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Impact of Irrigation Water on the Bacteriological Quality of Lettuce (*Lactuca sativa*) in the Market Garden Zone of Niamey, Identification of *Enterobacteria*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The urban community of Niamey is characterized by a prodigious development of urban and peri-urban agriculture. Lettuce (*Lactuca sativa*) is one of the main crops grown in these urban and peri-urban areas. The aim of this study is to assess the impact of irrigation water on the bacteriological quality of lettuce produced in the market gardening zone of the Niamey city. Three (3) market garden sites were studied (*Harobanda* (site 1), *Gamkallé* (site 2) and *Gounti yéna* (site 3). Eighteen (18) lettuce samples and twenty-nine (29) irrigation water samples were taken from the market garden sites and subjected to microbiological analysis using conventional methods appropriate to each indicator. Thus, five (5) indicators of contamiantion were searched, Total Mesophilic Aerobic Flora (TMAF), total coliforms (TC), faecal coliforms (FC), enterobacteria (Ent), Faecal streptococci (FS), *Escherichia coli* (*E. coli*) and *Clostridium perfringens* (CP). The results showed that irrigation water samples from site 1 were highly contaminated with Total Coliforms (TC) and Faecal Coliforms (FC) ($3.65\pm7.88.10^6$ and $1.41\pm3.04.\ 10^6$ CFU/100ml respectively), those from site 2 by Faecal Streptococci (FS) ($1.19\pm2.03.10^5$ CFU/100ml) and from site 3 by Enterobacteria (Ent) ($1.61\pm2.37.10^8$ CFU/100ml) and *Escherichia coli* ($8.22\pm11.86.10^5$ CFU/100ml). Thus, lettuce samples from site 1 are more contaminated with TC ($5.32\pm8.32.10^6$ CFU/g) and Ent ($3.10\pm0.95.10^7$ CFU/g). Faecal coliforms ($1.35\pm1.13.10^6$ CFU/g) and faecal streptococci ($5.30\pm8.21.10^5$ CFU/g) predominate on the site2 and finally site3 is more contaminated with *E. coli* ($2.43\pm0.38.10^5$ CFU/g). However, enterobacteria species such as *Citrobacter freundii, Enterobacter cloacae, Enterobacter sakazakii, Escherichia coli1, Klebsiella pneumoniae ssp ozaenae, Pantoea spp 1, Proteus mirabilis, Raoultella terrigena, Salmonella enterica arizonae, Salmonella spp, Serratia liquefaciens, Serratia marcescens and Serratia odorifera*1 were identified in irrigation water samples and lettuce at these sites. In fact, the quality of the irrigation water is not suitable for irrigation, and strongly influences the contamination of the lettuce produced by the bacteria. Consumption of this lettuce without the minimum of precautions could prove dangerous to consumers.

Keywords: Lettuce; watering water; contamination; Enterobacteria; Niamey/Niger.

1. INTRODUCTION

Over the past two decades, as a result of rapid urbanization and economic concentration, agriculture has expanded considerably in urban and peri-urban areas of West Africa [1,2]. In difficult contexts marked in particular by a lack of financial resources for the supply of drinking water, market gardeners are driven to use wastewater for irrigation and animal dung as fertilizer for the soil [2,3]. In most developing countries, crops are irrigated using treated, poorly treated, diluted or even raw wastewater [4]. The reuse of wastewater in market gardening and horticulture has thus become a common practice, responding among other things to demographic growth in cities and youth unemployment [5]. In fact, their reuse without prior treatment has negative impacts on both the environment and people's health [6]. From a health point of view, the greatest threat from wastewater reuse is the presence of pathogenic microorganisms. Viruses, bacteria and parasites can be present in wastewater in high concentrations and survive for long periods [7]. Spreading this water on vegetables can cause microbiological contamination of these products and the introduction of pathogenic microorganisms into the food chain [8].

The urban as a provider of fresh fruit and fruit and vegetables has gained in importance over the last 30 years in west Africa (Wognin, 2014). As is the case for many African cities, Niamey has seen the development of local agriculture, focused primarily on suppling the city with fruit and vegetables (Issa, 2018). The urban community of Niamey is characterized by inadequate drainage systems and the almost total absence of wastewater treatment plants. As a result, wastewater is discharged directly into the lowlands and other receiving areas, notably

the River Niger, without prior treatment [9]. Three (3) sources of irrigation water have been identified in Niamey: river water, well water and wastewater according to market garden sites [10]. Market gardeners on the *Gamkallé* corniche use river water, those on *Saga* use well water and those on *Gounti yéna*, a non-permanent tributary, use wastewater from toilets. Thanks to this water, market gardening is practicable throughout the 12 months of the year [10].

