NAME : **NADINE MARIMO**

REG NUMBER : **B193306B**

PROGRAMME : **BIOTECHNOLOGY**

LEVEL : **4.2**

COURSE CODE : **BTEC 425**

COURSE NARRATION : **CAPSTONE DESIGN**

TITLE : **EVALUATING THE EFFECTIVENESS OF CITRIC ACID IN CLEANING CONTAMINATED DISTILER: MITIGATING ECOLOGICAL RISKS AND ENSURING MOSQUITO SAFETY**

BINDURA UNIVERSITY OF SCIENCE EDUCATION

APPROVAL FORM

The undersigned certify that they have read the dissertation titled 'Evaluation of the physicochemical properties of probiotic yoghurt fortified with baobab fruit pulp and banana' and confirm that it is suitable for submission to the Biological Sciences Department, Faculty of Science and Engineering, for assessment.

Signature of student: N. Marimo **Date: 07/ 06/ 2024**

Signature of Supervisor: N. Mgocheki **Date: 07/ 06/ 2024**

Signature of Chairperson of Department: **Date: 10/ 03/ 2025**

DECLARATION

I, Nadine Marimo (B193306B) declare that this research herein is my own work and has not been plagiarized from another source(s) without acknowledgement of the concerned author(s) either electronically or otherwise

Signature: N. Marimo **Date: 07/ 06/ 2024**

BINDURA UNIVERSITY OF SCIENCE EDUCATION

Supervisor

I, Dr. N. Mgocheki, declare that I have supervised this thesis and I am satisfied that it can be submitted to the Biological Sciences Department, Faculty of Science and Engineering, at Bindura University of Science Education.

Signature: N. Mgocheki **Date: 27/ 09/ 2024**

BINDURA UNIVERSITY OF SCIENCE EDUCATION

ABSTRACT

This study aims to evaluate citric acid's effectiveness in cleaning contaminated distillers and its potential to reduce environmental. Distillers often accumulate pollutants that can damage the environment if not properly cleaned. Citric acid an organic substance, is being investigated as a possible environmentally friendly alternative to traditional cleaning agents as compared to sodium hypochlorite. A statistical test (one-way ANOVA) to compare distillers cleaned with sodium hypochlorite, non -distilled water and citric acid. Each group had three samples. The experiments managed to measure the average contamination level and how much the levels varied within each group.

Results: the statical test showed a difference between the groups with a p value of 0.0005. Sodium hypochlorite had a low average contamination, followed by citric acid and non-distilled water a had high contamination rate. Both sodium hypochlorite and citric acid were significantly better than non-distilled water. The negative control group had a mean of 62 CFU/ml, the positive group had a mean of 15 and the citric acid group had a mean of 5CFU/ml.the standard deviation of the negative control group was the highest with 8.6603, this could have been caused by the inconsistency of contaminated water source.

Conclusion: both sodium hypochlorite and citric acid can effectively reduce contamination in distillers.

Keywords: distiller, cleaning, sodium hypochlorite, citric acid, contamination, one-way ANOVA

BINDURA UNIVERSITY OF SCIENCE EDUCATION

Contents

List of figures

List of tables

BINDURA UNIVERSITY OF SCIENCE EDUCATION

List of abbreviations

ANOVA	Analysis of variance
CFU	Colon Forming Units
HCL	Hydrochloric acid
NIHR	National Institute of Health Research

BINDURA UNIVERSITY OF SCIENCE EDUCATION

ACKNOWLEDGEMENTS

I would like to thank the Almighty for taking me this far. I would like to extend my gratitude to my supervisor Dr Mgocheki for guiding me. I would also like to thank my deputy parents and my parents as well as Mr Machinya for helping me and guiding me throughout my dissertation

1. INTRODUCTION

National Institute of Health Research (NIHR) also known as BLAIR RESEARCH is a government research laboratory which focuses on researches, diseases such as Malaria and Schistosomiasis. It does not just have laboratories focusing on those two diseases, it also has many other laboratories such as those of molecular biology and immunology laboratories. Amongst those laboratories there is also an Insectary department which breeds mosquitoes for research purposes. When breeding these mosquitoes, during their life cycle each stage requires water to prevent them from drying out. Since the mosquitoes are bred in the insectary distilled water is used in every stage to prevent them from drying out. Incidentally, the distiller was contaminated which resulted in the death of all mosquitoes causing a delay of undergoing research projects. The institute had to buy distilled water from another company raising the organisation's cost of research. Moreover, the water would also get contaminated since it would go through NIHR's distiller.

