STUDY OF THE BACTERIOLOGICAL QUALITY OF SOME FRESH VEGETABLES SOLD IN TWO MUNICIPALITIES OF THE CITY OF LIKASI THAT CAN BE EATEN RAW.

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SUMMARY

Faced with the lack of food hygiene measures in the transport chain of some vegetables consumed raw, from the place of their production to their distribution site, we proposed to examine the bacteriological quality of vegetables sold in two communes of the city of Likasi (Panda and Likasi) that can be consumed raw.

For this purpose, samples of fresh vegetables belonging to fourteen different vegetable species were taken from the vendors during the 2019-2020 growing season, from March to May, and subjected to bacteriological analyses to determine the bacterial load of the different mesophilic germs, isolated and identified.

The bacterial load of the samples spread on the ground was higher than that of those on the shelves at the Panda Municipal Market. In the case of the samples from the municipality of Likasi, the bacterial load was higher in the samples bought at the Central Market than in those collected in a Supermarket.

After isolation, two germs of the Enterobacteriaceae family, showing faecal contamination, were identified: *Escherichia coli* (36.66%) and *Salmonella spp* (31.8%) for Panda. The percentages of these two germs were higher in vegetables from the municipality of Likasi, 56.25% for *Salmonella spp* and 43.75% for E. coli, then in vegetables from the municipality of Panda.

Key words: Bacteriological quality, fresh vegetables, faecal contamination.

1. INTRODUCTION

As the main source of vitamins in the diet, vegetables are an essential part of the human diet. It is known that a balanced diet rich in fruit and vegetables ensures good health and can reduce the risk of certain diseases. Their consumption plays a protective role in human health, against cardiovascular diseases and certain cancers. Vegetables have nutritional benefits linked to their chemical composition and above all their richness in micronutrients. They are therefore essential for a balanced diet (Delphine, 2003).

Despite the advantages linked to their consumption, there is a problem of food safety insofar as certain vegetables eaten raw have long been considered as sources of transmission of infectious diseases (Maistro, 2011). In addition, the microflora of these foods is dominated by spoilage bacteria, yeasts and moulds that can affect the organoleptic and commercial qualities of these products. In this respect, several studies carried out on fruit and vegetables have shown that they are contaminated with germs that are harmful to humans (Kasse, 2014; Kazwey, 2018; Delphine, 2003), which is why we have begun our investigations into the bacteriological quality of some fresh vegetables that can be eaten raw and that are sold in two municipalities in the city of Likasi, PANDA and LIKASI. The aim of this study is to determine the health risks for consumers of this type of vegetable.

2. STUDY ENVIRONMENT, MATERIALS AND METHODS

2.1. Study environment

This study was conducted in the city of Likasi during the growing season, from March to May 2020. For this initial phase, the study was limited to fresh vegetables sold in the Panda and Likasi communes of the city of Likasi and which are also consumed raw by the population of the said city in the province of Haut-Katanga, in the Democratic Republic of Congo.

Formerly known as Jadotville, the city of Likasi currently comprises four communes, namely: Kikula, Likasi, Panda and Shituru. Of these four communes, we chose two, Likasi and Panda, to carry out our investigations. Our choice was dictated by the fact that the two selected communes are mainly inhabited by fairly well-off families who are supposed to be large consumers of the raw vegetables in this study.

Two markets and a supermarket were selected for our research: the central market and a supermarket in the commune of Likasi, and a market in the commune of Panda. Samples were collected during the 2019-2020 growing season, specifically during the months of March, April and May 2020.

2.2 Biological material

The biological material consisted of two groups of eleven vegetables in each site. The bacteriological analysis of different samples was carried out at the biology laboratory of the Institut Supérieur Pédagogique de Lubumbashi (ISP-Lubumbashi). In table 1 are listed all the vegetables that we listed in the two study sites.

