

INVESTIGATING THE OCCURRENCE OF FUNGI AND BACTERIA IN FRUITS SPOILAGE IN SELECTED MARKETS IN BWARI AREA COUNCIL, ABUJA, Nigeria.

ABSTRACT

Aim: This study evaluated the microorganisms that cause spoilage of four different fruits sold in selected markets in Bwari Area Council, Abuja.

Study design: Factorial experimental design was employed for this study.

Place and Duration of Study: Department of Microbiology, Veritas University Abuja between July to September, 2020.

Methodology: Twenty-four samples (6 samples each) of four different fruits were obtained from Zuma, Kubwa and Bwari market, in Bwari Area Council. Standard microbiological method was used for the isolation, enumeration and identification of the bacteria and fungi species. Percentages were used to determine the distribution of the isolates in the different locations. Data collected were also subjected to Chi square test at $P=0.05$ level of probability to check significance difference between the fruit's location, abundance and occurrence of the isolates

Results: After microbiological analysis of the twenty-four samples, it was observed that the bacterial isolates include; *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella* spp while the fungal spp were of the genus *Aspergillus*, *Fusarium* and *Mucor*. The bacterial count ranged from 1.2×10^6 to 6.1×10^6 CfU/ml and that of fungi ranged from 3.1×10^6 to 5.4×10^6 CfU/ml while the most occurring bacterial species was *Staphylococcus aureus* and *Aspergillus* spp occurred most for the fungi isolates. The Statistical analysis showed that there was no significance difference between the occurrence and abundance of fungi and location.

Conclusion: Bacteria and fungi are associated with the spoilage of the selected fruits in Bwari Area Council and contamination rates are relatively high. The most common fungi associated with pawpaw, mango, banana and watermelon spoilage were mainly *Aspergillus flavus*, *Mucor species*, *Fusarium species* and *Aspergillus niger* while bacteria associated with these fruits were *Staphylococcus aureus*, *Klebsiella species*, and *Escherichia coli*. However, the most abundant species of bacteria and fungi associated with spoilage of fruits in Bwari Area Council were *Aspergillus species* and *Staphylococcus aureus* respectively.

Keywords: Fruits, spoilage, Bwari, Bacteria, Fungi

1. INTRODUCTION

Fruits are the comestible part of mature ovary of flowering plants which are normally eaten raw [1]. Fruits also include many structures that are not commonly called fruits such as bean pods, corn kernels, tomatoes, and wheat grains according to [1]. The importance of fruit in human nutrition cannot be overestimated as it provides essential growth factors such as vitamins and minerals necessary for proper body metabolism [2]. Humans and many animals have become dependent on fruits as a source of food.

However, fruits are easily spoilt and usually have active metabolism during the storage stage [3]. The high concentration of various sugars, minerals, vitamins, amino acids, and low pH also enhances the successful growth and survival of various forms of fungal and bacterial species. Annual reports have shown that 20% of fruits and vegetables produced are lost to spoilage especially during post-harvest stages. This has been associated with spoilage fungi and bacteria which can be toxigenic or pathogenic. Toxin-producing fungi and bacteria have been identified and isolated from spoilt fruits by previous researchers [2]. Pathogenic fungi and bacteria have been reported in cases of infections or allergies. *Aspergillus spp.* and *Cyanobacteria* produces mycotoxins and other toxic metabolites which can be harmful to humans and animals globally.

Micro-organisms, especially fungi, are known to destroy fruits, thereby reducing the quantity for consumption and the profits obtained from sales of fruits. There is need to identify these micro-organisms especially those that are pathogenic to humans so as to reduce the risk of contamination and infection arising from handling and consumption of fruits. Therefore, this study was undertaken to evaluate microbiologically spoilt fruits commonly sold in Bwari market, Abuja, and recommend appropriate control measure.

2. MATERIAL AND METHODS

Study Area

The study was conducted in Bwari, Abuja. Bwari is a district and an area council in the Federal Capital Territory (FCT) of Nigeria. The original inhabitants of the town are the Gbagyi speaking people. The paramount ruler is the Esu who is otherwise known as Sa-bwaya. However, with the establishment of FCT in Abuja so many changes occurred; such changes include the *turbaning* of late Musa Ijokoro (of Koro ethnic minority, and from Suleja Emirate where parts of Abuja's land were carved out) as District Head of Bwari in 1976, and his elevation as Sarki of Bwari to the position of a second-class status in 1997 by the Ministry of the Federal Capital Territory under the then minister, General Jeremiah Useni [4].

