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Physicochemical, Bacteriological and Parasitological Quality of Water Used to Wash Vegetables in Dschang, West Cameroon: Health Risk Assessment

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Abstract: The quality of water used for the cleaning fresh vegetables sold in Cameroonian cities is of little concern. The objective of this study was to determine the health risk associated with the water use for washing fruits and vegetables in the city of Dschang, in order to help develop strategies for the control waterborne diseases in Cameroon in general and in Dschang in particular. After a survey of 75 vegetable vendors in the Dschang market, 40 samples of washing water, 20 per season, were collected randomly throughout the main market in the city of Dschang. Physicochemical, bacteriological and parasitological analyses were carried out according to standard methods. Regardless of the season, bacteria indicative of faecal contamination were detected in the water used for washing fresh vegetables, with levels ranging from 0 to 8×10^4 CFU/100ml for *Escherichia. coli*, from 1.8×10^4 to 1.8×10^8 CFU/100ml for faecal coliforms, from 3×10^4 to 7.2×10^8 CFU/100ml for *Salmonella* spp., from 30 to 1.3×10^7 CFU/100ml for *Shigella* spp. and 20 to 7.5×10^5 CFU/100ml for faecal streptococci. *Ascaris* spp. eggs, *Entamoeba* spp. cysts and nematode larvae were detected in vegetable washing water, with a prevalence of 40%. Consumers of fresh vegetables washing by these waters, if raw and poorly washed, are at risk of bloody diarrhoea, gastroenteritis, typhoid and paratyphoid fever or inflammation of the colon tissue and severe necrosis. The authorities should introduce food hygiene in the markets as part of the strategies to combat waterborne diseases in this city. This solution is also valid for other cities in Cameroun.

Keywords: Vegetables Sold, Washing Water Quality, Health Risk, Dschang, Cameroon

1. Introduction

Water-borne diseases remain a major and current public health problem in Cameroon; they are endemic and certain diseases such as cholera which were considered eradicated are resurfacing. The most recent finding is that which was declared in 03 regions of the country in July 2018 with a toll of 158 cases and 11 deaths [1]. The city of Dschang is not immune to this problem. Indeed, in 2010 in the Dschang health

district (DSD), out of a population of 209,055 inhabitants, 24,232 patients were diagnosed and 2,000 patients suffered from water-borne diseases, i.e. a percentage of 8.25%; in 2018, epidemiological studies carried out in this same district showed that 78.78% of the population were exposed to typhoid fever, 33.02% to gastroenterics and 24.61% to bacterial dysentery and that women and children were the most exposed [2]. However, for years, the city council has been aware of the situation and, in an effort to eradicate these diseases, with the support of the state, has been carrying out multiple actions through the development of drinking water points and deworming campaigns. The hygiene and sanitation aspect is almost forgotten.

The food hygiene of fresh vegetables sold on the market, which represents a real public health problem, is completely forgotten, whereas the availability of healthy and nutritious food is one of the fundamental rights of the person, and an essential factor for an adequate state of health [3]. Indeed, in the market of the city of Dschang, the conditions of sale of fruits and vegetables are reproachable; water of unknown quality is used to maintain the fruits and vegetables all day long, and one may ask what is the health risk linked to this practice? In fact, the poor quality of vegetables and fruits has already been associated with the resurgence of several cases of waterborne disease worldwide [4-7]. The sales practices and quality of vegetables sold in the Dschang city market are known [8]. Unfortunately, no data are available on the quality of the water used to maintain the fruits and vegetables on the Dschang market, although it could be partly responsible for the poor sanitary quality of the vegetables observed on the market in this city. The aim of this study is to determine the sanitary risk linked to the use of water for washing fruits and vegetables in the city of Dschang, in order to help in the elaboration of strategies for the fight against waterborne diseases in Cameroon in general and in Dschang in particular.

