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# Determination of Heavy Metals and Biological Contaminants Present in Locally Processed Tomato, Pepper and Onion Puree Samples from Maiduguri Metropolis, Borno State, Nigeria

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## ABSTRACT

Contamination of food by heavy metals and microorganisms often occurs right from the farmlands and during processing. This endangers health by predisposition to diseases and factors that can initiate carcinogenesis. The levels of heavy metals and microbial contaminants in tomato, pepper and onion puree processed in some commercial milling points in Maiduguri metropolis was assessed. Twenty samples were obtained by random selection and analyzed for heavy metals by atomic absorption spectroscopy. Samples were cultured on nutrient, blood, MacConkey and mannitol salt agar media for 72 hours using streak plate method. The concentrations of heavy metals were within the range: mercury (0.158 ± 0.10 to 0.321 ± 0.27 mg/L), lead (0.167 ± 0.08 to 0.317 ± 0.25 mg/L), nickel (0.222 ± 0.11 to 0.574 ± 0.23 mg/L) and copper (0.032 ± 0.03 to 0.057 ± 0.03 mg/L) while cadmium was not detected in all the samples analyzed. Three pathogenic gram-negative (*Escherichia coli* Klebsiella species and *Proteus mirabilis*) and two less pathogenic gram-positive bacteria (*Bacillus subtilis*, *Coryne bacterium* specie) were isolated from the samples. The detection of very low concentrations of heavy metals was within WHO safety limits, while the presence of pathogenic bacteria rises concern over the health status of the general public. There is need to create awareness about good hygiene practices to operators of commercial milling machines within the study area.

**Keywords**: Cancer, Contaminants, Heavy metals, Pathogenic microorganisms

## INTRODUCTION

The aetiology of many diseases including cancers has been associated with environmental factors such as exposures to known toxicant metals and pathogens in the environment. This exposure may come from the food and water we eat/drink, the air we breathe, the surfaces we come in contact with and so on in the cause of our day-to-day activities. Heavy metals are non – biodegradable, have a long half-life and can accumulate in different parts of the body causing toxic effects (Jarup, 2003). The increase in heavy metals contamination of foods in developing countries is occurring due to unregulated urbanization and industrialization (Wong *et al*., 2003). Unregulated refuse disposal, refuse dump sites close to residential areas, rivers and farm lands have contributed largely to increased exposure to toxic heavy metals in Nigeria (Ferronato and Torretta, 2019).

Contamination of foods by heavy metals occur by the accumulation of chemicals: industrial wastes, fertilizers, pesticides and herbicides which leach out from the soil to the plant (Onakpa *et al*., 2018). At the time of processing milling machines are used and these machines are made from metals that work with the help of lubricants which may be source of contamination. Over time, these machines may produce metallic particles as a result of daily wear and tear. These particles are sometimes seen as tiny bits in milled flours and purees which may be removed during sieving of flours, however, purees or semi-liquid food samples which are not sieved will go directly into consumption after cooking. Heavy metals toxicity occurs when there is exposure or consumption of amounts that is more than the daily recommended limits. These levels are detrimental to health causing many complications including cancers.

Tomatoes, peppers and onions form the bulk of vegetables that are consumed daily in this part of the country. They are a source of vitamins, antioxidants, free glucose, free fructose and fructans that are beneficial to man. They are largely consumed and so cultivated almost all year round in different parts of Northern Nigeria by irrigation (Gidado *et al*., 2018). After harvest, fruits and vegetables are collected and transported to markets without any form of inspection by public health workers. Processing of these vegetables usually involves washing and removal of dirts and rotten parts, and milling at commercial milling points which is convenient and cheap due to the poor electricity supply in many homes. The processing

of these vegetables may be a source of contamination by microorganisms or heavy metals from the milling machines. Bacterial contamination may occur when fruits and vegetables are exposed on benches and baskets in open markets (Baiyewu *et al*., 2007). Some microorganisms are not destroyed by washing with water, they require treatments such as NaCl solution and heating to inactivate them, yet others still remain tolerant to this treatment and pose danger to human health by such resistance. Fruits and vegetables contain high moisture and thus easily susceptible to spoilage by microorganisms. Vegetables that are no longer fresh so called 'bage or baaje' in local dialect are sold at cheaper prices which may be a source of microbial contamination since they have lost membrane integrity of the fruits/vegetable stomata. Ogundipe *et al*., 2012; Obeng *et al*., (2018) reported microbial contamination of tomatoes by pathogenic microorganisms