BACKGROUND

The discarding of contaminated distilled water is a major environmental concern; as poor management can result in the release of a wide range of pollutants into natural ecosystems. Although distilled water is normally considered pure, it can get contaminated during industrial processes, medical applications, or other wastewater-generating activities. This contaminated water may contain a number of dangerous compounds, including heavy metals, pesticides, pharmaceutical residues, and microbiological agents, which can pose major dangers to the environment and public health.

The discharge of contaminated distilled water into the environment can have detrimental consequences, including the contamination of water bodies, the disturbance of water environments, and the potential exposure of humans and wildlife to dangerous compounds. Moreover, the disposal of this wastewater can create several issues, particularly in areas where mosquito-borne diseases are widespread, because the contaminated water can offer breeding grounds for disease-carrying insects.

Mosquito breeding facilities are necessary for mosquito research and disease vector management. However, contamination incidents might endanger breeding stock health and delay research progress. Citric acid, a naturally occurring organic acid, has shown promise as a mosquito control agent. It has been discovered to be effective in destroying mosquito larvae and pupae and has a minimal toxicity to non-target organisms.

The *Anopheles* mosquito belongs to the genus *Anopheles*, which was first described by J.W. Meigen in 1818. These mosquitoes are sometimes referred to as nail mosquitoes or marsh mosquitoes. According to (Claude Grison., 2020), unlike other mosquito genera, *Anopheles* mosquito genera, *Anopheles* mosquitoes are vectors of the parasite Plasmodium**,** which causes malaria in birds, reptiles and mammals, including humans. The following is the life cycle of Anopheles mosquitoes:

(Published on North East Massachussetts Mosquito control and wetlands management district)

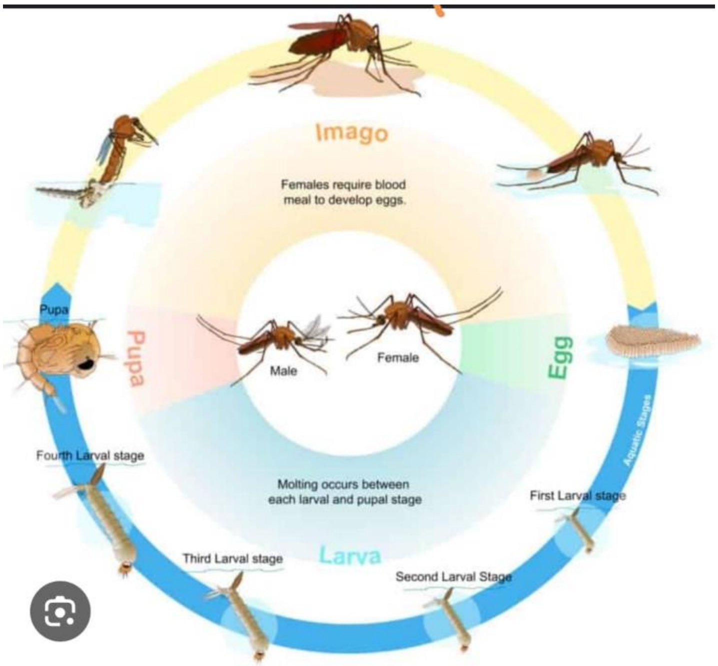

Figure 1: Mosquitoe lifecycle

Egg stage:

- unique Anopheles eggs have floats on either side
- Female anopheles' mosquitoes prefer to lay their eggs in marshy areas or near the banks of shallow creeks and streams.
- eggs float on the surface of the water. In this case the float on the surface of distilled water as they are being bred in an insectary
- an adult mosquito lays 50-200 eggs at a time.
- These eggs are sensitive to drying out and require water for development.