Table 1: Names and botanical families of the 14 vegetables that can be eaten raw and that were
inventoried in the markets of the communes of Panda and Likasi

Nº	Scientific name	Vernacular name	Ordre	Family
1	Abelmoscus esculentus	Gombo	Malvales	Malvaceae
2	Allium cepa	Onion	Asparagales	Amaryllidaceae
3	Allium porrum	Leek	Asparagales	Amaryllidaceae
4	Allium sativum	Garlic	Asparagales	Amaryllidaceae
5	Brassica oleracea var.botrytis	Cauliflower	Brassicales	Brassicaceae
6	Brassica oleracea	Soy cabbage	Brassicales	Brassicaceae
7	Capsicum annuum	Piment	Solanales	Solanaceae
8	Capsicum frutescens	Pepper	Solanales	Solanaceae
9	Cucumus sativus	Cucumber	Violales	Cucurbitaceae
10	Daucus carotta	Carrot	Apiales,	Apiaceae
11	Rumex acetosela	Oseille	Polygonales	Plygonaceae
12	Solanum lycopersicum	Tomato	Solanales	Solanaceae
13	Solanum melongena	African Eggplant	Solanales	Solanaceae
14	Zingiber officinale	Ginger	Zingiberales	Zingiberaceae

2.3 Methods

2.3.1. Sampling

The samples were collected in the municipalities of Likasi, in a Supermarket and at the Central Market, and from Panda to the Common Market. They were placed in sterile bags, carefully protected, labelled and then transported from Likasi to Lubumbashi to the biology laboratory at the Institut Supérieur Pédagogique de Lubumbashi where the analyses were carried out after receipt.

A total of forty-four samples were purchased from the various vendors, twenty-two from each commune. In each place of purchase, two purchasing groups were targeted. In the Panda market, we considered 11 vegetables displayed on the shelves and the same 11 vegetables displayed on the ground: *Rumex acetosela, Allium cepa, Brassica oleracea var.botrytis, Solanum melongena, Capsicum annuum, Solanum lycopersicum, Zingiber officinale, Abelmoscus esculentus, Capsicum frutescens, Brassica oleracea* and Allium sativum. Similarly, two groups of eleven identical vegetables were purchased respectively from the central market and a supermarket in the municipality of Likasi: *Daucus carotta, Brassica oleracea, Capsicum annuum, Solanum melongena, Cucumus sativus, Allium cepa, Zingiber officinale, Allium sativum, Capsicum frutescens* and *Solanum lycopersicum*. As can be seen, of the eleven vegetables collected from each site, there were only three that were not found in the other site. These were *Abelmoscus esculentus, Brassica oleracea var.botrytis, Rumex acetosela,* and *Capsicum porrum, Cucumus sativus, Daucus carotta.* The remaining eight vegetables were the same in both sites.

2.3.2. Bacteriological analysis

The bacteriological analysis consisted of four phases: enrichment, isolation, enumeration and identification of germs.

\rm Enrichment

10g of each sample was aseptically taken and crushed with a small pestle and then diluted in 100mL of sterile peptone water to obtain the dilution stock solution. This suspension containing the microorganisms was left to stand at laboratory temperature for 25-30 minutes to ensure their revivification. Decimal dilutions were made to facilitate counting. The first dilution was made by taking 1mL of the stock solution put in peptone water to form the 10^{-1} dilution, then 1mL of the 10^{-1} dilution was added in 9mL of peptone water to obtain the 10-2 dilution and so on to form the 10^{-3} , 10^{-4} , 10^{-5} dilutions (Diouf, 1992).

4 Isolation and enumeration of total coliforms

Germs were counted and searched for according to the horizontal methods of the standards, using nutritive agar as isolation medium (AFNOR, 1996). Total aerobic mesophilic flora (FMAT), faecal coliforms or thermo-tolerant coliforms, presumed pathogenic staphylococci (SPP), sulpho-reducing anaerobes (ASR), salmonella were counted.

The inoculation was done on the surface after allowing the medium to solidify and then incubated in an oven at 37° C for 24 hours. For inoculation at depth, 1mL was taken from solution 10^{-3} and placed in a sterile Petri dish to which 20mL of the nutrient agar medium was added. This solution was then

homogenised in a counter-clockwise direction, rested to solidify and the dish was turned over to avoid contamination (AFNOR, 1996).