Sample Collection

A total of 24 samples comprising of 6 samples each of the fruits; water melon, pawpaw, banana and mango were purchased from Kubwa market, Bwari market and Zuma Market in Bwari Area Council, Abuja. The samples were immediately transported to the microbiology laboratory using clean aluminum foil and analyzed within 24 hours of collection.

Isolation of microorganisms

The nutrient agar and potato dextrose agar media were used for the isolation of bacteria and fungi respectively. Each was prepared following manufacturer's instructions. These were sterilized by autoclaving at 121°C at 15 psi pressure for 15 minutes, after which they were removed from the autoclave and allowed to cool before pouring into sterile Petri dishes.

Ten (10) grams of each of the fruit samples were homogenized in 90 mls of sterile distilled water using an electric blender. After this, ten - fold serial dilution of the various samples was carried out [5]. From the appropriate dilution, 0.1 ml was plated onto the different media and the plates were incubated at 37°C for 48 hours for bacteria while the Potato Dextrose agar plates were incubated at 28°C for 72 hours to obtain the fungal growth. After incubation discrete colonies were sub-cultured into freshly prepared nutrient and potato dextrose agar plate for bacteria and fungi respectively. Total viable count was carried out and expressed as colony forming units per ml (CFU/ml).

Characterization and identification of the bacterial isolates

After incubation, each colony was sub cultured on freshly prepared nutrient agar medium to obtain pure culture. These were gram stained and subjected to preliminary identification using biochemical tests as described by [6].

Characterization and Identification of fungi isolates

A drop of lactophenol cotton blue stain was placed on a clean slide and with the aid of a mounted needle, a small portion of the mycelium from the fungal culture plates was removed and placed in the drop of the lactophenol cotton blue. This was teased on the slide with the aid of the two mounted needles and a cover slip was gently lowered on it. The slide was examined under the microscope of x40 objective. Morphological characteristics of the fungi such as type of hyphae and asexual reproductive structure was observed.

Calculation of Percentage frequency of Microbes

Percentage frequency of fungi in each fruit type will be calculated using the formula

$$\text{Percentage frequency} = \frac{\text{Total number of positive samples}}{\text{Total number of samples collected}} \times 100$$

Data analysis

Descriptive statistics such as percentages, proportions and frequency distributions were applied to compute some of the data. Data collected were also subjected to Chi square test at P=0.05 level of probability to check significance difference between the fruit's location, abundance and occurrence of the isolates [7].

3. RESULTS AND DISCUSSION

Results

Microbial Counts of fruits in Selected Markets in Bwari Area Council

From this study, total bacterial counts differed from location to location with highest values recorded for water melon from Kubwa market with mean counts of 6.1×10^5 and least count recorded in Banana from Zuma market with a total bacterial count of 1.20×10^5 . Water melon recorded mean bacterial counts of 2.30×10^6 , 6.1×10^6 and 5.8×10^6 in Bwari, Kubwa and Zuma markets respectively. Pawpaw recorded mean bacterial counts of 2.40×10^6 , $1.4.0 \times 10^6$ and 1.80×10^6 respectively for Bwari, Kubwa and Zuma markets. Banana recorded a mean bacterial count of 1.30×10^6 , 2.1×10^6 and $1.2.0 \times 10^6$ for Bwari, Kubwa and Zuma markets respectively while mango recorded mean bacterial counts of 4.20×10^6 , 3.90×10^6 and 2.11×10^6 respectively for Bwari, Kubwa and Zuma markets respectively [Table 1].

In terms of fungal counts, Water melon recorded total fungal counts of 5.40×10^3 , 7.30×10^5 and 4.60×10^6 for Bwari, Kubwa and Zuma markets respectively; Pawpaw recorded 4.3×10^5 , 6.00×10^3 and 5.40×10^3 , Banana recorded 2.40×10^5 , 2.90×10^5 and 1.90×10^5 while Mango recorded 3.20×10^5 , 2.90×10^5 and 3.11×10^5 counts for Bwari, Kubwa and Zuma markets respectively [Table 2].