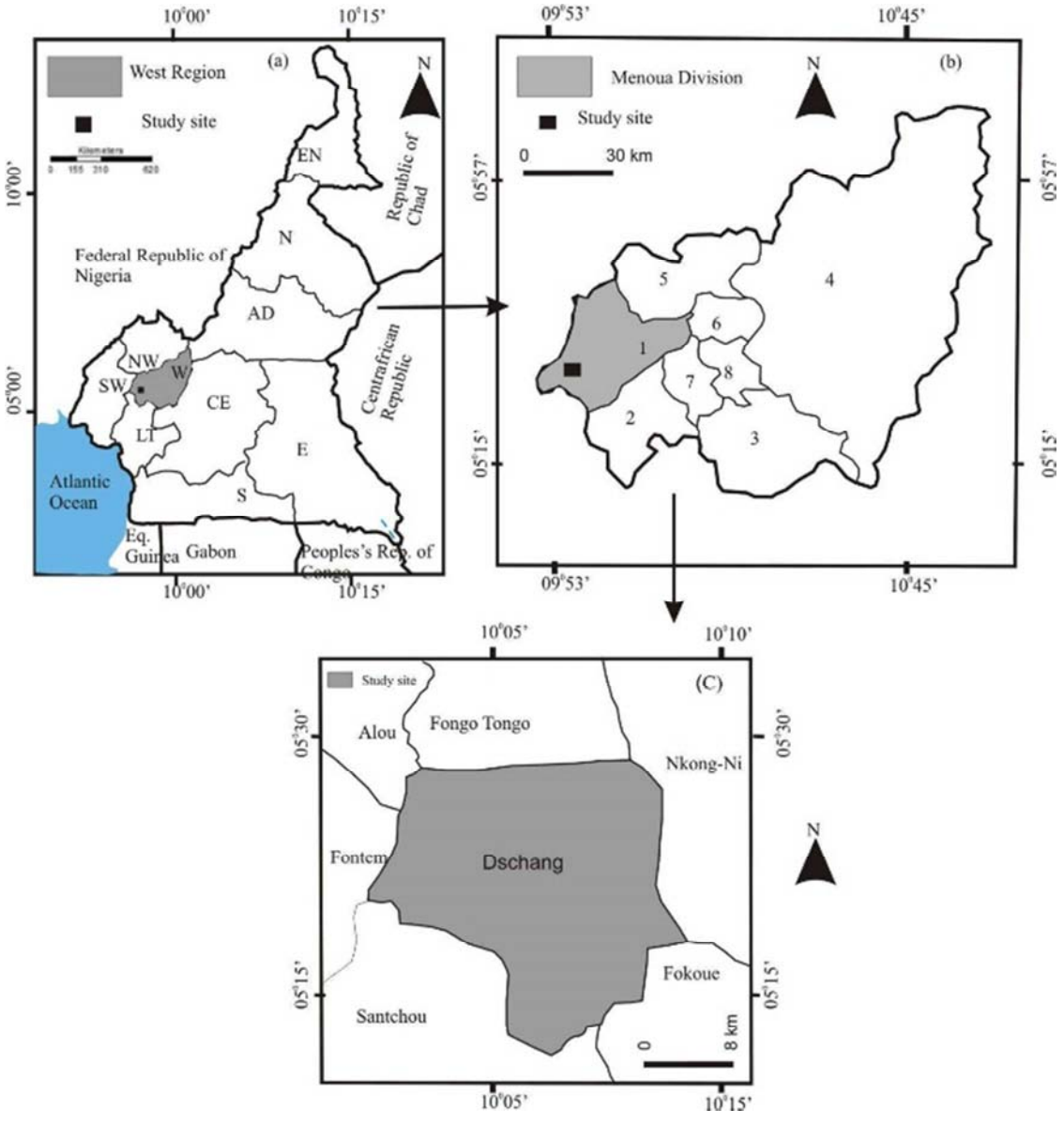

Figure 1. *Location of the city of Dschang.*

Figure 1 Location of the study site in Cameroon (a), in the West Region (b) and in the Menoua Division (c). EN–Exreme North Region; NW–North West Region; N–North Region; AD–Adamawa Region; CE-Centre Region; E–East Region; LT–Littoral Region; S–South Region; SW–South West Region; W–West Region. 1-Menoua Division; 2-Upper-Nkam Division; 3-Nde Division; 4-Noun Division; 5-Bamboutos Division; 6-Mifi; 7-Upper-Plateau Division; 8-Koung-Ki Division.