About 20% of cancer cases worldwide has been linked with infectious agents (Vandeven and Nghiem, 2014). Pathogenic microorganisms often infest fruits and vegetables in the farm or at the market causing losses and diseases to the consumers. These organisms are capable of causing mild to more severe diseases in the host when there are recurrences of infections over time leading to inflammations and compromise of the immune system. Microorganisms such as bacteria (Salmonella, Enterobacteria, *E. coli*, *H. pylori*, Listeria), viruses (hepatitis B and C viruses, human papilloma viruses) and fungi are carried from the soil, water, feces, humans and animals when we come in contact with them in our food and water.

Bioaccumulation of toxic heavy metals in fruits and vegetables in Northern (Akan *et al*., 2013; Barau *et al*., 2018; Sakiyo *et al*., 2020), South West (Aiwonegbe and Ikhuoria, 2007: Sobukola *et al*., 2010) and South East Nigeria (Uroko *et al*., 2019;) have been previously reported. However, there is no published work on the safety of daily consumed milled foods, fruits and vegetables within the study area especially with the high influx of people from the rural communities to the metropolis owing to unrest from the insurgency. Therefore, this study was carried out to assess the levels of contaminants in commercially milled tomatoes, pepper and onion puree from some locations within Maiduguri metropolis.

## MATERIALSANDMETHODS

### Sample Collection

The samples collection areas were University of Maiduguri Campus, Monday market, Gamboru market and Baga Road Maiduguri. Twenty samples of vegetable puree made of tomato, pepper and onion mixture were collected into dry bottles that were sterilized with 70% ethanol and tightly closed. Samples were transported to the laboratory for further treatment.

### Sample Pre-treatment for Heavy metal Assessment

As described by Shobha and Kalshetty (2017) with slight modification, 1g each of the samples were weighed and digested in a mixture of 5 $cm^3$ of dil. HCl, 2 $cm^3$ of Conc. $H_2SO_4$ and 20 $cm^3$ of Conc. $HNO_3$ in a conical flask under a fume hood. The content was mixed and heated gently at $180^oC$ for about 30 min on a hot plate until thick white fumes emanated. It was then heated for a further 30 min. and thereafter, the contents cooled before making up to the mark in 50 $cm^3$ volumetric flask. This digested solution was used to determine the quantities of Mercury (Hg), Lead (Pb), Nickel (Ni), Cadmium (Cd) and Copper(Cu)by using Atomic Absorption Spectrophotometer (Perkin Elmer Analyst 300). The instrument setting and operational conditions were done in accordance with the manufacturers' specifications.

### Sample Pre-treatment for Microbial Assessment

As described by Ogundipe *et al*. (2012) 10 g of each sample was weighed and homogenized into 90 $cm^3$ of sterile distilled water. About 1$cm^3$ from the tenfold dilution of the homogenized samples was inoculated in Nutrient, MacConkey, blood and mannitol salt agar for bacteria colony counts using the streak plate method.

### Preparation of Culture Media

The commercially prepared, Nutrient blood, MacConkey, mannitol salt agar powder was weighed as directed by the manufacturers dissolved in one-liter distilled water in a clean conical flask by shaking. The media were sterilized using autoclave machine at $121^0$ for 15 minutes and are allowed to be cooled. The Nutrient; blood, MacConkey, mannitol salt agar was poured into petri dishes at $55^0C$ except for Nutrient agar which is poured at $45^0C$ and are allowed to set.

