COMPARATIVE EFFECTS OF CONVENTIONAL AND NON-CONVENTIONAL FLOCCULANTS AND DISINFECTANTS ON MICROBIAL CONTAMINANTS IN WATER PURIFICATION

Abstract

Bioflocculants are microorganism-produced special natural inorganic macromolecule substances that can flocculate suspended solids, cells and colloidal solids. Bioflocculant purified from *Bacillus subtilis* isolated from the sediments of Onyearugbulem market stream inhibited the growth of coliform for well, stream and abattoir wastewater samples. On the other hand, alum sulfate when compared with bioflocculant at a dosage level of 200mg for well and stream waters and 800mg for abattoir waste water yielded flocculating activities of 87.33%, 78.42% and 24.60%. This indicates that purified bioflocculant was more effective than alum sulphate. Bioflocculant produced from *Bacillus subtilis* should be exploited in water treatment. *Moringa Oleifera* seed powder exhibited both flocculating and disinfecting potentials in water treatment but not effective in the treatment of abattoir wastewater.

Key Words Bioflocculant, *Moringa oleifera* seed powder, flocculating, disinfecting, water purification, *Bacillus subtilis*

Introduction

Forms by which water pollution can be accessed can be classified under three major headings: physical, chemical and biological methods (Onda *et al*., 2012).

This is characterized by the presence of objects and materials in water. These materials range from sediments/ particles to sticks both particulate and dissolved impurities. This evidence of water pollution can be accessed by measuring its turbidity. Water intended for drinking should not contain any foreign body; it must be clear and colourless. Other uses of (such as agricultural and aesthetic purposes) water may permit the presence of certain materials (Chapman and WHO, 2006).

This is characterized by a change in the chemical composition of the water. Water is made up of two atoms of hydrogen and one atom of oxygen. When there is an addition or subtraction from the chemical composition of water, such water is not fit for consumption. The following can be used to access the chemical composition of water: composition of trace metals and radionuclides, hardness, total dissolved solids and total soluble solids. Water fit for drinking must be tasteless and odourless (Flury, 1996).

This is characterized by the presence of microorganisms and organic materials that serve as nutrients for the growth of these microorganisms. These microorganisms are bacteria, fungi, protozoan and algae. Any water that contains these microorganisms is not safe for drinking (Hellawell, 2012).

An abattoir can be defined as any premise used for or in connection with the slaughter of animals whose meat is intended for consumption (Girards, 2005). Wastewaters from abattoirs are concentrated with the source of oxygen-consuming wastes. Abattoirs generally use large quantities of water for washing meat and cleaning process areas; they are usually located near large bodies of water for washing meat and processing (Nafarnda *et al*., 2006).

Flocculation is a stage in water purification which involves the gentle mixing of water. It brings about an increase in particle size from sub-microscopic micro-floc to visible suspended particles. On the addition of inorganic polymers such as alum and poly-aluminium chloride, floc size continues to build with additional collisions and interaction. Flocculation is a physical and chemical process used for the removal of the visible sediments and material from water which makes it a colloidal solution. This can be achieved through agitation or by the addition of flocculating agents (Moghimipour *et al*., 2014). Chemical flocculants include clarifying agents such as Iron (II) sulphate, Aluminium sulphate, and Iron (II) chloride in water treatment which results in the formation of colloids (Vasantharaj and Hemashenpagam, 2013).

Bioflocculants are microorganism-produced special natural inorganic macromolecule substances that can flocculate suspended solids, cells and colloidal solids (Zaki *et al.*, 2011). Several microorganisms which secrete flocculation biopolymer have been screened and isolated from activated sludge, wastewater, and soil (Zaki *et al.*, 2011). Species of microorganisms that have bioflocculant producing characteristics include bacteria (such as *Bacteroides*, *Bacillus* sp., *Bacillus mucilaginous*, *Bacillus subtilis*) fungi, actinomyces and algae (*Chlamydomonas reinhardtii, Chlorella minutissima)* (Okaiyeto *et al.*, 2013). Bioflocculants stands out among others as they have the advantage of innocuousness, biocompatibility, biodegradability and environmental friendliness, unlike organic and inorganic flocculants which are toxic and whose degradation intermediates are difficult to remove from the environment

(Okaiyeto *et al*, 2015). Besides, organic flocculants such as polyacrylamide and polyethene imine derivatives have been implicated in adverse human health effects (Nwodo *et al*., 2014). Conversely, the enormous advantages associated with bioflocculants motivate its consideration as an alternative, hence the vast interest in the scientific and industrial community worldwide (Nwodo *et al*., 2012).

Chlorination is defined as a form of chemical treatment of water that involves the addition of chlorine (Cl_2) or hypochlorite to water. Chlorination plays a key role in wastewater treatment of water processes by removing pathogens and other physical and chemical impurities. The effects of chlorine in wastewater treatment include disinfection, controlling odour and septicity, aiding scum and grease removal. Chlorination also helps to control activated sludge bulking foaming. It stabilizes waste activated sludge before disposal, destroys cyanides, removes phenols and ammonia (Hussain *et al*., 2002).

Description of the study area

Onyarugbulem abattoir got its name from the market (modern) and office complexes to the north of abattoir built by Navy Captain Anthony Ibe Onyearugbulem (1955-2002) who served as the military administrator of Ondo State during the regime of General Sani Abacha. Onyearugbulem abattoir was selected as the study area because of its location in the large expanse of the built-up area comprising of low, medium and high-income earners with residential buildings in the north by office complexes and west and east by private schools and ships. The abattoir is about 50 meters off the express (Ilesha-Owo) and covers about 1000m^2 landmass.

Materials and Methods

Separation and Purification of Bioflocculants

Purified isolates were introduced into 50ml of bio-flocculant production medium and then incubated for 3days. The culture broth was diluted into two volumes of distilled water and centrifuged at 4,000 rpm for 15 minutes. The supernatant was poured into three volumes of acetone (1:3) and added three times to precipitate the biopolymer flocculant. The precipitate was then centrifuged at 8000 rpm for 20mins and washed by ether. The crude obtained was dialyzed at $4^{o}C$ overnight in deionized water and vacuum dried overnight in a desiccator to obtain pure bio-flocculants (Elkady *et al*., 2011).

Jar test determination of bioflocculant dosage and measurement of Bioflocculaing activity

Different concentrations (0.1 to 1.0 mg/mL) of purified bioflocculant were prepared. Their flocculating activities were measured against 4gL kaolin clay suspension. A 3.0 mL of 1% (w/v) $CaCl_2$ was added to the different concentrations of the purified bioflocculant and mixed with 100 mL of kaolin clay suspension in 500ml beakers. The solution was rapidly mixed at 160 rpm for 2 minutes, followed by gradual flocculation at 40 rpm for 2 minutes and sedimentation for 5minutes. After sedimentation, 2 mL was gently withdrawn from the upper clarifying phase to measure the flocculating activity. The concentration dosage that gave the best flocculating activity was used for the subsequent experiment (Elkady *et al*., 2011).

Preparation of dialysis bag

Ethylenediaminetetraacetic acid (EDTA) (0.27g) was weighed into 100 mL of distilled water which was boiled. The dialysis bag was placed in the boiling water and was made to boil. The bag was removed and rinsed with distilled water. This process aids the easy opening of the dialysis bag (Elkady *et al.*, 2011).