2. Material and Methods

2.1. Presentation of the Study Area

Located in the West Region of Cameroon, Dschang Subdivision is the head quarter of the Menoua Division. The town extends over part of the villages of Foto and Foréké-Dschang between latitudes 5° 10' and 5° 38' North and between longitudes 9° 50' and 10° 20' East. It has an average altitude of 1400 m. This area is located in the South - West slope of the Bamoutos Mountains, and is dominated by low plateaus strongly dissected by small valleys sometimes swampy. The climate is characterized by a dry season from mid-November to mid-March and a rainy season from mid-March to mid-November (Figure 1).

2.2. Survey on Vegetable Washing Practices in the Dschang City Market

In order to obtain data on the washing practices of fresh vegetables in the city of Dschang, a survey form was drawn up and applied in the field to 75 fresh vegetable sellers. This form had four parts, namely: identification of the vendor, identification of the vegetables sold, vegetable cleaning techniques and finally the vendors' level of knowledge of the health risks linked to poor vegetable cleaning.

2.3. Sampling and Analysis of Fruit and Vegetable Washing Water

After processing the survey forms, the water used to wash lettuce, tomatoes, celery, basil and cucumbers was selected for the rest of the study. This is because these are the vegetables likely to be eaten raw that are most sold on this market. The water used for washing of these vegetables was sampled at a rate of four samples per type of vegetable and per season. A total of 40 water samples were taken (20 in the dry season and 20 in the rainy season) at random throughout the market. Samples for bacteriological analysis were taken using sterile 300 ml glass bottles. Those for parasitological analysis were collected in 1.5 litre plastic bottles. All samples were kept in a refrigerated cabinet until they reached the laboratory and were then subjected to bacteriological and parasitological analyses. The physicochemical parameters (pH, electrical conductivity, temperature and TDS) were measured directly in the field using a "Hanna" multi-parameter field meter.

The search for bacteria in the maintenance water samples was carried under rigorous aseptic conditions. It involved the identification and enumeration of faecal coliforms, *E. coli*, *Shigella* spp., faecal streptococci, *Salmonella* spp. by the membrane filter technique, followed by a culture in solid medium. *Salmonella spp*. and *Shigella* spp. were tested on Salmonella-Shigella agar medium, faecal coliforms and *Escherichia coli* on Triphenyl Tetrazolium Chloride (TTC) and tergitol 7 lactose agar medium, faecal streptococci on Slanetz medium, and then incubated at 37°C for 24 hours for *Salmonella spp*. *Shigella spp.*; 44°C for faecal coliforms and *E. coli*; 37°C for 48 hours for faecal streptococci. The identified colonies were enumerated by means of a colony counter and the data expressed as Log CFU/100ml.

The parasitological study was qualitative, the search for parasitic elements was done following the sedimentation technique used by Amamid [9]. This technique is based on the concentration of decantable elements of which eggs, larvae and cysts of parasites are part. One litre of water was left to stand for 8 hours, then the sedimented residues were centrifuged at 3500 rpm for 15 min and the resulting pellet was diluted 1:5 with acetoacetic acid buffer. Ether was then added in equal volume. After emulsification by strong agitation, the whole was centrifuged for 4 minutes at 1500 rpm, then the pellet was diluted with the buffer solution and observed under the microscope, between slide and coverslip, with objectives 10X, 40X, 100X successively after staining with lugol for the observation of cysts. The identification of the parasitic elements was based on morphological criteria such as: the shape, the nature of the shell or the membrane as well as the content of the cytoplasm. This parameter was calculated according to the formula below:

$$\text{prevalence} = \frac{Number\ of\ positif\ samples}{Number\ of\ studied\ samples} \times 100$$

2.4. Statistical Analysis

The collected data were entered and stored in Microsoft Excel 2016 and then transported to SPSS version 22 for analysis. The ANOVA test was used to compare the average bacterial contamination levels of the waters with each other. For all analyses, differences were considered significant at P values<0.05.

