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Comparative investigation of the efficacy of *Moringa oleifera* seed and potassium Aluminium Sulphate (ALUM) in remediation of effluent from Nigerian brewery, Kaduna

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Abstract
The study was carried out to compare the efficacy of *Moringa olefeira* seed and Potassium aluminium sulphate in remediation of effluent from the Nigerian Brewery, Kaduna. Treatment with both coagulants showed significant reductions in pH values in the treatment groups compared with the control group. From the physicochemical analyses, there was no significant difference at ($P > 0.05$) in the treatment groups compared with the control group. There was a significant decrease at ($P < 0.05$) in the values of all parameters in the entire treatment groups compared with the control group, and the effectiveness of the reduction is dose specific i.e. the higher the doses, the more effective the performance. Results from bacteriological analyses after treatment with 1g, 3g, 5g and 7g of *Moringa oleifera* seed revealed 1.8 x 104cfu/ml, 1.5 x104cfu/ml, 1 x 103cfu/ml and 0cfu/ml respectively from total bacterial count and 2MPN, <2MPN, <2MPN and <2MPN respectively from enumeration of total coliform. Treatment with Potassium aluminium sulphate revealed 2.6 x 104cfu/ml, 2 x 104cfu/ml, 1.8 x 104cfu/ml and 1.5 x 104cfu/ml respectively, from the total bacterial count and 1600MPN, 62MPN, 40MPN and 29MPN respectively, from the enumeration of total and feacal coliforms. The average bacterial count showed a significant difference at ($P < 0.05$) that existed between the coagulants doses as they related to bacterial recovery. The microorganism isolated from the effluent sample was Proteus mirabilis. From the above results, *Moringa oleifera* seed was found to be more effective in treating the effluent sample than Potassium aluminium sulphate.

Keywords: *Moringa oleifera* seed, Potassium aluminium sulphate, *Proteus mirabilis*, Brewery effluent

Introduction

In various parts of the world, water is treated conventionally to ensure good water quality and safe drinking water. This reduces the spread of waterborne diseases and combats their effects. Treatment processes include coagulation, flocculation, sedimentation, filtration and disinfection. The difficulty in adopting conventional water treatment technologies in developing countries is due to the high cost and scarcity of chemical coagulants and disinfectants (Ida *et al*., 2013) [12] as well as the lack of electricity in most sub-Saharan communities. Water treatment technology is difficult to develop in Africa's electricity-dependent water (Yongabi *et al*., 2011) [29]. In addition, the use of water treatment chemicals raises many safety concerns. Aluminum sulphate (alum), a commonly used water coagulant, produces acidic water, among other things, which is dangerous for pregnant women and causes predementia in some people (Yongabi *et al*., 2012) [28].

Coagulant of natural origin of plant origin is a simple, reliable and economical method of water purification that has been practiced in developing countries for years (Ida, 2013) [12]. The use of plant extracts with coagulant and antimicrobial properties has been shown to be safe for human health (Dalen et al., 2009) [7].

Of all the plants that have been used over the years, *Moringa oleifera* seeds (sticks) have proven to be one of the most effective for water purification. Its common names include Drumstick (derived from the shape of the pods), Horseradish (derived from the taste of the spice prepared from the roots), mother's best friend, miracle tree, and olive tree. In Nigeria it is locally called Zogale-gandi or zogale (Hausa), Eweile (Yoruba) and Okwe oyibo (Ibo) (Olayemi *et al.*, 1994) [21]. *Moringa oleifera* is a "multipurpose tree" possessing nutritive, medicinal, water purification abilities (Shittu *et al*., 2004) [25]. Almost every part of the plant (leaves, flowers, seeds, roots, barn and immature pods) can be used as food with medicinal and therapeutic purposes (Alo *et al*., 2012; Singh *et al*., 2012) [1], [26]. *Moringa oleifera* is edible; the leaves of *Moringa oleifera* are used as vegetables in soup preparation, the roots are used as spice (condiment) and garnish for food and oil extracted from the seeds are used for cooking (Onuoha *et al*., 2003)[22]. *Moringa oleifera* have been found to possess coagulating and antimicrobial activities, making it an effective water purification agent (Muyibi *et al*., 1994; Bichi *et al*., 2012; Mangale *et al*., 2012) [19], [5], [16]. Scientifically, the coagulation properties of *Moringa oleifera* seeds were first confirmed by the German scientist Samia Alazharia Jahn (Schwartz, 2000) [24]. The active agent is believed to be a protein but the exact form of the protein is not yet known. Research has identified proteins of sizes ranging from 3 to 60 kDa, all possessing coagulating ability. The proteins act as cationic polyelectrolyte, which attaches to the soluble particles and creates bindings between them, leading to large flocs in the water. Stirring and mixing accelerates the electrostatic flocculation, and the flocs condense the contaminants (Gottsch, 1992) [10].