Preparation of moringa seed powder, alum and chlorine for water treatment

Good quality *Moringa oleifera* seeds were harvested from FUTA area and made to dry in the sun. The pods and shell were removed after drying after which it was blended with the aid of an electric blender. The resulting powder was used in the water treatment process as a biological flocculant and disinfectant. This was used in varying quantities for the treatment of the water samples of 1000 mL in the following quantities: 0.1g, 0.2g and 0.4g. Three hundred grams (300g) of aluminium potassium sulphate purchased from Akure market was crushed using mortar and pestle. It was then employed in the water treatment process within varying quantities (0.1g, 0.2g, 0.4g) to 1000 mL of the waters sample. Sodium hypochlorite was used in concentrations of 1, 2 and 4 mL to disinfect 1000mL of stream water and 2,4 and 8mls to disinfect 1000 mL of abattoir water. (Amagloh and Benang, 2009).

Water treatment with the varying dosage of Alum, Biofloculant, Chlorine and *Moringa oleifera* Seed Powder

Test for best concentration of treatment agent

Different concentrations such as the recognized and recommended dosage, half of the recommended dosage and double dose of treatment agents (alum, bioflocculant, chlorine and moringa) were used to detect the dosage best fit for the treatment analysis. After allowing the water sample to sit for 30 minutes after collection, the supernatant was carefully collected in another clean container; 1000 mL of each water sample was in triplicate differently dosed with the recommended dosage, half of the recommended and double dose of the recommended. The optical density of the water samples was detected with the aid of a spectrophotometer 30minutes after treatment and 24hours after. The microbiological load was also detected after 30 minutes of treatment and 24 hours of treatment (Elkady *et al*., 2011).

The selected dose for each of the treatment agents (alum, bioflocculant, chlorine and moringa) were used to treat in triplicates1 litre of the collected water samples in sterilized containers the way it was done above. The treatment agents were used in the following order: each treatment agent: alum; bioflocculant; chlorine; moringa seed powder. Combinations of two treatment agents: bioflocculant and chlorine; bioflocculant and moringa seed powder; chlorine and moringa seed powder. Combinations of three treatments; alum, bioflocculant and chlorine; alum, bioflocculant and moringa seed powder; alum, chlorine and moringa seed powder; bioflocculant, chlorine and moringa seed powder. Then all the four treatment agents combined alum, bioflocculant, chlorine and moringa seed powder. Each of these water samples had control where the above-listed agents were not added (Amagloh and Benang, 2009; Elkady *et al*., 2011).

The optical density of the water samples was detected with the aid of a spectrophotometer at a wavelength of 550 nm 30minutes after treatment and 24hours after. The microbiological load was also detected after 30 minutes of treatment and 24 hours of treatment (Elkady *et al*., 2011; Cosa *et al*., 2013).

A twenty millilitre (20ml) of cell-free supernatant suspension of the bio-flocculant was added to 1 litre each water sample and was allowed to stand for 10 minutes. The optical density of the clarifying solution was then measured with a spectrophotometer at 550 nm.

The flocculating ability of the bacterium polymer was measured using the equation

$$; Flocculating\ Activity\ (\%) = \frac{(B-A)}{A}\ X\ 100$$

Where A is the absorbance of the sample experiment, B is the absorbance of the control experiment at 550 nm. (Cosa *et al*., 2013; Ugbenyen and Okoh, 2013).

Statistical Analysis

Data are presented as mean ± standard error (SE). Significance of difference between different treatment groups was tested using one-way analysis of variance (ANOVA) and significant results were compared with Duncan's multiple range tests using SPSS window 8 versions 20 software. For all the tests, the significance was determined at the level of $P<0.05$.

Results

Flocculating activities of water samples treated with alum recorded their highest flocculating activities with increased dosage. Flocculating activities increased after 24 hours of treatment as against the flocculating activities recorded after 30 minutes of treatment. The flocculating activities recorded when well and stream waters were treated had flocculating activities above average while that of abattoir wastewater was between the range of 21-25% (Table 1).

Table 2 shows the flocculating activities suggesting the best dosage of bioflocculant treatment for water samples. Highest flocculating activities were recorded with the highest bioflocculant concentration except for stream water after 30 minutes of treatment which it's recorded highest flocculating activities when 0.5mL of bioflocculant was used in water treatment. Abattoir wastewater recorded its highest flocculation after 30 minutes of treatment with 0.5mL, the flocculating activity reduced after 24 hours. Treatment with 1.0 mL of bioflocculant reduced flocculating activity after 24 hours of treatment.

Well, water, when treated with *Moringa oleifera* seed powder, had a steady increase in its flocculating activity over time with an increase in its doses. Increase in flocculating activity was also recorded after 24 hour treatment period. The case was also the same with stream and abattoir wastewaters. However, the flocculating activity of abattoir wastewater was low when placed side by side with well and stream waters (Table 3).

Table 1 **Flocculating activities suggesting the best dosage of alum treatment for the water samples**

Dosage	Well Water		Stream Water		Abattoir Waste Water	
(mg/L)	30 mins	24 hours	30 mins	24 hours	30 mins	24 hours
50	65.34±1.57[a]	75.34±0.58[a]	71.47±0.69[a]	75.50±1.15[a]	25.07±2.01[a]	23.45±0.58[a]
100	72.25±1.15[b]	76.53±0.53[a]	72.58±1.45[a]	76.53±0.53[a]	23.30±0.58[b]	21.48±0.00[b]
200	78.20±0.63[c]	87.33±0.58[c]	78.20±0.64[b]	87.33±0.58[b]	24.30±0.58[b]	24.60±0.15[b]

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same row are not significantly different ($P<0.05$).

Table 2 **Flocculating activities suggesting the best dosage of bioflocculant treatment for the water samples**

Dosage (ml/L)	Well Water		Stream Water		Abattoir Waste Water	
	30 mins	24 hours	30 mins	24 hours	30 mins	24 hours
0.5	63.24±1.21[a]	69.55±1.15[a]	95.35±0.00[a]	96.14±0.05[a]	25.08[c]±2.02[a]	23.45±0.58a

1.0	87.39±0.69^{b}	88.09±1.15^{b}	96.04±0.02^{b}	96.67±0.16^{b}	23.30±2.02^{b}	21.48±0.00^{b}
2.0	88.07±0.58^{b}	88.65±1.21^{b}	96.190.05^{b}9	96.93±0.17^{c}	24.30±0.58^{c}	24.60±0.15^{b}

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same row are not significantly different (P<0.05).

Table 3 Flocculating activities suggesting the best dosage of *Moringa oleifera* seed powder treatment for the water samples

Dosage (g/L)	Well Water		Stream Water		Abattoir Waste Water	
	30 mins	24 hours	30 mins	24 hours	30 mins	24 hours
50	63.82±0.58[a]	66.98±0.59[a]	65.82±1.15[a]	69.00±0.58[a]	47.40±0.88[a]	47.20±0.00[a]
100	66.20±1.53[a]	67.50±0.33[a]	67.20±1.15[a]	69.17±0.58[a]	52.37±1.15[b]	55.00±1.15[b]
200	66.00±0.58[b]	73.43±2.84[a]	68.10±0.51[a]	72.00±1.15[b]	55.50±1.15[b]	58.01±0.58[b]

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same row are not significantly different (P<0.05).

When treatment combinations alum, bioflocculant, chlorine, and *Moringa oleifera* seed powder was applied in the treatment of well water, treatment with alum alone yielded the least flocculating activity. Combined treatment of alum and bioflocculant had the highest flocculating activity. There was a slight increase in flocculating activity after 24 hours of treatment (Figure 1).

Singular treatment of stream water with chlorine yielded the lowest flocculating activity. Combined treatment of AB had the highest flocculating activity with B, BM, ABM, ACM, BCM and BM following closely. All these treatment combinations recorded a slight increase in its flocculating activity after 24 hours of treatment (Figure 2).