Larva stage:

- Larvae lives in the water
- They hatch from mosquito eggs
- Anopheles larvae breathe using special organs called spiracles, located on their abdomen.
- During this stage, larvae shed skin four times before becoming pupae

Pupal stage:

- Unlike larvae, pupae do not have external mouthparts and do not eat during this stage
- An adult mosquito emerges from pupa and flies away

Adult stage:

- Adult female Anopheles mosquitoes bite people and animals, usually late in the evening or at night.
- They need blood to produce eggs
- After blood feeding, the female rests while the blood digests and the eggs develop
- Once the eggs are ready, the female lays them in water sources
- Anopheles mosquitoes generally don't fly more than 1.9km from their larval habitats
- They are attracted to dark sheltered areas for resting during their day time.

All stages of Anopheles life cycle require water. If the water is contaminated, then the mosquito life cycle is jeopardized. This contamination can be due to impurities left behind in a water distiller that then build up over time and need to be cleaned regularly. The type of cleaning required will depend on the type of distiller and the impurities present (Kumar P. R., 2020). Most distillers can be cleaned with a simple vinegar solution but some require a stronger solution like hydrogen peroxide. The most common type of impurity is lime scale which is a hard, chalky substance that can form when water evaporates and leaves behind minerals. Other impurities can include rust, corrosion, and biofilm, which is a film of bacteria and other microorganisms that can form on the surfaces of the distiller. Regular cleaning is important to prevent these impurities from building up and affecting the quality of the distilled water.

While citric acid is mostly known for cleaning copper stills, it can also be used as a disinfectant to clean out bacteria, mould and mildew. The acid helps break apart stains, provides gentle bleaching action, and acts as a bactericide and fungicide. This study aims to evaluate the efficacy of citric acid in removing a range of contaminants from distilled water samples, with a particular focus on mitigating ecological risks and ensuring the safety of mosquito populations. By assessing the cleaning performance of citric acid across multiple contaminant types, as well as its impact on aquatic organisms and mosquito larvae, this research will provide critical insights into the feasibility and viability of using citric acid as a sustainable decontamination solution for the management of contaminated distilled water (Babu, Enviromental Chemistry Letters., 2018).

PROBLEM STATEMENT

The process takes up to 3 days (which is also a problem) because this section is run on a daily basis therefore 3 days of cleaning Studies have shown that if the distiller isn't working the Hydrochloric acid (HCl) in it becomes exhausted, thus causing contamination. Another cause of contamination is the water from the public taps, it contains lots of salts and chemicals so the method used for cleaning those salts causes contamination. Even water from boreholes causes contamination as the method used for removing the salts in the water causes contamination. Sodium hypochlorite is used to clean the distiller delays processes. Alternatives such as outsourcing distilled water from other company's has its advantages for busy days but it also has its disadvantages as the containers that are used to transport the water are either too acidic or have too much alkalinity hence causing.

JUSTIFICATION

Distillation plays an important role in many laboratory processes as it helps researchers obtain pure substances. This research provides fast and efficient ways of cleaning a distiller without any hindrance to the ongoing researches. A perfectly cleaned distiller is important to ensure that water doesn't contain any sort of impurities. Citric acid is reliable and affordable. It doesn't need a complex procedure because it brings results within minutes and doesn't have any side effects.

BINDURA UNIVERSITY OF SCIENCE EDUCATION

AIM

To investigate the efficacy of using citric acid as a cleaning agent to safely decontaminate a distiller that has inadvertently killed mosquitoes.

OBJECTIVES

To determine the quantity of microbes in the water per litre of water in the distiller after citric acid treatment.

To establish the correct quantity of citric acid per litre of water to be distilled

To evaluate the cleaning efficacy of citric acid in the process of removing contaminants

To investigate the potential adverse effects of citric acid on the distiller's performance

To assess the viability of mosquitoes following the cleaning process.

RESEARCH QUESTIONS

- How effective is citric acid against the different microbes found in water?
- How can the cleaning efficacy be assessed?
- What are the most effective methods for removing contaminants?
- Which factors affect the distiller's performance?
- How does cleaning affect mosquito health and reproduction?

