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Application of Moringa *oleifera* as a Natural Coagulant for the Treatment of wastewater from Bakery and Brewery Industries in Uyo, Akwa Ibom State, Nigeria.

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Abstract: *The use of Moringa oleifera as a natural coagulant for the treatment of bakery and brewery industrial wastewater was carried out using microbiological, phytochemical and physicochemical methods. The mean bacterial count obtained from the brewery wastewater ranged from 4.1 x 10^5 to 5.3 x 10^5 cfu/ml, The mean coliform count ranged from 2.2 x 10^4 to 3.1 x 10^4cfu/ml, while the mean fungal count ranged from 3.9 x 10^4 to 4.1 x 10^4cfu/ml Wastewater from the bakery had a mean bacterial count that ranged from3.9 x 10^5 to 4.2 x 10^5cfu/ml and the coliform count which ranged from 3.8 x10^4 to 6.3 x 10^4cfu/ml. There was a significant different (P > 0.05) between the microbial counts of the wastewater samples from the two sources. The mean bacterial count for brewery wastewater pre-treated with charcoal ranged from 3.0 x 10^5 to 3.9x10^5cfu/ml, mean coliform count ranged from 2.0 x 10^5 to 2.9 x 10^4cfu/ml, and mean fungal count ranged from 3.8 x 10^4 to 3.9 x 10^4 cfu/ml. The mean bacterial, coliform count and fungal counts of the bakery wastewater after pre-treatment with charcoal were within the following range, wastewater 3.7 x 10^5 to 4.0 x 10^5cfu/ml, 3.6 x 10^5 and 6.0 x10^5cfu/ml, and 3.0 x10^5 and 3.2 x 10^5cfu/ml. The microbial isolates obtained from the brewery, bakery, charcoal filtered brewery and bakery wastewater occurrence were; Bacillus sp, Enterobacter sp, Staphylococcus aureus, Proteus sp, Aspergillus sp, and Fusarium sp, Lactobacillus sp, Pseudomonas sp, Penicillium sp, Staphylococcus aureus 3(8.3%), Staphylococcus sp, Saccharomyces sp and Rhizopus sp. Results obtained from physicochemical analysis showed values for Physiochemical analyses showed : COD (0.38 ± 0.01 mg/L), (22.30 ± 0.11 ℃), turbidity (843 ± 0.20 NTU), DO (4.49 ± 0.01 mg/L), BOD (0.29 ± 0.01 mg/L) and pH (4.68 ± 0.10) for bakery wastewater, while the corresponding values for wastewater from the brewery industry were 0.23 ± 0.02 mg/L, 8.01 ± 0.08 ℃, 10.13 ± 0.03 NTU, 2.40 ± 0.01 mg/L, 0.13 ± 0.03 mg/L and 5.83 ± 0.30 respectively. Phytochemical analysis revealed the presence of saponins, cardiac glycoside, .flavonoids, tannins, alkaloids, terpenes and tannins. Terpenes were present in the seed while it was not detected in the flowering part. The treatment of the wastewater with, M. oleifera ground seed inhibited bacterial load in bakery wastewater but the inhibition for brewery wastewater started at 100 mg and the ground flower inhibited from 150mg.*

Keywords: *Brewery, bakery, wastewater, bacteriological and physicochemical analysis, treatment, Moringa oleifera*

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1.0 Introduction

Water is a resource that is essential for life and is required by almost every living organism. This resource is, becoming very limited in its pure state due to contamination from industrial and other anthropogenic sources (Eddy *et al*., 2004). Contamination of natural water by wastes has been globally viewed as one of the most serious threats to life (Briggs, 2003). Industries employ clean water at various production lines apart from its cleansing and solvent roles. However, water released after usage is no longer clean but contaminated with several impurities that may affect the physicochemical and bacteriological quality of the water. Discharge of such wastewater to the environment cannot guarantee the requirements that are necessary for the maintenance of the purity of the ecosystem. The brewery and bakery industries are among those that use a large volume of water in the course of production and subsequently discharge contaminated water into the environment (Somani *et al*., 2012).

Several treatments protocols have been implemented for industrial wastewater including adsorption, purification, chlorination, and others (Lea, 2010; Uchechukwu *et al*., 2018). Literature has extensively covered the advantages and disadvantages of each of the available methods and one of the major challenges is the absence of a completely green approach, which involves the use of materials that are non-toxic, inexpensive, easily accessible and eco-friendly (Uchechukwu *et al.*, 2015). One such method is the employment of biological coagulant against chemical coagulations that has been reported to leave chemical compounds in the water (Qasim *et al.*, 2000).

Coagulation is the process of coagulating colloidal particles due to the addition of synthetic materials to neutralize charged particles thus forming a precipitate due to the force of gravity. Coagulants can be synthetic materials such as ferrous sulfate $\{Fe(SO_4)\}$, aluminum sulfate or alum $\{Al_2(SO)_3\}$, and poly aluminum chloride (PAC) $\{Al_2(OH)_3Cl_3\}_{10}$. (Eddy and Ekop, 2007). Therefore to overcome the challenges associated with chemical coagulants, appreciable research efforts are currently focused on the use of natural coagulants for the treatment of wastewater. Therefore, the present study aims to use *Moringa oleifera* seed as a coagulant for the treatment of wastewaters from the brewery and bakery industries. Analysis of the phytochemical constituents of the plant material shall be carried out in addition to the analysis of the physicochemical, and bacteriological parameters of wastewater from brewery and bakery industries. *Moringa oleifera* (drumstick) is a cosmopolitan tropical, drought-tolerant tree, available throughout the year. It has been well documented for its

various pharmacological importance, namely, its analgesic, antihypertensive and anti-inflammatory effects (Joshi *et al*., 2012).

2.0 Materials and Methods

A completely randomized design method was used in this study. Physicochemical parameters of the wastewater were analysed using official and recommended methods. The microbiological quality of the wastewater from breweries and bakeries was carried out in the Department of Microbiology, University of Uyo, Uyo Akwa Ibom State.

The samples of brewery wastewater were collected from the Champion brewery in Uyo, Akwa Ibom State Nigeria at the points of entering the sewer system. The samples were stored in black plastic containers to preserve and further prevent changes in the characteristics of the effluent (Clesceri *et al.*, 1999) and transported to the Microbiology Laboratory, the University of Uyo in a cooler containing ice park. Bakery effluents were similarly collected from three industries.

