Int.J.Curr.Microbiol.App.Sci (2017) *6*(8): 2252-2266

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 8 (2017) pp. 2252-2266**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2017.608.265

Quantification of Microbiological and Physico-Chemical Variables during the Purification of the Wastewater from the Yaounde Slaughterhouse and the Receiving River System

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Keywords

Slaughter house, Ako'o water body, Test, Bacteria, Sulphate, Moringa, Starch.

Article Info

Accepted:
21 June 2017
Available Online:
10 August 2017

A B S T R A C T

A study was carried out from December 2016 to February 2017 with the aim of evaluating the effects of some coagulants (Aluminium sulphate, Moringa, Starch) on the effluent discharge of the Etoudi slaughterhouse and the Ako'o River which receives these waste products. Monthly water samples surveys were carried out on these two zones and physico-chemical analysis were done following the recommendations of Rodier and his collaborators. The effluents of this major slaughterhouse in the city of Yaounde are highly charged with diverse polluting substances and bacteria. The effects of aluminium sulphate, Moringa, Aluminium sulphate mixed with starch and Moringa mixed with starch were tested on these samples. Microbiological analyses on the other hand were carried out following the spread on the surface of a petri dish containing the culture medium of the water samples after successive dilution. On the bacteriological plan, the reduction rate offaecal coliform, total coliform and *Escherichia coli* were greater than 95% for the different bio-engineering techniques employed. As for the physico-chemical parameters, the rates of maximum reduction at 70%, 68% and 56% were obtained for the colour, turbidity and suspended particles respectively. Treatments realised with the addition of starch represented a higher efficiency as compared to that realised with sulphate and Moringa only.

Introduction

A slaughterhouse is an establishment or area where animals are killed and arranged purposely for consumption. In addition to slaughtering, they are equally made up of tripe plants for animal by products anda marketplace for selling meat. Throughout these activities, diverse waste products (fur, various fluids) are produced (IDE *et al.*, 2002). It is generally known that animals can be great reservoirs for pathogenic bacteria and other groups of organisms which are often very resistant to antibiotics (HERAU *et al.*, 2007). Water is the element used in cleaning and flushing these waste products into the adjoining environment. This water is often loaded with organic matter and thereby become an important source of pollution for the zone of reception (Ayo, 2012). If in some well developed countries, modern slaughterhouses are constructed with good purification stations for the waste water, this is not the case in most countries of the tropical regions where we observe a complete absence of treatment stations or well managed

outlet stations for the waste water in various slaughterhouses. Whenever theses exist, they are often in a dilapidating state ora non-functional system with the main consequence of being at origin of an increase discharge of parasites into the lands and water as stipulated in the findings of Markert *et al.,* (2003). This is coupled to the discharge of viral particles, bacteria and an enormous amount of biodegradable organic matter. Prior to the preceding element, it's worth developing new mechanisms so as to reduce the sanitary risks due to the arrival of these effluents into the environment (Ajeagah *et al.,* 2013; Ajeagah *et al.,* 2014). In general, increased levels of faecal coliforms provide a warning of failure in water treatment, a break in the integrity of the distribution system, possible contamination with pathogens. When levels are high there may be an elevated risk of waterborne gastroenteritis. Tests for the bacteria are cheap, reliable and rapid (1-day incubation).

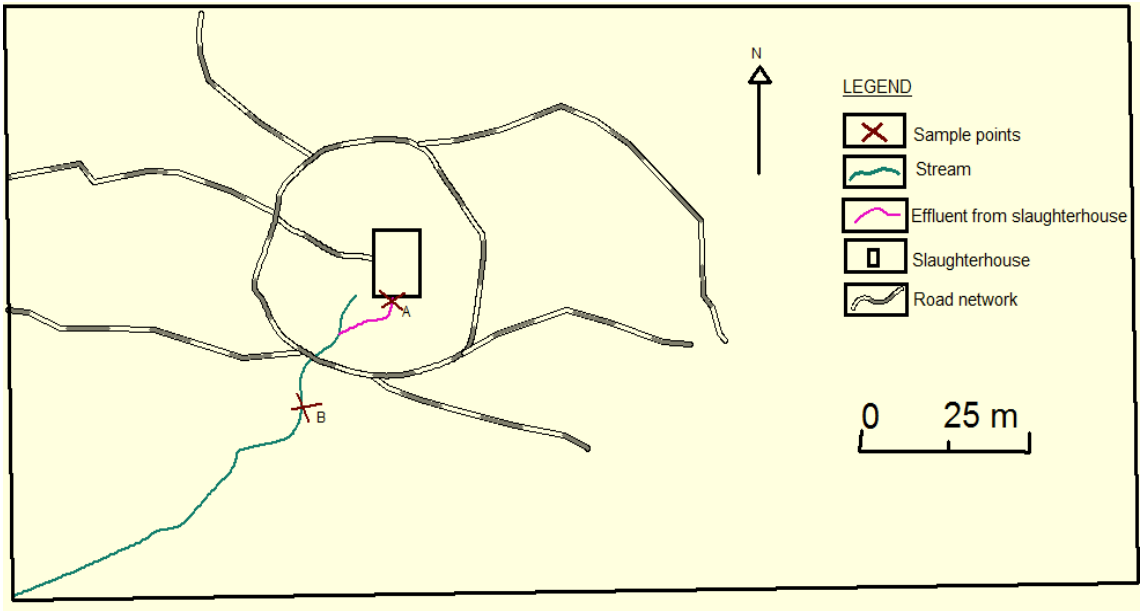
This study was initiated with the aim of testing the purification capacity of some substances on the bacteriological and physico-chemical quality of wastewater that is released from the Yaounde slaughterhouse and the Ako'o stream in which the waste water issued from this slaughterhouse is discharged.

Materials and Methods

Zone of study and sampling points

The study was carried out from December 2016 to February 2017 in the Yaounde slaughterhouse (SODEPA) which is situated at the Etoudi headquarters in the Yaounde I sub-division, with a surface area of about 10 hectares. This slaughter house has a slaughter capacity of 120 cows per day (Mandji, 2008) and is actually the only accredited abattoir in the city of Yaounde. The wastewater discharge is being received by the Ako'o water body of the Mfoundi river basin. Two sampling points were selected in our scientific study; the first station was chosen within the slaughter house, were monthly samples were taken on the wastewater produced by this zoo technical complex.