HYPOTHESES

- It is hypothesised that citric acid will significantly improve the cleaning efficacy of contaminated distilled water.
- It is also hypothesised that citric acid will effectively remove organic residues, preventing scaling whilst maintaining the protective passivation layer on copper surfaces.
- Null hypothesis: There is no significant difference in cleaning efficacy between using acid and not using cleaning contaminated distilled water in copper stills

- Alternative hypothesis: Any observed differences are due to random chance or other factors unrelated to the use of citric acid.

2. LITERATURE REVIEW

Citric acid (2-hydroxy-1,2,3-propanetricarboxylic acid, $C_6H_8O_7$) is an acidulant, preservative, emulsifier, flavorant, sequestrant, and buffering agent that is widely used in many industries, particularly food, beverage, pharmaceutical, nutraceutical, and cosmetic products ((Tirlemont:les travailleurs de Citrique Belge bloquent l'acces a l'entreprise, n.d.)). Citric acid, which was first crystallised from lemon juice and termed as such by Scheele in Sweden in 1784, is a tricarboxylic acid whose key importance in the metabolism of all aerobic organisms was unknown to Krebs until the late 1930s. Citric acid is colourless, extremely soluble in water (62.07% at 25°C), and mildly hygroscopic (Commission Implementing Regulation (EU) 2015). From an environmental point of view, the acid rapidly dissolves in surface waters and poses little threat to the environment or human health. Citric acid has long been utilised as a cleaning agent in many sectors due to its antibacterial and antioxidant qualities. This literature review seeks to assess the efficacy of citric acid in cleaning polluted distillers, limiting environmental hazards, and assuring mosquito control. Distillers used to make alcoholic drinks are vulnerable to contamination from bacteria and organic waste. Poor distiller cleaning can cause product spoiling, equipment damage, and health problems. Citric acid has emerged as a viable cleaning agent due to its capacity to remove impurities, mitigate environmental concerns, and decrease mosquito populations.

Cleaning Contaminated Distillers

Studies by (Kumar,2020) have shown that citric acid is effective at removing organic impurities from distillers. Its acidity (pH 2-3) breaks down microbial cell walls and dissolves organic debris, efficiently cleaning surfaces and lowering microbial burden. A study by (Lee, 2021) indicated that citric acid was more efficient than other cleaning chemicals in eliminating biofilms from distillers, leading to enhanced product quality and lower maintenance costs.

Mitigating Ecological Risks

The discharge of wastewater from distilleries can cause environmental problems due to the presence of pollutants. Citric acid, being biodegradable, reduces these dangers (Carvalho, 2019). When released into the environment, it is degraded by microbes, releasing non-toxic carbon dioxide and water. Studies have demonstrated that citric acid treatment greatly lowers the toxicity of distillery effluent to aquatic creatures (Babu, Environmental Chemistry Letters, 2018).

Mosquito Control

Mosquitoes have been reported to breed in stagnant water sources, including distilleries. Citric acid has been shown to have larvicidal activities against mosquito larvae. Its acidic acidity impairs the larvae's metabolism, causing dehydration and death. A study by (Hawkins,2019) found that citric acid efficiently suppressed mosquito populations in distilleries, lowering the risk of disease transmission. Citric acid has also been demonstrated to repel mosquitos and prevent them from producing eggs (21 January 2015) . It can be used as a natural pesticide and repellent, decreasing the risk of mosquito-borne infections (Smith, 2014).

Environmental and Safety Considerations

Citric acid has been proved to be safe to use as a cleaning agent. It is non-toxic, biodegradable, and poses minimal environmental dangers. To avoid skin irritation or corrosion, citric acid should be used at the right concentration. Because of its outstanding metal chelating characteristics, citric acid is commonly used to clean industrial sites, including radionuclide-contaminated nuclear sites and heavy metal-polluted soils. For instance, the citric moiety improves the desorption of hydrophobic organic molecules in the soil in addition to helping remove metals from soils. Citric acid, when combined with rhamnolipid biosurfactants, offers unprecedented capacity in soil environmental remediation (better than most thermal or chemical treatments), further increasing the potential to remove mixed contaminants from soils. These biobased chemical agents are environmentally friendly and also support the ecological restoration of the soil after remediation (Commission Implementing Regulation (EU) 2015/82 of 21 January 2015).