Singular treatment of alum recorded the lowest while the highest flocculating activity was recorded when the combined use of alum and bioflocculant was used in abattoir wastewater treatment (Figure 3).

Table 4 presents the number of coliform growth colonies on the agar plate. The number of growth colonies reduced with increased concentration and time in all the water samples treated. However, the lowest numbers of colonies were recorded in well water and highest in abattoir wastewater.

Total bacterial growth of water samples treated with varying concentrations of alum is presented in Table 5. The number of colonies reduced with increased concentration and time in the treatment of well and stream water samples. After 30 minutes of treatment with alum at 400mg concentration, flocculating activity increased beyond that of 200mg. the flocculating activity however reduced after 24 hours of treatment.

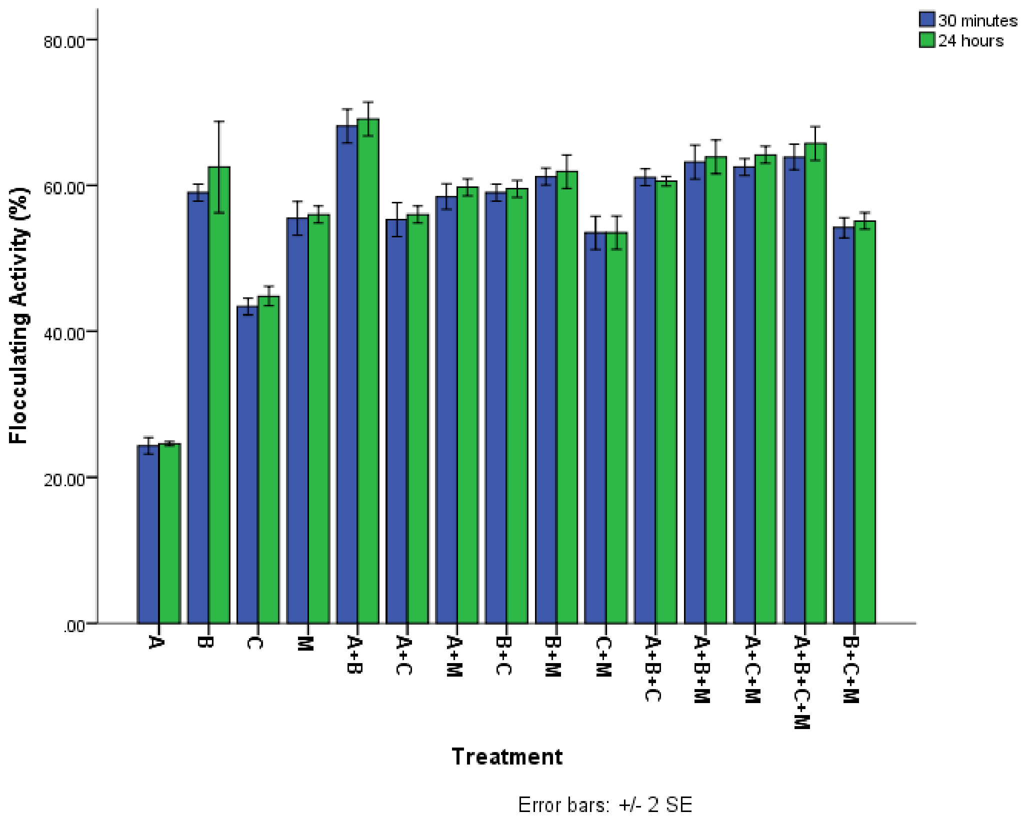

Figure 1 **Flocculating activities of well water samples treated with different combinations**

KEY A- Alum B- Bioflocculant C-Chlorine M-Moringa

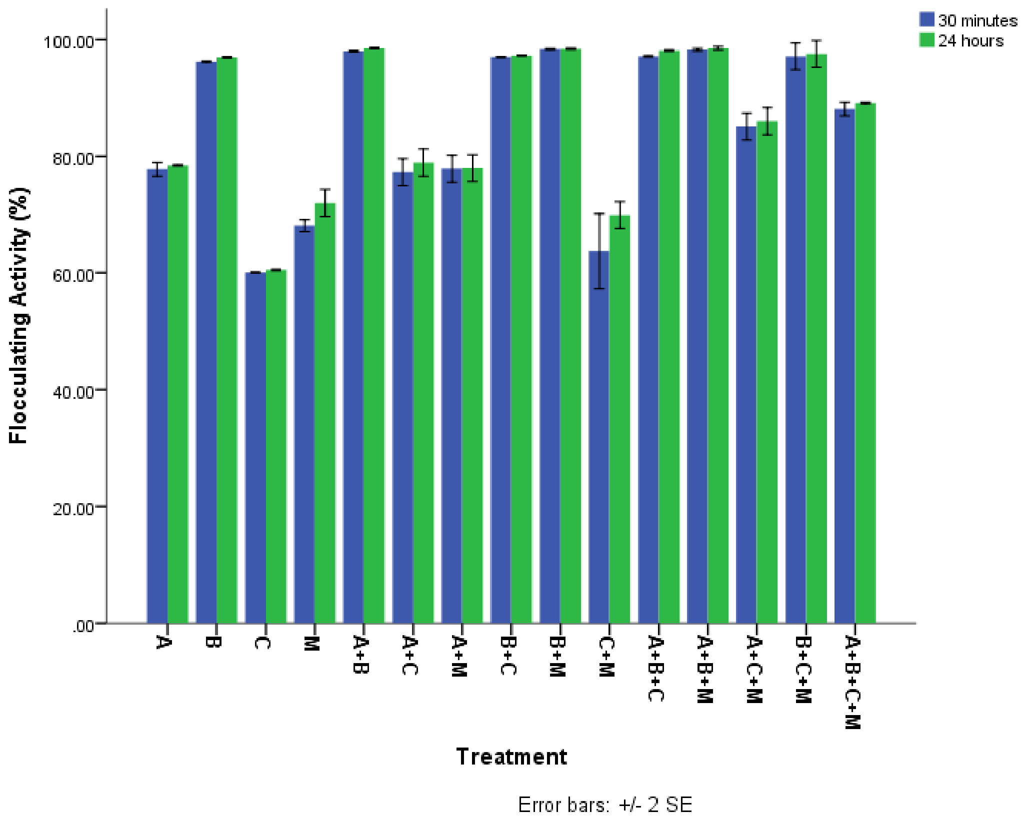

Figure 2 Flocculating Activities of a stream water sample with different treatment combinations