### Culture Preparation

The streak plate method was used for plating. Briefly, loops full of each sample was smeared over one corner of the solid medium which has sufficiently been sterilized by flames, cooled and used to make parallel streak from the main inoculated plate. The streaked plates were then incubated at $37^0C$ for 24 - 72 hours before checking for any colonies or growth.

### Identification of Microorganism

The isolates were identified by conventional methods starting with gram staining. Briefly, using a sterile wire loop a drop of distilled water was put on the center of grease free slide and a portion of colony was picked and emulsified in to drop of sample and allowed to air dry before fixing in to gram stain. Crystal violet was then applied for 3 minutes then replaced by iodine solution for 1 minute. This was rinsed with water, air dried and

viewed with microscope at X100 immersion oil objectives.

**Motility test** was carried out as described by Cheesebrough, (2006) tubes containing the motility medium were inoculated by making a hollow of about 1-2cm deep with a loopful of the culture. These tubes were then incubated at $37^0$C for 48hrs. Motility is observed by spreading of the organism outwards from the hollow point.

**Biochemical Characterization of Isolated Microorganisms**

**Indole test**: As described by Cheesebrough (2006), indole production by test organism was used to identify enterobacteria species. An aliquot of each isolate was inoculated onto 5cm$^3$ of sterile peptone-water enriched with 1% tryptophan and incubated at $37^0$C for 24 hours. Additionally, 0.5cm$^3$ of Kovac's reagent was introduced in the medium and gently stirred. A red colour indicated positive result while a yellow colour indicated negative test result of bacteria.

**Citrate Test:** As described by Cheesebrough (2006), an aliquot of each isolate was inoculated into Koser's citrate medium and incubated at $37^0$C for 72hours. A positive citrate utilization was confirmed by formation of bright blue colour while the retention of the initial green colour of the medium indicates a negative.

**Methyl Red/Voges Proskauer Test:** The ability to produce and maintain stable end product from glucose fermentation was used to further identify enterobacteria species as previously described (Ochei and Kolhatkar, 2000). An aliquot each of the isolates were inoculated into the glucose phosphate peptone water medium and incubated at $37^0$C for 48 hours. Few drops of methyl red were added to the culture, positive result was indicated by red colouration.

Voges-Proskauer test detects the neutral-reacting end products (acetoin) when cultivated in specific media. Enteric bacteria that ferment glucose, further metabolize pyruvic acid to end product acetyl-methyl carbinol (acetoin). This end product is converted to diacetyl, in the presence of atmospheric oxygen and 40% potassium hydroxide. Diacetyl is converted into a red complex, under the catalytic action of alpha-naphthol and creatine. This is a positive Voges-Proskauer (VP) test reaction. The VP test is used primarily to separate *Escherichia coli* (VP-negative) from the *Klebsiella-Enterobacter* groups (VP-positive).