The count was made by counting the colonies having grown on the agar after 24 hours' incubation in the oven. The number of colonies observed was thus multiplied by the dilution factor and then expressed in CFU/mL (colony-forming unit per millilitre) (AFNOR, 1996).

4 Identification of germs

To identify the germs, four culture media were used: CLED, Kligler, SIM (Sulfite Indole Mobile) and Simmons citrate.

Lysine will be used in the biochemical identification of the germs. This medium is useful for the diagnosis of enterobacteria. It allows the presence or absence of a lysine decarboxylase, a lysine deaminase and the production or not of hydrogen sulphide, which appears when the medium is blackened, to be demonstrated in a single tube.

The seeding on this medium was done in streaks on the slope and in punctures in the base. Then bring to the oven at 37°C for 24 hours (AFNOR, 1996).

- Reading on the slope

The appearance of the violet colour indicates that the germs do not possess lysine deaminize, while the burgundy red reveals the presence of lysine deaminize.

- Reading in the base

The presence of a lysine decarboxylase is determined by the appearance of the violet colour, which is indicated by a realization after a brief turn to yellow due to the fermentation of glucose. And when the colour is yellow, this means that there is an absence of lysine decarboxylase.

3. **RESULTS**

3.1 Isolation of germs

In tables 2, 3 and 4 the results of the sprouts that were isolated from vegetable samples from the communes of Likasi and Panda are presented respectively.

Table 2: Isolation of sprouts from vegetable sample	s collected in the Supermarket(S) and Central
Market (MC) of the Likasi commune	

N°	Vegetable species	Results per sa Vegetable species			Positive tests	
		MC	S	Headcount	%	
1	Allium cepa	+	-	1	50	
2	Allium porrum	+	-	1	50	
3	Allium sativum	+	+	2	100	
4	Brassica oleracea	+		2	100	
5	Capsicum annuum	Eto	-	2	100	
6	Capsicum frutescens	+	+	2	100	
7	Cucumus sativus	+	+	2	100	
8	Daucus carotta	-	-	0	0	
9	Solanum lycopersicum	+	-	1	50	
10	Solanum melongena	+	-	1	50	
11	Zingiber officinale	+	+	2	100	
	Total tests positifs	10	6	16		
	Pourcentage total	90,9	54,5	72,7		

Legend: (+): germ development; (-): no germ development.

Vertical reading of Table 3 shows that the samples from the central market were more contaminated (90.9%) than those from the supermarket (54.5%). On the other hand, horizontal examination of the same table indicates on the one hand that of all the vegetables under study, only the carrot, *Daucus carotta*, was not contaminated. On the other hand, the same horizontal reading shows, whether at the central market or the supermarket, that six vegetables were 100% contaminated (*Allium sativum*, *Brassica oleracea, Capsicum annuum, Capsicum frutescens, Cucumus sativus, Zingiber officinale*). The four 50% contaminated vegetables all refer to samples from the Central Market.

Table 3: Isolation of sprouts in samples of vegetables harvested at the market in the municipality ofPanda

N°	Espèces de légumes	Results per sampling site		Positive tests	
		Shelves	Floor	Staffing	Frequency
1	Abelmoschus esculentus	-	+	1	50
2	Allium cepa	+	+	2	100
3	Allium sativum		DE	0	0
4	Brassica oleracea	+	-	1	50
5	Brassica oleracea var.botrytis	+	-	1	50
6	Capsicum annuum	-	+	1	50
7	Capsicum frutescense	+	+	2	100
8	Rumex acetosela	-	+	1	50
9	Solanum lycopersicum	+	+	2	100
10	Solanum melongena	+	+	2	100
11	Zingiber officinale	+	+	2	100
	Total positive tests	7/11	8/11	15	
	Total percentage	63.6	72.7	68.2	

In contrast to the previous case, here the difference in positive tests in the two sampling groups is reduced: 63.6% for samples exposed on the shelves and 72.7% for those exposed on the floor. However, as in the commune of Likasi, here too we found an uncontaminated vegetable, *Allium sativum*. Furthermore, 100% contamination appears in 5 vegetables (*Allium cepa, Capsicum frutescense, Solanum lycopersicum, Solanum melongena, Zingiber officinale*) and 50% contamination in 5 other vegetables (*Abelmoschus esculentus, Brassica oleracea, Brassica oleracea var. botrytis, Capsicum annuum, Rumex acetosela*). Of the five cases of 50% contamination, three concerned vegetables exposed on the ground and two exposed on the shelves.