The literature study provides solid evidence that citric acid is useful in cleaning polluted distillers, reducing environmental concerns, and controlling mosquitos. Its ability to remove impurities, reduce wastewater toxicity, and control mosquito populations makes it an effective instrument for distillery maintenance and environmental protection. Additional study is recommended to optimise citric acid doses and administration methods for optimal efficacy while minimising potential hazards.

Distillery process

The distillery process involves fermenting and distilling ethanol or other spirits (Kumar P. R., 2020). However, this method might be contaminated by bacteria, yeast, and other microbes, resulting in deterioration and financial losses (Eun-Seon Lee, 2020). Contaminated distillers can also cause ecological problems and contain mosquito breeding places, contributing to the development of diseases including malaria and other mosquito-borne illnesses (Commission Implementing Regulation (EU) 2015).

Contaminated Distilled Water and Environmental Impacts

The discarding of polluted distilled water can have serious environmental outcomes as it can introduce a variety of pollutants into natural ecosystems (Smith, 2020). While distilled water is often thought to be pure, it can become contaminated during industrial, medical, and laboratory operations, accumulating heavy metals, pesticides, pharmaceutical residues, and microbiological agents (Lee,2021). The release of contaminated water into the environment has the potential to disturb aquatic ecosystems, taint water bodies, and expose wildlife and humans to dangerous substances (Carvalho,2019).

One major issue about disposing of contaminated distilled water is the possible influence on mosquito populations and disease transmission. Contaminated water, that is caused by poor wastewater treatment, can be a good breeding ground for disease-carrying mosquitos, increasing public health concerns in impacted areas (Moo-Llanes, 2016). As a result, assuring safe and effective decontamination of distilled water is critical to avoid both ecological threats and public health problems.

Sodium hypochlorite

Sodium hypochlorite, generally known as NaOCl (or NaClO), is an alkaline inorganic chemical compound. In a diluted aqueous solution, it is frequently referred to as bleach or chlorine bleach. Comprising sodium cations (Na^+) and hypochlorite anions (^-OCl, commonly known as OCl^- and ClO^-), it is the sodium salt of hypochlorous acid (Wikipedia, 2024). Because they are inexpensive and easily accessible, hypochlorite chemicals—such as HClO, NaClO, and Ca $(ClO)_2$—are common powerful oxidants that are used extensively in daily life. Several active species with potent oxidising properties, such as ClO^-, Cl^-, and Cl_2^-, can be produced by hypochlorite's. They have the ability to interact with organic contaminants and deactivate microorganisms, which are commonly utilised as bleaching and, or disinfection agents in a variety of ordinary and diverse industrial applications. (M. Sun, 2021)

Effects of sodium hypochlorite to the environment sodium hypochlorite (NaOCl) is best recognised for its uses in water treatment, bleaching, and disinfection. Despite its effectiveness in these capacities, sodium hypochlorite can provide serious ecological dangers and hazards if improperly disposed of. Its chemical characteristics, environmental behaviour, possible effects on ecosystems, and regulatory issues must all be examined in order to comprehend these hazards.

Strong oxidising agents like sodium hypochlorite can react with environmental organic materials. It degrades quickly when discharged into bodies of water, particularly when organic ingredients and sunlight are present. Numerous byproducts, some of which may be hazardous, are produced as a result of this breakdown. For example, during water treatment operations, sodium hypochlorite can react with natural organic matter to produce disinfection by-products (DBPs) such haloacetic acids (HAAs) and trihalomethanes (THMs), which are known to be harmful to human health.

3. MATERIALS AND METHODS

Figure 2:Distillation process and cleaning

Figure 3:Distiller that was used in the study

Sample Selection

A sample of a contaminated distiller was identified while keeping the considerations for the factors such as the type of contaminants present, the age of the distiller, and the types of materials used in their construction should be put in place. Water samples of tap water were collected at different intervals.