3. Results

3.1. Types of Water Used to Wash Vegetables in the Dschang City Market

The proportion of vendors who take the trouble to wash vegetables before selling is 45.34% and the proportion who never wash is 54.66%. The water used for washing comes either from the river, rain, boreholes or wells. Well water is the most used (38.23%).

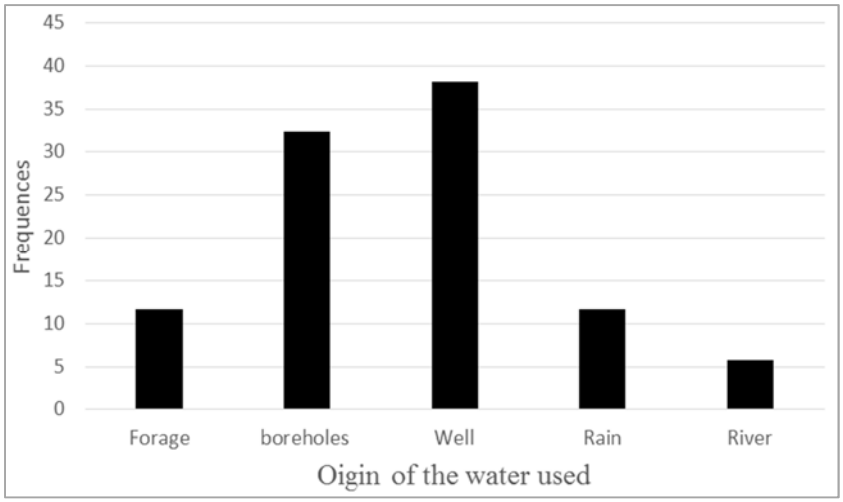

Figure 2. *Distribution of vendors according to the origin of the water used to wash fresh vegetables.*

3.2. Proportion of Vendors Who Take the Trouble to Change the Wash Water Throughout the Day

Vendors' opinions diverge with regard to the frequency of changing the water used to wash vegetables throughout the day. Among the 45.33% who take the trouble to wash the vegetables before selling (69.69%) say they do not change the wash water during the day, (24.24%) say they change the wash water at least once during the day.

Figure 3. *Some vegetables and maintenance water.*

3.3. Knowledge of Health Risks

The proportion of vendors who take the trouble to wash vegetables before selling is 45.33% and those who never wash is 54.66% (Figure 3). Among those who wash, 68.96% say that the water is clear and therefore of good quality, while 31.04% are aware that the water is of poor quality but since this is what is available they use it. 44.11% said that they wash vegetables to remove mud, 23.52% to remove chemicals; 26.47% to make them fresh. 55.88% of them said that they are aware that after washing these vegetables are not of good sanitary quality.

3.4. Physicochemical Quality of Vegetable Washing Water (Rainy Season)

The temperature ranged from 22.0°C to 22.4°C (Table 2); the pH ranged from 3.8 to 7.27, the electrical conductivity from 7 µS/cm to 406 µS/cm and the TDS from 3 to 207 ppm.