3.2 Identification and counting of sprouts

The results of the identification and counting of germs are recorded in Tables 4-8.

3.2.1. Samples from the Likasi commune

Table 4: Identification and counting of germs in samples from the Supermarket of the Municipalityof Likasi

N°	Vegetable species	Samples	Identified sprouts	Total sprouts in CFU/ml
1	Allium cepa	Oi S	EIV	-
2	Allium porrum	Poi S	-	-
3	Allium sativum	Ai S	Salmonella spp	8*10 ³
4	Brassica oleracea	Cp S	Salmonella spp	35*10 ³
5	Capsicum annuum	Pv S	Salmonella spp	33*10 ³
6	Capsicum frutescens	Pi S	Esherichia coli	19*10 ³
7	Cucumus sativus	Co S	Esherichia coli	27*10 ³
8	Daucus carotta	-	-	-
9	Solana lycopersicum	To S	-	-
10	Solana melongena	Au S	-	-
11	Zingiber officinale	Gi S	Esherichia coli	$26*10^3$

Legend: Ai S = garlic supermarket, Au S = eggplant supermarket, Cc S = cabbage supermarket, Cp S = cabbage head supermarket, Gi S = ginger supermarket, O i S = onion supermarket, Os S = sherry supermarket, Pi S = chilli supermarket, Pv S = pepper supermarket, To S= tomato supermarket

The main information that emerges from Table 4, in the samples from the Supermarket of the Municipality of Likasi is that the bacterial load varies from $8*10^3$ (*Allium sativum*) to $35*10^3$ CFU/ml (*Brassica oleracea*).

If we calculate the average of this bacterial load in the contaminated samples, we find a value of $24.67*10^{3}$ CF/ml.

Table 5: Identification and enumeration of germs in contaminated samples from the central market
of the municipality of Likasi

N°	Vegetable species	Samples	Identified sprouts	Total sprouts in CFU/ml
1	Allium cepa	Oi MC	Escherichia coli	$23*10^{3}$
2	Allium porrum	Poi MC	Esherichia coli	15*10 ³
3	Allium sativum	Ai MC	Salmonella spp	39*10 ³
4	Brassica oleracea	Ср МС	Esherichia coli	15*10 ³
5	Capsicum annuum	Pv MC	Salmonella spp	$27*10^{3}$
6	Capsicum frutescence	Pi MC	Salmonella spp	$10*10^{3}$
7	Cucumus sativus	Co MC	Salmonella spp	$20*10^{3}$
8	Daucus carota			-
9	Solana lycopersicum	To MC	Esherichia coli	100*10 ³
10	Solana melongena	Au MC	Salmonella spp	$32*10^3$
11	Zingiber officinale	Gi MC	Salmonella spp	$100*10^{3}$

Legend: Oi MC = *Allium cepa*, Poi MC = *Allium porrum*, Oi MC = *Allium cepa*, Poi MC = *Allium porrum*, Ai MC = *Allium sativum*, Cp MC = Head cabbage central market, Pv MC = Pepper central market, Pi MC = Pepper central market, Co MC = *Cucumber* central market, To MC = Tomato central market, Au MC = eggplant central market, Gi MC = Ginger central market

Table 5 shows that the bacterial load in the samples from the central market of the municipality of Likasi analysed varies from 10,103 in *Capsicum frutescens* to 100,103 CFU/ml in *Solanum lycopersicum and Zingiber officinale*. As a result, the bacterial load of the Central Market samples is higher than that of the Supermarket. In Central Market, the average value of the bacterial load for the ten contaminated vegetables is 38.1*10³ CFU/ml. And the germs identified after isolation in the two Likasi markets are *Salmonella spp and Esherichia coli*.