Fig.1 Map of the slaughterhouse and Ako'o River presenting the sampling points



The second station was chosen on the Ako'o River which serves as the receptor of the waste products from this slaughter house. Various samples were then transported to the laboratory of Hydrobiology and Environment of the University of Yaounde 1, for the physico-chemical and bacteriological analysis in relation to the application of decontamination engineering to be applied.

Bacteriological parameters

The bacteriological analysis was based on the research and isolation of fecal coliform (CF), Total coliforms (CT) and the *Escherichia coli* specie which is considered as a classical indicator of recent pollution. In this effect, water samples meant for these analyses were withdrawn from various study points in samplers which were first labelled and sterilised at 120° C for over 15 minutes at a sealed pressure. These samples were brought back to the laboratory in a refrigerated container, then the search of the microorganisms was conducted in the next 2 to 4 hours that followed their withdrawal from the study points (Rodier, 2009). The number of bacteria in solution was quantified by using the spread plate technique. In this technique, the sample is appropriately diluted and a small aliquot transferred to an agar plate. The bacteria are then distributed evenly over the surface by a streaking technique by moving a sterile loop once or twice through the region and then making more streak lines that neither overlap nor enter that specific region on the petri dish of 90 mm diameter Marchal *et al.*, 1991. After colonies are grown, they are counted and the number of bacteria in the original sample calculated (Fig. 1). The end point of our analysis is the number of colony forming units per mL (CFU/mL) since we are counting the number of colonies rather than the actual number of bacteria. We are assuming that each viable bacteria in the suspension will form an individual colony, which is a valid assumption if we do all the techniques properly. CFU/mL is actually a more useful determination than counting all the bacteria under a microscope because in many bacterial populations some significant number will be dead cells and thus of no interest**.** The inoculation was done by withdrawing after homogenisation, 50 mL of the diluent at 1/10th of each of the samples with the help of a micropipette.

Total coliforms

The culture is done on an endo midium for endobateria in order to control the quality of water and confirm the presumptive tests of confirmation of the water by coliforms. For total coliforms, incubation is done at 37°C. The colonies recorded in our findings are dark purple in colour. The Total coliforms group includes Fecal Coliform bacteria such as *Escherichia coli* (*E. coli*) as well as other types of Coliform bacteria that are naturally found in the soil.

Fecal coliforms

These fecal coliform are part of the bacteria flora of the colifoms. They originate mainly from feaces in the sample. The incubation is done at 44°C for over 24hrs on an endomedium and the colonies are red in colour. A faecal coliform is a facultative anaerobic, rod-shaped, gram-negative, non-sporulation bacterium. Coliform bacteria generally originate in the intestines of warm-blooded animals. Faecal coliforms are capable of growth in the presence of bile salts or similar surface agents, are oxidase negative, and produce acid and gas from lactose within 48 hours at 44 ± 0.5°C.The term thermotolerant coliform is gradually gaining acceptance over "faecal coliform".

Escherichia coli

The culture is done on an endo medium and

the incubation is done at 44°C for 24h. The *Escherichia coli* appears in a red colony with a metallic brightness. The enumeration of the bacterial load was done through the direct counting of colonies. The values were expressed in units forming colonies per millimetre (UFC/mL) per sample assessed. *E. coli* is a Gram-negative rod-shaped bacteria, which possesses adhesive fimbriae and a cell wall that consists of an outer membrane containing lipopolysaccharides, a periplasmic space with a peptidoglycan layer, and an inner, cytoplasmic membrane. Some strains are piliated and capable of accepting and transferring plasmid to and from other bacteria but it remains a non-spore forming bacteria. Such property enables *E. coli* under bad/stress conditions to survive for a longer period. *E. coli* is a rod shaped, Gram-negative, facultative anaerobe, lactose-fermenting, non–endospore-forming microorganism. Its cell measures 1–2 µm in length and 0.1–0.5 µm in diameter with its ten flagella grouped in a peritrical arrangement.

Physico-chemical parameters

Potential hydrogen (pH),

The pH measure expressed in Conventional Units (C.U) and the salinity measure expressed in mg/L were done *in situ* with the help of portable multiparameters of the mark HANNA modèle 9829.

Electrical conductivity and total solid dissolved (TDS), resistivity

Electrical conductivity reveals the degree of mineralisation of water as a proportion to the quantity of ions in water. Measures of the conductivity, of the STD, and of the resistivity are directly carried out on the field with the help of a portable multiparameter of the mark HANNA modèle 9829 with a precision of 0.1 and the subsequent results are expressed in µS/cm and in mg/LinΩ.

Colour, turbidity and suspending products (MES)

The colour, turbidity and water content in suspending products or matter is measured in the laboratory on the raw samples, with the help of the DR 2010 spectrophometer following the wavelengthsλ = 455, 450 and 810 nm respectively, with the subsequent results expressed in Platinium-Cobalt (Pt-Co), in FTU (Formazine Turbidity Unit) and in mg/L respectively.

Process of treatment using the Jar test

The Jar Test is an experiment that is aimed at determining on a given sample, the compared efficiency of a coagulant in relation to the injected doses. The coagulants used in this case are; Moringa, Alluminium sulphate and starch. It consists of measuring with the help of a balance, a mass of 10 g of Moringa, 10 g of Aluminium sulphate and 10 g of starch. These masses are first mixed with a litre of distilled water. On the main solutions, the proportions of 10 mg/L of Moringa and 10 mg/L of Aluminium sulphateare withdrawn, then 10 mg/L of Moringa are introduced in two Erlenmeyer containing 500 mL of the sample from the slaughterhouse and the Ako'o river respectively. 10 mg/L of Aluminium sulphate are also introduced into two other Erlenmeyer containing respectively 500 mL of the samples from the slaughter house and the Ako'o River. The witness Erlenmeyer contains 500 mL of the raw sample from the slaughterhouse and the river consecutively. The total sample is now carried on a magnetic agitator for a rapid agitation for over 3 minutes followed by a rapid agitation that lasts 15 minutes and a phase of decantation that lasts 30 minutes during which destabilised flocs are carried to the bottom. After this decantation, the floating elements of every sample are recovered and the physico-chemical and biological parameters are re-measured. Then, to these

four testing samples are added 10 mg/L of an additive starch solution. The total is then agitated and then left for decantation and the physico-chemical and biological parameters are measured afresh.

