salts, there was a considerable rise in TDS and EC in the treatment groups compared to the control group. This supports Nweke's (2009) [15] conclusion that the TDS and EC of a coagulant increase with the amount of organic and inorganic salt present. The values of every parameter in the complete treatment groups decreased significantly ($P \leq 0.05$) when compared to the control group, and the efficiency of the reduction was dose-dependent, meaning that the performance improved with greater dosages.

Hardness and nitrate, which both saw a substantial increase ($P \leq 0.05$) in the treatment groups compared to the control group, were the only two variables that did not. The calcium and magnesium ions present in the water sample could have disintegrated, which would explain why the hardness has increased. The combined coagulants undoubtedly improved the effluent sample's BOD readings, bringing them down from 2.54 mg/l to 1.25 mg/l, proving that the primary coagulant did a great job of capturing particles or structural organic matter.

Bacteriological status of the effluent sample before and after treatments with graded doses of the coagulants

As the doses of the coagulants were increased, there was a progressive reduction in the total bacterial count and total coliform count in the bacteriological examinations as shown in table 1 when comparing the effects of different coagulant dosages on bacterial recovery, the average bacterial count revealed a significant difference ($P \leq 0.05$). According to Eilert *et al*. (1981) [8], an antibacterial compound called 4(-L-rhhamnosloxy) benzyl isothiocynate may be the cause of the antibacterial action of *Moringa oleifera* coagulants.

Another explanation for this phenomenon is that the seed contains a recombinant cationic protein that may flocculate both Gram-positive and Gram-negative bacterial cells and promote the aggregation of negatively charged particles in suspension, including bacterial cells, clay, and silicate microspheres.

Conclusions

With an increase in dose, it was discovered that the combined coagulant was effective on the physicochemical parameters of the effluent sample since, following treatment, the majority of the parameters were within NIS/WHO permitted limits. Following treatment with graduated doses of the mixed coagulant, the bacterial burden in the effluent sample was significantly reduced.

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