Materials and Methods

Sampling Point

The water sample was collected from the effluent of Nigerian Brewery Kakuri, Kaduna. It was collected from a point where the effluent was thoroughly mixed and close to the discharging premises outlet. The Brewery is located between latitude 10°27'32.13"N - 10°27'33.39"N and longitude 7°24'46.39"E - 7°24'43.83"E. (KEPA, 1998) [14].

Collection and Identification of Plant Materials

Fifty gram dried seeds of *Moringa oleifera* were collected from a single tree at the main Botanical garden of Ahmadu Bello University, Zaria. Identification was established at the Herbarium unit of the Faculty of Life Sciences, Ahmadu Bello University Zaria, with the aid of treatise and regional flora (Dutta, 1979) [8] and by comparison with herbarium sheets of the authentic species.

Preparation of *Moringa oleifera* Seed Powder

The collected seeds were de-shelled by hand to remove the kernels. The seed kernels were further dried at ambient temperature (23ºC - 25ºC) for a period of five days. Direct sunlight was avoided to prevent photo-degradation of some of the plant's phytochemical constituents. The dried kernels were pulverized using laboratory mortar and pestle to obtain its powdered form. The powder was then sieved with a plastic strainer of 2.5mm^2 size to obtain fine powder. The fine powder obtained was stored in a sterile air-tight container in a dark place to prevent photo-oxidation and for further analyses (Alo, *et al*., 2012) [1].

Collection of effluent sample

Effluent sample was collected aseptically according to recommended standard method described by (APHA, 2005) [3]. The effluent sample was collected by lowering a sterile container of 25litres nominal capacity into the water body about 30cm deep, and allowed to overflow before closing the cap and withdrawing from the water. The container was then transported to the laboratory for further analyses.

Experimental Design

Ten (10) 1000ml sterile plastic containers were labeled and arranged on a working desk. A plastic container containing effluent sample was used as negative control and another container with distilled water was used as positive control. Eight (8) plastic containers with effluent sample were used for treatments with graded doses of *Moringa oleifera* seed and Potassium aluminium sulphate.

- The physicochemical parameters and bacteriological quality of the positive and negative control were determined.
- 1g, 3g, 5g and 7g of the powdered *Moringa oleifera* seed were added to the 4 plastic containers containing 1000ml effluent sample each.
- The same graded doses of powdered Potassium aluminium sulphate were added to another 4 plastic containers containing 1000ml effluent sample each.
- After adding the varying amounts of coagulants in grams to the eight (8) plastic containers with effluent samples, the containers were stirred for 60 seconds using a glass rod which was followed by slow and gentle mixing for 5 minutes.
- The slow gentle mixing accelerated the electrostatic flocculation and allowed the larger particles (flocs) to "condense the contaminants" (Bergman and Arnoldsson, 2008) [4].
- The contents were allowed to settle for 6hr. After sedimentation, the supernatants were carefully decanted and then physicochemical parameters and bacteriological status of the treated samples and negative control were determined and compared with values of Nigerian Industrial Standard (APHA, 2005) [3].