Mature *Moringa oleifera* seeds were gotten from Ikot Ekpene Main Market and identified in the department of Science Technology, Biology Unit in Akwa Ibom State Polytechnic. The seeds were de-shelled; air-dried to constant weight and ground to a powdered form using a manual grinding machine. (Emel model 242) Various concentrations of the seed powder were prepared by dilution of the appropriate amount of the stock solution. to obtain 10,000, 20,000 and 30,000 mg/L (Alo *et al.,* 2012). Each of these concentrations was used as coagulants in the treatment of wastewater.

2.1 Wastewater treatment

500 ml of water sample was added to each beaker. A known concentration of the extract was added and allowed to stand for an hour, after which the 200 ml of the water sample was taken for further analysis. . Dissolved oxygen was determined in situ using the portable DO meter, Jenway 9071 model due to fast-changing nature of the parameter.

2.2 Chemical analysis

The pH of each sample was determined using the Hach sensor 3 pH meter. The pH meter was first calibrated with buffer solutions.

The turbidity test of the effluent water samples was measured using a 2100Q (HACH) turbidity meter and compared before and after treatment (Amagloh and Benang, 2009).

BOD was measured using dissolved oxygen (DO) meter. The measurement of dissolved oxygen was done before and after five days of incubation. From the measured values of DO before and after five days, the BOD was calculated using equation 1

$$BOD\ (mg/L) = \frac{(DO_1 - DO_5)V_b}{V_s} \qquad (1)$$

where: DO_1 is the DO at the point of sample collection, DO_5 is the DO after five days, V_b is the volume of the BOD bottle and Vs is the volume of the sample

Chemical oxygen demand was determined using a titrimetric method, 0.4g $HgSO_4$ was placed in a refluxing flask, 200 ml of the sample was added. Two milligrams of sulphuric acid were added to remove NO_2^- N present in the sample. With the aid of a pipette, 10 ml $K_2Cr_2O_7$ solutions were connected to a condenser. A blank was also prepared. The mixture was refluxed for hours and cooled. A blank was washed with distilled water into an Erlenmeyer flask and diluted to about 150mls and cooled to room temperature. The excess dichromate was titrated with standard FAS using two drops of ferroin as an indicator.

2.3 Microbiological analyses

One milliliter of the sample was pipette and serially diluted to 10^{-6} using the pour plate method.

One milliliter each from 1:1000000 was cultured on nutrient Agar for the total heterotrophic bacterial count, MacConkey Agar (MCA) for the total coliform count, Sabouraud dextrose agar (SDA) for the total mycological count. Plates of Nutrient agar and MacConkey agar were incubated at 37 ^{0}C for 24–48 h while plates of Sabouraud dextrose agar were incubated at room temperature for 3

to 5 days (Collins *et al*., 2004). The emerging colonies were enumerated, sub-cultured and identified using standard microbiological and biochemical methods.

2.4 Phytochemical analysis

Phytochemical analysis was carried out using the methods reported elsewhere (ref). The analysed parameters included alkaloids, flavonoids, and others

2.5 Antibacterial essay test

The antibacterial assay was carried out to investigate the microbiological quality of the water sample. The pour plate method was used to examine the reduction of the bacterial population from wastewater samples according to the method reported by Arafat and Mohamed (2013).

2.6 Test of the efficacy of ground seeds of Moringa oleifera

A set of five sterile containers containing 500 ml each of bakery and brewery wastewater was set up. The following, 0.5, 1, 1.5, 2.0 and 2.5 g of ground seed each was introduced to the set up respectively and mixed with a sterile glass rod. The water was allowed to settle for 20 minutes, and the supernatant sample was withdrawn with a sterile pipette for analysis. This procedure was repeated with the bakery and brewery wastewater pre-treated with charcoal. Finally, all treatment was analyzed microbiologically using pour plate technique described by Collins *et al*. (2004).

3.0 Results and Discussion

Table 1 shows the cultural, morphological and biochemical characteristics of bacterial isolates. The organism has *Bacillus* sp. is a Gram positive aerobic rod in pairs, with an endospore which are motile and catalase positive, while *Lactobacillus* sp though Gram positive, they are non-motile, non-sporulation and catalase negative and also rods shape bacterium. The table also has *Staphylococcus aureus* as the only coagulase positive organism; it is a Gram positive organism that forms clusters and catalase positive. *Streptococcus* sp was also isolated and indicated as Gram positive cocci, catalase negative, coagulase negative, MR, oxidase positive and non-motile.

The fungi isolated with their morphological characteristics in this analysis as shown in Table 2 include *Fusarium* spp which had colony colour as cottony whitish, types of somatic hyphea is filamentous, nature of hyphea is septate, the specific vegetable structure is short branched condiospores, an asexual reproductive spore is microconidis, *Rhizopus* sp morphological characteristics include colony colour as cream coloured, an asexual reproductive spore is globes, *Penicillium* spp had greenish-blue and the types of somatic hyphea is aerial while the nature of hyphea is septate. The asexual reproductive spore is oval while *Aspergillus niger* had colony colour as brownish colony becoming darker with agar, types of somatic hyphea are filamentous, nature of hyphea is septate, the specific vegetable structure is foot cell, the asexual reproductive spore is globes conidia as morphological characteristics.

The total microbial load of brewery wastewater and bakery wastewater before treatment are presented in Table 3. The total microbial load counts obtained from brewery 1 wastewater were total herotrophic count (THBC) $4.10 \pm 0.10 \times 10^5$ cfu/ml, total coliform count (TCC) $2.20 \pm 0.04 \times 10^4$ cfu/ml and total fungal count (TFC) $3.90 \pm 0.04 \times 10^4$ cfu/ml, while microbial load in brewery 11 wastewater were THBC $5.30 \pm 0.03 \times 10^5$ cfu/ml, and TFC $4.10 \pm 0.08 \times 10^4$ cfu/ml, the total average microbial counts obtained from bakery 1 wastewater were THBC $3.90 \pm 0.21 \times 10^5$, TCC $6.30 \pm 0.11 \times 10^4$ cfu/ml and TFC $6.30 \pm 0.11 \times 10^4$ cfu/ml, while microbial load obtained from bakery 11 wastewater were THBC $4.20 \pm 0.14 \times 10^5$ cfu/ml, TCC $3.80 \pm 0.12 \times 10^4$ cfu/ml and TFC $3.30 \pm 0.06 \times 10^4$ cfu/ml.