Table 1: Mean bacterial Counts (CFU/ml) in fruits sold in Bwari Area Council

Fruit	Location		
	Bwari	Kubwa	Zuma
Water Melon	2.30 x10 ⁶	6.1 x10 ⁶	5.8 x10 ⁶
Pawpaw	2.40 x10 ⁶	1.40 x10 ⁶	1.80 x10 ⁶
Banana	1.30 x10 ⁶	2.1 x10 ⁶	1.20 x10 ⁶
Mango	4.20 x 10 ⁶	3.90 x 10 ⁶	2.11 x 10 ⁶

Table 2: Mean Fungal Counts (CFU/ml) in fruits sold in Bwari Area Council

Fruit	Location		
	Bwari	Kubwa	Zuma
Water Melon	5.40 x10 ³	7.30 x10 ⁵	4.60 x10 ⁶
Pawpaw	4.3 x10 ⁵	6.00 x10 ³	5.40 x10 ³
Banana	2.40 x10 ⁵	2.90x10 ⁵	1.90 x10 ⁵
Mango	3.20 x 10 ⁵	2.90 x 10 ⁵	3.11 x 10 ⁵

Distribution of bacteria in fruits sold in Bwari Area Council

The result of the findings revealed that water melon samples had 16.66 % *E. coli*, 66.66 % *Staphylococcus aureus* and 16.67 % *Klebsiella species*. Pawpaw samples had 33.33 % *E. coli*, 66.66 % *Staphylococcus aureus* and 0 % *Klebsiella species*; Banana recorded 0.00 % *E. coli*, 83.33% *Staphylococcus aureus* and 16.67% *Klebsiella species* while Mango recorded 33.33% *E. coli*, 33.33% *Staphylococcus aureus* and 33.33% *Klebsiella species* [Table 3].

Table 3: Distribution of bacteria in fruits sold in Bwari Area Council

Fruits	Bacteria			Total
	<i>E. coli</i>	<i>Staphylococcus aureus</i>	<i>Klebsiella spp</i>	
Water melon	1(16.66%)	4(66.66%)	1(16.6%)	6(25.0%)
Pawpaw	2(33.33%)	4(66.66%)	0(0.00%)	6(25.0%)
Banana	0(0.00%)	5(83.33%)	1(16.67%)	6(25.0%)
Mango	2(33.33%)	2(33.33%)	2(33.33%)	6(25.0%)

Total	5(20.83%)	15(62.50%)	4(16.67%)	24(100.0%)
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Distribution of fungi in fruits sold in Bwari Area Council

Aspergillus flavus was observed to be the most dominant fungal species encountered in all the four types of fruit samples collected. Percentage occurrence of *Aspergillus niger* was 37.50%, *Fusarium* species was 8.33%, *Aspergillus flavus* 41.67% and *Mucor* species 12.5%. Water melon had 83.33 % *Aspergillus niger*, 0 % *Fusarium* species, 16.67 % *Aspergillus flavus* and 0 % *Mucor* species. Pawpaw had % 33.33 % *Aspergillus niger*, 16.67 % *Fusarium* species, 50.00% *Aspergillus flavus* and 0.00%% *Mucor* species; Banana recorded 16.67 % *Aspergillus niger*, 0 % *Fusarium* species, 66.67 % *Aspergillus flavus* and 16.67% *Mucor* species while Mango had 16.67 % *Aspergillus niger*, 16.67 % *Fusarium* species, 33.33 % *Aspergillus flavus* and 33.33 % *Mucor* species.

Table 4: Distribution of fungi in fruits sold in Bwari Area Council

Fruits	Fungi				Total
	<i>Aspergillus niger</i>	<i>Fusarium</i> species	<i>Aspergillus flavus</i>	<i>Mucor</i> species	
Water melon	5(83.33%)	0(0.00%)	1(16.67%)	0(0.00%)	6(25.0%)
Pawpaw	2(33.33%)	1(16.67%)	3(50.00%)	0(0.00%)	6(25.0%)
Banana	1(16.67%)	0(0.00%)	4(66.67%)	1(16.67%)	6(25.0%)
Mango	1(16.67%)	1(16.67%)	2(33.33%)	2(33.33%)	6(25.0%)
Total	9(37.50%)	2(8.33%)	10(41.67%)	3(12.5%)	24(100.0%)