The frequency of these two germs has been calculated and presented in Table 6.

Germ	Frequency	Percentage
E.coli	7	43,75%
Salmonella spp	9	56,25%
Total	16	100,00%

 Table 6: Cumulative frequency of germs identified in contaminated samples from Likasi commune

These results show that the frequency of the Salmonella germ (56.25%) in the samples from two sites is higher than that of Escherichia coli with a frequency of 43.75%.

3.2.2. Samples from the municipality of Panda

Table 7: Identification and enumeration of germs from samples on the shelves in Panda municipality

N°	Vegetable species	Samples	Identified sprouts	Total sprouts in CFU/ml
1	Abelmoschus esculentus	Go E	0	0
2	Allium cepa	Oi E	Salmonella	$46*10^{3}$
3	Allium sativum	Ai E	0	0
4	Brassica oleracea	Cp E	Salmonella	$85*10^{3}$
5	Brassica oleracea var.botrytis	Cc E	Escherichia Coli	88*10 ³
6	Capsicum annuum	Pv E	0	0
7	Capsicum frutescence	Pi E	Escherichia coli	19*10 ³
8	Solana lycopersicum	To E	Escherichia coli	61*10 ³
9	Solanam melongena	Au E	Salmonella	$40*10^{3}$
10	Rumex acetosela	Os E	0	0
11	Zingiber Officinale	Gi E	Escherichia coli	64.10 ³

Legend: Ai E = garlic spread, Au E = eggplant spread, Cc E = cauliflower spread, Cp E = head cabbage spread, Gi E = ginger spread, Oi E = onion spread, Os E = sorrel spread, Pv E = pepper spread, Pi E = chilli pepper spread, To E = tomato spread

\mathbf{N}°	Vegetable species	Samples	Identified sprouts	Total sprouts in CFU/ml
1	Abelmoschus esculentus	Go S	Escherichia coli	71*10 ³
2	Allium cepa	Oi S	Escherichia Coli	8*10 ³
3	Allium sativum	Ai S	0	0
4	Brassica oleracea	Cp S	0	0
5	Brassica oleracea v .botrytis	Cc S	0	0
6	Capsicum annuum	Pv S	Salmonella spp	96*10 ³
7	Capsicum frutescence	Pi S	Escherichia coli	23*10 ³
8	Solanam lycopersicum	To S	Salmonella	$10*10^{3}$
9	Solanam melongena	Au S	Salmonella	74*10 ³
10	Rumex acetosela	Os S	Salmonella	$27*10^{3}$
11	Zingiber officinale	Gi S	Escherichia coli	34*10 ³

Table 8: Identification and germ count of samples spread on the market floor

Legend: Ai S = garlic on the floor, Au S = eggplant on the floor, Cc S = cauliflower on the floor, Cp S = cabbage on the floor, Gi S = ginger on the floor, Oi S = onion on the floor, Os S = sorrel on the floor, Pi S = chilli pepper on the floor, Pv S = pepper on the floor, To S = tomato on the floor

Analysis of the results in Tables 7 and 8 reveal the absence of contamination in four and three samples, respectively, displayed on the shelves and on the ground in Panda market. In terms of bacterial load, it is interesting to note that in the first case it varies from $19,10^3$

UFC/ml in *Capsicum frutescens* to $88,10^3$ UFC/ml in *Brassica oleracea* and in the second case from $8,10^3$ UFC/ml in *Allium cepa* to $96,10^3$ UFC/ml in *Capsicum annuum*.

As in Table 6, the cumulative frequencies of the identified germs, after isolation, *Salmonella spp* and *Escherichia coli* have been compiled in Table 9.

Table 9: Cumulative frequency of identified and enumerated germs (Escherichia coli and
Salmonella) in the municipality of Panda

Germ	Frequency	Percentage
E. coli	8	53.3
Salmonella	7	46.7
TOTAL	15	100

It can be seen from this table that 53.3%, or 8 cases, of *Escherichia coli* were found in the samples under study, compared to 46.7%, or 7 cases, of *Salmonella spp* in all eleven vegetable samples analysed in the two sites.