The microbial mean count from bakery/brewery effluents after pre-treated with charcoal is presented in Table 4**.** The total mean

count from brewery 1 effluent were total heterotrophic bacterial count (THBC) 3.9 ± 0.10 X 10^5 cfu/ml, total coliform count (TCC) 2.0 ± 0.02 x 10^4 cfu/ml and total fungal count (TFC) 3.9 ± 0.03 x 10^4 cfu/ml, while microbial load in brewery 11 wastewater were THBC 3.0 ± 0.00 x 10^5 cfu/ml, TFC 2.9 ± 0.01 x 10^4 cfu/ml and TFC) 3.9 ± 0.03 x 10^4 cfu/ml. The total heterotrophic bacterial count from brewery 1 effluent were THBC 3.7± 0.10 X 10^5 cfu/ml, TCC 6.0 ± 0.01 x 10^4 cfu/ml and TFC 3.2 ± 0.06 x 10^4 cfu/ml, while the total counts obtained from brewery 11 were THBC 4.0 ± 0.12 x 10^5 cfu/ml, TCC 3.6 ± 0.11 x 10^4 cfu/ml and TFC 3.0 ± 0.02 x 10^4 cfu/ml.

The mean counts from bakery/brewery wastewater after treatment with ground seeds and flower of *Moringa oleifera* is presented in **Table 5**. The mean microbial counts obtained from bakery/brewery wastewater after treatment with *Moringa oleifera* ground seeds were as viz: TFC ranged from 2.0 ± 0.21 x 10^3 (cfu/g) to 5.0 ± 0.10 x 10^3 (cfu/g), TCC and THBC was not indicated in Brewery 1, while THBC was 1.0 ± 0.01 x 10^4 (cfu/ml), TFC 3.0 ± 0.01 x 10^4 (cfu/ml) and TCC was not indicated in brewery 11 effluent. The mean counts obtained from Brewery 1 wastewater after treatment with *Moringa oleifera* ground flower were THBC 2.0 ± 0.21 x 10^4 (cfu/ml), TCC 1.0 ± 0.003 x 10^3 cfu/ml and TFC 3.0 ± 0.12 x 10^3 cfu/ml to 10.0 ± 0.02 x 10^3 cfu/ml, while the mean counts obtained from Brewery 11 wastewater after treatment with *Moringa oleifera* ground flower were THBC 1.1 ± 0.02 x 10^4 cfu/ml, TFC ranged from 1.0 ± 0.12 x 10^3 cfu/ml to 8.0 ± 0.12 x 10^3 cfu/ml, while TCC was not indicated. The microbial mean counts obtained from Bakery 1 wastewater after treatment with *Moringa oleifera* seeds, TFC ranged from 1.0 ± 0.11 x 10^3 cfu/ml to 7.0 ± 0.01 x 10^3 cfu/ml, THBC and TCC was not indicated, while the microbial mean counts obtained from Bakery 11 wastewater were as viz: TFC ranged from 3.5 ± 0.01 x 10^3 cfu/ml to 8.0 ± 0.01 x 10^3 cfu/ml, THBC and TCC were not indicated (i.e. no growth).

Table 1 Characterization and identification of bacterial isolates

Sample source	Cell shape	Coagulase	Gram staining	Catalase	Urease	Spore	Motility	MR	VP	Indose	Oxidase	Citrate	Lactose	Glucose	Maltose	Sucrose	Probable organism
BRW 1	Rod	-	+	+	+	+	+	-	+	-	-	-	AG	AG	AG	AG	*Bacillus* sp
BRW I	Rod	-	+	-	+	-	-	-	-	-	-	+	AG	AG	AG	AO	*Lactobacillus* sp
BRW II	Cocci rod	-	+	-	-	-	-	+	-	-	+	+	AO	AG	AG	AG	*Streptococcus* sp
Brk II	Cocci	+	+	-	-	-	-	-	+	-	-	-	AG	AG	AG	AG	*Staphylococcus*
Brew I	Rod	-	-	-	-	-	-	-	+	-	-	-	OO	AG	AG	AG	*Proteus* sp
Brew II	Rod	-	-	-	-	-	-	-	-	-	-	-	AO	AG	OO	AG	*Pseudomonas* sp
Brew 2	Rod						-						AO	AG	AG	AG	*Fusarium* sp
Brk 1	Cocci	-	-	-	-	-	-	-	-	-	-	-	AG	AO	AG	AO	*Enterococcus* sp

Keys: AG = acid and gas, AO = acid and gas production, OO = No acid No gas, + positive, - = negative

The microbial means counts from Bakery 1 wastewater after treatment with *Moringa oleifera* ground flower for THBC was 2.0 ± 0.05 x 10^4 cfu/ml and 4.0 ± 0.11 x 10^4 cfu/ml,

TFC ranged from $4.0 \pm 0.08 \times 10^3$ cfu/ml to $8.0 \pm 0.12 \times 10^3$ cfu/ml. Microbial load obtained from Bakery 11 wastewater for THBC ranged from $1.0 \pm 0.1 \times 10^4$ cfu/ml and $6.0 \pm 0.14 \times 10^4$ (cfu/ml), TFC ranged from $3.0 \pm 0.01 \times 10^3$ cfu/ml to $10.0 \pm 0.03 \times 10^3$ cfu/ml, while TCC was not indicated.

Table 6 shows the microbial isolates obtained from brewery and bakery wastewater. Microbial isolates were obtained from Brewery and Bakery wastewater. The presence of isolates is indicated with a positives (+) sign while absence is indicated with a negative (-) sign.