Results and Discussion

Biological parameters

Qualitative aspect

The colonies of *E. coli* enteropathogens observed on Endo medium are of medium size, the colour red with metallic gilded shine and regular contours. While the colonies of faecal and total coliforms observed on Endo medium are of medium size, pink in colour with no gilded metallic reflection and irregular contours.

Quantitative aspect

Feacal coliforms

The value obtained for the witness sample differs significantly to that obtained in the treated samples. The highest value (424666 CFU/ml) was obtained with the sample from the river and the lowest (0 CFU /ml) were obtained on the samples of the river treated with Aluminium sulphate + starch and the slaughterhouse treated with Aluminium sulphate+ starch (Fig. 2).

Total coliforms

During the study, the highest values were obtained with the witness samples (69666 UFC/ml for the slaughter house and 44000 CFU /ml for the river) and the lowest values (0 CFU /ml) was registered with samples from the slaughterhouses and the river is treated with Aluminium sulphate + starch. The values obtained with witness samples differs significantly from that obtained with the treated samples ($p<0.05$) (Fig. 3).

Escherichia coli

During the study, the highest value was obtained based on the witnessed sample of the river (106566 CFU /ml) and the lowest values (0 CFU /ml) were registered at the sample of the slaughter house that is treated with Moringa only; sample of the river treated with Aluminium sulphate only; sample of the slaughterhouse and the river treated with Moringa + starch; sample slaughterhouse and water body with Aluminium sulphate +starch. The values obtained with the witness samples differs significantly from those obtained with the treated samples ($p<0.05$) (Fig. 4).

Physico-chemical parameters

Colour

Throughout the period of study, the values of the colour obtained in relation to the witness sample differs significantly ($p<0.05$) from that obtained in other samples. Generally, we observe that the values obtained decreases from the witness sample till the treated sample with Moringa plus starch. Similarly, it decreases from the witness values till the sample treated with Aluminium sulphate. Contrarily, the values obtained from the sample of the river treated with Aluminium sulphate and starch is higher as compared to that of the sample of the river treated with Aluminium sulphate only (Fig. 5).

Turbidity

The values of the turbidity obtained with the witness sample differ significantly of ($p<0.05$) as compared to those obtained in other samples. Excepted the sample treated with Aluminium sulphate only, the higher values were observed from the samples of the slaughter house. These values decrease progressively from witness sample from the treated samples with Moringa and starch + Moringa as well as those treated with

Aluminium sulphate and Aluminium sulphate and starch (Fig. 6).

Suspension materials

The values of the particles of suspension decreases progressively from the witness samples to the treated samples with Moringaand Moringa + starch, these values decrease equally from the treated samples Aluminium sulphate and Aluminium sulphate + starch. Apart from the treated sample with Moringa and starch, these values are higher along the values of the slaughter house (Fig. 7).

pH

Throughout the period of study, the values of the pH obtained do not vary significantly (p=0.6). The highest value (8.80 U.C) was obtained with the sample of the river treated with Moringa + starch and the lowest value was obtained with the sample of the slaughterhouse with Aluminium sulphate only (6.92 U.C) (Fig. 8).

Electrical conductivity raw sample

Throughout the period of study the highest conductivity values (1538µS/cm) was obtained with the sample of the treated water from the river with Moringa + starch and the lowest value was obtained at a sample of treated sample of the river with Moringa only. Except for the treated sample with Aluminium sulphate, the values obtained are higher than the samples treated as compared to the raw sample. The variations of this parameter are not quite significant (p=0.91) (Fig. 9).

TDS

Throughout the period of study the highest values of the TDS (778.38mg/L) was obtained with the help of the sample of the river treated with Moringa and starch while the lowest value was obtained from the analysis of the sample of the river treated with Aluminium sulphate only. Globally, the highest values are obtained based on the samples treated with Moringa and starch and Aluminium sulphate and starch. The values of TDS obtained do not vary significantly (p=0.91) (Fig. 10).

Resistivity

Throughout the study, the values of the resistivity were higher in the case of the samples of the river except from the sample treated with Moringa only. The highest value (3345.33Ω.cm) was obtained based on the witness sample of the river while the lowest value (1119.33Ω.cm) was obtained on the sample of the slaughterhouse treated with Moringa and starch (Fig. 11).

Calculation of reduction rate =Initial value-Residual value/Initial value X100

Statistical analysis

Correlation analysis

The application of the Spearman correlation test with physico-chemical variables presents a highly significant correlation with a threshold of 1% and 5 % threshold. At the threshold 1%, the pH is positively and significantly correlated to the TDS(r=0.830; p=0.003). The TDS are positively and significantly correlated to the conductivity (r=0.855; p=0.002).The SS are positively and significantly correlated to the colour(r=0.927; p=0.000).Significant correlations between physico-chemical and biological variables were equally obtained in this effect, 5% threshold, the SS are positively and significantly correlated to total coliforms (r=0.754; p=0.012) and at *E. coli* (r=0.761; p=0.011). The turbidity is correlated to faecal coliforms (r=0.646; p=0.044). The colour is correlated to faecal coliforms (r=0.751;

p=0.012), (r=0.754; p=0.012) and at *E. coli* (r=0.706; p=0.023). At the threshold 1%, the SS were positively and significantly correlated to faecal coliforms (r=0.886; p=0.001).The turbidity was positively and significantly correlated at *E. coli*(r=0.811; p=0.004).

Analysis of the principal components

The principal component analysis (ACP) was realised to determine the physico-chemical and biological characteristics of different groups formed. The matrix analysed was made up of 7 physico-chemical parameters and 3 biological variables. The essential of the total variance is furnished in the first two factorial axes F1 (65.294 %) and F2 (32.790 %) that cumulate 98.084 % of the total inertia value (Figure 13 A). On the correlation circle (Figure 13 B), the colour (cou), the turbidity (Tur), suspension particles (MES), the resistivity (res), the faecal coliforms (CF), total coliforms (CT), and *E. coli* (EC) are partly positively and significantly correlated between them and on the other hand they are correlated on the F1 axis. The potential hydrogen(pH), the total dissolved (TDS), the electrical conductivity (con) are on one hand positively and significantly correlated between them and on the other hand they are positively correlated at the F2 axis.