Determination of Physicochemical Parameters of the effluent sample

Physicochemical parameters of the raw water were determined before and after treatments. pH, temperature, Total suspended solids (TSS), Electrical conductivity (EC), were measured in situ using Combo Hanna multiparametric meter. While other physical and chemical parameters where determined using the methods described in details by APHA (2005) [3].

Bacteriological Analysis of Effluent Sample

The bacteriological analysis of the effluent sample was determined before and after treatment with *Moringa oleifera* seed and Potassium aluminium sulphate (Alum). It was carried out by determining the total bacterial count.

Total Bacterial Count

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 feacal coliform

Presumptive test: Total coliform and faecal coliform were enumerated by multiple tube fermentation tests as described by APHA (2005) [3]. Coliform count was obtained using the three tube assay of the Most Probable Number (MPN) technique. Presumptive coliform test was carried out using MacConkey broth (Oxoid). The first set of the five tubes received sterile 10ml double strength broth while the second and third sets received 5ml single strength broth. Durham tubes were placed in inverted position in all the tubes before sterilization. The three sets of tubes received 10ml, 1ml and 0.1ml effluent samples using sterile pipette. The tubes were carefully labeled and incubated at 37ºC for 24hours for estimation of total coliforms and at 44.5ºC for 24hours for estimation of faecal coliforms and examine for acid and gas production. Acid production was determined by colour change in the broth from reddish purple to yellow while gas production was checked for by entrapment of gas in the Durham inverted tubes. The MPN for the three set of tubes were determined from the MPN table.

Confirmed test: Confirmed test was carried out on all primary fermentation tube that showed gas formation after 24hr and 48hr incubation. It was carried out by transferring a loopful of culture from a positive tube from presumptive test into a tube containing Brilliant Green Lactose Bile (BGLB) broth (oxoid) with Durham tubes. The tubes were incubated at 37ºC for 24hours for total coliform and 44.5ºC for faecal coliforms and observed for gas production.

Completed test: Completed test was carried out by streaking a loopful of broth from a positive tube of the confirmed test onto Eosine Methylene Blue (EMB) agar plate for pure colonies. The plates were incubated at 37ºC for 24hours. Colonies that developed on EMB agar were further identified by Gram staining.

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 (AMSCUE, 2005) [2].

Biochemical Tests

All colonies identified after Gram staining above were further screened biochemically using procedures described in detailed by (APHA, 2005) [3].

Table 1: Physico-chemical parameters of untreated and *Moringa oleifera* seed treated effluent

