Microbial contamination in palm oil selected from markets in major cities of Ghana

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\textbf{ABSTRACT}

The study assessed the microbiological contamination of palm oil sold in the major cities of Ghana's oil-producing regions. Seventy samples (10 samples from each region) were randomly collected in sterile bottles and transported aseptically to the laboratory for analysis. AOAC standard methods and procedures were used to isolate and identify bacteria and fungi based on their cultural, morphological, and biochemical characteristics. The results were analysed using One-Way ANOVA with 5% significance level, using GraphPad Prism, version 5.0 for windows, and the results presented in graph and tables. The quality of oils was moderately good with total Coliform counts of $2.0 \times 10^1$ to $6.03 \times 10^3$ CFU/g and $1.72 \times 10^3$ to $6.66 \times 10^3$ CFU/g. Microbial counts from the selected regions were statistically different at $P<0.05$. Findings established the absence of yeast and moulds in the oils in addition to extremely pathogenic Coliforms such as Salmonella and Shigella species. \textit{Staphylococcus aureus}, \textit{Staphylococcus epidermidis}, \textit{Escherichia coli}, and \textit{Pseudomonas aeruginosa} were highlighted as dominant coliforms found in the oils after the assay. The overall findings suggest that the oil from the Greater Accra region was of best quality and safest for consumption. Oil samples from the Central and Ashanti regions were of relatively poor quality recording the highest dominant coliforms. Nonetheless, the presence of the isolated potentially harmful microorganisms in the palm oil samples points to hygienic issues and poses a relative health hazard to consumers.

1. Introduction

The wide versatility of palm oil has made it one of high demand globally. Nonetheless, 77\% of palm oil produced worldwide is consumed as food (Nesaretnam, 2017) and the remaining 23\% used in manufacturing biodiesel, drugs, cosmetics, polish, and detergent (Berger, 2010; Ohimain et al., 2013). Unfortunately, the safety of palm oil, as a food ingredient for meal preparation has come under serious attack globally due to fraudulent activities. In order to mask undesirable characteristics and to reduce the cost of processing palm oil, manufacturers and retailers intentionally add the Sudan IV dye, which is harmful to health. Incidentally, Ghana has been caught up in this menace as traces of the carcinogenic Sudan IV dye was detected in palm oil being exported from Ghana to the United Kingdom (UK). This led to a ban on palm oil from Ghana to the UK in 2005 (Rapid Alert System for Food and Feed, 2005). Other researches have confirmed the presence of the Sudan IV dye in palm oil (Amoako-Mensah, 2017; MacArthur et al., 2020). Aside the fraudulent activities that characterise the production and sale of palm oil, one major food safety issue that has implication for consumer health has been downplayed.

Palm oil is widely used in its crude form (CPO) across Africa and Asia (Albert and Astride, 2013). In its crude state, palm oil has most of its vitamin E and carotenoids intact making it a better option than refined palm oil in terms of nutrition and health (Berger, 2010). Globally, palm oil is processed using either the traditional or industrial method (Dongho et al., 2017). Basically, the two methods adopt the same procedure, with the only difference being the technology and the scale of production employed. However, it has been observed that the technology, which informs the level of processing, used greatly influences the quality of the oil produced (Ofosu-Budu and Sarpong, 2013). In West Africa, where palm oil is a major ingredient in most meals, palm oil production is done by smallholder producers who are often ignorant about modern techniques that yield safe palm oil (Dongho et al., 2017; Izah and Ohimain, 2013; Osei-Ampomah et al., 2012).

Oil palm production and palm oil processing in Ghana are dominated by small scale enterprises who use 77\% of land to produce 39\% of...
national output and processes 80% of CPO respectively (Ofosu-Budu and Sarpong, 2013). Most of the palm oil processed for human consumption is processed by these small-scale farmers who use traditional methods (Ofosu-Budu and Sarpong, 2013). The study by Osei Amponsah et al. (2018) on the role of small-scale enterprises in agricultural development agendas in Ghana concluded that the sector does not feature prominently in the current development plan of Ghana due to the quality of palm oil produced. Available literature implicates traditional methods of production, which is associated with poor sanitary and storage practices, in the poor microbiological quality of CPO (Madhusudhan et al., 2015; Okechalu et al. 2011; Tagoe et al., 2012). Additionally, CPO tends to have some amount of moisture and impurities due to inadequate processing and these factors invariably enhance the activities of microorganisms (Dongho et al., 2017; Morcillo et al., 2013). As a major food commodity, microbial contamination of CPO should be a source of worry since it is widely consumed in food, animal feed, and traditional medicine (Dongho et al., 2017).