Résultat de l'Analyse en factorial analysis and Dendogram

The factorial map (Picture 13 A) presents a distribution of the 5 samples relative to their biological and physico-chemical parameters. Three main groups of stations are issued from this factorial plan. The F1 Aaxe discriminates the group I constituted the samples treated with Moringa plus the addition of starch and the samples treated with Moringa + starch and the treated samples with Aluminium sulphate +starch, the F2 axe discriminates in the negative part of group II constituted the treated samples with moringa only (Mo); Aluminium sulphate only and in the positive part it discriminates the group III comprising the witness sample that has not undergone treatment (RS). The hierarchical classification analysis (ACH) enabled us to the raw sample (RS) is far higher than the two samples that underwent a treatment (Moringa; Aluminium sulphate) and these three samples are far away that underwent two treatments (Moringa and starch; Aluminium sulphate and starch).

The values of the colour of wastewater from the slaughterhouse passed from 6322 Pt. Co (witness sample) to1104 Pt. Co (treated sample with Aluminium sulphate + starch). The values of the colour of the sample of the Ako'o water body passed from 6177Pt.Co (raw sample) to 719Pt.Co (sample treated with Moringa + starch) with rates of reduction of the pollution load varying between 70 to 91%. The rates of purification are close to (90%) for those obtained through the work of Gadoum (2014) on the effect of a coagulant (aluminium sulphate) on the abiotic properties of water. The values of turbidity of the sample of the slaughterhouse passed from 1503 FTU (raw sample) to 248 FTU (sample treated with Moringa + starch). Meanwhile, the values of turbidity of the sample withdrawn from the water body passed from 1223 FTU (witnessed sample) to 130 FTU (sample treated with Aluminium sulphate + starch) with despondency rates of charge comprised between 68 to 98%. The high values of turbidity obtained from witnessed samples could be explained by the presence of diverse particles originating from the cleaning of animals at the abattoir. Rodier *et al.,* (2009) indicate that, the assessed values of the turbidity of residual effluents and polluted waters are generally very high.

Similarly, the suspending particles of the sample issued from the slaughterhouse passed from 864 mg/l (witnessed sample) to 74 mg/l (sample treated with Moringa + starch).

Meanwhile, the values of the suspending matter in the water body passed from 820 mg/l (witnessed sample) to 158 mg/l (sample treated with Aluminium sulphate and starch). The witness values are largely greater than those obtained (111 mg/l) by the works of Reounodjien (2015) on the effluents of the same slaughter house. This difference could be explained by the increase in the slaughtering activity with the principal consequence being the increase in the quantity of dirt produced. The rates of despondency oscillated between 75 to 91% in the wastewater analysed. The high rates of despondency obtained could be explained by the fact that the reactive used could have led to the coagulation and decantation of particles in suspension. These rates are similar to those obtained by IDE *et al* 2002 who using the techniques of screening and grit remove also as to purify the samples issuing from the slaughter house, obtained purification rate ranging from 50 to 80 % for suspending particles.

Generally, for these three parameters, the sanitization rates that are most elevated were obtained through the treated samples with the addition of an additive (Moringa +starch; Aluminium sulphate + starch) in reverse to those treated alone (Moringa or Aluminium Sulphate). The increase of this purification rate could be explained by the action of starch which was added as an additive which could have reduced the residual load. Similar observations were done by Ide *et al.*, in 2002; Metahri (2012) who obtained a despondency rate of over 60% in flocculation, and of over 95% in flocculation with the addition of a chemical additive.

The values of the pH are in the range pH (6-9 U.C) recommended by the ministry of environment for the effluents issued from slaughter houses. They oscillate between 6.92 U.C (sample treated with Aluminium sulphate) and 8.81 U.C (sample treated with Moringa + starch). Samples treated with Moringa+ Aluminium sulphate have a low content in pH. This could be explained by the fact that, Aluminium sulphate by reacting with water molecules liberates H^+ ions that provoke the acidification of the milieu. Meanwhile, those treated with the addition of an additive present a slight basicity. These variations of the pH could be linked to the nature of the substances liberated by starch which could have led to an increase in the pH. The values of the electrical conductivity did not evolve in a significant manner in all the different samples assessed. Nevertheless, the highest values were obtained through the samples treated with sulphate and starch. This high mineralisation could be linked to the Aluminium sulphate employed in our assessment. The variation profile of the TDS is similar to that of the conductivity. The positive and significant correlations ($r=0.927$; $p=0.000$) were therefore obtained between the conductivity and the TDS.

As concerns the biological parameters, the faecal coliform values(163666 CFU /ml for the slaughter house; 424666 CFU /ml for the river water), total coliforms (69666 CFU /ml for the slaughter house; 44000 CFU /ml for the water body) and *Escherichia coli*(171666 CFU /ml for the slaughter house; 1065666 CFU /ml for the water body) obtained were very high and the values of faecal coliform were higher than those obtained (7800 CFU /ml) by Reounodji in 2015 on the effluents of the same slaughter house. For these three groups of bacteria, the rates of despondency are higher than 95%. This indicates the efficiency of the coagulants such as aluminium sulphate, Moringa and the additive starch used in the reduction of bacteria load as presented by Alakaparampil *et al.*, 2013. These soluble molecules of the products applied in our study are positively charged and can easily cross the plasma membrane to form a ligand with the cationic proteins of the membrane which are charged negatively and

thereby favouring the process of sedimentation (Alakaparampil *et al.*, 2013). The treatment with aluminium sulphate + starch was more efficient with a despondency rate of 98% for all the three groups examined. This was followed by the treatment with Moringa and starch whose despondency ratios comprised between 98 and 100%.

Generally, Aluminium sulphate + starch has always been very efficient followed by Moringa + starch (Fatombi *et al.*, 2013). The load of different elements measured in the various witness samples notably, the slaughter house and the river is similar. This depicts that the quasi totality of pollution load at the level of the slaughter house reaches river Ako'o that receives the effluents of the slaughter house because this slaughter house does not possess a system of treatment of waste water. More so, the canal that leads to the effluent of the slaughterhouse is cemented thereby enabling a reduced auto-purification, consequently the totality the pollutants produced are found in the river Ako'o. According to Ajeagah *et al.*, (2012), failing septic systems can allow contaminants in the effluent to flow into the water table, aquifers, drainage ditches and nearby surface waters.