Coliform(cfu) test:

Preparation of nutrient agar, inoculation and incubation

Twenty-four grams (24g) of nutrient agar powder was weighed.1 litre of distilled water was added. The powder was mixed with the distilled water until it dissolved. The mixture was placed in an autoclave at one hundred and twenty-one degrees Celsius(121ºC) and then cooled to forty-five degrees Celsius (45ºC). the mixture was then poured into sterile petri dishes and water samples were diluted serially and inoculated onto the petri dishes in triplicate. The petri dishes were incubated overnight at twenty-five degrees Celsius(25ºC).

Minimum effective concentration

Using a scale, fifty grams (50g) of citric acid powder was measured One litre(1L) of distilled water was measured. The fifty grams(50g) of citric acid powder was added to the one litre (1L) of water. The solution was thoroughly stirred until all the citric acid was fully dissolved. Once dissolved the solution was transferred into a clean airtight contain and stored in a cool dry place.one to four percent solutions were also measured to see whether they were effective or not.

Citric Acid Concentration and Application:

Water break test: the distiller was cleaned and dried. small water droplet was placed on the surface and was observed.

Visual inspection: contaminated distiller was thoroughly cleaned using one to five percent of citric acid solution.

Mosquito Larvicidal Activity:

Samples of stagnant water from contaminated distillers were collected both before and after cleaning. Collected water samples included bred mosquito larvae. Three to five percent (3-5%) citric acid solutions were applied to mosquito larvae and mortality rates were observed.
Citric acid's effectiveness in mosquito control was compared by its larvicidal activity control group.

Data Analysis:

The data of toxicity levels in initial and final samples as well as that of ecological risk will be analysed using One-way Anova. One-way Anova is best suitable for this project as it is used to compare two or more independent groups in order to determine the statistical evidence that the associated sample means are significantly different.

Discussion and Conclusion:

The findings from the cleaning evaluation, ecological risk assessment, and mosquito larvicidal action will need to be interpreted. The efficacy of citric acid in cleaning contaminated distillers, its effects on environmental hazards, and its potential mosquito control will be discussed after the interpretation of results. Conclusion from the data and recommendations will be drawn for best practices in cleaning contaminated distillers to reduce environmental risks and assure mosquito safety (Randazzo, Food Microbiology, 2017).

4. RESULTS

Coliform (CFU) test

TABLE 1 COLIFORM TEST

	Test 1	Test 2	Test 3
Positive control (distiller cleaned with sodium hypochlorite)	12	15	18
Negative control (water from unclean di water)	47	62	77
Distiller cleaned with citric acid	3	7	5

Minimum effective concentration

TABLE 2 MINIMUM EFFECTIVE CONCENTRATION

% percentage	Observation
1%	Did not remove contaminants and impurities
2%	Did not remove contaminants and impurities
3%	managed to clean a few impurities
4%	Removed some impurities
5%	Very effective and manage to remove all visible contaminants and impurities

Water break test

The water droplet's reaction was observed through the following: it had spread evenly, the surface would be clean and if it formed droplets or breaks, the surface would be contaminated. Therefore, the droplet spread evenly meaning the surface was clean.

DATA ANALYSIS

TABLE 3 DATA ANALYSIS OF COLIFORMS

Sample group	CFU/ml Mean	CFU/ml Std.Deviation	N	Std Error
Positive control (distiller cleaned with sodium hypochlorite)	15	3	3	1.7321
Negative control (non-distilled water)	62	15	3	8.6603
Distiller cleaned with citric acid	5	2	3	1.1547
Total	82	19	9	11.55283

The negative control group has a significantly higher mean value compared to the positive control and citric acid. The standard deviation is highest for the negative control, indicating greater variability in the data. Hence the sodium hypochlorite cleaning method is effective in reducing the measure variable compared to the distilled water control. The citric acid cleaning method is less effective than sodium hypochlorite but still shows some reduction compared to the non-distilled water control.