Parameters	Before Treatment	After Treatment				NIS/WHO
	Control	1g (Mean ± S.E)	3g (Mean ± S.E)	5g (Mean ± S.E)	7g (Mean ± S.E)	
PH	9.30 ± 0.23^{b}	6.80 ± 0.00^{a}	6.55 ± 0.14^{a}	6.5 ± 0.17^{a}	6.45 ± 0.144^{a}	6.5 - 8.5
TEMP	25.40 ± 0.80	25.25 ± 0.43	25.30 ± 0.46	25.30 ± 0.34	24.80 ± 0.75	40°C
TDS	581.28 ±0.04^{a}	632.28 ±0.12^{b}	665.28 ± 0.12^{c}	727.64 ± 0.01^{d}	765.37 ±0.32^{e}	1500mg/l
EC	1055.67 ± 0.88^{a}	1150.09± 0.05^{b}	1210.27 ± 0.14^{c}	1323.27 ± 0.14^{d}	1391.17 ± 0.16^{e}	1000µS/cm
PHOSPHATE	2.50 ± 0.00^{c}	2.45 ± 0.29^{ab}	2.40 ±0.00^{b}	2.40 ± 0.00^{b}	2.00 ± 0.00^{a}	0.3mg/l
SULPHATE	385.00 ± 8.66^{d}	290.00 ± 0.00^{c}	28O.00 ± 0.00^{c}	200.00 ± 0.00^{b}	100.00 ± 0.00^{a}	100mg/l
NITRATE	27.50 ± 4.33^{a}	30.00 ± 0.00^{a}	35.00 ± 0.00^{ab}	40.00 ± 0.00^{b}	40.00 ± 0.00^{b}	50mg/l
CHLORIDE	260.08 ± 0.03^{e}	130.17 ± 0.04^{d}	125.01 ± 0.00^{c}	80.06 ± 0.03^{b}	65.05 ± 0.03^{a}	250mg/l
CALCIUM	80.16 ± 0.00^{e}	62.52 ± 0.00^{d}	52.10 ± 0.00^{c}	44.09 ± 0.00^{b}	37.67 ± 0.00^{a}	75mg/l
HARDNESS	280.13 ± 0.05^{a}	400.09 ± 0.02^{b}	480.09 ± 0.01^{c}	560.12 ± 0.06^{d}	800.09 ± 0.05^{e}	150mg/l
ALKALINITY	188.12 ± 0.05^{d}	130.11 ± 0.01^{c}	113.13 ± 0.04^{b}	80.05 ± 0.03^{a}	80.08 ± 0.03^{a}	120mg/l
TURBIDITY	125.00 ± 0.00^{d}	80.00 ± 0.00^{c}	77.00 ± 0.00^{b}	75.00 ± 0.00^{b}	62.00 ± 1.15^{a}	5NTU
TSS	3.01 ± 0.00^{c}	1.09± 0.03^{a}	1.21 ± 0.01^{b}	1.23 ± 0.01^{b}	1.08 ± 0.02^{a}	
DO	4.03 ± 0.00^{c}	3.96 ± 0.02^{c}	3.14 ± 0.01^{b}	3.16 ± 0.02^{b}	2.53 ± 0.01^{a}	
BOD	2.54 ± 0.02^{d}	1.94 ± 0.03^{c}	1.62 ± 0.01^{b}	1.62 ± 0.01^{b}	1.35 ± 0.02^{a}	300mg/l
COD	8.36 ± 0.16^{d}	6.15 ± 0.03^{c}	5.43± 0.24^{b}	5.11± 0.04^{b}	4.22 ± 0.06^{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: Physico-chemical parameters of untreated and Potassium aluminium sulphate treated effluent