Table 2: Characterization of fungal isolates

Samples	Colony	Types of somatic cells	Natural hyphea	Special vegetative structure	Asexual spores	Special reproductive structure	Conidial	Vesicle shape	Confirmed organisms
Bre 1	-	Filamentous	Septale	Foot cell	Chlamydosphores	Sporandochis absent	Globes	Globes	*Fusarium* sp
Bre 2	Compact white or yellow based	Filamentous	Septale	Foot cell	Globse conidial	Smooth wall erect conidosphore	-	-	***Aspergillus niger***
Bre 1	Dance gray green	Filamentous	Septale	Broom-like appearance	Globes cham	Erect conidosphore	-	-	*Penicillium* spp
Brk 1	Whitish colony becoming brown	Filamentous	Coemocyil	Sloten rhizoids	Ovid sporangosphores	Erect sporangiosphore not in grump	-	-	*Rhizopus* spp
Brk2	Milky colour moist	Absent	Septale	Bidding	ballisdosphille	Telispore	-	-	*Saccharomyces cerevisiae*

Keys: Bre I = Brewery I, Bre II = Brewery II, Brk I = Bakery I, Brk 11= Bakery II

Table 3: Total microbial count before treatment (cfu/ml)

Effluents sources	THBC x 10^5	TCC x 10^4	TFC x 10^4
Brw 1	$4.10 \pm \times 10^5$	2.2 ± 0.04	3.9 ± 0.04
Brw 2	5.30 ± 0.03	3.1 ± 0.01	4.1 ± 0.04
Bkr 1	3.90 ± 0.21	6.3 ± 0.11	3.4 ± 0.08
Bkr 2	4.20 ± 0.14	3.8 ± 0.12	3.3 ± 0.06

Keys: THBC = Total Heterotrophic Bacterial Count , TCC = Total Coliform Count , TFC = Total Fungal Count, Brw1 = Brewery Wastewater 1, Brw2 = Brewery Wastewater 2, Bkr1 =Bakery Wastewater 1, Bkr 2 = Bakery Wastewater 2

Table 4: Microbial mean count of brewery and bakery pre-treated with charcoal filtered (cfu/ml)

Effluents sources	THBC x 10^5	TCC x 10^4	TFC x 10^4
Brw 1	3.9 ± 0.10	2.0 ± 0.02	3.9 ± 0.03
Brw 2	3.0 ± 0.0	2.9 ± 0.01	3.8 ± 0.07
Bkr 1	3.7± 0.22	6.0 ± 0.01	3.2 ± 0.06
Bkr 2	4.0 ±0.12	3.6 ±0.11	3.0 ± 0.02

Keys: THBC = Total Heterotrophic Bacterial Count , TCC = Total Coliform Count, TFC = Total Fungal Count, Brw1 = Brewery Wastewater 1, Brw2 = Brewery Wastewater 2, Bkr1 = Bakery Wastewater 1, Bkr2 = Bakery Wastewater 2

Table 5: Mean counts from Baker/Brewery wastewater after treatment with ground seed and flower of *Moringa oleifera*

Effluents Sources	Concentration	Ground *M. oleifera* seeds			Ground *M. oleifera* Flower		
		THBC x 10^4 (cfu/ml)	TCC x 10^3 (cfu/ml)	TFC 10^3 (cfu/ml)	THBC x 10^4 HBC x 10^4 (cfu/ml)	TCC x 10^3 (cfu/ml)	TFC 10^3 (cfu/ml)
BW1	0.5	-	-	5.0 ± 0.10	2.0 ± 0.01	-	1.0 ± 0.01
	1.0	-	-	3.0 ± 0.02	-	-	8.0 ± 0.11
	1.5	-	-	3.0 ± 0.11	-	-	7.0 ± 0.003
	2.0	-	-	2.0 ± 0.21	-	-	4.0 ± 0.04
	2.5	-	-	-	-	-	8.0 ± 0.12
BW 2	0.5	1.0 ± 0.01	-	3.0 ± 0.01	1.1 ± 0.02	-	5.0 ± 0. 03
	1.0	-	-	2.0 ± 0.02	-	-	3.0 ± 0.05
	1.5	-	-	2.0 ± 0.03	-	-	3.0 ± 0.11
	2.0	-	-	1.0 ± 0.03	-	-	1.0 ± 0.01
	2.5	-	-	1.0 ± 0.03	-	-	1.0 ± 0.12
BK 1	0.5	-	-	7.0 ± 0.01	4.0 ± 0.11	-	8.0 ± 0.12
	1.0	-	-	5.0 ± 0.03	2.0 ± 0.05	-	7.0 ± 0.03
	1.5	-	-	3.0 ± 0.02	-	-	5.0 ± 0.11
	2.0	-	-	2.0 ± 0.01	-	-	5.0 ± 0.20
	2.5	-	-	2.0 ± 0.01	-	-	4.0 ± 0.08
BK2	0.5	-	-	8.0 ± 0.01	6.0 ± 0. 14	-	10.0 ± 0.03
	1.0	-	-	6.0 ± 0.03	2.0 ± 0.04	-	8.0 ± 0.04
	1.5	-	-	3.6 ± 0.03	1.0 ± 0.11	-	5.0 ± 0.12
	2.0	-	-	3.5 ± 0.01	-	-	5.0 ± 0.04
	2.5	-	-	-	-	-	3.0 ± 0.01

Keys: Brw1: Brewery Wastewater 1, Brw2: Brewery Wastewater 2, Bkr1: Bakery Wastewater 1 Bkr2: Bakery Wastewater 2

Table 7 shows the phytochemical screening of the extract (seed and flower) of *Moringa oleifera* revealed the various chemical components in each of the plant parts as shown **in Table 7.** The Phytochemical screening indicated the presence of alkaloids, tannins, saponins, flavonoids, cardiac glycoside and terpenes and other metabolites. Tannins were absent in the seed while terpenes was absent in the flower.

Table 6: Microbial isolates obtained from Brewery and Bakery wastewater

Isolates	Brw 1	Brw 2	Brk 1	Brk 2
Bacillus spp	+	+	++	-
Enterobacter spp	+	++	+	-
Lactobacillus spp	+	-	-	+
Streptococcus spp	+	+	+	-
Pseudomonas spp	-	+	+	-
Staphylococcus aureus	++	+	+	++
Proteus spp	+	-	-	-
Staphylococcus spp	-	-	+	-
Aspergillus niger	+	++	+	-
Fusarium spp	+	+	-	+
Penicillium spp	+	+	-	-
Rhizopus spp	-	-	+	+
Saccharomyces cerevisiae	-	-	+	+

Keys : Brw1 = Brewery Wastewater 1, Brw2 = Brewery Wastewater 2, Bkr1 = Bakery Wastewater 1, Bkr2 = Bakery Wastewater 2, + = Positive, - = Negative