This study aims to assess the impact of irrigation water on the bacteriological quality of lettuce produced in the market gardening zone of the city of Niamey.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in the urban community of Niamey. The city of Niamey is located in the south-western part of Niger between 13° 24' and 13°35'N latitude and, 2°00' and 2°15'E longitude with an altitude between 160 and 250 m. Its administrative boundaries extend over 552.27km², including around 297.46km² of urbanized area [11]. Niamey's population is estimated at around 1,407,635. The city of Niamey is subdivided into five (5) communal arrondissements, whose population distribution by communal arrondissement is as follows: Niamey I: 287,902 inhabitants; Niamey II: 338,455 inhabitants; Niamey III: 223,685 inhabitants; Niamey IV: 376,271 inhabitants; Niamey V: 181,321 inhabitants [12]. The climate is Sahelo-Sudanian, with a long dry season from October to May and a short rainy season from June to September. The cold dry season is the most favorable for vegetable production, during which most crops are grown. This study has been focused on three (3) major vegetable-growing sites in the urban community of Niamey.

- Site1, located in Niamey's commune V (*Harobanda*), stretches along the river over a length of around 2km and a width varying from 200m to 700m depending on the extent of the riverbed.
- Site2, located right in the center of town, the *Gamkalé* corniche forms part of the left bank of the River Niger. It is bounded on one side by the Kennedy Bridge and on the other by the Niamey abattoir.
- Site3 is the *Gounti yéna* valley, covering an area of around 38.1 ha. The environment around this valley is complex: less than 2 km from the valley and often within it, there are roads with high traffic density; a wastewater treatment plant collecting urban water and open dumps [11].

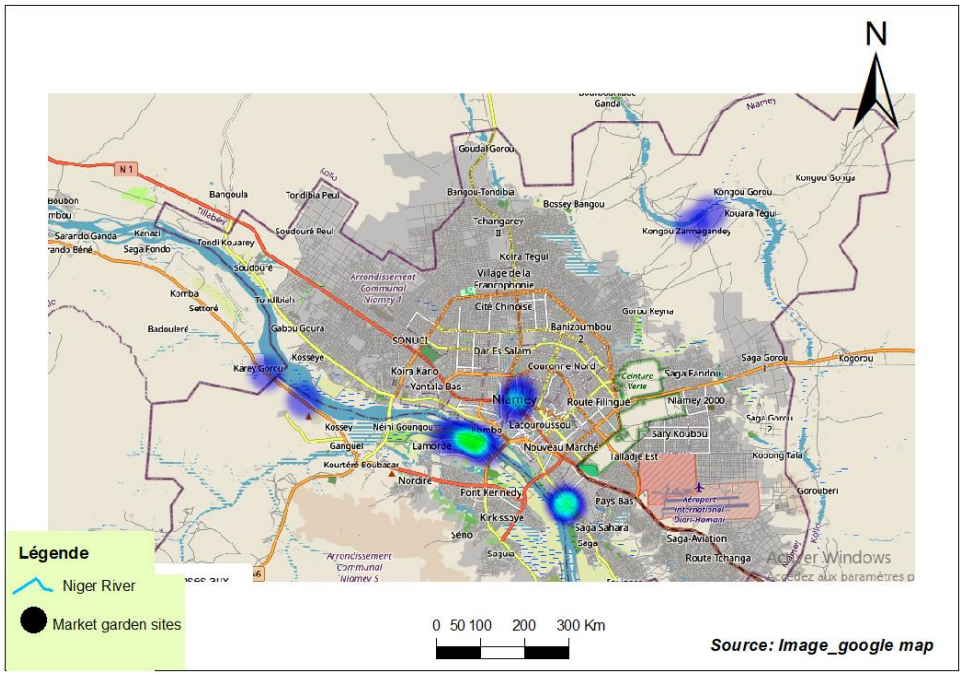

Fig. 1. Geological map of the urban community of Niamey presenting the market garden sites of study

a: watering water source1

b: watering water source 2

c: field of lettuce

d: lettuce dishes

Fig. 2. some sources of irrigation water (a; b) and lettuce dishes (c; d) at the Harobanda site