Table 1. *Physicochemical quality of fresh vegetable washing water in the rainy season.*

		washing water used by type of vegetable					
		celeri	**Basyl**	**Cuncumber**	**Tomatoes**	**Lettuce**	**Standard**
T°C	Min	22,0°C	21,7°C	21,7°C	22,1°C	22,0°C	<28°C
	Max	22,4°C	22°C	21,9°C	21,8°C	22,1°C	
	M±SD	22.1±0,18	21.8±0,12	21.7±0,1	21.7±0,1	22.0±0,05	
pH	Min	6,9	6,8	6,8	3,8	6,7	6.5°C
	Max	7,5	7,3	7,3	6,3	7,0	9.5°C
	M±SD	7.1±0,2	7±0,2	7.1±0,2	5.3 ±0,1	6.8±0,1	
EC (µs/cm)	Min	48	14	50	298	44	50 (µs/cm)
	Max	54	308	406	581	322	2000 (µs/cm)
	M±SD	52±2,8	161±150,5	266.5±152,0	463,7±137,6	182.5±159,9	
TDS (PPM)	Min	24	7	26	152	22	
	Max	26	157	207	296	164	1000 (PPM)
	M±SD	26±1,4	81.7±76,3	136±77,2	236,25±70,1	92.75±81,6	

Min: Minimum; Max: Maximum; M±SD: mean ± standard deviation, EC=elecric conductivity.

3.5. Physico-chemical Quality of Vegetable Water in the Dry Season

The temperature values vary between 22.0°C and 26.9°C; the temperature differences recorded are relatively small (Table 2); the pH between 3.8 and 8.6, the electrical conductivity fluctuates between 7 µS/cm and 114 µS/cm and the TDS between 3 and 164 ppm.

Table 2. *Physicochemical quality of fresh vegetable washing water in the dry season.*

		washing water used by type of vegetable					
		celeri	**Basyl**	**Cuncumber**	**Tomatoes**	**Lettuce**	**Standard**
T°C	Min	22,0°C	26,3°C	26,7°C	21,2°C	26,0°C	<28°C
	Max	22,9°C	26,8°C	26,9°C	21,8°C	26,6°C	
	M±SD	75.4±1.9	26.6±0.2	26.8±0.9	21.7±0.1	22.05±0.1	
pH	Min	6,15	5,4	6,3	3,8	5,02	6.5°C
	Max	8,6	6,29	6,8	6,31	6,67	9.5°C
	M±SD	6.7±0.6	5.7±0.4	6.3±0.4	5.3±1.1	6.1±0.8	
EC (µs/cm)	Min	22	31	7	7	32	50 (µs/cm)
	Max	34	54	24	114	72	2000 (µs/cm)
	M±SD	30.7±2.7	47.2±10.9	34.7±27.7	62.5±44.4	48.5±16.8	
TDS (PPM)	Min	14	16	3	3	22	
	Max	17	27	15	164	163	1000 (PPM)
	M±SD	15.5±1.1	23.5±5.1	11±5.4	31.7±22.7	61.2±59.0	

Min: Minimum; Max: Maximum; M±SD: maen ± standard deviation; EC=elecric conductivity.

3.6. Bacteriological Quality of Water Used for Vegetable Care

With the exception of *E. coli*, which was not detected in the water used to wash celery in the rainy season, all the undesirable bacteria tested were detected in all water samples (Figure 4, Figure 5). The water was generally statistically more contaminated in the dry season. In general, *Salmonella spp*. were the most present bacteria in the dry season and faecal coliforms the most present in the rainy season. In both dry and wet seasons, *E. coli* was the least represented bacterium.

In the rainy season, undesirable bacteria were detected in the fresh vegetable water with bacterial loads of 2×10^4 to 1.8×10^6 CFU/100ml for faecal coliforms, 90 to 6.1×10^3 CFU/100ml for faecal streptococci, 5×10^5 to 1×10^9 CFU/100ml for *Shigella* spp. from 3×10^6 to 108×10^7 CFU/100ml for *Salmonella* spp. and from 0 to 2×10^4 CFU/100ml for *Escherichia coli.* In the dry season, this ranged from 1.8×10^5 CFU/100ml to 108×10^7 for faecal coliforms, from 40 CFU/100ml to 1.5×10^7 CFU/100ml for faecal streptococci, from 3×10^3 CFU/100ml to 1×10^6 CFU/100ml for *Shigella* spp., from 1×10^3 CFU/100ml to 1.1×10^5 CFU/100ml for *Salmonella spp.* and from 0 to 2×10^4 CFU/100ml for *Escherichia coli*.