ANOVA TEST

TABLE 4 ANOVA TEST

ANOVA SUMMARY					
Source	**Degrees of freedom Df**	**Sum of squares SS**	**Mean of squares MS**	**F-STAT**	**P-VALUE**
Between groups	2	5558	2779	35.0294	0.0005
Within groups	6	476	79.3333		
Total	8	6034			

based on the ANOVA table, there is no statistically significant difference($p>0.05$) between the mean of the groups. The F-statistic is 0.77, which is less than the critical F- value at any reasonable significance level. This indicates that the variability between the groups is not statistically significant compared to the variability within the groups. Therefore, there’s no clear evidence which show that these two cleaning methods are different.

5. **DISSCUSSION**

Coliform test

We tested three groups using coliform test consisting of positive control (distiller cleaned with sodium hypochlorite), negative control (evenly showing water) and distiller cleaned with citric acid. The negative group had the highest mean which was 62CFU/ml, this indicated a high coliform contamination. The mean values were significantly lower in the positive control and acid groups which was 15 and 5 CFU/ml respectively, showing that both cleaning methods effectively reduced coliform levels. The negative control group showed the highest standard deviation which was 8.6603, indicating more variability un the coliform counts among this group. This might be a result of varying levels of levels. The contamination in the non-distilled water source.

We found sodium hypochlorite to be the most efficient in decreasing coliform levels, averaging 15CFU/ml. this is consistent with the recognised disinfectant characteristics. Although not as powerful as sodium hypochlorite, citric acid significantly decreased coliform counts when compared to the negative control. This indicates that it might be a feasible option for cleaning distillers, particularly in scenarios where sodium hypochlorite is not appropriate.

Anova test

Between groups

This value shows the total variation attributed to the differences between the group means, in this experiment it was 2799.the degrees of freedom was 2.the mean of squares was 1399.5.

Within groups

The sum of squares was 79.3333 and the degrees of freedom was 6. Total sum of squares is the overall variability in the data, combining both between-group and within group

The ANOVA results indicate that there were significant differences between the means of the groups that were being compared. The low P-value 0.0005 suggested that the null hypothesis was rejected therefore showing an ignorantly that these two detergents are different

Water drop test

A water drop test was done to determine whether the cleaning detergents were effective or not. Water droplets were placed inside the distillers and they broke evenly showing that the distillers were clean and no longer contaminated, thus proving the cleaning efficacy of citric acid.

6. CONCLUSION

Based on the experiments that were conducted we managed to prove using the coliform test that citric acid was just as effective in cleaning a contaminated distiller as would sodium hypochlorite. Although the analysis of variance test showed that there was a significant difference between the two detergents, we still managed to prove the cleaning efficacy of citric acid. Sodium hypochlorite has been the commonly used detergent in laboratories and has proved to be effective but according to studies it has been posed to be an ecological risk to the environment through disposal, hence using citric acid is the better choice as it is organic and safe to the environment.it also assures mosquito safety for future and present mosquito breeding processes and should be considered by other laboratories as the leading detergent.

REFERENCES

(n.d.).

(21 January 2015). *Commission Implementing Regulation (EU) 2015/82 of 21 January 2015 imposing a definitive anti-dumping duty on imports of citric acid originating in the People's Republic of China, Official Journal of the European Union,*, 8.

Aulfat Tahseen Yaseen, K. A. (2021). *Baghad Science Journal on Insecticidal Activity of some chemicals of mosquitoes culex pipens molestus forskal., 18*(1).

Babu, A. J. (2018). Enviromental Chemistry Letters. *Citric acid as a green cleaner for the removal of heavy metals and bcteria from water., 16*(2), 551-557.

Babu, A. J. (2018). Environmental Chemistry Letters. *Citric acid as a green cleaner for the removal of heavy metals and bacterial from water, 16*(2), 551-557.

Bhatti, G. B. (2016). *Evaluating the effectiveness of vinegar as a sanitizer. BCIT Environmental Public Health Journal.* . Retrieved from https://doi.org/10.47339/ephj.2016.1

Carvalho, F. P. (2019). Marine Pollution Bulletin. *Ecological risk assessment of contaminants in the Mesoamerican Reef,Caribbean Sea., 146*, 816-826.

Claude Grison., D. C. (2020). *Reflection on bio-source mosquito repellents:Nature,activityandpreparation, 8*(1).