Table 5: Biochemical Characteristics of Bacterial Isolates

Biochemical Tests

Gram Reaction	Motility	Catalase	Coagulase	Indole	Citrate	Suspected Organism
+	-	+	+	-	+	<i>Staphylococcus aureus</i>
-	+	+	-	+	-	<i>Escherichia coli</i>
-	+	+	-	-	+	<i>Klebsiella species</i>

Regional Distribution of bacteria in fruits sold in Bwari Area Council

Occurrence of bacteria in selected fruits in Bwari area council differed from region to region with 60.0%, 20.0% and 20.0% *Escherichia coli* recorded in Bwari, Kubwa and Zuma markets respectively. 26.67%, 26.67% and 46.67% *Staphylococcus aureus* was recorded in Bwari, Kubwa and Zuma markets respectively while 25.00%, 0.00% and 75.00% *Klebsiella species* were recorded in Bwari, Kubwa and Zuma markets respectively (Figure 1).

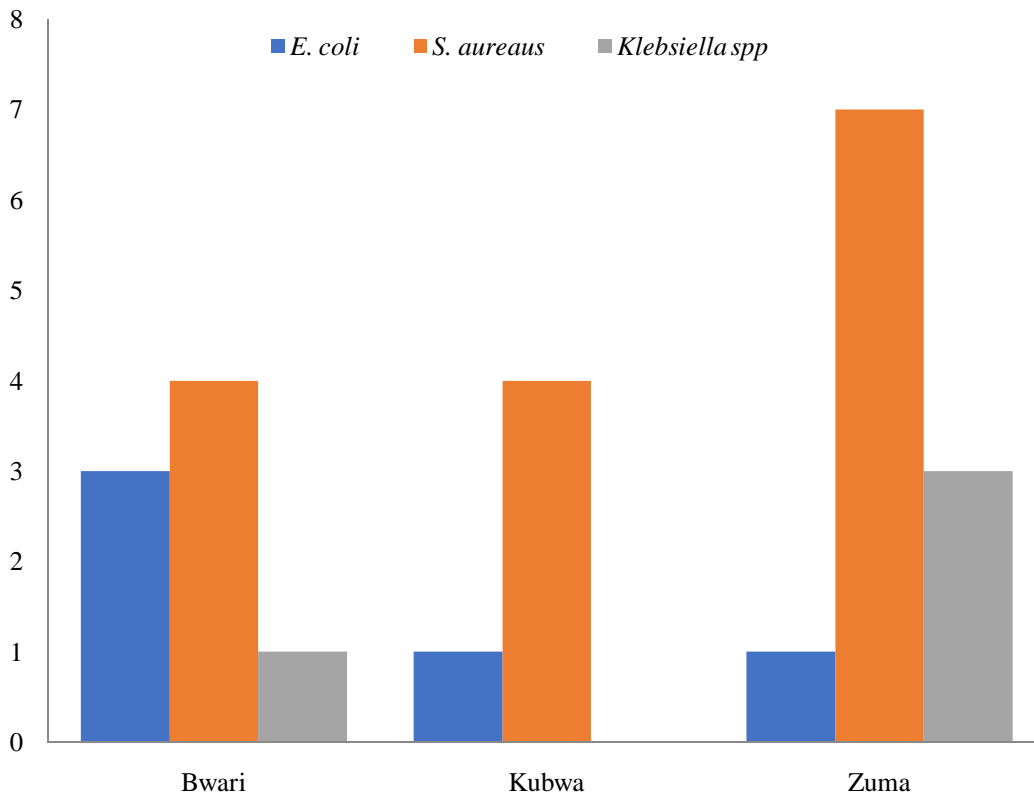


Figure 1: Regional distribution of bacteria in fruits sold in Bwari Area Council

Regional Distribution of fungi isolated from fruits in Bwari Area Council

a percentage occurrence of 30.00%, 33.33%, 0.00% and 33.33% respectively for *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporium*, and *Mucor* species. Kubwa market had percentage occurrence of 20.00%, 44.44%, 0.00% and 66.67% for *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporium*, and *Mucor* species respectively while Zuma market had percentage occurrence of 50.00%, 22.22%, 100.00% and 0.00% each for *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporium*, and *Aspergillus* species respectively.