KEY A- Alum B- Bioflocculant C-Chlorine M-Moringa

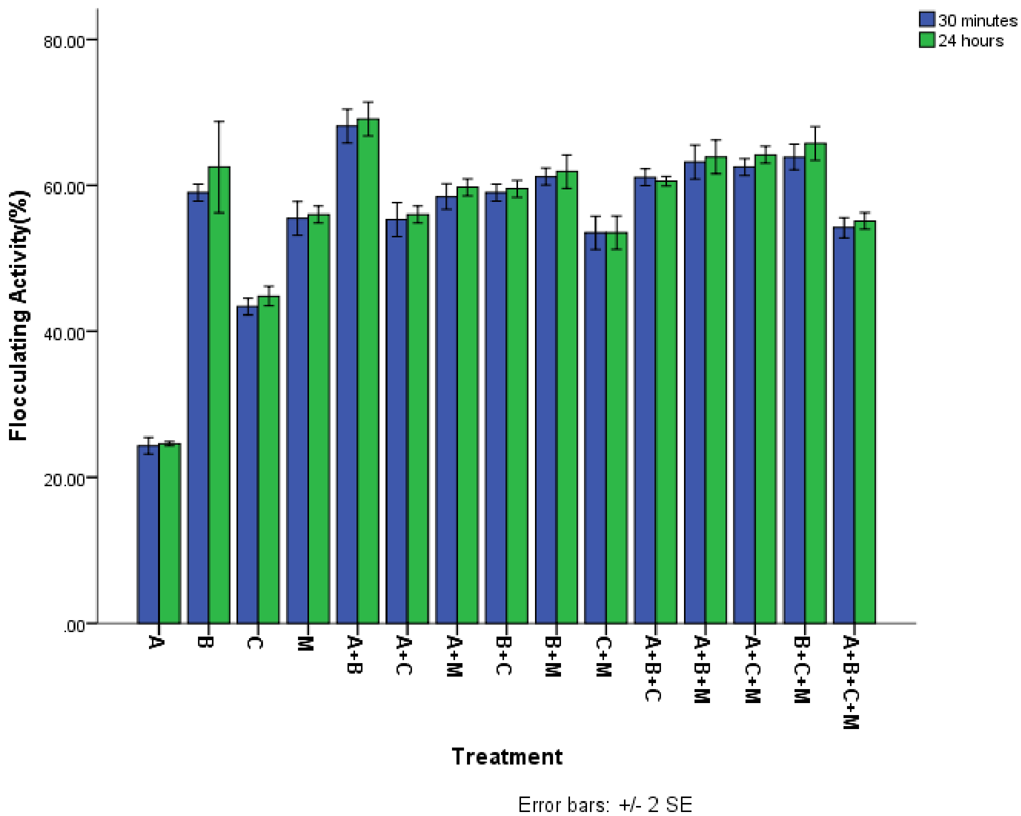

Figure 3 Flocculating Activities of abattoir wastewater sample with different treatment combinations

KEY A- Alum B- Bioflocculant C-Chlorine M-Moringa

Table 4 Coliform Growth of water samples treated with alum using varying concentrations

Dosage	Well Water		Stream Water		Abattoir Waste Water	
(mg/L)	30 mins	24 hours	30 mins	24 hours	30 mins	24 hours
50	14.33±1.20[a]	11.66±0.88[a]	24.00±0.57[a]	22.00±0.57[a]	64.66±1.45[a]	61.00±0.57[a]

100	14.00±0.58[ab]	11.00±0.58[ab]	21.00±0.58[b]	20.67±0.88[a]	57.67±0.88[b]	54.00±0.57[b]
200	11.00±0.58[b]	10.00±0.58[b]	22.33±0.88[b]	19.33±0.33[a]	47.00±1.15[c]	44.00±0.57[c]

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same row are not significantly different (P<0.05).

Table 5 Total bacterial Growth of water samples treated with alum using varying concentrations

Dosage	**Well Water**		**Stream Water**		**Abattoir Waste Water**	
(mg/L)	**30 mins**	**24 hours**	**30 mins**	**24 hours**	**30 mins**	**24 hours**
50	22.33±0.88[a]	20.00±0.58[a]	33.33±0.88[a]	29.67±0.88[a]	75±1.73[a]	69.33±0.33[a]
100	21.67±0.88[a]	19.67±0.67[a]	30.00±0.58[b]	28.00±1.00[a]	66±1.15[b]	61.00±0.58[ab]
200	20.00±0.58[a]	19.00±0.58[a]	27.33±0.33[c]	27.33±0.33[a]	73±0.58[b]	41.00±10.50[c]

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same row are not significantly different (P<0.05).

Bioflocculant treatment yielded a significant reduction in the numbers of coliform colonies with increased dosage and time when treated with stream water. Abattoir wastewater followed the same sequence. However, in the treatment of AWW, the number of colonies were greatly increased (Table 6).

Treatment of water samples with bioflocculant yielded a steady decrease in the flocculating activities of the water samples with increased concentration of treatment agent and time (Table 7).

About 90% reduction was recorded when *Moringa oleifera* seed powder was used in the treatment of water samples in the number of coliforms concerning concentration and time (Table 8).

The steady decrease was recorded in the bacterial population with increased concentration and treatment time (Table 9).

Figure 4 shows the effect of treatment combinations on the number of coliform for well well water sample. Treatment combinations BM, CB, ABC, ABM and ACM yielded no growth. Singular treatment with alum yielded the highest number of the colony after 30 minutes of treatment which further reduced after 24 hours.

Singular treatment of well water with chlorine yielded no bacterial growth. No colony growth was recorded with AM, AC, BC, CM, ABC, ACM, BCM, and ABCM. The highest number of the colony was recorded after 24 hours of treatment with BCM (Figure 5).

Table 6 **Coliform Growth of water samples treated with bioflocculant using varying concentrations**

Dosage (ml/L)	Well Water 30 mins	Well Water 24 hours	Stream Water 30 mins	Stream Water 24 hours	Abattoir Waste Water 30 mins	Abattoir Waste Water 24 hours
0.5	15.67±0.67[a]	12.33±1.45[a]	7.00±0.58[a]	5.33±0.88[a]	183.67±0.88[a]	181.00±0.58[a]
1.0	7.3±1.45[b]	5.00±1.15[b]	3.33±0.88[a]	2.67±0.88[a]	180.00±0.58[b]	178.00±1.52[b]
2.0	0.67±0.67[c]	0.00±0.00[c]	4.00±0.58[b]	1.33±0.33[b]	78.33±0.33[c]	77.00±0.58[b]

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same row are not significantly different (P<0.05).

Table 7 **Total bacterial Growth of water samples treated with bioflocculant using varying concentrations**

Dosage (ml/L)	Well Water 30 mins	Well Water 24 hours	Stream Water 30 mins	Stream Water 24 hours	Abattoir Waste Water 30 min	Abattoir Waste Water 24 hours
0.5	30.006±0.58[a]	28.00±0.58[a]	26.00±2.08[a]	24.68±1.76[a]	200.00±0.00[a]	198.00±0.58[a]
1.0	27.00±1.15[b]	25.00±0.58[b]	20.00±0.58[b]	18.33±0.33[b]	199.00±0.50[a]	199.33±0.88[b]
2.0	24.00±0.58[c]	22.00±1.15[c]	18.00±0.58[c]	16.67±0.89[c]	93.33±0.88[a]	87.33±0.88[c]

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same row are not significantly different (P<0.05).

Table 8 Coliform Growth of water samples treated with *Moringa oleifera* seed powder using varying concentrations

Dosage	Well Water		Stream Water		Abattoir Waste Water	
(mg/L)	30 mins	24 hours	30 mins	24 hours	30 mins	24 hours
50	56.33±0.88^{a}	52.33±1.45^{a}	95.67±1.20^{a}	70.00±2.51^{a}	185.67±0.33^{a}	178.67±8.17^{a}
100	52.67±1.20^{b}	49.67±1.20^{b}	32.67±1.45^{b}	30.00±4.58^{b}	182.33±1.20^{b}	169.33±0.88^{b}
200	8.00±0.58^{c}	7.33±0.33^{b}	21.00±0.58^{c}	18.00±0.58^{c}	40.00±0.58^{c}	35.33±0.88^{b}

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same row are not significantly different (P<0.05).

Table 9 Coliform Growth of water samples treated with *Moringa oleifera* seed powder using varying concentrations

Dosage	Well Water		Stream Water		Abattoir Waste Water	
(mg/L)	30 mins	24 hours	30 mins	24 hours	30 mins	24 hours
50	94.67±0.88^{a}	90.00±0.58^{a}	121.67±1.20^{a}	140.67±2.17^{a}	197.67±0.33^{a}	195.09±0.58^{a}
100	80.33±0.88^{b}	70.00±0.58^{b}	73.00±1.15^{b}	62.00±1.15^{a}	186.67±2.84^{b}	176.67±2.19^{b}

200	18.33±0.33[c]	16.33±0.33[c]	26.00±0.58[c]	24.00±0.58[b]	70.00±0.58[c]	66.00±0.58[c]

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same row are not significantly different (P<0.05).