Parameters	Before Treatment	After Treatment				NIS/WHO
	Control	1g (Mean ± S.E)	3g (Mean ± S.E)	5g (Mean ± S.E)	7g (Mean ± S.E)	
PH	9.30 ± 0.23^{b}	5.74 ± 0.15^{a}	5.28 ±0.20^{a}	5.21 ± 0.17^{a}	5.14 ± 0.13^{a}	6 - 8.5
TEMP	25.40 ± 0.80	25.65 ± 0.66	25.55 ± 0.66	25.20 ± 0.40	25.35 ± 0.37	40°C
TDS	581.56 ± 0.22^{c}	631.22 ± 0.02^{b}	665.20 ± 0.05^{a}	728.11 ± 0.06^{d}	786.29 ± 0.19^{e}	1500mg/l
EC	1056.33 ± 0.33^{c}	1169.06± 0.13^{a}	1210.07 ± 0.44^{b}	1324.11 ± 0.10^{d}	1395.15 ± 0.15^{e}	1000µS/cm
PHOSPHATE	2.33 ± 0.16^{b}	0.52 ± 0.00^{a}	0.50 ± 0.00^{a}	0.31 ± 0.00^{a}	0.31 ± 0.00^{a}	0.3mg/l
SULPHATE	395.00 ± 2.88^{d}	390.02 ± 0.01^{d}	370.01 ± 0.02^{c}	300.10 ± 0.06^{b}	250.02 ± 0.02^{a}	100mg/l
NITRATE	27.50 ± 4.33^{c}	30.00 ± 0.01^{a}	30.03 ± 0.02^{a}	35.07 ± 0.04^{ab}	40.05 ± 0.03^{b}	50mg/l
CHLORIDE	260.01 ± 0.01^{e}	240.04 ± 0.01^{d}	225.01 ± 0.01^{c}	200.07 ± 0.03^{b}	180.05 ± 0.03^{a}	250mg/l
CALCIUM	80.16 ± 0.00^{e}	52.01 ± 0.01^{d}	42.48 ± 0.00^{c}	36.07 ± 0.00^{b}	34.43 ± 0.03^{a}	75mg/l
HARDNESS	280.01 ± 0.01^{a}	520.01 ± 0.01^{b}	720.01 ± 0.01^{c}	840.04 ± 0.02^{d}	920.04 ± 0.03^{e}	150mg/l
ALKALINITY	188.11 ± 0.06^{d}	176.01 ± 0.01^{c}	105.04 ± 0.03^{b}	60.04 ± 0.02^{a}	60.05 ± 0.03^{a}	120mg/l
TURBIDITY	125.02 ± 0.01	97.01 ± 0.01	93.01 ± 0.01	95.06 ± 0.02	73.03 ± 0.02	5NTU
TSS	3.02 ± 0.01^{c}	3.03 ± 0.03^{c}	2.02 ± 0.02^{b}	1.01 ± 0.01^{a}	1.02 ± 0.01^{a}	
DO	4.03 ± 0.03^{c}	3.05 ± 0.26^{b}	2.55 ± 0.02^{ab}	2.55 ± 0.03^{a}	2.25 ± 0.87^{a}	
BOD	2.51 ± 0.01^{b}	2.51 ± 0.01^{b}	2.45 ± 0.86^{b}	2.25 ± 0.02^{ab}	2.00 ± 0.11^{a}	300mg/l
COD	8.52 ± 0.01^{e}	7.50 ± 0.00^{d}	6.94 ± 0.15^{c}	3.51 ± 0.01^{b}	3.02 ± 0.12^{c}	4mg/l

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

Table 4: Bacteriological characteristics of untreated and treated effluent with graded doses of the coagulants

	Control	1g	3g	5g	7g	NIS/WHO
TBC of MOS	26000.24 ± 0.144^{e}	18000. 17 ± 0.17^{d}	4000.27 ± 0.17^{c}	1000. 23 ± 0.23^{b}	0.53 ± 0.27^{a}	10
MPN	>1800.00	2	<2	<2	<2	0
TBC of PAS	26000.07 ± 0.07^{e}	20000.40 ± 0.21^{d}	18000.12 ± 0.07^{c}	15000.10 ± 0.10^{b}	1000.23 ± 0.12^{a}	10
MPN	>1800.00	1600	62	40	29	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 parameters of untreated and *Moringa oleifera* seed treated effluent

As observed in table 1 there was significant reduction in pH value in the treatment groups compared with the control group. When effluent with a pH value of 9.3 was treated with 1g/l, 3g/l, 5g/l and 7g/l of *Moringa oleifera* seed, the pH value dropped to 6.5. This is in consonance with the results of Muyibi and Evison (1995) [19] which showed that the pH of water softened with *Moringa oleifera* seed and was within the recommended NIS/WHO standard. Katayon *et al*., (2004) [13] also reported that the decrease in pH when using MO seed in water treatment could be due to hydrogen ions of the weak acidity of *Moringa oleifera seed*, which balances the hydroxide ions in the raw water. There was no significant difference in temperature at ($P > 0.05$) between the treatment groups compared with the control group. However, there was significant increase in TDS and EC in the treatment groups compared with the control group. The values of TDS and EC were more pronounced with increase in doses of the coagulant. This could be due to high organic salt contents of the coagulant. It could also be due to solubility of mineral elements, charged macromolecules or other ionic compounds that dissolved in the treated sample. There was significant decrease at ($P \leq 0.05$) in the values of all parameters in the entire treatment groups compared with the control group and the effectiveness of the reduction is dose specific, i.e. the higher the doses the more effective the performance. The only exception was seen in hardness and nitrate in which a significant increased at ($P \leq 0.05$) was observed in the treatment groups compared with the control group. The high value of hardness could be due to the fact that the coagulant contains calcium, magnesium and other hardness causing substances. Also the BOD value of the effluent sample before treatment was found to be 2.54mg/l which represents low concentration of organic materials. However, after treatment with graded doses of *Moringa oleifera* seed the BOD value was significantly degraded to 1.35mg/l. This finding suggested that BOD value decreases with increase in doses of the coagulant which also agrees with the results of (Luanmanee *et al*., 2002) [15] and (Hamoda *et al*., 2004) [11].