Large quantities of faecal coliform bacteria in water are not harmful according to some authorities, but may indicate a higher risk of pathogens being present in the water. Some waterborne pathogenic diseases that may coincide with polluted animal wastewater include ear infections, dysentery, typhoid fever, viral and bacterial gastroenteritis, and hepatitis A.

Fig.1 Photography of colonies *E. coli* (A) and faecal coliform and total (B)

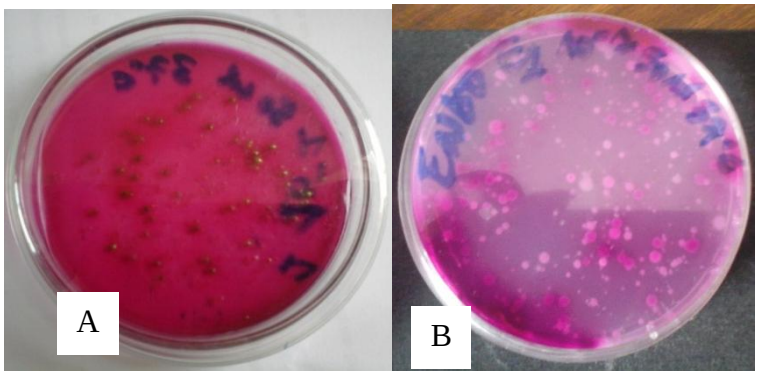


Fig.2 Variation profile of faecal coliforms in the samples assessed

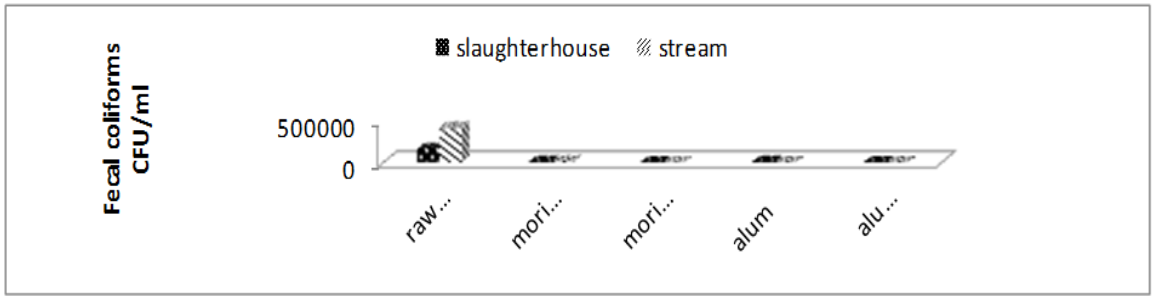