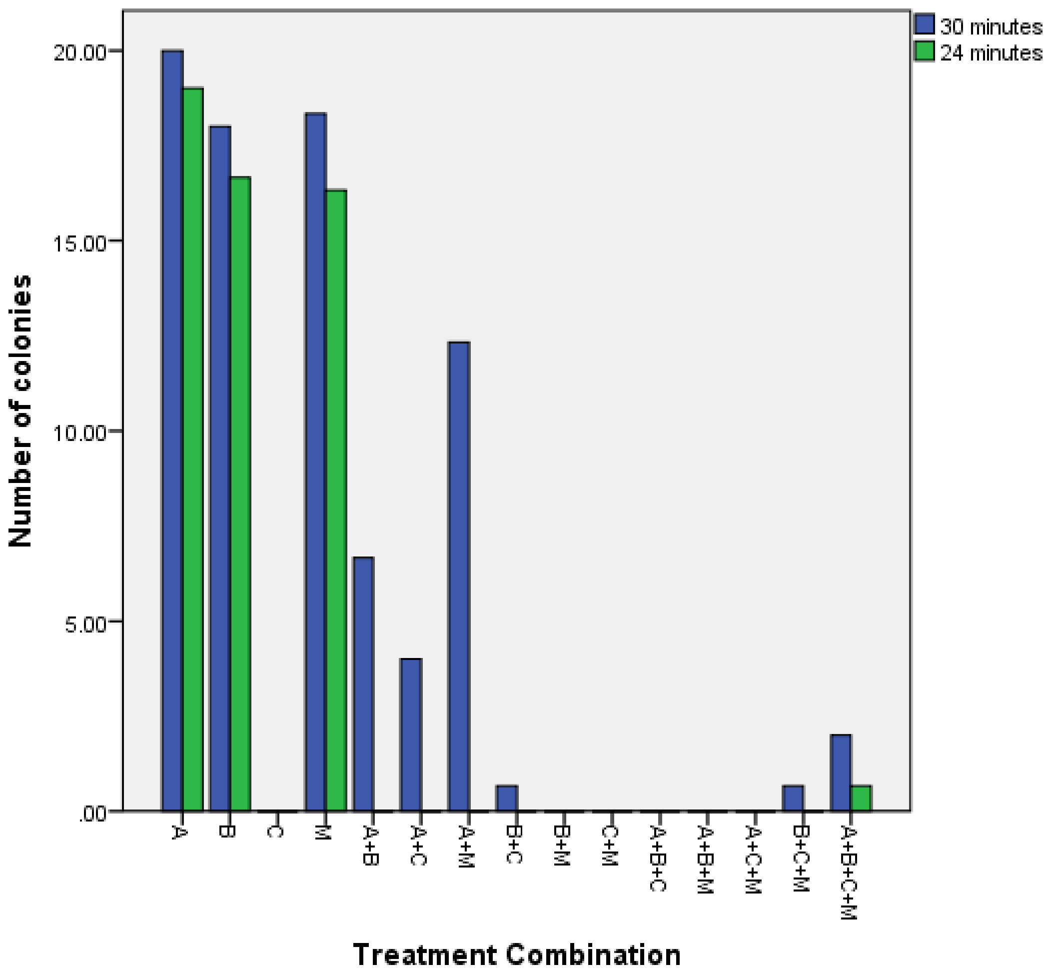

Figure 4 **Effect of various treatment combinations on the number of coliform bacteria for well water sample**

KEY A- Alum B- Bioflocculant C-Chlorine M-Moringa

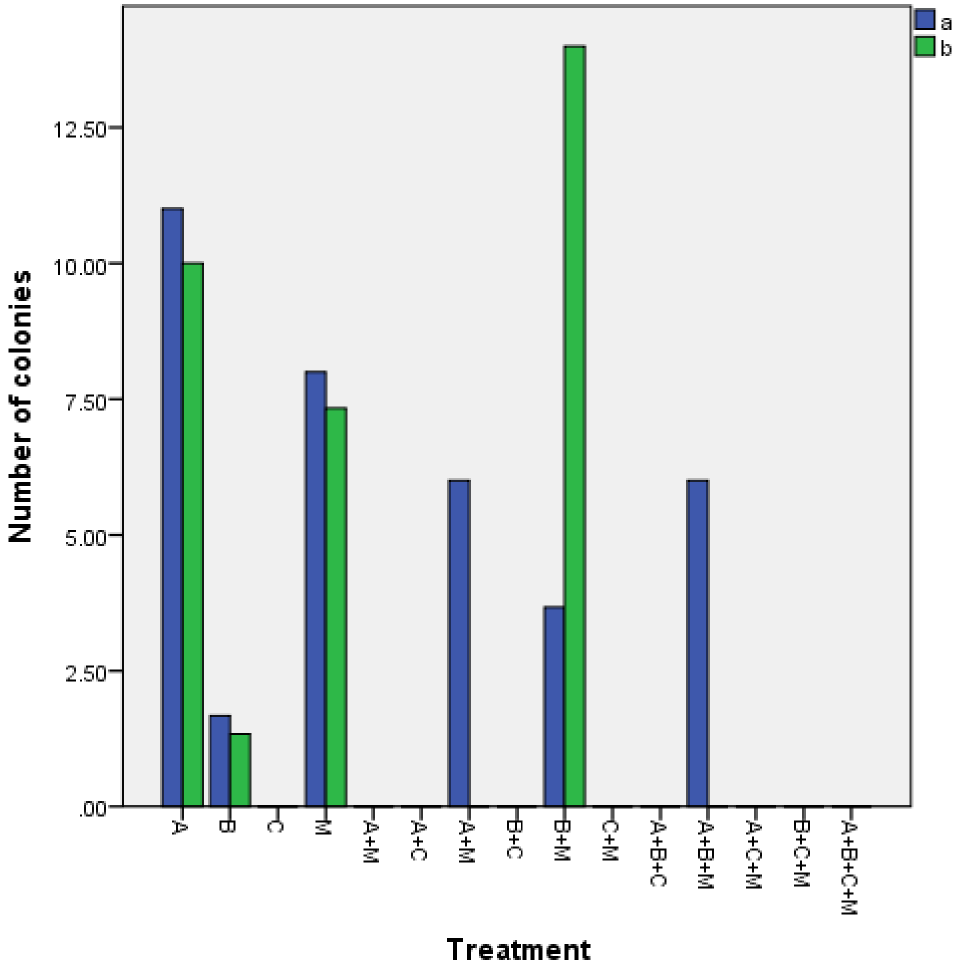

Figure 5 **Effect of various treatment combinations on the number of bacteria for well water sample**

KEY A- Alum B- Bioflocculant C-Chlorine M-Moringa

In figure 4, the highest number of colonies was gotten after 30 minutes of treatment with alum which subsequently reduced after 30 minutes. No coliform growth was observed with BM, CM, ABC, ACM, BCM, ABCM.

Figure 5 shows that no bacterial growth occurred with the following treatment patterns: C, CM, ABC, ACM. The highest number of bacterial growth was recorded with A.

No coliform growth was recorded in the treatment of AWW with AC, CM, ABC, ABM, BCM and ABCM. The highest FA was recorded in alum and AM after 30 minutes of treatment with significant reduction after 24 hours (Figure 6).