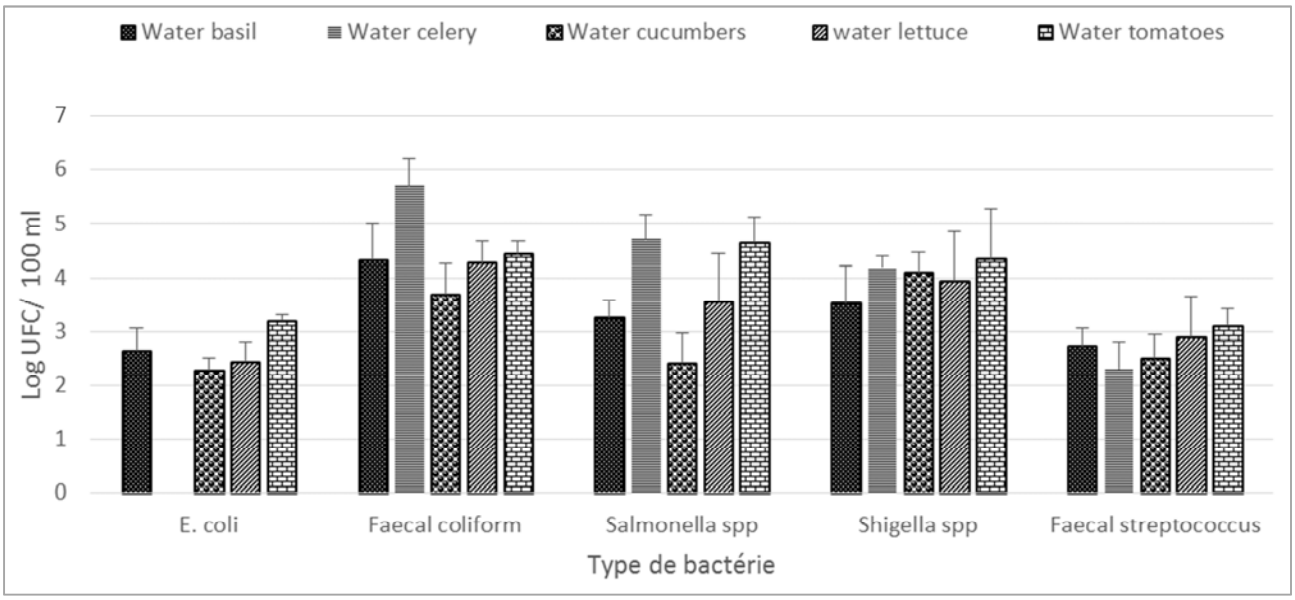

Figure 4. *Bacteriological quality of water used for washing vegetables in the rainy season.*