Palm oil is usually used in cooking at temperatures adequate to destroy pathogenic organisms that are likely to be present in the oil. Nevertheless, CPO is also eaten raw in dishes such as “gari” and beans, and “abomu” (warmed raw palm oil poured on mashed cooked vegetables) especially in rural communities in Ghana. Again, in traditional communities and among some city dwellers, when people have diarrhoea, they are made to drink raw palm oil even though there is no scientific evidence and literature to support the potency of the oil in stopping the frequent defaecation. It is possible for the palm oil produced in Ghana to have microbial contaminants since poor handling and production practices have been reported (Adjei-Nsiah and Klerkx, 2016). Nonetheless, very few studies have been documented on microbiological contamination of palm oil in Ghana. The limited studies mainly focused on factors that affect palm oil quality, precisely determining physico-chemical properties such as free fatty acids (Osei-Amponsah et al., 2012). Studies on microbial quality of CPO in neighbouring countries have isolated biological contaminants of public health interest (Madhusudhan et al., 2015; Okogbenin et al., 2014; Okechalu et al. 2011).

Therefore, it is important to investigate the microbial quality of CPO processed in Ghana to protect the health of consumers who do not apply heat to the oil they consume as food or traditional medicine. This study was carried out to ascertain the microbial quality of CPO processed and sold on the Ghanaian market. If contaminated, organisms responsible were isolated and identified based on their cultural, morphological, and biochemical characteristics.

2. Materials and methods

2.1. Study area

The study was undertaken in seven regions of Ghana namely: Greater Accra, Ashanti, Eastern, Brong Ahafo, Volta, Western and Central regions of Ghana. Apart from Greater Accra region which was selected based on the fact that it is the hub of commercial activities, the other six regions process crude palm oil. Palm oil samples were collected from the markets within the capital towns of the selected regions.

2.2. Study design

The study employed an analytical cross-sectional design for sampling the palm oil. The study was carried out from August–December, 2019.

2.3. Sample collection

2.3.1. Sampling procedure and preparation

With the help of a clean 250 mL plastic bottle, ten palm oil samples each were randomly collected from the hub of all commercial activities in the capital towns of the study areas, making seventy samples. Sampling was done once, from August–December, 2019 between 6:00 am, and 9:00 am. The sampling bottles were tightly covered and appropriately labelled. They were immediately transported at room temperature in a box to the Teaching and Research Farm of the School of Agriculture, University of Cape Coast. The samples were stored at room temperature and away from light to prevent undesirable series of chemical reactions involving oxygen and light at the laboratory. After all the samples had been collected, they were sent to the Microbial Biotechnology Laboratory of the Department of Biochemistry, Kwame Nkrumah University of Science and Technology (KNUST) for analysis. The process carried out in quantifying, isolating, and identifying the microorganisms is summarised in a block diagram as shown in Figure 1.

2.4. Reagents preparation

2.4.1. Mannitol Salt Agar

One hundred and eleven grams (111 g) of Mannitol Salt Agar powder was weighed using an electronic balance (Golden Mettler 3003) into one litre (1L) media bottle (Pyrex). A litre of distilled water was measured and added to the weighed sample, stirred and brought to boil at 100 °C and allowed to dissolve completely. The solution was then sterilized by autoclaving at 121 °C for 15 minutes.

2.4.2. MacConkey agar

Twenty-six (26) grams of MacConkey Agar were weighed using an electronic balance (Golden Mettler 3003) and 500 ml of distilled water measured with a measuring cylinder and added to it. The mixture was brought to a boil to dissolve completely. The solution was then sterilized by autoclaving at 121 °C for 15 minutes and allowed to cool down to 50 °C. The cooled mixture was then poured into sterile Petri dishes.

2.4.3. Malt extract agar

Fifty (50) grams of Malt Extract Agar was weighed using Electronic balance (Golden-Mettler 3003) into Media bottles. Five hundred (500) ml of distilled water was measured using a beaker and added to the powder and mixed. The mixture was brought to a boil to dissolve completely and sterilized by autoclaving at 121 °C for 15 minutes. The sterilized mixture was cooled down to 50 °C and poured into sterile Petri dishes.