Treatment with M, CM, ABC, ABM, ACM, BCM and ABCM did not yield any growth in the treatment of AWW. The highest FA was recorded with treatment after 24 hours. Treatment with bioflocculant did not bring about any change with time (Figure 7).

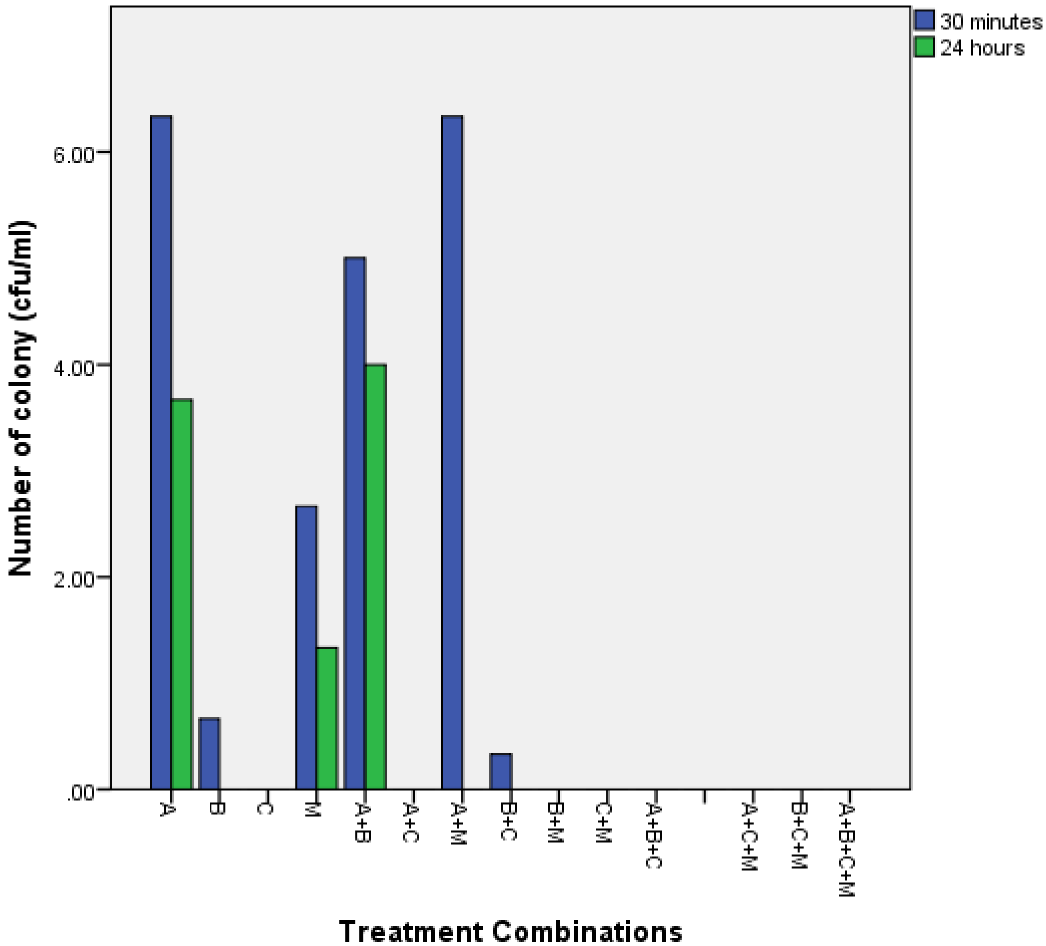

Figure 6 **Effect of various treatment combinations on the number of coliform bacteria for stream water sample**

KEY A- Alum B- Bioflocculant C-Chlorine M-Moringa

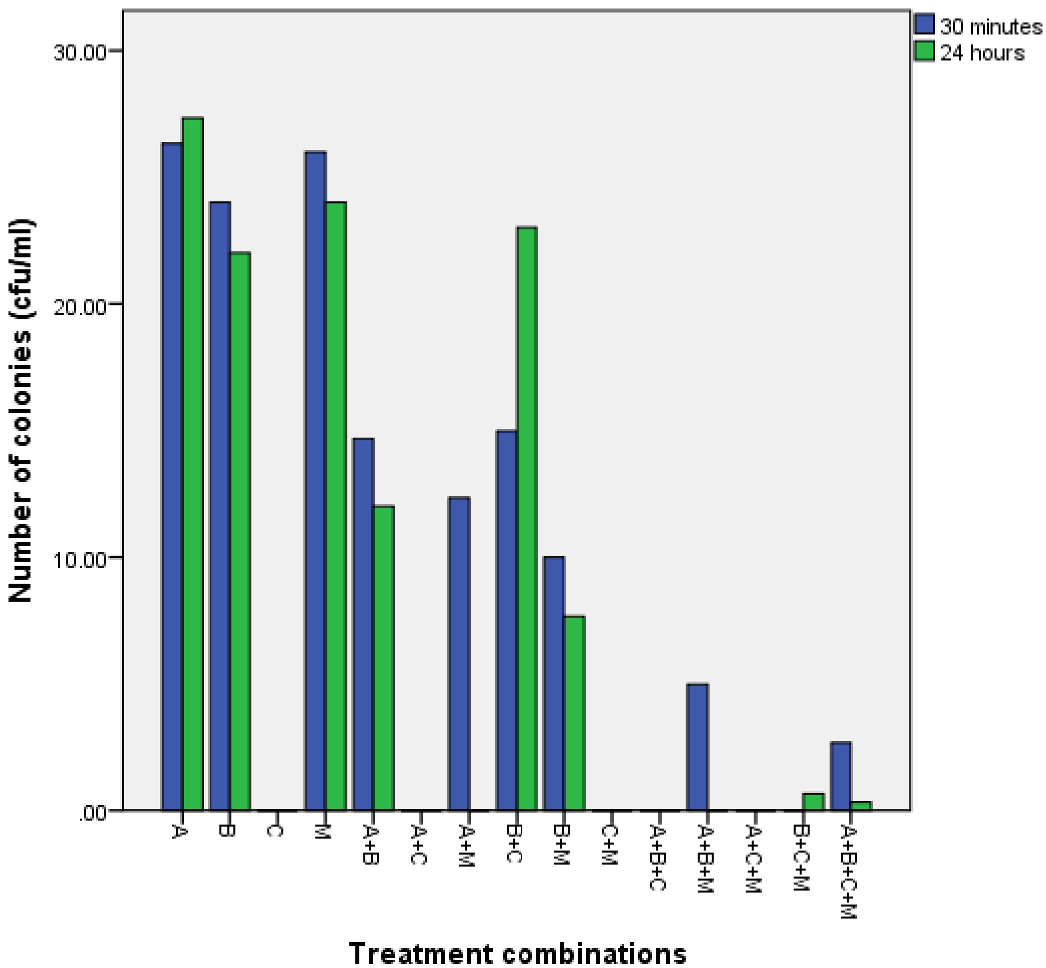

Figure 7 **Effect of various treatment combinations on the number of bacteria growth on NA for stream water sample**