2.2 Sampling

Lettuce and irrigation water samples were taken from three (3) production sites in the urban community of Niamey. Samples were taken under sterile conditions, using single-use sterile gloves and alcohol to avoid external contamination. A lettuce sample corresponds to three (3) bunch of lettuce, weighing around 150g, taken at random from different corners of the field. Water samples were taken at production sites. Sterile 100ml bottles were used for sampling. In fact, the sample of irrigation water corresponds to 100ml of water taken from the source by dipping the bottle directly into the pool. An information sheet was attached to each sample. A total of eighteen (18) lettuce samples (8; 6; and 4 samples respectively for site1; site2 and site3) and twenty-nine (29) irrigation water samples were taken (ten (10) samples for site 1, ten (10) samples for site2 and nine (9) for site3).

2.3 Transport Conditions

Each sample of lettuce collected was well packaged in a polyethylene bag, then carefully labeled. The samples were then transferred to the microbiology laboratory of the Faculty of Science and Technology (FAST), after being packaged and placed in a cooler containing carboglass to keep the temperature down to around 4°C.

2.4 Microbiological Analysis of Samples

2.4.1 Preparation of stock solutions

The stock solution was prepared by grinding the lettuce sample in a sterile polyethylene bag around the flame of a Bunsen burner. Then, 25 grams of the crushed material were taken and introduced directly into 225 mL of previously prepared and sterilized buffered peptone water. Next, 1 mL of each stock solution was taken and introduced into a test tube containing 9 mL of buffered peptone water to perform the various decimal dilutions in accordance with ISO 6887-V08-010-6 (2013) [13]. All water samples were homogenized by vigorously shaking the bottles in a vertical motion. In fact, 1 mL of each water sample was taken and introduced into a test tube containing 9 mL of buffered peptone water to perform the various decimal dilutions.

2.4.2 Bacterial culture

- Total Aerobic Mesophilic Flora (TAMF) was enumerated in accordance with ISO V08-051(1992) / ISO 4833. This flora was counted on PCA (Plat Count Agar). Incubation was carried out at 37° C for 24 hours.
- Coliforms were tested on VRBL medium by incubation at 37°C for 24 hours for total and 44°C for fecal. All characteristic colonies were counted in accordance with ISO 4832 (February, 2006). Coliforms showed purplish colonies equal to or greater than 0.5 mm in diameter after 24 hours' incubation. Plates containing more than 15 characteristic colonies and less than 300 total colonies were retained.
- Fecal Streptococcus enumeration was carried out on Kanamycin-Asculin-Azid-Agar (KAA) agar in accordance with the AFNOR NF 190-0411 standard (1989). Inoculation was carried out using the surface spreading method. Incubation was at 37°C for 24 hours. After 24 hours, faecal streptococci appeared as small translucent colonies surrounded by black halos.
- *Clostridium perfringens* enumeration was carried out on TSN agar according to the ISO 7937 standard. A first reading was taken after 24 hours to prevent total blackening of the tube, followed by a second reading after 48 hours when the large colonies visible in the tube were counted.
- *E. coli* were counted on EMB agar (Bio-rad, France) in accordance with ISO 18140. After incubation at 37°C for 24 hours, characteristic *E. coli* colonies (green with a metallic sheen) were counted.
- Enterobacteria were counted on Mac Conkey agar, in accordance with ISO Standard 21528-1. Inoculation was carried out by spreading 0.1 mL of each dilution onto Mac Conkey agar, which had previously been poured into Petri dishes. The plates were then incubated at 37°C for 24 hours. Red colonies characteristic of Enterobacteria were counted.

2.4.3 *Enterobacteria* identification

Enterobacteria from water and lettuce samples were randomly selected and streaked onto nutrient agar. Biochemical characterization was carried out on Api 20E strips. Strains were identified by comparing their characteristics with those of known taxa as described in the bio Mérieux SA manual.

2.5 Interpretation

Bacterial load was calculated in accordance with ISO 7218 (2007) using the following formula:

$$N= \frac{\sum C}{(n1+0,1xn2)v.d}$$ [13]

- Σc = Total number of colonies counted in plates with colony counts between 15 and 300.
- n1 = number of plates counted in the first dilution;
- n2 = number of plates counted in the second dilution;
- d = dilution factor at which 1st counts were made;
- v = inoculum volume.