Table 7: Phytochemical Screening of *Moringa oleifera*

Test	Observation	Inference
***Moringa oleifera* seeds**		
Saponin frothing test	Stable foam form	+++
Phlobatannin	No ppt formed	-
Athraquinone	No pink colour	-
Terpenes	Pink colour	+++
Deoxy sugar	Violet ring observed	++
Cardiac glycoside	Reddish-brown observed	+++
Flavonoids	Pale yellow colouration seen	+
Tannin	Blue-green ppt formed	_
Alkaloids Mayer's reagent	Milky ppt formed	+++
***Moringa oleifera* flower**		
Saponin frothing test	Stale foam formed	+++
Phlobatannin	no ppt formed	_
Anthraquinone	no pink colour	_
Terpenes	no pink colour observed	_
Deoxy sugar	Violet ring observed	++
Cardiac glycoside	Reddish-brown	+++
Flavonoids	Pale yellow colouration	+
Tannin	Blue-green ppt	++
Alkaloids Mayer's reagent	Milky colour ppt	+++

Keys:+ + + = Abundant, ++ = Moderate , + = Scanty, - = Negative

Table 8 shows the potency of ground *moringa oleifera* seeds and flowers on brewery and bakery wastewater pre-treated with charcoal. The potency of ground *Moringa oleifera* seeds and flowers on brewery and bakery wastewater pre-treated with charcoal. The potency of ground *Moringa oleifera* seeds and flowers on brewery and bakery wastewater pre-treated with charcoal revealed the activity of *Moringa oleifera* seeds and flowers at different concentrations. The ground seeds inhibit the growth of THBC and TCC, while fungal growth

is reduced are their concentration increases, both in bakery and brewery wastewater. The ground flower inhibits the growth of THBC and TCC at a higher concentration in brewery wastewater. It inhibits at the concentration of 100 to 250, while bakery wastewater is inhibited at a concentration of 150 to 250, while reduction is observed as the concentration increases.

Table 8: The potency of ground *Moringa oleifera* seeds and flower on brewery and bakery wastewater pre-treated with charcoal

Samples Source	Concentration	Ground *M. oleifera* seeds			Ground *M. oleifera* Flower		
		THBC x 10	TCC x 10	TFC x 10	HBC x 10	TCC x 10	TFC x 10
Brw 1	50	-	-	5.0 ± 0.10	2.0 ± 0.02	2.0 ± 0.12	9.0 ± 0.03
	100	-	-	4.0 ± 0.12	-	-	7.0 ± 0.01
	150	-	-	5.0 ± 0.08	-	-	5.0 ± 0.04
	200	-	-	2.0 ± 0.10	-	-	4.0 ± 0.11
	250	-	-	-	-	-	2.0 ± 0.05
Brw 2	50	-	-	2.0 ± 0.02	1.0 ± 0.01	-	6.0 ± 0. 01
	100	-	-	1.5 ± 0.02	-	-	4.0 ± 0.01
	150	-	-	1.4 ± 0.01	-	-	2.0 ± 0.01
	200	-	-	1.0 ± 0.11	-	-	1.0 ± 0.01
	250	-	-	-	-	-	-
Bkr 1	50	-	-	7.0 ± 0.01	3.0 ± 0.00	2.0 ± 0.05	10.0 ± 0.08
	100	-	-	5.0 ± 0.10	1.0 ± 0.01	-	7.0 ± 0.12
	150	-	-	3.0 ± 0.11	-	-	5.0 ± 0.04
	200	-	-	2.0 ± 0.11	-	-	4.0 ± 0.11
	250	-	-	1.0 ± 0.08	-	-	2.0 ± 0.03
Bkr2	50	-	-	6.0 ± 0.05	3.0 ± 0. 12	1.0 ± 0.03	10.0 ± 0.09
	100	-	-	4.8 ± 0.01	1.0 ± 0.11	-	7.0 ± 0.05
	150	-	-	2.9 ± 0.01	-	-	4.0 ± 0.04
	200	-	-	2.8 ± 0.01	-	-	3.0 ± 0.03
	250	-	-	-	-	-	2.0 ± 0.01

Values are means ± standard deviation of 3 replicates, Keys:Brw1 = Brewery Wastewater 1, Brw 2 = Brewery Wastewater 2, Bkr1 = Bakery Wastewater 1 Bkr2 = Bakery Wastewater 2

Table 9 shows the physicochemical properties of brewery and bakery wastewater before and after treatment with charcoal. Investigation of physicochemical properties of Brewery and Bakery wastewater before and after treatment with charcoal was carried out. The results show that the concentration of COD, Turbidity, DO and BOD in all the samples reduced when treated with activated charcoal, while, temperature and pH increased when the samples were treated with activated charcoal.

Table 10 shows the physicochemical properties of charcoal filtered wastewater of bakery with 250mg of ground seeds and flowers of *Moringa oleifera.* The results indicate that bakery 1 sample treated with seeds of *Moringa oleifera* revealed an increase in the concentration of pH, DO and COD in the sample treated with seeds, while, temperature, BOD and turbidity increases when treated with flower. Furthermore, the Bakery 2 sample treated with seeds of *Moringa oleifera* revealed an increase

in the concentration of pH and DO, while other parameters increase when treated with flowers. Table 11 shows the physicochemical properties of charcoal filtered wastewater of brewery with 250mg of ground seeds and flowers of *Moringa oleifera.* The concentration of pH, DO, COD, and BOD in Brewery 1 increases when treated with the seeds of *Moringa oleifera*, while, temperature and turbidity increase when treated with flower. For brewery 2 the concentration of pH, temperature and DO increases when treated with the seeds while, turbidity, BOD and COD increase with the treatment with flower.

Samples of wastewater from brewery and bakery industries in Akwa Ibom were analyzed. The analysis of the wastewater before treatment showed the total average microbial load of brewery wastewater and bakery wastewater before treatment.

The total microbial load counts obtained from brewery 1 wastewater were total heterotrophic count (THBC) $4.10 \pm 0.10 \times 10^5$ cfu/ml, total coliform count (TCC) $2.20 \pm 0.04 \times 10^4$ cfu/ml and total fungal count (TFC) $3.90 \pm 0.04 \times 10^4$ cfu/ml, while microbial load in brewery 11 wastewater were THBC $5.30 \pm 0.03 \times 10^5$ cfu/ml, and TFC $4.10 \pm 0.08 \times 10^4$ cfu/ml, the total average microbial counts obtained from bakery 1 wastewater were THBC $3.90 \pm 0.21 \times 10^5$, TCC $6.30 \pm 0.11 \times 10^4$ cfu/ml and TFC $6.30 \pm 0.11 \times 10^4$ cfu/ml, while microbial load obtained from bakery 11 wastewater were THBC $4.20 \pm 0.14 \times 10^5$ cfu/ml, TCC $3.80 \pm 0.12 \times 10^4$ cfu/ml and TFC $3.30 \pm 0.06 \times 10^4$ cfu/ml. Statistical analysis (ANOVA) shows that there was a significant difference ($p > 0.05$) between the microbial count of the wastewater samples.