KEY A- Alum B- Bioflocculant C-Chlorine M-Moringa

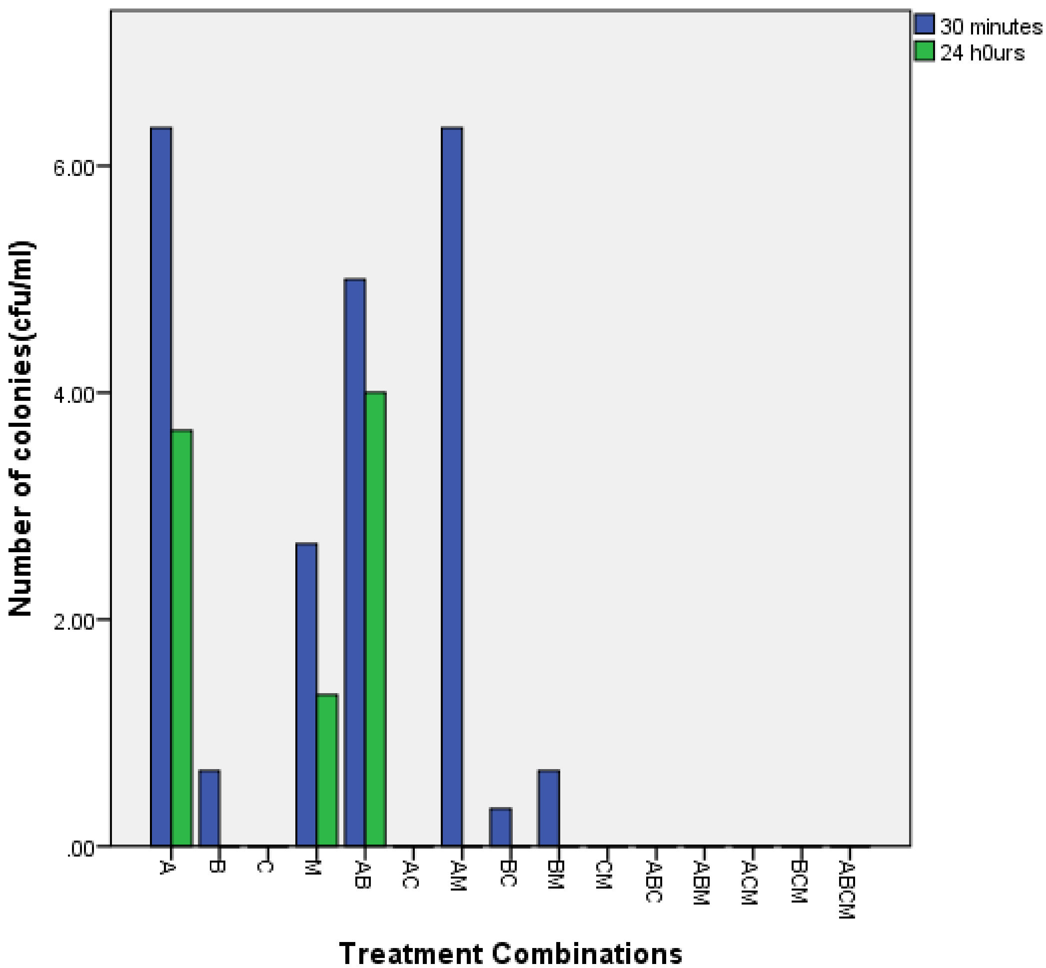

Figure 8 Effect of various treatment combinations on the number of coliform bacteria for abattoir wastewater sample

KEY A- Alum B- Bioflocculant C-Chlorine M-Moringa

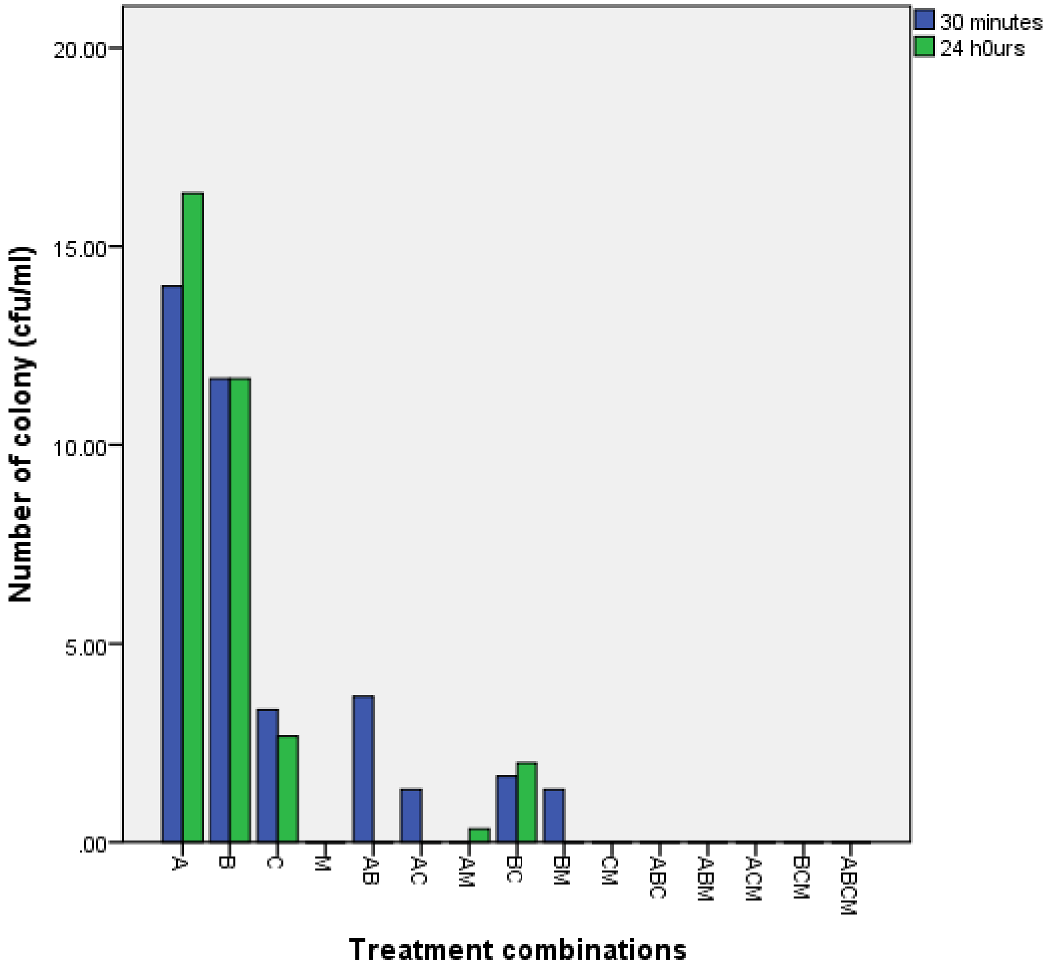

Figure 9 Effect of various treatment combinations on the number of bacteria growth on NA for abattoir wastewater sample

KEY A- Alum B- Bioflocculant C-Chlorine M-Moringa

Discussion

The array of bacteria isolated from the well water sample are typical of well water resident bacteria. Ngwa and Chrysathus (2013) isolated *Klebsiella* sp, *Escherichia coli*, *Salmonella* sp, *Enterobacter* and *Proteus* sp from well water sources in Bambui, Cameroon student residential area. Agwaranze *et al*., (2017) also isolated E. *Coli*, *Staphylococcus aureus*, *Pseudomonas* sp, *Enterococcus* sp and *Enterobacter* sp from well water sources in Wakuri, Nigeria. Ngwa and Chrysathus (2013) stated that most of the bacterial species isolated during their study are faecal related organisms. This indicates that these organisms might have been introduced into well water by faecal contamination. Nafarnda *et al*, (2012) isolated E. *coli* and Fecal *Streptococcus* from stream water bodies with which abattoir waste effluents are discharged. Benka-Coker and Ojior (1995). Isolated bacterial species from the genus *Salmonella*, *Escherichia*, *Shigella*, *Klebsiella*, *Streptococcus*, and *Staphylococcus* from Ikpoba river, Nigeria. The presence of these organisms was as a result of faecal discharge into the water body in that the abattoir wastewater contains a high percentage of coliform bacteria because of the contents of the gastrointestinal tracts of the slaughtered animals are discharged directly into the water. Nafarnda *et al*, (2012) stated that the presence of faecal coliform may also indicate the presence of pathogenic viruses and protozoa.