Fig.3 Variation profile of total coliforms in the samples assessed

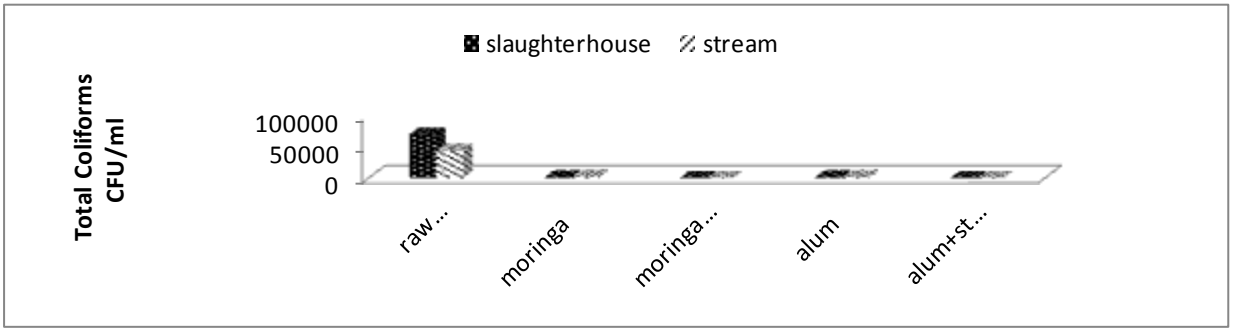


Fig.4 Variation profile *Escherichia coli* in the samples assessed

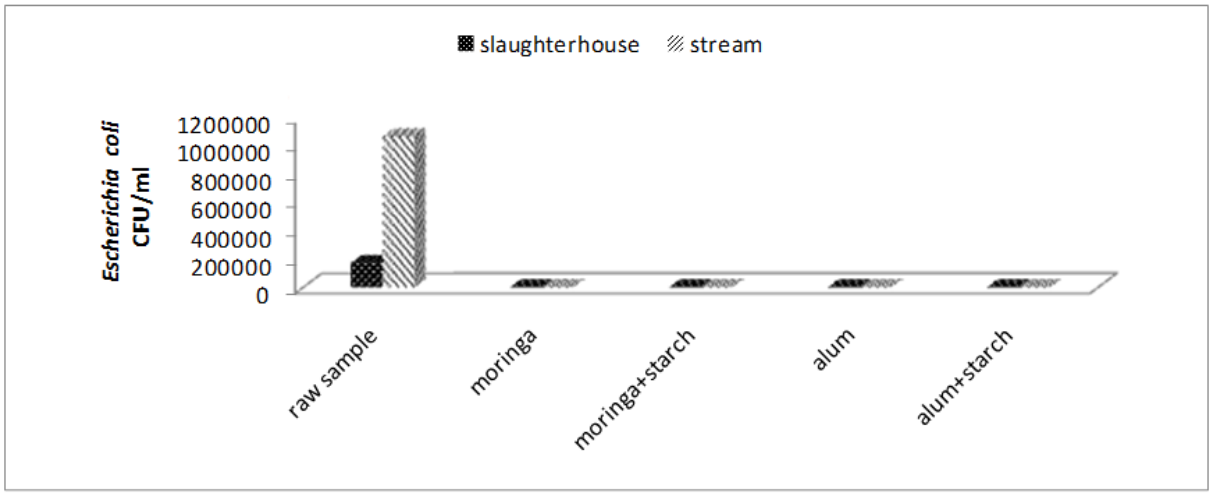


Fig.5 Variation profile of the colour in the samples assessed

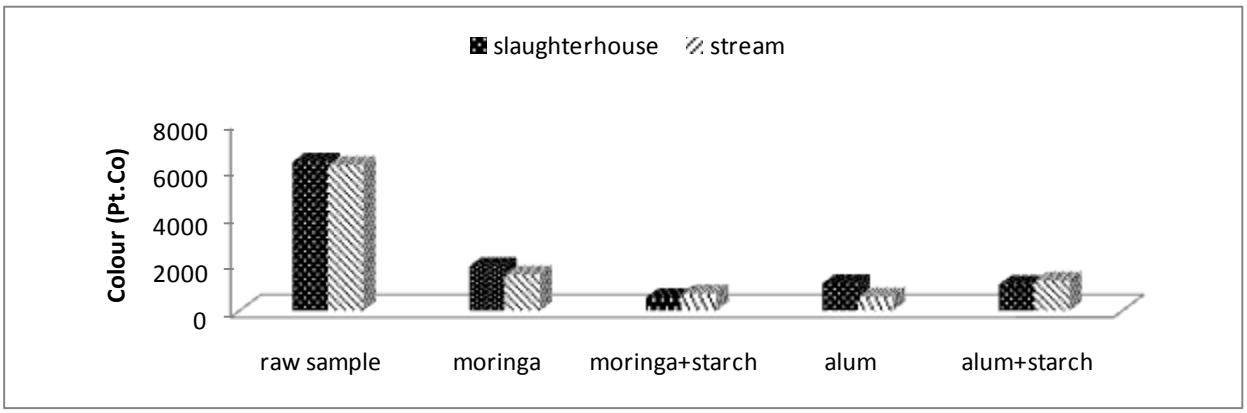


Fig.6 Variation profile of the turbidity in the samples assessed

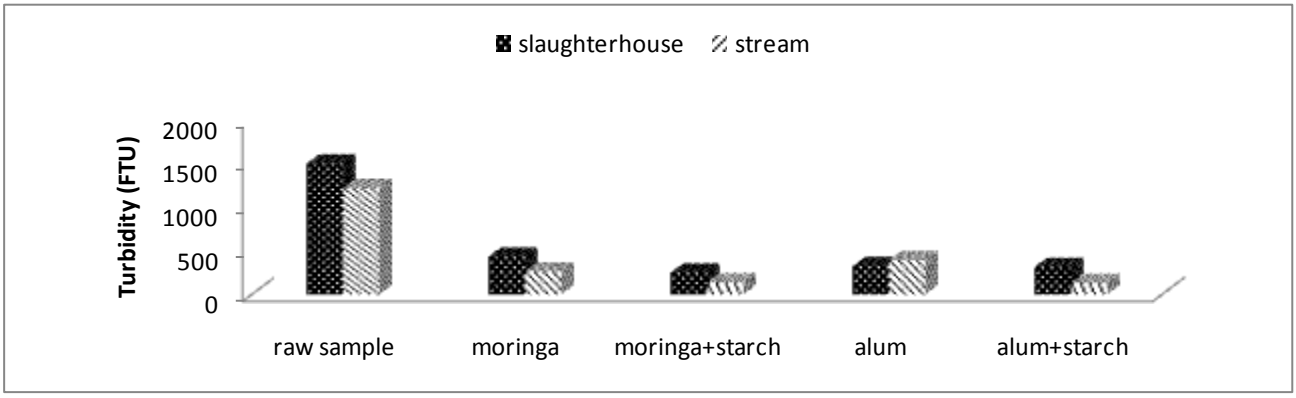


Fig.7 Variation profile of the suspension solids in the samples assessed

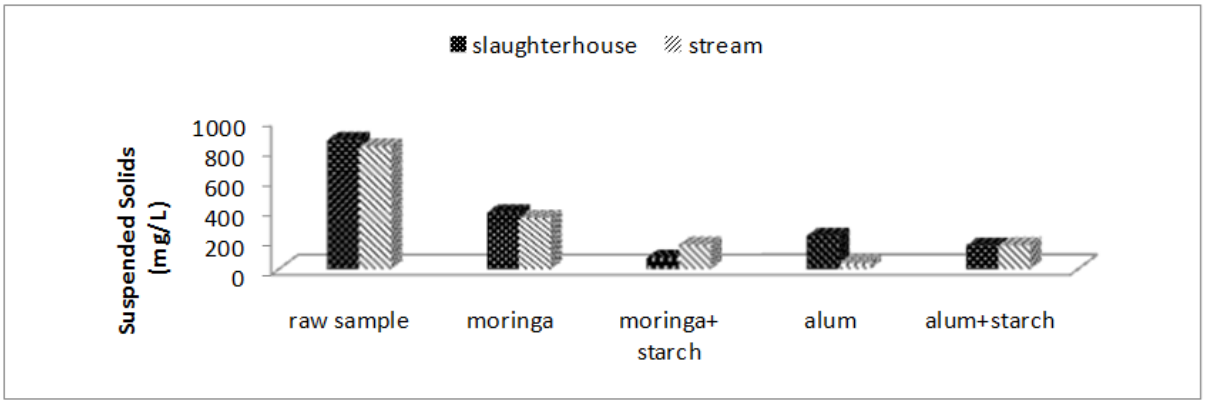


Fig.8 Variation profile of the pH in the samples assessed