Physico-chemical parameters of untreated and Potassium aluminium sulphate treated effluent

As observed in table 2 there was significant reduction in pH value in the treatment groups compared with the control group. The addition of alum to the effluent sample reduced the pH value below the NIS/WHO acceptable limit of 6.5. Graded doses of alum caused changes in pH value of the effluent sample, i.e. from a pH value of 9.3 to a settled value of 5.14. The tendency towards increase in acidity could be due to trivalent cation of aluminium which serves as a Lewis acid that can accept a lone pair of electrons (Miller *et al*., 1984) [18]. It could also be due to sulphuric acid that was released during the treatment process. However, high dose of alum in water treatment even though a better coagulant may lead to high acidity raising health concerns about alum related diseases as reported by (Miller *et al*., 1984, Martyns *et al*., 1998 and Najm *et al*., 1998) [18],[20]. There was no significant difference in temperature at ($P > 0.05$) between the treatments groups compared with the control group. There was significant increase in TDS and EC in the treatment groups compared with the control group. Alum increased the TDS and EC of the effluent sample and drastically reduced the pH

in response to increase in doses of the coagulant. The high values of TDS and EC of the treated samples could be due to dissolution of aluminum ions. However, since alum is a double salt of aluminium sulphate, it is expected to cause increase in TDS and EC as they are positively correlated. This agrees with the findings of Ordonez *et al*., (2010) [23] and Alo *et al*., (2012) [1] which indicated that TDS and EC increase as more coagulants are added to water. There was significant decrease at ($P \leq 0.05$) in the values of all parameters in the entire treatment groups compared with the control group and the effectiveness of the reduction is dose specific, i.e. the higher the doses the more effective the performance. The only exception was also seen in hardness and nitrate in which a significant increase at ($P \leq 0.05$) was observed in the treatment groups compared with the control group. The one-way analysis of ANOVA revealed that variation in the doses of Potassium aluminium sulphate did not have significant effect on BOD values of the treated samples at ($P > 0.05$). This is because all the treated samples had almost the same BOD values after treatment with graded doses of the coagulant.

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

With regards the bacteriological investigations in table 3, there was progressive decrease in total bacterial count and total coliform count with increase in doses of the coagulants in which the highest reduction was found in *Moringa* coagulant. The average bacterial count showed significant difference at ($P \leq 0.05$) that existed between coagulants doses as they relate to bacterial recovery. The antibacterial activity of *Moringa oleifera* coagulants could be due to the presence of antibacterial agent known as 4(α-L-rhhamnosloxy) benzyl isothiocynate as reported by (Eilert *et al*., 1981) [9]. It could also be due to the presence of a recombinant cationic protein in the seed which is able to flocculate Gram-positive and Gram-negative bacterial cells and to mediate the aggregation of negatively charged particles in suspension, such as bacterial cells, clay and silicate microspheres as reported by (Broin *et al*., 2002) [6] and (Suarez *et al*., 2003) [27].

Conclusions

Moringa oleifera seed was found to be more effective on the physicochemical parameters of the effluents sample than Potassium aluminium sulphate and the higher the doses the more effective the performance. There was significant reduction in the bacterial load of the effluents sample after treatment with graded doses of *Moringa oleifera* seed than with Potassium aluminium sulphate.

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