2.4.4. Serial dilution

Five (5) grams of each sample was weighed using Electronic balance (Golden-Mettler 3003) into dilution bottles and 45 ml of peptone water measured and added to the weighed sample and mixed. To help reduce the dense culture of cells formed to a lighter more useable concentration, it was serially diluted to 10⁻¹, 10⁻², 10⁻³, and 10⁻⁴ as portrayed in Figure 2.

For isolation of fungi, Malt Extract produced by OXOID Laboratories Basingstoke Hampshire, England was used. Other agars like Mannitol Salt Agar and MacConkey agar were used to isolate Staphylococcus species and to obtain the total Coliform count. All chemical reagents used were of analytical grade.

![Flow chart for quantifying, isolating and identifying microorganisms.](image)
2.5. Laboratory analysis

2.5.1. Microbial parameters
Parameters monitored in the laboratory included Staphylococcus aureus, total Coliform count (TCC), and fungi. Standard methods and procedures were used for the laboratory determination of all samples. The spread plate method was used to isolate and enumerate the various organisms. The determinations were done in duplicate. One millilitre aliquot from each of the dilutions was inoculated into already prepared petri dishes of the medium. The inoculum was evenly spread with a sterile bent rod and allowed to dry for 15 minutes and incubated for a specified period of time.

2.5.1.1. Determination of Staphylococcus aureus. Staphylococcus species were isolated and enumerated on salt mannitol agar (SMA). The plates of the dried inoculum were inverted and incubated at 35 °C for 24 hours (Albuquerque et al., 2007). After incubation yellow colonies formed on the plate were immediately counted and recorded as Staphylococcus counts.

2.5.1.2. Determination of total Coliform count (TCC). TCC was isolated and enumerated on MacConkey agar (MA) (Thunberg et al., 2002). The plates of the dried inoculum were inverted and incubated at 35 °C for 24 hours. Colonies formed were counted and recorded.

2.5.1.3. Determination and isolation of fungi. The fungi assay was carried out on malt extract agar (MEA). The plates of the dried inoculum were inverted and incubated. The plates of the dried inoculum were inverted and incubated were inverted and incubated at 25 °C for 120 hours. Colonies formed were counted and recorded.

2.6. Sub-culturing

Sub-culturing is done to transfer cultures from one medium into another medium by inoculation. Streak plating helps to isolate microorganisms from a mixed culture to obtain a pure culture. In examining the morphology, single colonies of microorganisms were identified and picked as unique isolates. These isolates were further picked and streaked into poured fresh nutrient agar plates and incubated for 24 hours to obtain pure isolates for further testing (Fung, 1985).

2.7. Biochemical tests

The Biochemical tests were done to confirm the isolated microorganisms’ identities and predict their fermentative profiles and prowess. For this study, the catalase, triple sugar iron (TSI), citrate, and Gram staining tests were carried out.

2.7.1. Catalase test
Catalase test was carried out to distinguish among Gram-positive cocci, differentiate aero tolerant strains of Clostridium (catalase-negative) and Bacillus strains (catalase-positive) (Fung, 1985). Five (5) ml of 3% H2O2 was prepared and a drop of it was placed on a glass slide. A loop was used to transfer a small amount of the colony growth onto the surface of the clean, dry glass slide.

2.7.2. Citrate utilisation test
Simmons citrate test probes the ability of microorganisms to utilise citrate as their carbon source. Organisms that can utilize citrate as their sole carbon source use the enzyme citrase to transport citrate into the cell. A sterilised straight inoculation needle was used to touch the top of a well-isolated colony. Simmons citrate agar was inoculated on the slant through the centre to the bottom and then streaked unto the agar slant. The agar slant was incubated at 35 °C.

2.7.3. Gram staining and microscopy
Gram stain is used to distinguish between two large groups of bacteria based on their different cell wall constituents. The gram stain distinguishes gram-positive groups from gram-negative groups by staining red or violet. A small amount of well-isolated colony was placed in a drop of physiological saline water on a dry slide. The slide was flame killed to kill microorganisms and fix unto the slide. Crystal violet staining reagent was placed on the heat fixed cell for about 1 minute. The slide was gently washed with distilled water. Gram A mordant, gram iodine was placed on the slide and washed using alcohol as a decolourising agent. Safranin was used as a counterstain and placed on the side then washed with distilled water until the colour disappeared. A drop of immersion oil was placed on each slide before observing it under the microscope (Fung, 1985).