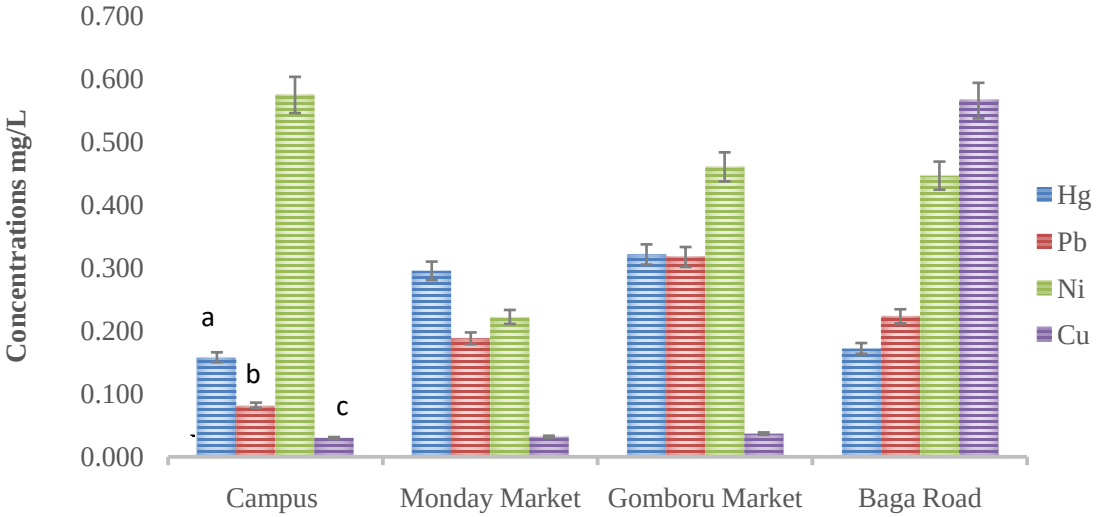
**Catalase Test:** This was used to differentiate Staphylococcus from streptococcus spp.which are both catalases producing bacteria. As described by Cheesebrough (2006) an aliquot of the bacterial isolate was transferred with a sterilized wire loop to a drop of hydrogen peroxide on a clean glass slide. The presence of catalysis observed by bubbling indicated a positive test for staphylococcus spp. while absence of bubble indicated negative.

## RESULTS AND DISCUSSION

### Concentrations of Heavy Metals in Commercially Milled Tomato/Pepper/Onion Puree in Some Locations Within Maiduguri Metropolis

Metals are naturally occurring elements that have a high atomic weight and a density greater than that of water. They have many applications in agriculture, industrial, medical and life support for health and well-being in the form of macro- and micro- elements needed by the body. Nickel, chromium and cadmium are among the heavily utilized industrial metals classified by the International Agency for Research on Cancer as Group 1 carcinogens. The concentrations of some heavy metals in commercially milled tomato, pepper and onion puree in selected locations within Maiduguri metropolis was assessed. Figure 1 represents the mean concentrations of heavy metals in samples from various locations within Maiduguri metropolis. The observed concentrations of heavy metals were within the range: mercury (0.158 – 0.321 mg/L), lead (0.082 – 0.317 mg/L), nickel (0.222 – 0.574 mg/L) and copper (0.030 – 0.565 mg/L) while cadmium was absent in the samples in all the areas of sampling. The highest concentration of mercury (0.321 ± 0.16 mg/L) was recorded in sample collected from Gomboru market. However, these values are not significantly different ($p > 0.05$) from those observed in samples from the campus, Monday market and Baga road. Nickel in samples from the campus (0.574 ± 0.22 mg/L), lead in samples from Gomboru market (0.317 ± 0.20 mg/L) and copper in samples from Baga road (0.565 ± 0.03 mg/L) were the most concentrated heavy metals in all the samples analyzed from the study area. Ametepey *et al*., (2018) reported lead concentrations in green pepper, tomatoes and onions from Tamale, Ghana below the limits of 0.3 mg/kg of WHO/FAO (2007). Nickel concentration in samples from the campus is significantly higher ($p < 0.05$) than mercury, lead and copper from same location. It is also significantly higher ($p < 0.05$) than copper from all the other 3 sampling sites (Monday market, Gomboru market and Baga road).Heavy metal contaminations in fruits, vegetables and fodder have been previously reported (Wang *et al*., 2005; Intawongse and Dean, 2006; Ametepey *et al*., 2018).Vegetable gardening along river beds and dump sites are one of the major sources of heavy metal contamination in vegetables and other foods (Olayiwola *et al*., 2018). Toxicity by these metals can induce oxidative stress, DNA damages and cell death thereby increasing the risk of cancer and related diseases (Kim, Kim and Seo, 2015). Metal toxicity occur when there is an ingesting or