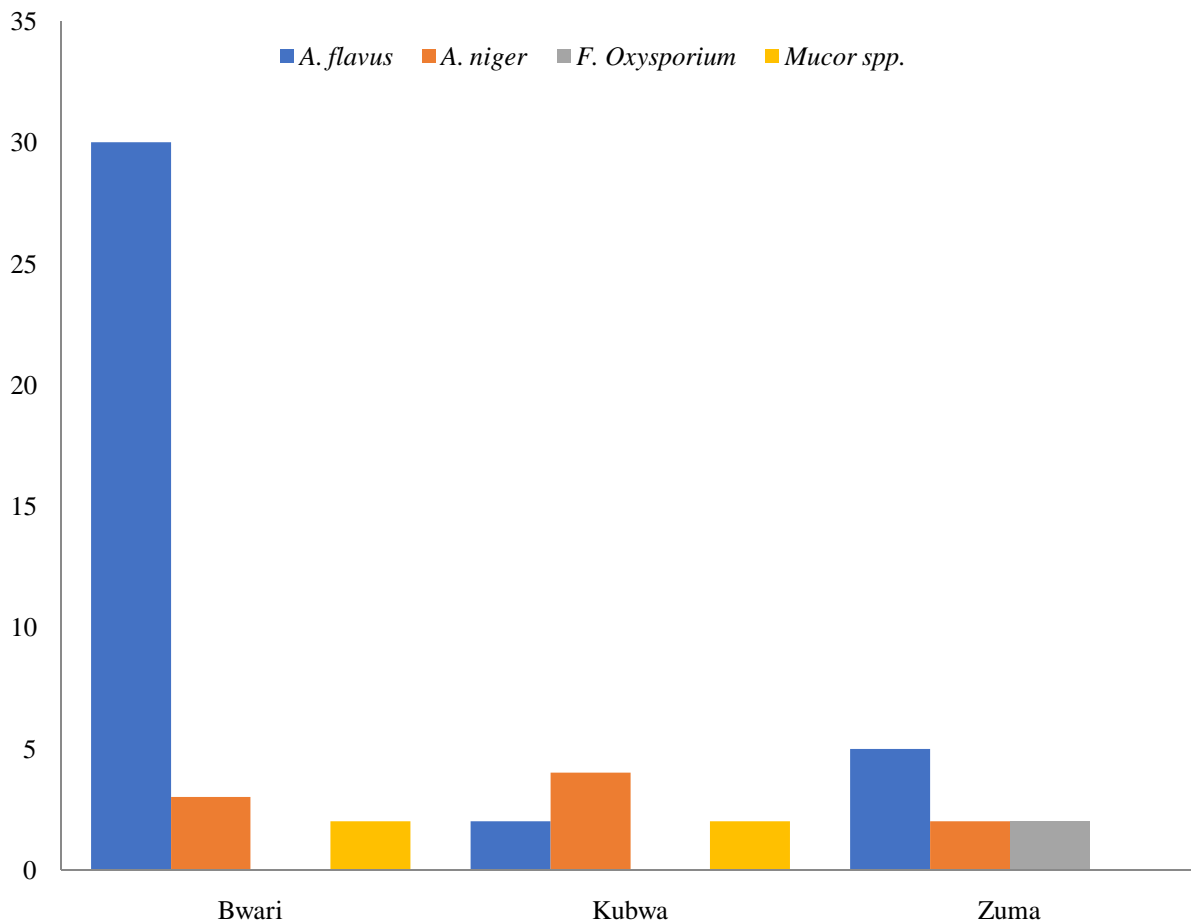


Figure 2: Regional Distribution of fungi in fruits sold in Bwari Area Council

DISCUSSION

This study was aimed at investigating the occurrence of fungi and bacteria on selected fruits namely pawpaw, mango, banana and watermelon in Bwari Area Council. All samples collected had fungal and bacterial growth. Three bacteria species namely *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella species* were isolated in this study. This finding is similar to those of [8; 9; 10;11].