2.8. Data analysis

Statistical analysis was performed by One-Way ANOVA with a 5% level of significance, using GraphPad Prism, version 5.0 for Windows. All statistical analyses were expressed as mean ± SEM. All graphs were constructed using GraphPad Prism version 5.0. Additionally, in cases where the error bars are shorter than the height of the symbol, GraphPad Prism does not draw the error bars.

3. Results

3.1. Quantitative assessment of Coliform contamination of oils

The outcome of the total Coliform count assay indicated the presence of Coliforms in oil samples from six out of the seven regions. Figure 3 portrays the results of total Coliform count on palm oil sampled from the regions used for the study. As indicated in the figure, CPO from the Central and Ashanti regions was the most contaminated followed by the
Volta and Brong Ahafo regions. None of the samples from the Greater Accra region was contaminated with the Western region having the least contaminants. In all, about 24% of the total number of samples used for the study was compromised with all (100%) of those samples having total Coliform counts that exceeded the acceptable maximum limit of $1.0 \times 10^2$ CFU/g for ready to eat foods. The total Coliform counts in palm oil samples recorded a maximum of $2.0 \times 10^2 \pm 6.03$ CFU/g and a minimum of $1.72 \times 10^3 \pm 6.66$ CFU/g as shown in Table 1. The statistical computation of the data however indicated a statistically significant differences ($p < 0.05$) in the recorded Coliform counts among the regions. The results suggest that the oil samples from the Ashanti and Central regions of Ghana were of poor microbial quality, while those from the Greater Accra and Western Regions were of a relatively better and acceptable microbial quality.

As depicted in Table 1, four samples each from the Ashanti and Central regions were contaminated with Coliform. However, Coliform count of samples from the Ashanti region scored the highest ($7.2 \times 10^2 - 1.5 \times 10^3$), followed by the Volta ($2.7 \times 10^2 - 3.7 \times 10^2$) and Western ($2.0 \times 10^2 - 3.1 \times 10^2$) regions respectively, even though they had comparatively fewer compromised samples. That notwithstanding, limits of comprised samples from all regions exceeded the acceptable threshold for ready to eat food. Data on the total Coliform count of compromised samples from the seven regions used for the study is provided in Appendix 1A and 1B.

### 3.2. Staphylococcus aureus determination

The assay for the determination of *Staphylococcus aureus* indicated *Staphylococcus aureus* in samples collected from five (Ashanti, Central, Western, Eastern, and Brong Ahafo) out of the seven regions used for the study. Volta and Greater Accra were the regions that recorded no *Staphylococcus aureus* in all the samples collected from those two regions. Among the detected *Staphylococcus aureus* were some *Staphylococcus epidermis* in some of the assayed samples.

### 3.3. Morphological characterization of isolates

The colonies were characterised using the shape, colour, margin, elevation, and optical characteristics on the agar plate as an index. Six colonies were obtained from the oils as repeating across samples with their morphological details as shown in Table 2.

### 3.4. Biochemical characterization of isolates

The biochemical profile of the isolated organisms featured covered their catalase and oxidase responses, citrate utilization, and sugar fermentation profiles as summarized in Table 3. The results in the table indicate that all the isolates were catalase and citrate positive. The sugar fermentation profile implied that they all ferment glucose, sucrose, and lactose to produce acids, and this resulted in a colour change of the red phenol indicator in the triple sugar iron test with one (1) isolate producing gas. The results also show that one (1) isolate was oxidase positive while all the others were negative.

Reconciliation of the morphological and biochemical characteristics with a standard database on bacteria identification indexed six unique species of bacteria in the oil samples. The phylogenetic index used in this study was not exhaustive but sufficient to point to the genus and moderately precise suspected species of the organism under classification. The details of the identities of the various isolates are provided in Table 4.