exposure of amounts that is detrimental to life. This again is dependent on dose, route of exposure and chemical species involved as well as the age, gender, genetics and nutritional status of exposed individuals. WHO/EFA (1996) and WHO/FAO (2007) have documented the safety limits of these metals in humans/animals at concentrations of mercury (0.1µg/kg BW), lead (2mg/kg), Nickel (10mg/kg) and copper (30mg/kg). The occurrence of heavy metals is considered normal if in the following ranges: Pb 0.5 – 30 mg/kg; Cu 2.5 mg/kg; Ni 0.02 – 50 mg/kg and Cd < 2.4 mg/kg). The concentration of heavy metals in this study were found to be lower than the WHO/FAO (2007) standards and are within acceptable, safe limits for human survival.




Values are mean ± SD (n = 5) Values with alphabets (a, b, c) are significantly different ($p < 0.05$) from each other

**Figure 1: Concentration of Heavy Metals in Locally Processed Tomato, Pepper and Onion Puree in Selected Locations within Maiduguri Metropolis**

**Bacteria Isolated in Selected Samples within Maiduguri Metropolis**

The incubation of media plates with the various samples at 37 for 24 – 72h showed growth of both gram positive and gram-negative bacterial colonies (Table 1). Three (3) gram negative bacteria (*Escherichia coli*, Klebsiella spp and *Proteus mirabilis*) were identified by their inability to stain with crystal violet after washing with alcohol but picked up the red-brick colour of iodine solution used for counterstaining. Gram negative bacteria are pathogenic and infest fresh vegetables like tomatoes and onions that are consumed raw. All these microbial isolates were similarly reported as contaminants in tomatoes by Wogu and Ofuase, (2014) and Obeng *et al*. (2018). *E. coli* can survive under many environmental conditions, contaminate vegetables at any time pre - or post-harvest (Luna-Guevara, 2019) and becomes difficult to control by regular processing techniques. Exposure to pathogenic *E. coli* varieties causes serious food poisoning, septic shock, meningitis, or urinary tract infections in humans (*Abebe et al.,* 2000). Unlike normal flora *E. coli*, the pathogenic varieties produce toxins and other virulence factors that enable them to reside in parts of the body normally not inhabited by *E. coli* and to damage host cell. These pathogenic traits are encoded by virulence genes carried only by the pathogens (*Abebe et al.,* 2000).

The percentage of bacterial colonies isolated in the commercially milled tomato/pepper/onion puree in this study is presented (Figure 2). About 38% of total bacteria identified was Corynebacterium specie, a gram-positive bacterium with negative motility. Corynebacterium species are recognized as members of the normal human flora. They are isolated from the skin, mucous membranes, and gastrointestinal tract and occur among those with prosthetic and medical devices. Corynebacterium species are implicated in food spoilage (Alibi *et al*., 2016) and have not been reported to be linked with carcinogenesis, even though these microorganisms were isolated from cancer patients as a result of compromise of the immune system in end stage cancer patients (Daisuke *et al*., 2017).Eighteen (18%) percent of the total bacteria isolated were *Bacillus Subtilis.* These bacterial spores can survive extreme heat during cooking. Some strains of *B. subtilis* are responsible for causing ropinessa sticky, stringy consistency caused by bacterial production of long-chain polysaccharides in spoiled bread dough. For a long time, bread ropiness was associated uniquely with *B. subtilis* species by biochemical tests. *Staphylococcus aureus* food poisoning (SFP) is usually not life-threatening. Most cases of SFP do not require treatment because the condition will pass on its own. *S. aureus* has a high salt tolerance, and can grow in ham and other meats, and in dairy products. The toxins that the

bacteria produce is also heat resistant and cannot be destroyed through cooking making it a challenge for humans. *Proteus mirabilis* is part of the Enterobacteriaceae family that can also cause wound infections, sepsis and pneumonia in hospitalized patients (Majeed *et al*, 2019). *P. mirabilis* strains are resistant to first-generation cephalosporins and ampicillin (Gonzalez *et al*, 2016) however processing methods such as heat may potentially inhibit their capacities.