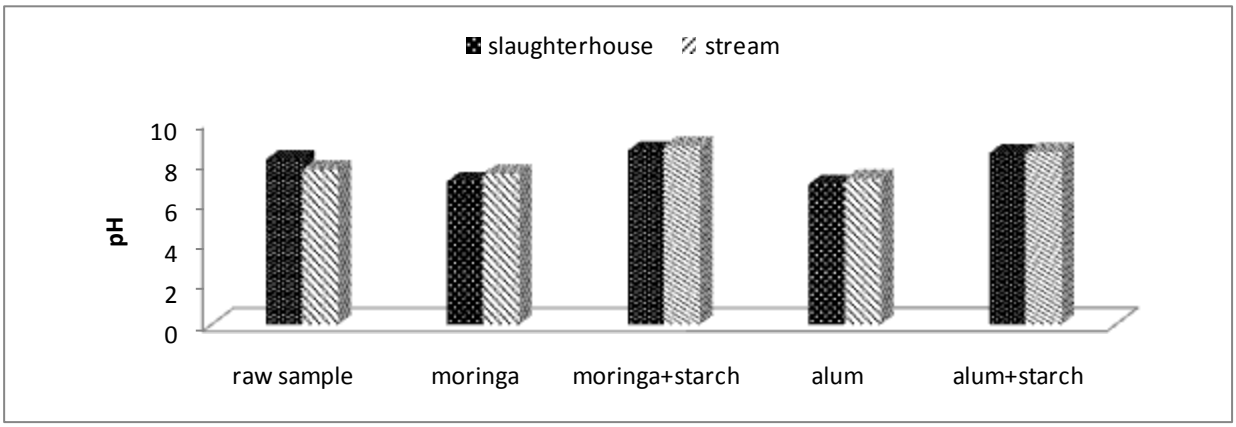


Fig.9 Variation profile of the electrical conductivity in the samples assessed

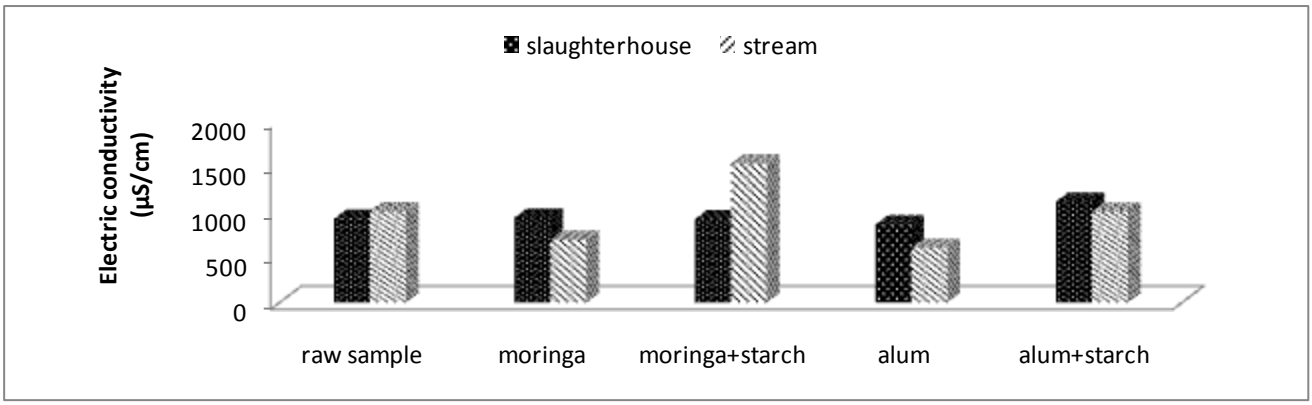


Fig.10 Variation of the profile of the resistivity in the samples assessed

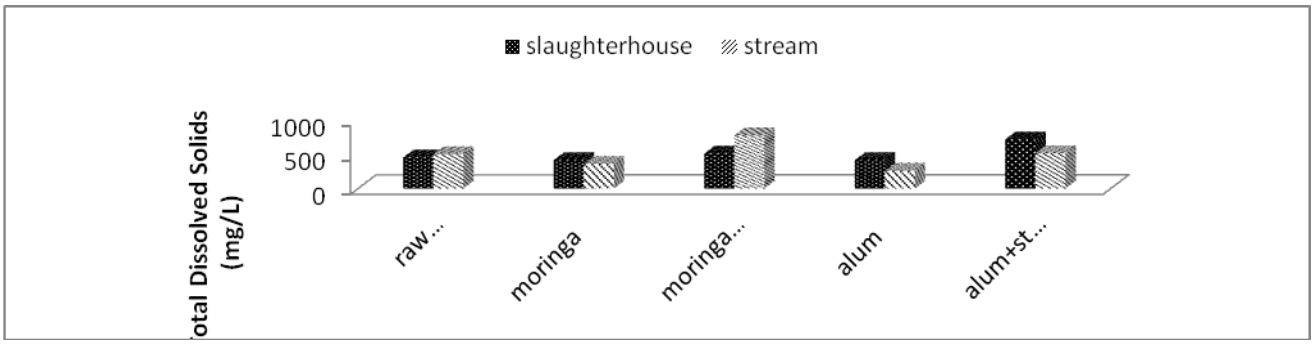


Fig.11 Variation profile of the resistivity in the samples assessed

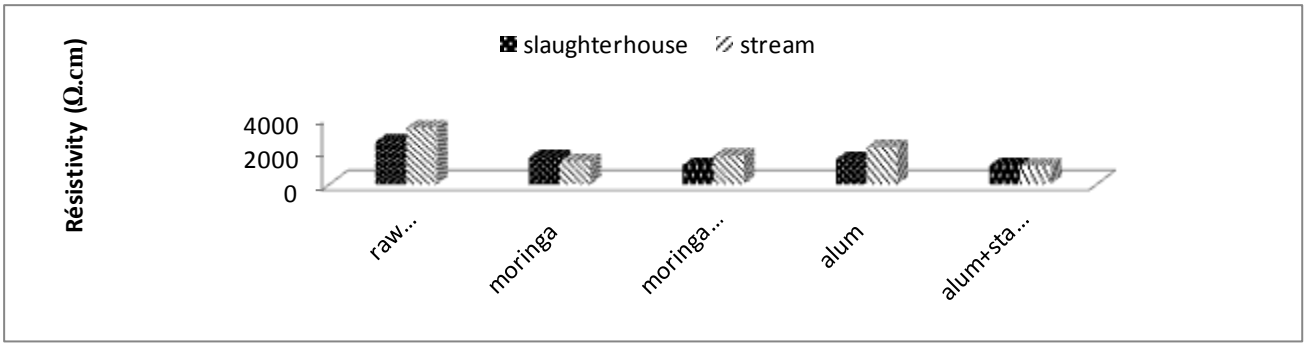


Fig.12 Result of the analysis in principal components (ACP) done by different variables measured in different stations during the study area: (A) Histogram of values; (B) correlation between the variables and the factorial axes F1 and F2