2.5.1 Frequency calculate

The frequency of enterobacteria species was calculated using the following formula:

$$\mathbf{F} = \frac{n}{N_T} \times 100$$

- F: Frequency of germ isolation
- n : number of each species of enterobacteria identified
- N_T : number of total species identified

2.5.2 Determination of fecal contamination origin

Table 1. Criteria for determination of fecal contamination origin

Ratio CF/SF (R)	Origin of contamination
R<0,7	Strictly animal
0,7<R<1	Mixed predominantly animal
1<R<2	Uncertain
2<R<4	Mixed predominantly human
R>4	Strictly human

(Borrego et Romero, 1982; Wognin, 2014)

2.6 Statistical Analysis

The data were analyzing using SPSS 23.0.0.0. The results were expressed as means±standard deviation for bacterial loads and percentage for frequency. The statistical differences among the means of data calculating using one-way analysis of variance and Duncan's test (DMRT). Differences were considered significant for values of $P<.05$. The document was drafted using Microsoft Word. Arc Gis software was used to design the geographical map of the study area.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Water contamination

The Total (TC) and faecal (FC) coliforms, faecal streptococci (FS), enterobacteria (Ent) and *Escherichia coli* were tested in samples of irrigation water. The microbiological characteristics of the irrigation water are shown in Table 2. Average total coliform loads ranged from $1.53±3.15.10^6$ to $3.65±7.88.10^6$ CFU/100ml of water for sites 2 and 1 respectively. Fecal coliform loads varied from $4.29±7.88.10^5$ CFU/100ml (site 2) to $1.41±3.04.10^6$ (site 1). Enterobacteria have the highest values of all indicators. These range from $4.34±6.84.10^7$ (site1) to $1.61±2.37.10^8$ CFU/100ml (site3). Average E. coli loads ranged from $3.77±5.68.10^5$ (site1) to $8.22±11.86.10^5$ CFU/100ml (site3). Faecal streptococci averages loads ranged from $2.18±5.25.10^4$ (site1) to $1.19±2.03.10^5$ CFU/100ml (site2) of irrigation water. Indeed, site1 is more loaded with total and faecal coliforms, site2 with faecal streptococci and site3 with enterobacteria and E. coli. The values obtained for all irrigation water samples are above the threshold values.

3.1.2 Contamination of lettuce samples

Table 3 shows the bacteriological characteristics of lettuce samples according to production site. The Total Aerobic Mesophilic Flora (TMAF), Total (TC) and faecal (FC) coliforms, faecal streptococci (FS), enterobacteria (Ent), *Escherichia coli* (E. coli) and *Clostridium perfringens* (CP) were tested in samples of lettuce. The level of contamination ranged from $1.60±0.52.10^5$ (site3) to $5.32±8.32.10^6$ CFU/g (site1) of lettuce for total coliforms and $1.65±0.92.10^5$ (site3) to $1.35±1.13.10^6$ CFU/g (site2) of lettuce for faecal coliforms. *Enterobacteria* recorded the highest level of contamination. Loads ranged from $1.13±0.09.10^7$ (site3) to $3.10±0.95.10^7$ CFU/g (site1) of lettuce. In addition, a predominance of Total coliforms ($5.32±8.32.10^6$ CFU/g) and Enterobacteria ($3.10±0.95.10^7$ CFU/g) was observed at site1. Site2 was heavily contaminated with faecal coliforms ($1.35±1.13.10^6$ CFU/g) and faecal streptococci ($5.30±8.21.10^5$ CFU/g), while site3 was more heavily loaded with *E. coli* ($2.43±0.38.10^5$ CFU/g lettuce). The level of contamination of all lettuce samples, whatever the site, was higher than the French standards that vegetables for human consumption must meet.

3.1.3 Comparative analysis of irrigation water and lettuce contamination levels

Fig. 3 shows the variation curves in contamination levels for irrigation water and lettuce. It can be seen that all loads are above standards. For all indicators, the irrigation water samples are more contaminated than the lettuce samples for enterobacteria. A certain similarity is observed in the variation of loads according to indicators between water and lettuce samples (Total Coliforms and Faecal streptococci).