Carcinogenic pathogens such as *Helicobacterpylori*, *S. haematobium*, human papilloma viruses and hepatis viruses are causes of chronic inflammation and immune suppression during persistent infections (Pathogens and Cancer, accessed 2020). With the exception of *S. haematobium*, these pathogenic microorganisms are common causes of stomach, liver and cervical cancers. Microorganisms isolated in this study rise concerns over their pathogenesis, however none is linked with the initiation of carcinogenesis even though some of the microorganism have been found to infect cancer patients as a result of their low immunity status. Furthermore, during processing of the milled puree, the possibilities of inactivation of these organisms by heat will limit the virulence for infections on consumers. Though, inactivating heat resistant *B. subtilis* and *Staphylococcus aureus* by routine processing methods in the household still remains a problem to be solved.

**Table 1: Bacterial Isolates in Selected Samples**

| S/NO | Morphology of Bacterial Colonies | Gram Reaction | Microorganism Isolated | Motility |
|---|---|---|---|---|
| 1. | Positive rodlike colonies | Negative | *Escherichia coli* | Negative |
| 2. | Cocci shaped, clustered colonies | Positive | *Staphylococcus aureus* | Negative |
| 3. | Rod shaped colonies | Positive | *Bacillus subtilis* | Positive |
| 4. | Negative rodlike colonies | Negative | *Klebsiella specie* | Negative |
| 5. | Negative rodlike colonies | Negative | *Proteus mirabilis* | Positive |
| 6. | Whip handle appearance on gram staining | Positive | *Corynebacteria specie* | Negative |