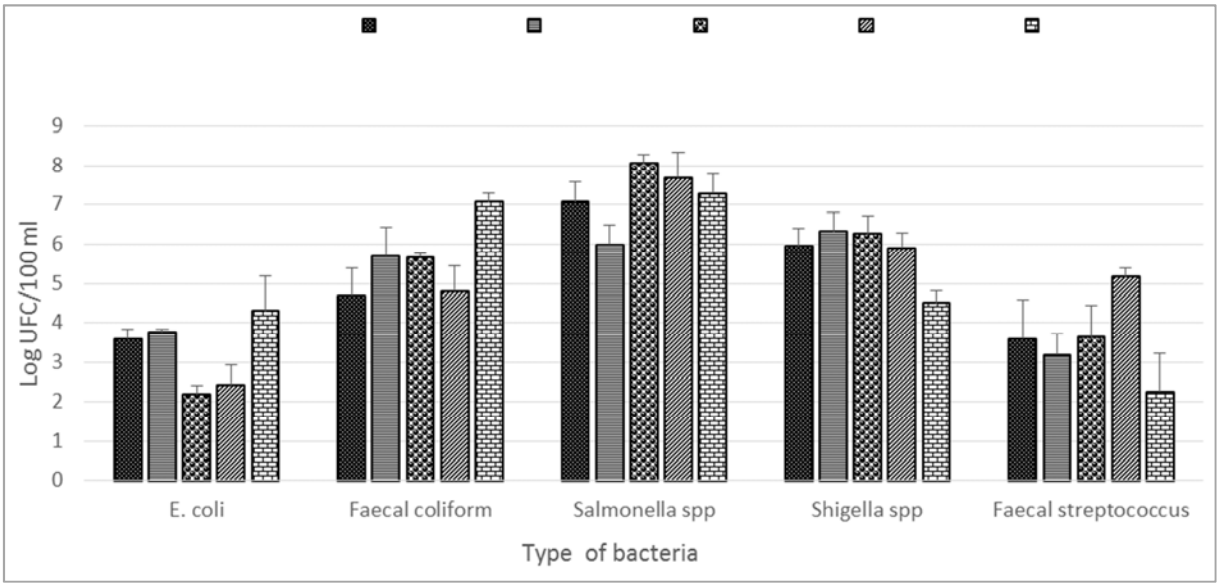

Figure 5. *Bacteriological quality of water used for washing vegetables in the dry season.*

3.7. Parasitological Quality of Water Used for Washing Fresh Vegetables

Ascaris spp. eggs, *Entamoeba* spp. cysts and nematode larvae were detected in vegetable washing water (Figure 6). *Ascaris* spp. eggs were detected in 40% of the sample, *entamoeba* spp. cysts in 60% of the sample and nematode larva in 80% of the sample of water used to wash vegetables that could be consumed raw. Water used to wash lettuce has always been the most contaminated, this of basil and cucumber the least contaminated.

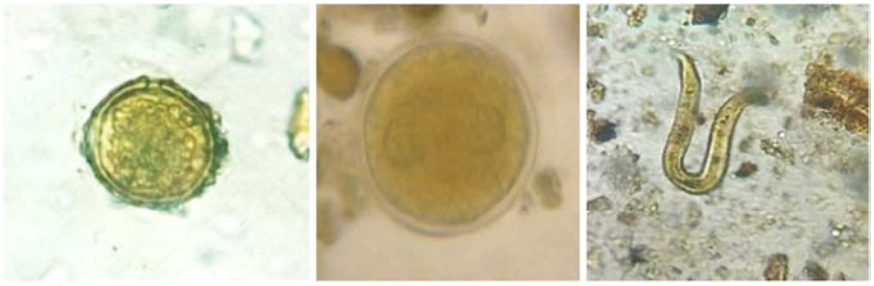

Figure 6. *Parasitic elements observed under the microscope in vegetable maintenance water samples A): Egg of Ascaris* spp. *B): Cyst of Entamoeba* spp.*; C: Nematode larva.*

Table 3. *Parasitological quality of water used for washing vegetables*

	Quality of water used by type of vegetable					
	Basil n=4	**Celeri n=4**	**Cuncumber n=4**	**Lettuce n=4**	**Tomatoes n=4**	**Total**
Eggs of d'*Ascaris* spp.	A	P	A	P	P	40%
Cyst of *Entamoeba* spp.	A	P	A	P	A	60%
Nematode larva	P	A	P	P	P	80%
Positive sample	1	2	1	3	2	9
Prevalence (%)	25,0%	50,0%	25,0%	75,0%	50,0%	40%

A=absent, P=present.