4. **DISCUSSION**

4.1. Bacterial contamination

In the municipality of LIKASI, the contamination rate was 90.9% for samples purchased from the Central Market(TM) and 54.5% for those from the Supermarket(S). The fact that the samples from the Supermarket were less contaminated than those from the Central Market is certainly justified by the very poor hygienic conditions in the former site(s) than in the latter. It is this same cause that explains the superiority of the contamination of vegetables exposed on the ground (72.4%) over those exposed on the shelves (63.6%) in the municipality of PANDA.

All these results are in agreement with those in the literature according to which the vegetables in the central market of the city of Lubumbashi and in some European cities were more contaminated than those in the supermarkets (Delphine.2003; Kazwey.2018).

It should be noted, however, that among the vegetable samples we analyzed, one in the commune of Likasi was carrot (*Daucus carotta*) and the other one in the commune of PANDA was garlic (*Allium sativum*), which were uncontaminated. This could be a result of the antiseptic and antimicrobial properties of the garlic on the one hand, and on the other hand, for the carrot under treatment conditions before being exposed (it is cleaned before being spread because it is considered as fruit by sellers and buyers) (Kazwey, 2018).

4.2 Identification of sprouts

The identification of two germs of the Enterobacteriaceae family, *Escherichia coli* and *Salmonella spp*, with a frequency of 43.75% and 56.25% respectively in the municipality of Likasi and 53.3% and 46.7% in the municipality of Panda, allowed us to deduce that the contamination thus revealed came from faecal matter, since the two germs mentioned above are witnesses to faecal contamination (Delphine, 2003). As we know, ingestion of these two bacteria is a source of many diseases such as typhoid fever, diarrhoea, food contamination resulting in abdominal cramps and vomiting (Lambert, 1989, Gentillini, 1993).

4.3. Enumeration of germs

The bacterial load in the samples analyzed showed that the total bacterial count in the contaminated vegetables at the two sites in the municipality of Likasi was between $8,10^3$ and $100,10^3$ CFU/ml in the Central Market and between $8,10^3$ and $35,10^3$ CFU/ml in the Supermarket. In the first case, the

average value of the bacterial load was $42.3*10^3$ CFU/ml and in the second case it was $24.3*10^3$ CFU/ml.

If the bacterial load is higher in Central Market vegetables than in Supermarket vegetables, this is also due to the very poor hygienic conditions in Central Markets in developing countries. This can be demonstrated by visiting, for example, any market in cities in the DRC.

As for the bacterial load in contaminated vegetables from the commune of Panda, it led to an average value of $57.6,10^3$ UFC/ml for samples exposed on the shelves and $54.9,10^3$ UFC/ml for those exposed on the ground. Given that the two values are close; this seems to indicate that all the vegetable display areas in the central markets of our municipalities have a high degree of contamination. It is therefore very necessary to decontaminate them before serving them as salads. For this reason, before raw vegetables and fruit are eaten, they should be cleaned using a solution of chlorinated water with a concentration of between 100 and 150 parts per million of total chlorine or between two and seven ppm residual chlorine (www.inspection.gc.ca.com accessed on 16/01/2021).

5. CONCLUSION

Our work focused on the bacteriological quality of some fresh vegetables sold and that can be eaten raw in the city of Likasi.

The results obtained showed that the vegetables from the central markets of our municipalities are of poor hygienic quality, resulting in the abundant presence of *Salmonella spp* and *E. coli*. This constitutes a real danger if these vegetables are eaten raw without complete decontamination.

Moreover, the presence of these germs implies that the contamination found is of faecal origin and is one of the causes of diarrhea and typhoid fever. However, it would be interesting that further studies be carried out to possibly highlight the existence of other germs, sources of contamination of these foodstuffs.

The problem of contamination of vegetables thus raised is a challenge to all of us and especially to our leaders to set up strategies to prevent the contamination of these foodstuffs.

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