### 4. Discussion

Microorganisms are ubiquitous and diverse and thus can be found in a diversity of environments thriving under varying conditions. Palm oil is a rich source of carbon and when supplied with vitamins and minerals, it provides a suitable medium for microorganisms to exist and thrive under the right conditions of pH, temperature, aeration, and pressure. Considering the fact that it is relatively difficult to detect microorganisms of interest when the population is low, the prevalence of pathogens was carried out by initial estimation of hygiene indicator organisms (FAO/WHO, 2016). The findings of this study indicate that there was seemingly high Coliform contamination in oil samples from all regions used for the study and most of the samples recorded counts exceeding the maximum threshold of $1.0 \times 10^2$ CFU/g by a factor of $10^2$ which is quite commendable even though it is a cause for concern.

Coliflora, broadly classified into lactose fermenters and non-lactose fermenters, are a diverse group of microorganisms with some associated degree of pathogenicity and toxicity, particularly with some species such as *Salmonella*. This explains why Coliflora are of interest in food safety and quality analysis, particularly as an index of hygiene and safety. The Coliform counts recorded in this study though seemingly high, qualify them as the incidence of moderate risk since these organisms are heat sensitive and labile thus may fade out when heat is applied. Admittedly, palm oil is mostly used in heat-related processes such as frying, in this part of the continent and therefore the tendency for the microbial load to reduce drastically before consumption as far as there is
perturbed accumulation in the body system can trigger some physiological (pneumonia), or other parts of the human body if consumed after storage conditions (FAO/WHO, 2016). Ideally, adequate and effective to ineffectiveness of the processing methods used and possibly poor environmental factors and conditions that prevail in the respective regions. which could be attributed to the differences in sources of the raw ma-

no recontamination. Nonetheless, microbial contamination of palm oil poses health risk to those who consume palm oil without applying heat. Surprisingly, the Ashanti region where palm oil is predominantly eaten raw in a delicacy (“abomu” - mashed “kontomire”/garden eggs in tomato sauce), registered the highest Coliform count. “Abomu” is also enjoyed by people of Central and Brong Ahafo regions where Coliform counts were also relatively high.

The statistical analysis on Coliform counts reflected significant difference (P < 0.05: P = 0.0226) in the loads across the various regions, which could be attributed to the differences in sources of the raw material (palm fruits), mode of processing and packaging, as well as environmental factors and conditions that prevail in the respective regions. Generally, the presence of Coliform, which is a hygiene indicator, points to ineffectiveness of the processing methods used and possibly poor storage conditions (FAO/WHO, 2016). Ideally, adequate and effective heat processing should render a food free of any hygiene indicators. Thus, the presence of Coliform in the oil samples could be an indication of the fact that there were issues with the processing procedure. This presupposes that palm oil samples from the Greater Accra region received adequate and effective heat treatment and the opposite is the case for the other regions, especially the Central and Ashanti regions. Okogbenin et al. (2014) isolated food pathogens from freshly milled palm oil in their investigation on the effect of sterilization on palm oil quality in Edo State, Nigeria. The authors however attributed the contamination of the oils to post production handling practices. Casual observation of palm oil processors and retailers revealed that new oil may be poured into a used bottle or mixed with old stock of oil. These practices may lead to recontamination of otherwise sterile oil.

P. aeruginosa, S. aureus, S. epidermidis, and E. coli were found to be present in the oil samples collected in this study. P. aeruginosa is the only common bacterium isolated in the study of Tagoe et al. (2012) on factors influencing the quality of palm oil produced at the cottage industry level in Ghana and the present study. The presence of these organisms in oils has implications for the oil and ultimately human health. Advancement in microbiological studies has established P. aeruginosa and S. aureus as lipolytic organisms associated with pathogenicity (Izah and Ohimain, 2013). P. aeruginosa for instance, causes infections in the blood, lungs (pneumonia), or other parts of the human body if consumed after surgery.

Again, the consumption of pathogenic levels of these organisms and their accumulation in the body system can trigger some physiological responses in the consumer. Staphylococcus aureus for instance is known to produce enterotoxins which can result in food poisoning as well as gastroenteritis especially when the oil is consumed raw without further adequate heat treatment and processing (Tesfaye et al., 2015). The danger is that these enterotoxins can persist in the oil for prolonged periods, thus rendering it an issue of public health concern. With regard to the oil, the production of these lipases ultimately leads to the cleavage of triglycerides into free fatty acids in the oils which results in the initiation of rancidity and ultimately compromised oil quality and rapid spoilage. This unique action of bacterial lipases affects the physicochemical properties such as free fatty acid value, peroxide value and iodine value as well as rancidity.