Virtually all the bacteria species isolated from stream water were found resident in the Onyearugbulem abattoir waste water. The reason cannot be farfetched based on the fact that the bacterial species found in the stream were sourced from the abattoir effluents. Mittal (2004) stated that abattoir wastewater contains several million colony forming units (cfu) /100 ml of total coliform, faecal coliform, and *Streptococcus* groups of bacteria. The bacterial species isolated from abattoir slaughtering site include *Escherichia coli*, *Citrobacter* sp, B. *subtilis*, *Bacillus cereus* (Habtamu *et al*., 2013), *Micrococcus leteus* and *Klebsiella pneumoniae.* (Nandita *et al*., 2015). The bacterial species isolated from the stream bank are in relation to the ones isolated by Onuoha, (2017). They are *Staphylococcus aureus, Escherichia coli*, *Pseudomonas* sp, *Bacillus cereus*, *Proteus* sp. The bacteria species isolated from the stream sediments correlates with the ones isolated by Eniola and Olayemi (2008). They include *Staphylococcus aureus*, *Klebsiella* sp, *Clostridium* sp, *Shigella* sp, *Pseudomonas* sp, *Escherichia coli*, *Streptomyces* sp, *Salmonella* sp, *Bacillus cereus*, *Enterobacter* sp. Ntozonke *et al*. (2017) isolated his bioflocculant producing bacteria from the sediments of a stream. This agrees with the findings of this research where two bacterial species (*Bacillus subtilis* and *Streptomyces somaliensis)* were isolated from Onyearugbulem stream sediments.

Nwodo *et al.* (2014) used a consortium of *Streptomyces* and *Cellulomonas* isolated from Tyurine river in the eastern cape province of South Africa to produce bioflocculant which was capable of initiating flocculating activities between 78 -91%. Shimofuruya *et al.* (1996) isolated and produced biopolymer from *Streptomyces griseus*. This biopolymer was capable of aggregating kaolin particles to form small flocks. Manisvasgan *et al*., (2015) produced bioflocculant from *Streptomyces* sp MBRC-91.

The best flocculating activity was achieved with the highest concentration and time when alum, bioflocculant purified from *Bacillus subtilis* and *Moringa oleifera* seed powder was used in the treatment of water samples. Yuliastri *et al* (2016) used 80mg/l and 100mg/l of *Moringa oleifera* seed powder in groundwater and wastewater treatment. The choice of dosage for *Moringa oleifera* seed powder is relative. *Bacillus mucilaginous* MBFA9 isolated from soil sample had a good flocculating capability which achieved a flocculating rate of 99.6% for kaolin suspension at a dosage of 0.1ml/l (Deng *et al*., 2003). A 20 mg/l bioflocculant dosage was sufficient in providing more than 85% humic acid removal (Zouboulis, 2004). The dosage of bioflocculant used in water treatment is dependent on the source, composition and the method of purification of the bioflocculant.

Well, water when treated with different combinations of alum, bioflocculant, chlorine and *Moringa oleifera* seed powder. There was a general increase in the flocculating activities of each treatment combination after 24 hours of treatment. The combined therapy of alum and bioflocculant had the highest flocculating activity followed by the combined therapy of alum, bioflocculant, chlorine and *Moringa oleifera* seed powder. Alum recorded the lowest flocculating activity. The increase in the flocculating activity with time could be as a result of enough contact time of alum with the particles resident in the water. High flocculating activity recorded with the combined use of alum and bioflocculant could be as a result of the fact that both alum and bioflocculants are flocculants. The difference only lies in the physical and chemical nature. The combined treatment of stream water also witnessed an increase in the flocculating activity of each treatment parameter, the same was also experienced in the treatment of abattoir wastewater. B proved to be the most effective flocculating agent for stream water while C brought about the lowest flocculating activity. The reason behind this cannot be farfetched as chlorine itself is a disinfecting agent and not a flocculant.

The coliform count of the water samples treated with alum, there was a general reduction in the growth of coliform concerning increased dosage and time. Bioflocculant purified from *Bacillus subtilis* eliminated coliform in well water after 24 hours of treatment. The coliform count of stream water was reduced to one. *Moringa Oleifera* proved more effective in the elimination of coliform than alum. After the treatment of the water samples with alum, the total bacterial count was 20.00±0.58, 27.00± 0.33 & 41.00±1.52 respectively for well, stream and abattoir wastewater. Treatment of well water with bioflocculant yielded 0.00±0.00, 1.33±0.33 and 77.0±0.58 for well, stream and abattoir wastewater respectively. 22.00±1.15, 16.00±0.89 and

87.0±0.88 were the values gotten for the treatment of the above-listed water samples with *Moringa oleifera* seed powder.

Singular chlorine therapy; combined therapy of bioflocculants and *Moringa oleifera* seed powder, combined therapy of bioflocculant and *Moringa oleifera* seed powder; combined therapy of chlorine and *Moringa oleifera* seed powder; combined therapy of alum, bioflocculant and chlorine; combined therapy of alum, bioflocculant and chlorine; combined therapy of alum, bioflocculant and *Moringa oleifera* seed powder totally eliminated the coliform growth for well water sample. Singular Chlorine therapy; combined therapy of alum and *Moringa oleifera* seed powder; bioflocculant and *Moringa oleifera* seed powder; alum, bioflocculant and *Moringa oleifera* seed powder; alum, chlorine and *Moringa oleifera* seed powder; bioflocculant, chlorine and *Moringa oleifera* seed powder; and alum, bioflocculant, chlorine and *Moringa oleifera* seed powder worked effectively in the removal of coliform from stream water. Chlorine; alum and chlorine; chlorine and *Moringa oleifera* seed powder; alum, bioflocculant and chlorine; alum, bioflocculant and *Moringa oleifera* seed powder; alum, chlorine and *Moringa oleifera* seed powder; bioflocculant, chlorine and *Moringa oleifera* seed powder; and alum, bioflocculant, chlorine and *Moringa oleifera* seed powder totally eliminated coliform from abattoir wastewater. Chlorine; alum and *Moringa oleifera* seed powder; alum and chlorine; chlorine and *Moringa oleifera* seed powder; alum, bioflocculant and chlorine; alum, chlorine and *Moringa oleifera* seed powder, bioflocculant, chlorine and *Moringa oleifera* seed powder; and alum, bioflocculant, chlorine and *Moringa oleifera* seed powder totally eliminated bacterial growth in well water. Alum and chlorine; chlorine and *Moringa oleifera* seed powder; alum, bioflocculant and *Moringa oleifera* seed powder eliminated bacterial growth in stream water. *Moringa oleifera* seed powder; chlorine and *Moringa oleifera* seed powder; alum, bioflocculant and *Moringa* *oleifera* seed powder; alum, chlorine and *Moringa oleifera* seed powder; bioflocculant, chlorine and *Moringa oleifera* seed powder eliminated bacterial growth in abattoir wastewater.

Conclusion

Bioflocculant produced from *Bacillus subtilis* isolated from the stream sediment of Onyearugbulem market has exhibited maximum coagulating effect on well, stream, and abattoir water samples. The bioflocculant also exhibited an inhibitory effect on the bacterial population in the water samples especially coliform bacteria.

This bioflocculant has proven to be more effective than its chemical counterpart, alum sulphate in water-sediment coagulation and the inhibition of bacteria.

Moringa oleifera seed powder exhibited both coagulating and inhibitory effects on the water samples. Chlorine, a chemical disinfectant still proved more effective than *Moringa oleifera* seed powder.

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