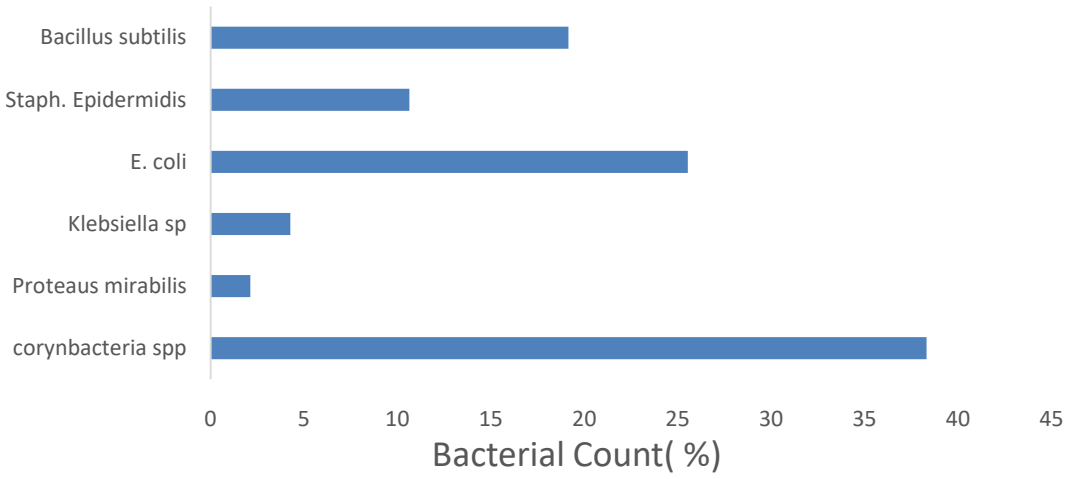



**Figure2: Bacteria Colonies Count (%) in selected samples**

**Table 2: Biochemical Identification of Bacterial Isolates in Selected Samples**

| Location | Sample | Indole Test | Methyl red test | Voges Proskauer | Citrate Test | Catalase Test | Coagulase Test | Bacteria Identified |
|---|---|---|---|---|---|---|---|---|
| CAMPUS | H1 | - | - | - | + | - | - | *Corynbacteria* |
| | H1 | - | - | + | + | - | - | *Bacillus subtiis* |
| | H1 | - | - | - | - | + | + | *S. aureus* |
| | H2 | + | + | - | - | - | + | *E. coli* |
| | H2 | - | - | - | + | + | - | *P. mirabilis* |
| | H2 | - | - | - | + | - | - | *Corynbacteria* |
| | H3 | - | - | - | + | - | - | *Corynbacteria* |
| | H4 | - | - | - | + | - | - | *Corynbacteria* |
| | H4 | - | - | + | + | - | - | *Bacillus subtilis* |
| | H5 | + | + | - | - | - | + | *E. coli* |
| | H5 | - | - | - | + | - | - | *Corynbacteria* |
| MONDAY MARKET | H6 | - | - | - | + | - | - | *Corynbacteria* |
| | H7 | - | - | - | + | - | - | *Corynbacteria* |
| | H8 | + | + | - | - | - | + | *E. coli* |
| | H8 | - | - | + | + | - | - | *Bacillus subtilis* |
| | H9 | - | - | - | + | - | - | *Corynbacteria* |
| | H9 | - | - | - | + | - | - | *Corynbacteria* |
| | H10 | - | - | - | - | + | + | *S. aureus* |
| | H10 | - | - | + | + | - | - | *Bacillus subtilis* |
| | H10 | - | - | - | + | - | - | *Corynbacteria* |
| GOMBORU MARKET | H11 | + | + | - | - | - | + | *E. coli* |
| | H11 | - | - | + | + | - | - | *Bacillus subtilis* |
| | H11 | - | - | - | + | - | - | *Corynbacteria* |
| | H12 | + | + | - | - | - | + | *E. coli* |
| | H12 | - | - | + | + | - | - | *Bacillus subtilis* |
| | H13 | + | + | - | - | - | + | *E. coli* |
| | H13 | - | - | - | + | - | - | *Corynbacteria* |
| | H14 | + | + | - | - | - | + | *E. coli* |
| | H14 | - | - | - | - | - | - | *Klebsiella spp* |
| | H14 | - | - | - | + | - | - | *Corynbacteria* |
| | H15 | - | - | - | + | - | - | *Corynbacteria* |
| | H15 | - | - | - | - | + | + | *S. aureus* |
| | H16 | + | + | - | - | - | + | *E. coli* |
| | H16 | - | - | - | - | - | - | *Klebsiella spp* |
| | H16 | - | - | - | - | + | + | *S. aureus* |
| BAGA ROAD | H17 | + | + | - | - | - | - | *E. coli* |
| | H17 | - | - | + | + | - | - | *Bacillus subtilis* |
| | H17 | - | - | - | + | - | + | *Corynbacteria* |
| | H18 | + | + | - | - | - | - | *E. coli* |
| | H18 | - | - | - | + | - | - | *Corynbacteria* |
| | H18 | - | - | + | + | - | - | *Bacillus subtilis* |
| | H19 | + | + | - | - | - | + | *E. coli* |
| | H19 | - | - | - | + | - | - | *Corynbacteria* |
| | H19 | - | - | + | + | - | - | *Bacillus subtilis* |
| | H20 | + | + | - | - | - | + | *E. coli* |
| | H20 | - | - | - | + | - | - | *Corynbacteria* |
| | H20 | - | - | - | - | + | + | *S. aureus* |

## CONCLUSION

Contamination of foods by heavy metals and microorganism is a likely event which may occur during planting as a result of practices such as herbicides and pesticides treatments. It can also occur during harvesting, transportation or processing of the transported produce. Heavy metals and pathogenic microorganisms' contamination are of concern to ascertain the safety of processed foods. This study has shown presence

of heavy metals in relatively safe concentrations for human exposure while pathogenic bacteria *E. coli* and *Proteus mirabilis* which are both gram negative bacteria and *Bacillus subtilis*a heat-resistant, gram-positive bacteria were found in the test samples. There is need to create awareness on good hygiene practices by operators of commercial milling machines within the study area to ensure food safety and healthy status.

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