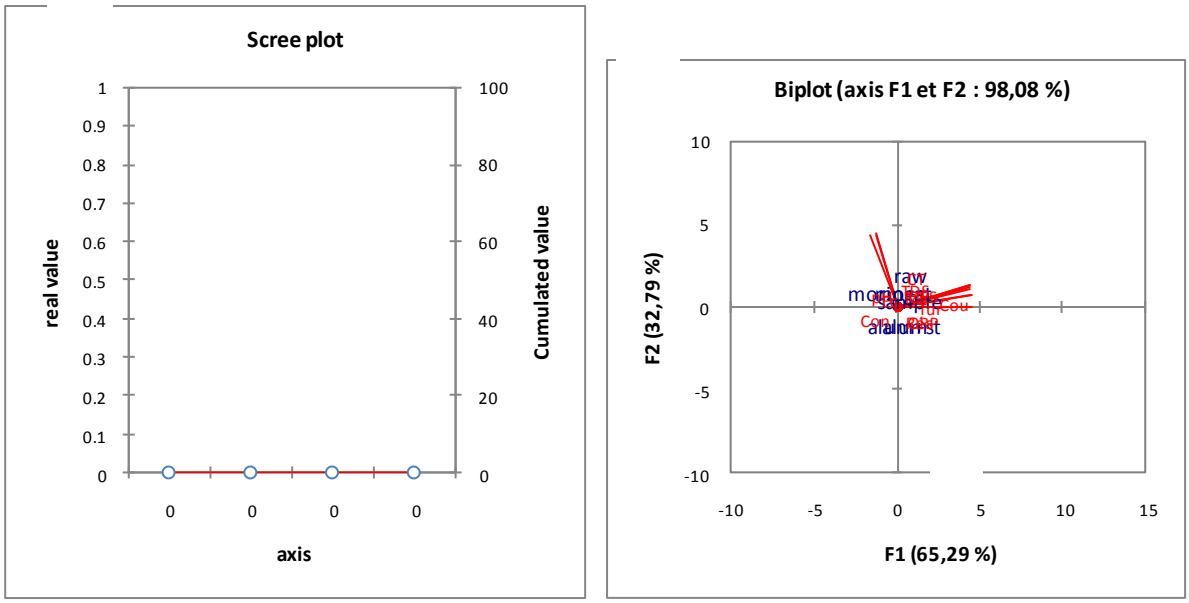
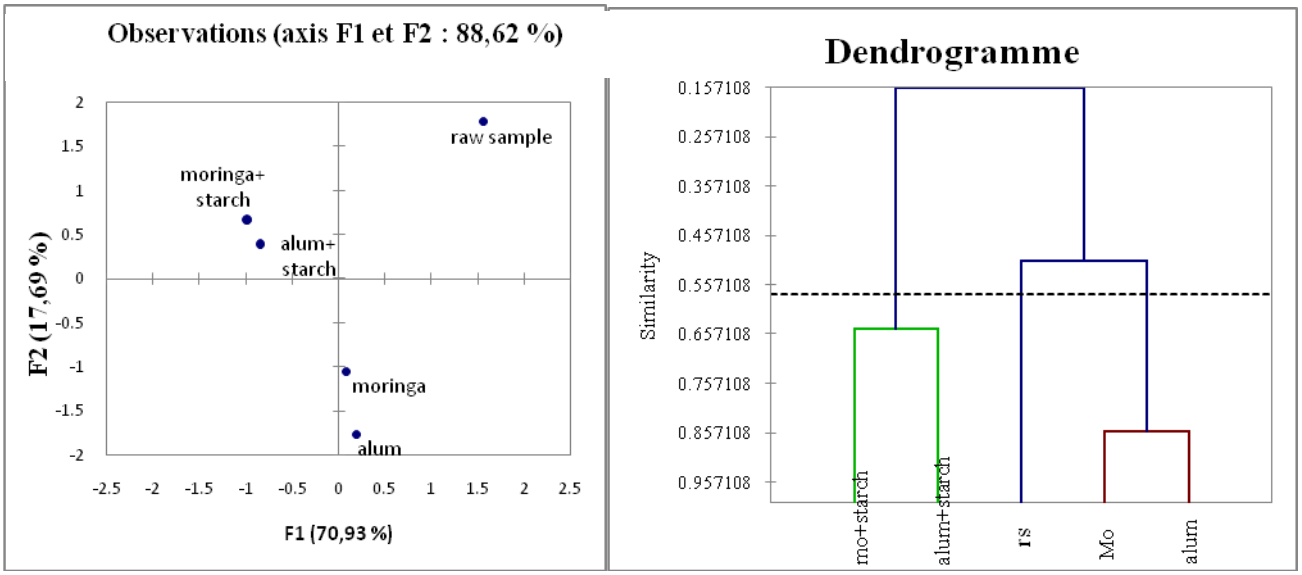


Fig.13 (A) Biplot illustrating the distribution of stations in relation to their characteristics on the factorial plan F1 X F2; (B) Hierarchical classification of sample stations based on the values of parameter measured during the period of study



Pronost *et al.*, (2002) and Satin and Selmi (1995) presents the aerobic decomposition of wastewater from industrial and domestic effluents as a possible method to reduce polluted water discharged into the rivers or waterways. Zogo *et al.*, (2011) indicate that a reduction of the pollution load in waste water purification stations may require the use of chlorine, oxidants and other forms of disinfectants.

In conclusion, this study enabled us to evaluate the purification potentialities of the waste water from the Etoudi slaughterhouse and from the receiving water body through the use of diverse coagulants and flocculants such as Moringa, Aluminium sulphate and starch. The analysis of the waste water of the Etoudi abattoir and the Ako'o water body revealed that, a great proportion of measured physico-chemical and biological parameters greatly overpass the expected values. These studies revealed that, these effluents were rich in microorganisms that are potentially pathogenic to humans. The water treatments engineering employed to reduce the polluting load are very efficient with the outcome producing water with purification rates close to 97% which can then be released into the environment.

Acknowledgement

We thank the laboratory of Hydrobiology and Environment of the University of Yaounde 1 for reagents and collaboration in the realisation of this research.

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How to cite this article:

Zanga Adalbert Donatien, Ajeagah Gideon Aghaindum and Ngassam Pierre. 2017. Quantification of Microbiological and Physico-Chemical Variables during the Purification of the Wastewater from the Yaounde Slaughterhouse and the Receiving River System. *Int.J.Curr.Microbiol.App.Sci.* 6(8): 2252-2266. doi: https://doi.org/10.20546/ijcmas.2017.608.265