3.1.4 Origin of faecal contamination

The origin of faecal contamination was determined according the criteria defined by (Borrego and Romero, 1982). If the ratio FC/FS is greater than 4, the fecal contamination was strictly of human origin. The Table 4 shown FC/FS ratios for lettuce and watering water samples. Fecal contamination of irrigation water samples was of human origin at all sites (FC/SF >4). Fecal contamination of human origin was also observed in lettuce samples from sites 1 and 3. Contamination of lettuce samples from site 2 was mixed but predominantly human (4 >FC/SF> 2).

3.1.5 Diversity of *Enterobacteria* isolated at production sites

Table 5 shows the diversity of *Enterobacteriaceae* isolated at the production sites. A total of thirteen (13) species belonging to nine (9) genera were identified. The species identified were *Citrobacter freundii, Enterobacter cloacae, Enterobacter sakazakii, Escherichia coli1, Klebsiella pneumoniae ssp ozaenae, Pantoea spp 1, Proteus mirabilis, Raoultella*

Table 2. Level of contamination of irrigation water by production site

	Means loads ± standard deviation of irrigation water samples (CFU/100ml)				
Sites	**TC**	**FC**	**Ent**	***E. coli***	**FS**
Site 1	$3.65\pm7.88.10^{6a}$	$1.41\pm3.04.10^{6b}$	$4.34\pm6.84.10^{7c}$	$3.77\pm5.68.10^{5b}$	$2.18\pm5.25.10^{4a}$
Site 2	$1,53\pm3,15.10^{6a}$	$4,29\pm7,88.10^{5b}$	$8.15\pm12.32.10^{7c}$	$5.29\pm8.96.10^{5b}$	$1.19\pm2.03.10^{5a}$
Site 3	$1.47\pm4.33.10^{6a}$	$2.33\pm4.64.10^{5b}$	$1.61\pm2.37.10^{8c}$	$8.22\pm11.86.10^{5b}$	$3.70\pm8.23.10^{4a}$
Total	$2.37\pm5.75.10^{6}$	$7.64\pm20.15.10^{5}$	$9.24\pm15.91.10^{7}$	$5.64\pm8.83.10^{5}$	$5.20\pm1.19.10^{4}$

TC : Total coliform ; FC : Faecal Coliform ; Ent : Enterobacteria ; E. coli : Escherichia coli ; FS: Faecal Streptococcus. Site1 : Harobanda ; site2 : Gamkalé ; Site3 : Gounti yéna. Values with the same letter in the same column are not significantly different (P>.05).

Table 3. Microbial contamination levels of lettuce samples by production site

	Means loads ± standard deviation of lettuce samples (CFU/g)						
Sites	**TMAF**	**TC**	**FC**	**Ent**	**FS**	**CP**	***E .coli***
Site1	$2.49\pm2.50.10^{7a}$	$5.33\pm8.47.10^{6a}$	$2.14\pm1.19.10^{6b}$	$9.05\pm13.54.10^{6a}$	$4.56\pm7.23.10^{5a}$	$3.80\pm6.13.10^{4a}$	$1.83\pm1.10.10^{5a}$
Site2	$5.31\pm1.63.10^{7b}$	$8.30\pm8.36.10^{5a}$	$1.35\pm1.21.10^{6ab}$	$3.20\pm1.22.10^{6a}$	$6.57\pm9.40.10^{5a}$	$1.85\pm1.94.10^{4a}$	$2.00\pm2.10.10^{3b}$
Site3	$3.03\pm1.76.10^{6a}$	$1.60\pm1.26.10^{5a}$	$1.65\pm0.99.10^{5a}$	$4.40\pm2.17.10^{5a}$	-	-	$1.95\pm1.17.10^{5b}$
Total	$2.94\pm2.66.10^{7}$	$2.68\pm5.98.10^{6}$	$1.44\pm1.28.10^{6}$	$5.19\pm9.47.10^{6}$	$4.22\pm7.33.10^{5}$	$2.30\pm4.35.10^{4}$	$1.26\pm1.24.10^{5}$

TMAF: Total Mesophilic Aerobic Flora; TC: Total coliform; FC: Faecal coliform; Ent: Enterobacteria; E. coli: Escherichia coli; FS: Faecal Streptococcus; CP: Clostridium perfringens. Site1: Harobanda; site2 : Gamkalé ; Site3 : Gounti yéna. Values with the same letter in the same column are not significantly different (P>.05).