P. aeruginosa is found commonly in soil and water, E. coli in the intestines of humans and animals and in the environment, and S. aureus on human skin, in the nose, armpit, groin, and other areas. S. aureus is an indicator organism for hygiene as it is mostly transferred from humans to food via handling and packaging just as E. coli. The detection of these indicator organisms in the palm oil samples thus point to probable poor hygienic practices during processing and packing by producers and handling by distributors and retailers (Tesfaye et al., 2015). By inference, and based on the conclusion of Okogbenin et al. (2014), it stands to reason that poor hygienic practices can expose even sterile oil to contamination. Other microbes that have been isolated in similar studies and scientific reviews on microbial quality of palm oil are Aspergillus spp., Salmonella sp., Penicillium sp. Enterobacter, Bacillus, Proteus, Micrococcus, Trichphyton schoenleinii, Microsporum canis, Candida, and Mucor (Enyoh et al., 2018; Izah et al., 2018; Nganjoh et al., 2020; Okogbenin et al., 2014; Tagoe et al., 2012).

The absence of yeast and moulds in the palm oil points to the freshness of the palm oil used for the study. Oils generally accumulate fungal loads upon prolonged storage, especially under unhygienic conditions. The fungal load could also be high when rotten palm fruits are used in the production of the oil. Fungi, particularly moulds develop spores that can withstand harsh treatments and persist in the palm oil after extraction and processing. The absence of fungi in the samples thus reflects the probable quality of raw materials and the effectiveness of the processing method used in the production of the oils.

### Table 3. Biochemical profile of isolated bacteria from oil samples from 7 regions in Ghana.

| Isolate | Catalase | Oxidase | Citrate | Glucose | Lactose | Sucrose | Gas |
|---------|----------|--------|---------|---------|---------|---------|-----|
| 1       | +        | -      | +       | +       | +       | +       | -   |
| 2       | +        | -      | +       | +       | +       | +       | +   |
| 3       | +        | +      | +       | +       | +       | +       | +   |
| 4       | +        | -      | +       | +       | +       | +       | +   |
| 5       | +        | -      | +       | +       | +       | +       | -   |
| 6       | +        | +      | +       | +       | +       | +       | -   |

| Isolate | Inference |
|---------|-----------|
| 1       | Staphylococcus aureus |
| 2       | Staphylococcus aureus |
| 3       | Escherichia coli |
| 4       | Pseudomonas aeruginosa |
| 5       | Staphylococcus aureus |
| 6       | Staphylococcus epidermidis |
5. Conclusion

The oils sampled from the selected regions in Ghana were of moderately good quality even though count of organisms of all samples were above acceptable limit. Total Coliform counts ranged from $2.0 \times 10^3$ ± $6.0^2$ CFU/g to $1.72 \times 10^2$ ± $6.66$ CFU/g with statistically significant differences ($P < 0.05$) in the observed loads among the regions. There was absence of yeast and moulds in the oils as well as extremely pathogenic Coliforms such as Salmonella and Shigella spp. S. aureus, S. epidermidis, E. coli and P. aeruginosa were the dominant Coliforms found in the oils. Overall, findings showed (Table Appendix 1A and Appendix 1B) that oil from the Greater Accra region was of the highest quality and safe for consumption whereas the palm oil from the Central and Ashanti regions were of the most poor quality. The presence of the isolated microbes could be an indication of unhygienic handling of the oil since the oil proved to be fresh with the absence of yeast and moulds. Thus, the contamination is likely to be from the environment and unhygienic handling practices.

In Ghana, palm oil is widely consumed as food and in some cases without any further heat treatment. In view of that the microbial quality of the oil is of public health importance. It is therefore imperative for a further research that extends the study to cover other markets and smallholder palm oil processors for a conclusive evidence. Additionally, an audit tool should be developed to observe production and retail practices of palm oil handlers. In the meantime, it is imperative to educate consumers to further process palm oil before eating it.

Declarations

Author contribution statement

Roseline Love MacArthur: Performed the experiments; Analyzed and interpreted the data and Wrote the first draft manuscript.