The result of the present study showed bacterial load which ranged from 1.20×10^6 to 5.8×10^6 , which is higher than the report of [10] and however lower than the report of [11]. A possible reason of variation in bacterial counts between the findings of this study and previous studies may be location which affects the extent to which fruits are exposed to microbial contaminants.

Staphylococcus aureus recorded the highest occurrence in this study with a percentage of 62.50%. This finding is in agreement with [10; 11] who reported a similar trend in their studies. A high occurrence of this bacterium is a pointer to the unhygienic conditions of the study locations since occurrence of this bacterium is usually linked to how clean or dirty an environment is [8]. The presence of *Escherichia coli* which is an indicator organism might however be as a result of **unclean hands of the vendor, contact with sewage and contaminated water [12]** and this might have adverse effect on the health of consumers.

Four fungal species namely *Aspergillus niger*, *Fusarium species*, *Aspergillus flavus* and *Mucor species* were isolated from the fruits sampled. This is an indication that these pathogens could grow and survive in these fruits which implies that

water melon, pawpaw, banana and mango are prone to infections by a variety of fungal pathogens. The fungi species mainly *Aspergillus* with *Aspergillus flavus* having the highest percentage occurrence [41.67%] were the most encountered in this study. This is similar to the report of [13] whose findings revealed the existence of *Aspergillus species* as the most abundant fungal species in Nsukka and also similar to the findings of [14] who reported *Aspergillus flavus* as the most dominant fungal species in fruits sold in Wuse Market, Abuja, Nigeria. The findings also agree with the report of [15] who reported highest prevalence of *Aspergillus flavus* in fruits in Ibadan, South-Western Nigeria. The high occurrence of *Aspergillus species* in this study could be attributed in part to the nature of these fungi that usually produce numerous airborne sessile spores that can easily land on the fruits while on display in the markets for sale as well as before storage in refrigerators. The possession of suitable nutrients on fruits which supports growth of microbes might be another possible reason for the abundance of fungal species in this study.

The high rate of isolation of fungi species from some fruits in this study showed that fungi are responsible for post-harvest deterioration of some edible fruits in Bwari Area Council. This is similar with the reports of [16] who reported that fungi are responsible for post-harvest losses of edible fruits in Enugu. It is also similar to the reports of [17] who reported fungi as the spoilage organism of some edible fruits and vegetables in North-east Nigeria. According to [16], losses due to post harvest spoilage or pathological decay are as a result of latent infection in the field that become active following harvest or of cross contamination during harvest, cleaning, storage and distribution. This further supports the findings of this study.

Generally, spoilage fungi are considered toxigenic or pathogenic and toxigenic fungi have been isolated from spoiling fruits in the past [2]. During refrigeration, some fungi may produce mycotoxins which could cause infections or allergies and *Aspergillus species* which was most abundant in this study indicates the tendency of production of toxic metabolites which can be of detrimental effect to human health.

In terms of location, Zuma market of Bwari Area council recorded highest fungal and bacterial contamination of fruits sold. This is not just a coincidence but an eye-opener to the need for adoption of good hygienic practices in this market by vendors and buyers too. Statistical analysis using Chi-square test showed that there is no significant relationship between occurrence of fungi and location at $P > 0.05$. There was also no significant relationship between abundance of fungi and location. In terms of fruits and bacteria isolated, there was no significant association observed between fruits and abundance of bacteria at $P > 0.05$.

4. Conclusion

Bacteria and fungi are associated with selected fruits in Bwari Area Council and contamination rates are relatively high. The most common fungi associated with pawpaw, mango, banana and watermelon are mainly *Aspergillus flavus*, *Mucor species*, *Fusarium species* and *Aspergillus niger* while bacteria associated with these fruits are *Staphylococcus aureus*, *Klebsiella species*, and *Escherichia coli*. However, the most abundant species of bacteria and fungi associated with spoilage of fruits in Bwari Area Council are *Aspergillus species* and *Staphylococcus aureus* respectively.

High occurrence of *Aspergillus species* in this study is an indication of the possibility of aflatoxin contamination in the near future, if necessary, precautions are not taken and implemented. This implies that the fruits examined in this study requires necessary public health attention. Also, location seemed to have no effect on the distribution of microbial contaminants in fruits sold in Bwari Area Council. This was evident from statistical analysis.

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