Table 4. Faecal contamination origin

	Ratio faecal coliforms /faecal streptococci		
	Site 1	**Site 2**	**Site 3**
Lettuce	69,68	2,55	9,43
Origin of faecal contamination	**Human**	**Mixed predominantly human**	**Human**
Water	59,80	12,50	20,56
Origin of faecal contamination	**Human**	**Human**	**Human**

Site1 : Harobanda ; site2 : Gamkalé ; Site3 : Gounti yena.

Table 5. Diversity of enterobacteria species isolated at production sites

Species identified	**Distribution by production site (n)%**			**Total**
	site 1	**site 2**	**site 3**	
Citrobacter freundii	-	-	(2)8,70[a]	(2)8,70
Enterobacter cloacae	(1)4.35[a]	(1)4.35[a]	-	(2)8,70
Enterobacter sakazakii	(1)4.35[a]	-	-	(1)4,35[a]
Escherichia coli1	(2)8,70[a]	-	(1)4.35[a]	(3)13,04
Klebsiella pneumoniae ssp ozaenae	-	(1)4.35[a]	-	(1)4.35[a]
Pantoea spp 1	-	-	(1)4.35[a]	(1)4.35[a]
Proteus mirabilis	-	(1)4.35[a]	-	(1)4.35[a]
Raoultella terrigena	(1)4.35[a]	-	-	(1)4.35[a]
Salmonella arizonae	-	-	(1)4.35[a]	(1)4.35[a]
Salmonella spp	(1)4.35[a]	(2)8,70[a]	(3)13.04[a]	(6)26.09
Serratia liquefaciens	(1)4.35[a]	-	(1)4.35[a]	(2)8.70
Serratia marcescens	-	(1)4.35[a]	-	(1)4.35[a]
Serratia odorifera 1	-	(1)4.35[a]	-	(1)4.35[a]
Total	**(7)30.43**	**(7)30.43**	**(9)39.13**	**(23)100**

Site1 : Harobanda ; site2 : Gamkalé ; Site3 : Gounti yéna. Values with the same letter in the same column are not significantly different (P>.05).

Table 6. Diversity of species isolated from irrigation water

Species of enterobacteria identified	**Distribution (n)%**			**Total**
	Site1	**Site2**	**Site3**	
Citrobacter freundii	-	-	(2)14.28[a]	(2)14.28[a]
Enterobacter cloacae	(1)7.14[a]	-	-	(1)7.14[a]
Enterobacter sakazakii	(1)7.14[a]	-	-	(1)7.14[a]
Escherichia coli1	(2)14.28[a]	-	(1)7.14[a]	(3)21.43
Raoultella terrigena	(1)7.14[a]	-	-	(1)7.14[a]
Pantoea spp 1	-	-	(1)7.14[a]	(1)7.14[a]
Salmonella spp	(1)7.14[a]	1)7.14[a]	(1)7.14[a]	(3)21.43[a]
Serratia liquefaciens	(1)7.14[a]	-	(2)14.28[a]	(3)21.43[a]
Total	**(7)50.00**	**(1)7.14**	**(6)42.85**	**(14)100,0**

Site1 : Harobanda ; site2 : Gamkalé ; Site3 : Gounti yéna. Values with the same letter in the same column are not significantly different (P>.05).