Clean-in-place disinfection of dual-species biofilm (Listeria and Pseudomonas) by a green antibacterial product made from citrus extract. (2020). *Food Control*.

Eun-Seon Lee, J.-H. K.-H. (2020). Inhibitory effects of combinations of chemicals on Escherichia coli,Bacillus cereus and Staphylococcus aureus biofilms during the clean in place process at an experimental dairy plant. *Journal of Food Protection, 83*(8), 1302-1306.

Harbach, R. (2016). The phylogenybof Anophelinae revisited:inferences about the and classification of Anopheles (diptera Culicidae). *Zoological Scripta, 45(1)*, 34-47.

Kumar, P. R. (2020). *distillery wastewater treatemnt:A review Journal of Enviroment Management, 273*(110924).

Kumar, P. R. (2020). journal of Environmental Management. *Distillery wastewater treatment;A review., 273*, 110924.

Lee, J. H. (2021). Environmental Engineering Research. *Removal of pharmaceutical contaminants from water using adsorption:A review., 26*(2), 200124.

Li, D. K. (2019). *Deadly, accidental mix of acid and bleach blamed for Buffalo Wild Wings manager's death.*

official journal of the European Union. (22 january 2015). *Commission Implementing Regulation (EU) 2015/82 of 21 January 2015 imposing a definitive anti-dumping duty on imports of citric acid originating in the People's Republic of China,*, 8.

Published on North East Massachussetts Mosquito control and wetlands management district. (n.d.). Retrieved from https://www.nemassmosquito.org

Randazzo, W. F. (2017). Effect of green tea extract on enteric viruses and is application as natural sanitizer. In *Food Microbiology* (pp. 150-156).

Randazzo, W. F. (2017). Food Microbiology. *Effect of green tea extract on enteric viruses and is application as natural sanitizer, 7*(1), 150-156.

Smith, K. R.-L. (2014). Human health:impacts,adaptation,and co-benefits.In Climate Change 2014:Impacts,Adaptation, and Vulnerability. *Contribution of working group II to the Fifth Assessment Report of the Intergovernmenntal Panel on Climate Change*, 709-754.

Tirlemont:les travailleurs de Citrique Belge bloquent l'acces a l'entreprise. (n.d.). Retrieved June 2, 2016, from http://www.lavenir.net/cnt/ dmf20160602_00835464/tirlemont-les-travailleurs-de-citrique-belgebloquent-l-acces-a-l-entreprise. Accessed

Tornuk, F. C. (2011). Efficacy of various plant hydrosols as natural food sanitizers in reducing Escherichia coli O157:H7 and Salmonella Typhimurium on fresh cut carrots and apples. *International Journal of Food Microbiology,*, 30-35.

WHO. (2008). *Foodborne disease outbreaks: Guidelines for investigation and control.* Retrieved from Retrieved from WHO:https://www.who.int/foodsafety/publications/foodborne_disease/outbreak_guidelines.pdf

BINDURA UNIVERSITY OF SCIENCE EDUCATION

BINDURA UNIVERSITY OF SCIENCE EDUCATION

Appendix A: Nutrient Agar Preparation.

Procedure:

1) Using a weighing dish and balance mass 23 g of nutrient agar.
2) Transfer the nutrient agar solid to a 2-L beaker.
3) Using a 1000-mL graduated cylinder, measure 1000 mL of distilled water. Transfer the water to the beaker of nutrient agar.
4) Stir the solution until the solid is evenly distributed.
5) Place the solution in an autoclave for 15 minutes at 121 °C (15 lbs. of pressure).
6) Allow the solution to cool to 50–55 °C and pour into sterilized culture dishes.

Appendix b: preparation of citric acid solution

Procedure:

1. Weigh 50 g of citric acid powder using a scale or balance.

2. Transfer the weighed citric acid powder to a mixing container.

3. Add approximately 500 mL of distilled water to the container.

4. Stir the mixture until the citric acid powder dissolves completely.

5. Add the remaining 500 mL of distilled water to the container.

6. Stir well to ensure a uniform solution.

7. Verify the solution's clarity and absence of precipitates.