Table 9: Physicochemical Properties of Brewery and Bakery Wastewater before Treatment and after Treatment with Charcoal

Wastewater sources	COD (mg/l)	Temperature (0C)	Turbidity (NTU)	DO (mg/l)	BOD (mg/l)	pH
Brw 1	0.23± 0.02	8.01± 0.08	10.13±0.03	2.40±0.01	0.13±0.03	5.88±0.30
Brw 1 with charcoal	0.03±0.001	22.30±0.030	7.63± 0.30	1.85±0.15	0.82±0.20	7.69±0.11
Brw 2	0.43±0.11	24.00±0.03	10.02±0.07	1.49±0.30	0.33±0.07	3.0±0.01
Brw 2 with charcoal	0.33 ±0.003	23.50±0.003	7.55±0.001	1.33±0.02	0.17±0.001	6.48±0.001
Bkr 1	0.38±0.01	22.30±0.11	8.43±0.20	4.49±0.01	0.29±0.01	4.68± 0.10
Bkr 1 with charcoal	0.03±0.000	24.60±0.30	7.92±0.11	2.13±0.01	0.19± 0.04	7.44± 0.50
Bkr 2	0.40± 0.02	24.10± 0.50	9.10± 0.10	3.22± 0.68	0.27± 0.88	5.10± 0.50
Bkr 2 with charcoal	0.40±0.001	25.00± 0.003	8.80±0.001	1.21± 0.02	0.21± 0.09	7.15± 0.04

The microbial mean counts of brewery and bakery wastewater pre-treated with charcoal shows the total means count of brewery 1 wastewater as total heterotrophic bacterial count (THBC) of $3.9 \pm 0.10 \times 10^5$ cfu/ml, total coliform count (TCC) $2.0 \pm 0.02 \times 10^4$ cfu/ml and total fungal count (TFC) $3.9 \pm 0.03 \times 10^4$ cfu/ml, while the microbial load obtained from brewery 11 wastewater were THBC $3.0 \pm 0.00 \times 10^5$ cfu/ml, TCC $2.9 \pm 0.01 \times 10^4$ cfu/ml and TFC $3.9 \pm 0.03 \times 10^4$ cfu/ml.

Table 10: Physicochemical Properties of Charcoal Filtered Wastewater of Bakery with 250 mg of Ground Seeds and Flowers of *Moringa oleifera*

	Bakery 1		Bakery 2	
Parameters	**Flowers**	**Seeds**	**Flowers**	**Seeds**
Temperature (0^C)	21.31± 0.11	20.40± 0.012	24.00±0.001	23.70± 0.004
pH	5.10±0.001	60.00±0.011	5.25±0.002	5.35± 0.001
Turbidity (WTU)	8.75± 0.02	7.01 ±0.002	7.02± 0.003	6.81± 0.001
DO (mg/l)	2.31± 0.03	2.33± 0.001	1.99± 0.02	2.26±0.03
BOD (mg/l)	0.39±0.01	0.35± 0.01	0.41±0.001	0.28± 0.001
COD (mg/l)	0.29± 0.01	0.30± 0.03	0.39±0.002	0.23± 0.001

Table 11: Physiochemical Properties of Charcoal Filtered Wastewater of Brewery with 250 mg of Ground Seeds and Flowers of *Moringa oleifera*

	Brewery 1		Brewery 2	
Parameters	**Flowers**	**Seeds**	**Flowers**	**Seeds**
Temperature (0C)	24.1± 0.012	23.20± 0.03	20.10±0.04	21.10±0.03
pH	5.67 ±0.02	6.10±0.03	6.75±0.065	6.85± 0.001
Turbidity (WTU)	6.35±0.002	6.00±0.001	8.34±0.003	7.43± 0.001
DO (mg/l)	2.51±0.01	3.03±0.002	2.10±0.001	2.87± 0.06
BOD (mg/l)	7.61±0.002	6.75 ±0.002	7.10±0.001	6.19± 0.001
COD (mg/l)	0.03 ±0.01	0.23±0.002	0.31± 0.002	0.28±0.02

The total heterotrophic bacterial counts from bakery 1 wastewater were THBC 3.7 ± 0.22 x 10^5 cfu/ml, TCC 6.0 ± 0.01 x 10^4 cfu/ml and TFC 3.2 ± 0.06 x 10^4 cfu/ml, while the total counts obtained from bakery 11 wastewater were THBC 4.0 ±0.12 x 10^5cfu/ml, TCC 3.6 ± 0.11 x 10^4 cfu/ml and TFC 3.0 ± 0.02 x 10^4 cfu/ml. Statistical analysis (ANOVA) shows that there was no significant different ($p< 0.05$) between brewery and bakery wastewater pretreated with charcoal. The prevalent isolates were *Bacillus* spp, *Enterobacter* spp, *Lactobacillus* spp, *Streptococcus* spp, *Staphylococcus aureus, Proteus* sp, *Pseudomonas s*pp, *Staphylococcus* spp, *Aspergillus* niger, *Fusarium* spp, *Penicillium* spp, *Rhizopus* spp and *Saccharomyces cereviciae*. This agrees with the work of Henze *et al.* (2001) who isolated similar organisms from industrial layout, and also stated that this could be might be attributed to the fact that pollutants can be discharged directly or indirectly into the environment since wastewater from industry may include sanitary waste of employees, processing waste from plants and water from floor washing. The quality of brewery effluent can fluctuate significantly as it depends on various processes and the organic components in brewery effluent since it is easily biodegradable. There was no significant difference between isolates of brewery and bakery wastewater.