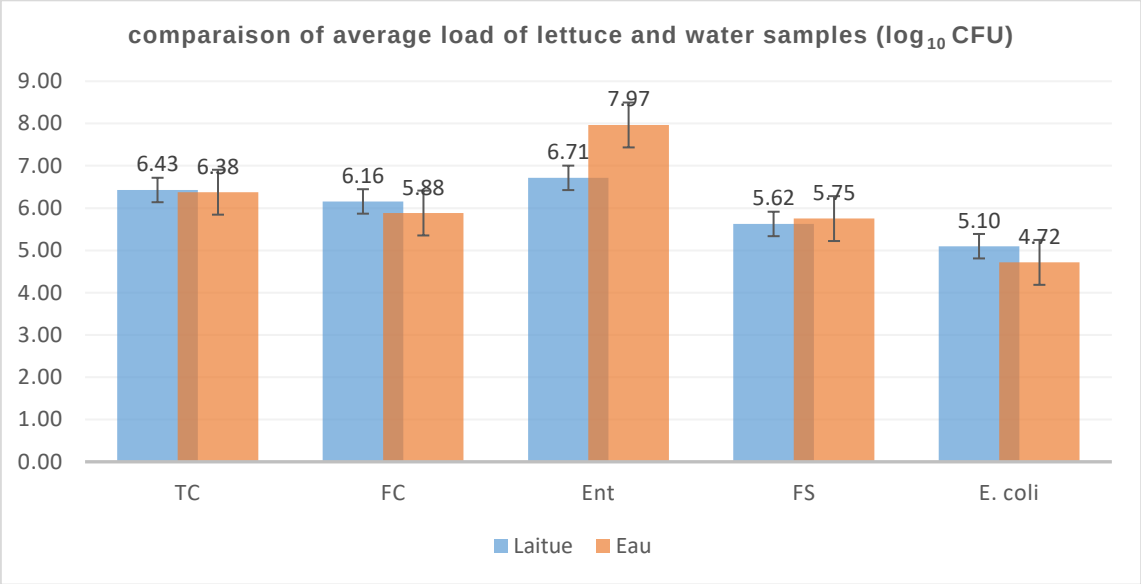

Fig. 3. Level of contamination of irrigation water and lettuce samples

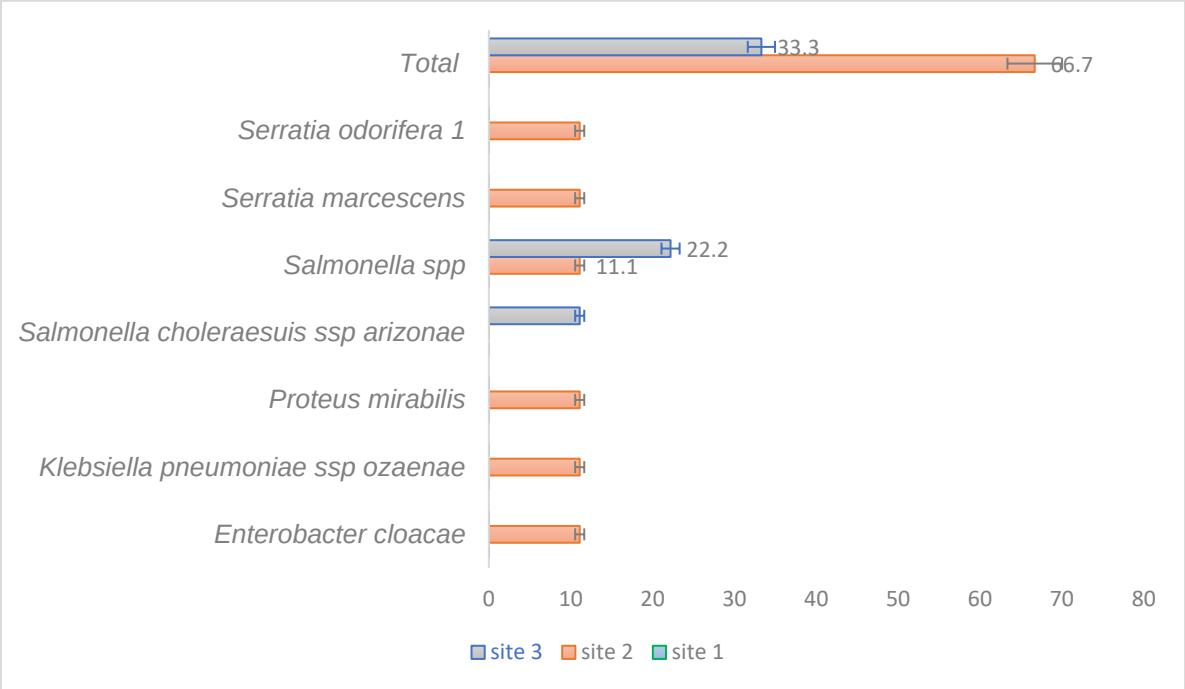

Fig. 4. Frequency of *Enterobacteria* species isolated from lettuce depending on market garden site

terrigena, Salmonella arizonae, Salmonella spp, Serratia liquefaciens, Serratia marcescens and Serratia odorifera 1. On all sites, Salmonella spp, Escherichia coli1, Citrobacter freundii, Enterobacter cloacae, Enterobacter sakazakii and Serratia liquefaciens predominate (with 26.1%, 13.0% and 8.70% respectively for each of the last 4 species).