4. Discussion

Regardless of the season, undesirable bacteria, indicative of faecal contamination, were detected in the water used for washing vegetables, with levels ranging from 0 to 8×10^4 CFU/100ml for *E. coli*, from 1.8×10^4 to 1.8×10^8 CFU/100ml for faecal coliforms, from 3×10^4 to 7.2×10^8 CFU/100ml for *Salmonella* spp., from 30 to 1.3×10^7 CFU/100ml for *Shigella* spp., and 20 to 7.5×10^5 CFU/100ml for faecal streptococci. While according to current standards none of these bacteria should be detected in 100 ml of service water [10, 11]. High

levels of these bacteria in fresh vegetable water pose a risk to consumers of these vegetables if they are poorly washed and eaten raw. The risk is higher in the dry season than in the rainy season because of the reduced water supply during this period. Indeed, the risk of microbiological contamination associated with fresh vegetables is very high and can occur at any stage of production. This includes the environment in which they are produced, transported, sold, stored, prepared and consumed. The personal hygiene of handling and the type of raw material are also potential sources of contamination [12, 13]. The poor quality of these waters may be due to insufficient drinking water points in the market, which causes vegetable vendors to sometimes use the same water for several days, or to vendor ignorance. Also, during vegetable washing, water contamination or cross-contamination by equipment, vegetables or handlers are likely to be the main risk factors for contamination of washing water. Indeed, in this city wash water is used all day long; the bucket containing the water when not properly washed can accumulate bacteria, forming biofilms that can contaminate the water during the next collection. Since the action of washing is not to make their vegetables clean but mainly for good presentation and to attract customers, vendors do not pay particular attention to the quality of the water used. This finding is in line with that of Foming [14] in the markets of Bafoussam, who indicated that the water at the collection point is of good quality but of poor quality in the market due to multiple washing of vegetables or the poor quality of collection containers. In developing countries, vegetable vendors are often neither licensed nor trained in food hygiene and sanitation practices and therefore engage in serious unsanitary practices [15].

40% of the samples of housekeeping water analysed contained at least one parasitic element *Ascaris spp eggs, Entamoeba spp* cysts and nematode larvae. The contamination of these cleaning waters with these parasites can be explained either by the origin of the water used for washing or by the contamination of previously washed vegetables. These results obtained during the studies are close to the prevalence of 23.3% obtained by Mohamed [16] in Sudan on the parasitological quality of washing water used by traders.

The main factors of water quality pollution in general are, among others, the lack of environmental sanitation and the precarious hygiene conditions of disadvantaged populations in cities. The climatic and physical conditions of the environment influence the level of water pollution [17]. The use of these waters therefore constitutes a threat to the health of the user populations and human contamination is then achieved through the consumption of food contaminated by the water [5, 18]. Other studies [19] showed that the different qualities of water used in the markets were of poor quality and at least one type of parasite was found and this posed a risk to consumers. Similarly studies revealed the presence of several parasites (*strongyloides stercoralis; G. lamblia, A. lumbricoides*) in the washing water used by vendors [20].

5. Conclusion

Water-borne diseases persist in the city of Dschang and the quality of the water used to wash vegetables has also been questioned. The objective of this study was to determine the health risk associated with the use of fruit and vegetable washing water in the city of Dschang. *Salmonella* spp., *Shigella* spp., faecal streptococci*, E. coli*, faecal coliforms, *Ascaris* spp. eggs, *Entamoeba* spp. cysts and nematode larvae were found in the washing water of fresh vegetables at levels well above the WHO standard. These bacteria and parasitic elements contained in the water can contaminate the vegetables and in the chain the consumers of these vegetables if they are badly washed and eaten raw. The risk is even higher in the dry season than in the rainy season. All of these bacteria are evidence of faecal contamination. Consumers of these fresh vegetables, if poorly washed and raw, are at risk of bloody diarrhoea, gastroenteritis, typhoid and paratyphoid fever, or inflammation of the colon tissue and severe necrosis. It is therefore urgent for the public authorities to introduce food hygiene in the markets in their strategies to combat waterborne diseases in Dschang. But it is important to note that this recommendation is important for all cities in Cameroon.

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