The results also showed that at 2.5 g concentration of the treatment, the mean total count of bacteria for brewery 1 wastewater treated with ground seeds was completely inhibited, while brewery 11 at the lowest concentration were of 0.5g recorded the bacteria count of 1.0 ± 0.01 x 10^4 cfu/ml and at 1.0mg to the highest concentration of 2.5mg, there was complete inhibition of the bacterial count, fungal count of brewery 1 and 11 were reduced and ranged from 0.1 ± 0.03 x 10^3 cfu/ml to 5.0 ± 0.01 x 10^3 cfu/ml as the highest concentration of 2.5mg inhibited the count in brewery1. The coliform counts of wastewater pretreated with charcoal were completely inhibited, while mean fungal counts were

inhibited only at the highest concentration of 2.5g. The treatment with ground flower showed that at the lowest concentration of 0.5g brewery 1 and brewery 11 recorded $1.1 \pm 0.02 \times 10^4$ cfu/ml, $2.0 \pm 0.01 \times 10^4$ cfu/ml for total heterotrophic and coliform count as $1.0 \pm 0.001 \times 10^3$ cfu/ml, while fungal count recorded from $1.0 \pm 0.12 \times 10^3$ cfu/ml to $10.0 \pm 0.02 \times 10^3$ cfu/ml. The treatment with charcoal and the ground flower for brewery 1 and 11 wastewater recorded $2.0 \pm 0.02 \times 10^4$ cfu/ml and $1.0 \pm 0.017 \times 10^3$ cfu/ml for heterotrophic bacterial count, while $2.0 \pm 0.12 \times 10^3$ cfu/ml was for coliform count.

Also, bakery 1 and bakery 11 wastewater treated with ground seeds inhibited the heterotrophic and coliform counts from the least concentration of 0.5mg though the fungal count was only inhibited at the highest concentration of 2.5mg for bakery 11, whereas it was reduced as the concentration reduced. The ground flower of the bakery wastewater treatment was able to inhibit at the concentration of 1.5mg for bakery 1 and 2.0mg for bakery 11, while the fungal count was also reduced but not inhibited even at the highest concentration. Bakery 1 and 11 wastewater pretreated with charcoal and ground seeds inhibited the total heterotrophic bacterial count, the fungal count was reduced as shown in table 8, while the ground flower treatment was also able to reduce from 1.5mg concentration for bakery 1 and 11. From the results, the reduction and inhibition effects of the microbial growth with ground seeds of *Moringa oleifera* could be attributed to the presence of antimicrobial activity such as the bioactive component which is responsible for rendering a definite action on microbial quality of the wastewater. The seeds of *Moringa oleifera* contain some chemical compounds of the antimicrobial peptide such as lectin, which exhibit effective antibacterial activity (Foidle *et al.,* 2001). The team also recorded that the water-soluble *Moringa oleifera* showed bactericidal effects against *Bacillus cereus, Micrococcus* sp, *Pseudomonas* sp, *Serratia marcescens* and *Staphylococcus aureus* by affecting their growth and survival.

The potency of *Moringa oleifera* on bakery wastewater pretreated with charcoal and *Moringa oleifera* seeds as shown o table 8 also indicated complete inhibitory effects of total heterotrophic and coliform counts from the least concentration, fungal count ranged from $1.0 \pm 0.08 \times 10^4$cfu/ml to$7.0 \pm 0.07 \times 10^4$cfu/ml, but bakery 1 wastewater inhibited at the highest concentration, while wastewater pretreated with charcoal and ground flowers of *Moringa oleifera* inhibited the total heterotrophic bacterial count at 1.5mg concentration for bakery 1 and 11 wastewater, total coliform inhibited from 1.0mg while there was no inhibition for fungal growth which reduction that ranged from $2.0 \pm 0.01 \times 10^4$cfu/ml to $10.0 \pm 0.08 \times 10^4$ cfu/ml.

The results have shown that pretreating the wastewater with charcoal has remarkable effects on the microbial population. It has an adsorption capacity of the dissolved gaseous liquid compound and dissolved substance thus the ability to absorb some organic compound in the wastewater thereby limiting microbial nutrients which could have interfered with their growth and multiplication Johnson (2005). The resistance of fungal growth in all samples might have been attributed to the finding of Latge (2010) that the fungal cell wall plays a critical role in physiology as, in addition to its structural function in defending cell shape and integrity which has a dynamic function in the interaction of fungal with their surroundings. Hence, fungal pathogens evolved various strategies to protect their cell walls and prevent the elicitation of cell wall-triggered immune responses in their host. The inhibition of the fungal growth at 2.5g concentration, is an indication that plants use several strategies of overcoming fungal attacks, including the production of antimicrobial portions and peptides. In general, these defense-related proteins found in plants and seeds can inhibit fungal growth as they bind to and disrupt the

fungal proper function of chitin a key component of the fungal cell wall as stated by Adelina *et al.* (2014).

The physicochemical parameter of the quality of the wastewater was measured before and after treatment to evaluate the removal efficiency of the major pollutants of concern in wastewater treatment such as BOD, COD, DO, turbidity, pH and temperature. Our results show that *Moringa oleifera* seeds are efficient as a primary coagulant in wastewater treatment for the removal of these pollutants. There is also an indication that activated charcoal can be used as an absorbent for the treatment of wastewater before disposal. The results show that the concentration of COD, Turbidity, DO and BOD in all the samples as shown in table 10, reduced when treated with activated charcoal, while, temperature and pH increased when the samples were treated with activated charcoal. Bakery 1 sample treated with seeds of *Moringa oleifera* revealed an increase in the concentration of pH, DO and COD in the sample treated with seeds, while, temperature, BOD and turbidity increases when treated with flower. Bakery 2 sample treated with seeds of *Moringa oleifera* revealed an increase in the concentration of pH and DO, while other parameters increase when treated with flower. The concentration of pH, DO, COD, and BOD in Brewery 1 increases when treated with the seeds of *Moringa oleifera*, while, temperature and turbidity increase when treated with flower, while in brewery 2 the concentration of pH, temperature and DO increases when treated with the seeds and turbidity, BOD and COD increases with the treatment with flower. This result reveals the efficacy of the flower and the seed of *M. oleifera* for the treatment of industrial wastewater in agreement. According to Saroj *et al.,* (1995) and Dalen *et al.*(2010), it has the capability of restoring the chemical nature of wastewater to a normal or portable stage in conformation with Saroj *et al.* (1995).

The preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, cardiac glycosides, saponins, deoxy sugar, while terpenes were present in ground seeds but not in the ground flowers and tannins in the ground flower but not in ground seeds. These are believed to be responsible for the observed efficacy effects. Rahman *et al.* (2009) also attributed their observed antimicrobial effects of these plant extracts to the presence of these secondary plants' metabolites. The high inhibition action on bacteria associated with wastewater also reveals that the plant has a broad spectrum effect as reported by Foidle *et al*. (2001). Though the ground seed has high efficacy than the ground flower, the flower and seed part of *M. oleifera* are active and low cost means of treatment of industrial wastewater.