Ernest Teye: Conceived and designed the experiments, Supervised the experiment, Reviewed the first manuscript.

Sarah Darkwa: Supervised, Analyzed and interpreted the data, Reviewed the manuscript.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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Appendix

Appendix 1A. Total Coliform count of compromised samples from Volta, Western, Brong Ahafo and Ashanti Regions

| Volta region          |       |       |       |       |       |       |       |
|-----------------------|-------|-------|-------|-------|-------|-------|-------|
| Sample                | Rep   | $10^3$ | $10^2$ | $10^3$ | $10^4$ | $10^5$ | CFU/g |
| 1                     | 1     | 33     | 3      | -      | -      | -      | 3.7 $\times 10^2$ |
|                       | 2     | 42     | 5      | -      | -      | -      | 2.7 $\times 10^2$ |
| 2                     | 1     | 21     | 1      | -      | -      | -      | 2.0 $\times 10^2$ |
|                       | 2     | 32     | 4      | -      | -      | -      | 2.0 $\times 10^2$ |
| Western region        |       |       |       |       |       |       |       |
| 1                     | 1     | 23     | 3      | -      | -      | -      | 3.1 $\times 10^2$ |
|                       | 2     | 39     | 2      | -      | -      | -      | 2.0 $\times 10^2$ |
| 2                     | 1     | 19     | 1      | -      | -      | -      | 2.0 $\times 10^2$ |
|                       | 2     | 21     | 2      | -      | -      | -      | 2.0 $\times 10^2$ |
| Brong Ahafo region    |       |       |       |       |       |       |       |
| 1                     | 1     | 23     | 3      | -      | -      | -      | 2.9 $\times 10^2$ |
|                       | 2     | 35     | 7      | -      | -      | -      | 1.9 $\times 10^2$ |
| 2                     | 1     | 15     | 5      | -      | -      | -      | 1.5 $\times 10^2$ |
|                       | 2     | 23     | 2      | -      | -      | -      | 1.5 $\times 10^2$ |
| 3                     | 1     | 11     | -      | -      | -      | -      | 1.5 $\times 10^2$ |
|                       | 2     | 19     | 2      | -      | -      | -      | 1.5 $\times 10^2$ |
| Ashanti region        |       |       |       |       |       |       |       |
| 1                     | 1     | 38     | 6      | -      | -      | -      | 3.2 $\times 10^2$ |
|                       | 2     | 26     | 9      | -      | -      | -      | 2.0 $\times 10^2$ |
| 2                     | 1     | 82     | 15     | -      | -      | -      | 7.2 $\times 10^2$ |
|                       | 2     | 66     | 22     | 1      | -      | -      | 2.5 $\times 10^2$ |
| 3                     | 1     | 34     | 5      | -      | -      | -      | 2.5 $\times 10^2$ |
|                       | 2     | 21     | 2      | -      | -      | -      | 2.5 $\times 10^2$ |
| 4                     | 1     | 138    | 48     | 3      | -      | -      | 1.5 $\times 10^2$ |
|                       | 2     | 154    | 35     | 8      | -      | -      | 1.5 $\times 10^2$ |
Appendix 1B. Total Coliform count of compromised samples from Eastern and Central Regions

| Sample | Rep | 10^1 | 10^2 | 10^3 | 10^4 | 10^5 | cfu/g |
|--------|-----|------|------|------|------|------|-------|
| Eastern region | | | | | | | |
| 1 | 1 | 25 | 9 | - | - | - | 1.9×10^5 |
| | 2 | 14 | 2 | - | - | - | |
| 2 | 1 | 19 | 1 | - | - | - | 2.0×10^5 |
| | 2 | 21 | 2 | - | - | - | |
| Central region | | | | | | | |
| 1 | 1 | 71 | 16 | - | - | - | 2.0×10^5 |
| | 2 | 59 | 19 | 3 | - | - | |
| 2 | 1 | 185 | 55 | 9 | - | - | 1.7×10^5 |
| | 2 | 163 | 62 | 11 | - | - | |
| 3 | 1 | 34 | 5 | - | - | - | 3.1×10^5 |
| | 2 | 28 | 8 | - | - | - | |
| 4 | 1 | 38 | 6 | 3 | - | - | 3.5×10^5 |
| | 2 | 34 | 5 | - | - | - | |

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