4.0 Conclusion

The results indicate that ground seeds of *Moringa oleifera* from the least to the highest concentration were effective to inhibit bacterial load while the fungal load was only able to inhibit at the highest concentration of 2.0mg in bakery and brewery 1 and 11 wastewater, while the ground flower was able to reduce at the lowest concentration, but inhibited the load from 1.0mg concentration and fungal load at 2.5mg. This was possible because of the presence of water soluble cationic protein in the seeds of *Moringa oleifera* which aided in the coagulation. The present study reveals that *Moringa oleifera* seeds and flower powder is efficient and economically viable coagulant to treat wastewater. From the results, the optimum dosage for the treatment of wastewater with *Moringa oleifera* seeds and flowers should be 0.5mg/L.

5.0 References

Adelina, J., Jorge, C. G., Gabriela, R. B., Salvado, S., Benit, D. M. & Jain, T. (2014). Microbiology and pharmacological evaluation of the micro-propagated *Rubus liebermanni* medical plant. *Evidence Based Complimentary and Alternative Medicine*, 201, pp. 115-120.

Alo, M., Ukpai, A. E. & Chukwudi, A. (2012). antibacterial activities of water, ethanol and

methanol extract of *Ocimum gratissium, Veronia amygdalina* and *Afromomum leguera. Advanced in Applied Science Research,* 3, 2, pp. 844-848.

Amagloh, F. K. & Banang. B. (2009). Effectiveness of *Moringa oleifera* seeds as coagulant for water purification. *African Journal of Agriculture Research*,4, 1, pp. 119-123.

Arajat, M. G. & Mohamed, S. O. (2013). preliminary study on the efficacy of leaves, seeds and back extract of *Moringa oleifere* in reducing bacterial load in the water. *International Journal of Advance Research,* 6, pp. 2320-5407.

Briggs, D. (2003). Environmental pollution and the global burden of disease. *Britain Medicinal Bulletin*, 68, pp. 1–24.

Clecieri, L. S., Greenberg, A. E. & Eaton, A. D. (1999). *Standard methods for the examination of water and wastewater.* (20th ed.). Washington, DC: American Public Health Association, USA. pp. 211-214.

Collin, C. H., Lyne, P. M., Grenga, J. M. and Falleinern, J. O. (2004). *Microbiological methods.* (8th ed.). London: Arnold Publisher, p. 384.

Dalen, J., Smith, B. W., Shelley, B. M., Sloan, A. L., Leahign, L. & Begay, D. (2010). Mindful eating and living (MenL), weight, eating behavour and psychological outcomes associated with a mindfulness-based intervention for people with obesity. *Journal of Behavour, Health and Social Issues,*8, 2, pp. 260-264.

Eddy, N. O., Udoh, C. O. & Ukpong, I. J. (2004). Heavy metals in the sediment of the Cross River Estuary at Oron, South Eastern Nigeria. *African Journal of Environmental Pollution and Health,* 3, 1, pp. 6-10.

Eddy, N. O. & Ekop, A. S. (2007). Assessment of the quality of water treated and distributed by the Akwa Ibom Water Company. *E. Journal of Chemistry.* 4, 2, pp. 180-186

Foidle, N., Makkar, H. P. S. S. & Beckar, K. (2001). The potential of *Moringa oleifera* for agricultural and industrial uses. In: what development potential for *Moringa oleifera* product?. *http://miracletree.org/moringa.doc/the potential of moringa oleifera*

Henze, M., Haresmoes, P., Lacar, J. N. & Arvin, E. (2001). *Wastewater treatment biological and chemical process.* (3rd ed.). Springer-Varlag Berlin and Heidelberg Gimbn and Co. Kg (Germany), pp. 33-37.

Johnson, B. C. (2005). clinical perspectives on the health effects of *Moringa oleifera.* A promising adjunct for balance nutrition and better health. Kos health publication. http://www.koshealthpib.com.article.pdf/clinical-perpectives-moringa-aug.-05/pdf.

Joshi, D., Sanmontha, J., King, U., Alice, R. & Cynthia, S. W. (2012). Higher gamma-amino butyric and neutron density in the white matters of orbital frontal cortex in schizophrenia. *Neuroscience Research Australia,* 72, pp. 725-733.

Latge, J. P. (2010). Tasting the fungal all wall cell. *Cell Microbiology*, 12, pp. 863-868.

Lea, M. (2010). Bioremediation of turbid surface water using seeds extracts from *Moringa oleifera* Lam drumstick tree. *Current Protocol in Microbiology,* 20, pp. 1321-221.

Qasim, M., Gulzar, S., Shinwani, Z. K. & Aziz, I. (2000). Modeling of conventional water supply treatment. *Journal of Biological Science,* 39, pp. 52-59.

Rahman, M. M., Sheikh, M. M., Sharmin, S. A., Islam, M. S., Rahma, M. A., Rahman, M. M. and Alam, M. F. (2009). Antibacterial activity of leaf juice and extract of *Moringa oleifera* lam against some human pathogenic bacteria. *JJournal of Natural Science,* 8, 2, pp. 219-227.

Saroj, K. P., Pulk, K. M. and Saha, B. P. (1995). Studies on the antiulcer activity of *Moringa oleifera* leaf extract on gastric ulcer models

in rats. *Phytotherapy Research,* **9**(6):463-468.

Solowora, A. (2006). *Medicinal plants and traditional medicine in Africa*. (2nd ed.). Ibadan, Nigeria: Spectrum Book Limited, pp. 150-153.

Somani, A., Bealin-Kelly, F., Axcell , B. & Smart, K. A. (2012). Impact of storage temperature on lager brewing yeast viability, glycogen, trehalose, and fatty acid content. *Journal of America Society of Brewery Chemistry,* 70:, pp. 123–130.

Uchechukwu, O. F., Azubuike, O. S., Odoemelam, S. A. & Eddy, N. O. (2018). Kolanut pot husk: a potential biosorbent for Cd^{2+}, Ni^{2+} and Pb^{2+}. *African Journal of Environment and Natural Science Research,* 1, 2, pp. 1-9.

Uchechukwu, O. F., Azubuike, O. S. & Eddy, N. O.(2015). Biosorption of Cd^{2+}, Ni^{2+} and Pb^{2+} by the shell of *Pentaclethra macrophylla.* Equilibrium isotherm study. *Journal of Science, Technology and Environmental Informatics, 2, 1, pp. 26-35*

Conflict of Interest

The authors declared no conflict of interest