3.1.6 Frequency of enterobacteria species isolated from irrigation water

Enterobacteriaceae species isolated from irrigation water and their frequencies are presented in Table 6. A total of eight (08) species belonging to seven (7) genera were identified from irrigation water. *Escherichia coli 1,*

Salmonella spp and *Serratia liquefaciens* were isolated with a frequency of 21.4% (three (3) species each). Of all the species, only one was identified at site 2 (*Salmonella spp*).

3.1.7 Frequency of species isolated from lettuce samples by site

Fig. 4 shows the species identified from lettuce samples according to production site. A total of seven (7) species belonging to five (5) genera were identified in the lettuce samples. Of these, six (6) were identified at site 2 (11.1% each) and three (3) at site 3 (11.1% for *Salmonella ssp arizonae* and 22.2% *Salmonella spp*). No enterobacteria species were identified at site1.

3.2 Discussion

This study assessed the impact of irrigation water on the bacteriological quality of lettuce produced in the market-garden zone of the urban community of Niamey. Three (3) market garden sites in the urban community of Niamey were studied. The production sites concerned are: *Harobanda* (site1), *Gamkallé* (site2) and *Gounti Yéna* (site3). Total and faecal coliforms, faecal streptococci, enterobacteria and *Escherichia coli* were tested in samples of irrigation water and lettuce. The study continues with the characterization of some enterobacteria species isolated from water and lettuce.

The loads of all the indicators tested in the irrigation water samples exceeded the values set in the WHO guide (10^5 CFU/100 ml) for non-restrictive irrigation [14]. A similar finding was made in Senegal by (Ndiaye et al.) [8]. Total coliform loads ranged from $1.47±4.33.10^6$ to $3.65±7.88.10^6$ CFU/100ml of irrigation water. In Côte d'Ivoire Coulibaly-Kalpy et al., reported results slightly higher than these [15]. The values obtained by these authors range from $2.19.10^7$ CFU/100ml to $2.74.10^7$ CFU/100ml. On the other hand, these results are slightly higher than those obtained in Burkina Faso by Koussakou [7]. Fecal coliform loads were in order of $1.41±3.04.10^6$ CFU/100ml of irrigation water. These results corroborate those reported by *Djegbe* et al. [4]. Faecal streptococci loads ranged from $2.18±5.25.10^4$ to $1.19±2.03.10^5$ CFU/100ml. Koffi-Nevry et al. found almost similar results ($2.1.10^4 ± 2.7.10^3$ CFU/100ml) [2]. Heavy contamination of Gourou basin water [15].

The irrigation water of Site1 is more loaded with total and faecal coliforms (respectively $3.65±7.88.10^6$ and $1.41±3.04.10^6$), and site3 with enterobacteria and *Escherichia coli* (respectively $1.61±2.37.10^8$ and $8.22±11.86.10^5$ CFU/100ml). Site2, on the other hand, was more loaded with faecal streptococci ($1.19±2.03.10^5$ CFU/100 ml). This higher level of contamination in site3 can be explained by the nature of the water used for watering, which is wastewater from the city. This water is discharged directly into the lowlands and other receiving areas, notably the River Niger, without any prior treatment. Before flowing into the river, this water passes through a number of market garden sites, notably via watercourses, the most important of which in Niamey is the *Gounti Yena* outfall [9]. It should be noted that the loads of these indicators are higher than the values set (10^3 CFU/ 100 ml) by WHO guidelines for the safe discharge of wastewater into the environment [16]. A similar finding has been made by other authors in Africa by (Aboulouafa; El Addouli; Adjahouinou; El Ouali; Coulibaly) [15,17,18,19,20].

In fact, the microbiological quality of lettuce samples, regardless of the production site considered, is not fit for human consumption. All loads are well above the values recommended by the French Standardization Association [21,22]. This level of contamination could be explained not only by poor production practices, but also by the use of wastewater as irrigation water. Indeed, the level of TAMF contamination varies from $3.03±1.24.10^6$ CFU/g (site3) to $5.31±1.27.10^7$ CFU/g of lettuce (site2). The variation in load is significant between these sites (P = .000). The higher level of contamination in lettuce samples from site2 could be explained by the proximity of this site to industrial zones, which discharge their wastewater directly into the lowland through this site which contribute to water pollution. Thus, Djibo H. reported the use of poor condition water at this site [10]. These results are similar to those obtained by Wognin et al. in Côte d'Ivoire [22]. Poor exposure and storage conditions for vegetables (dust, insects, rodents, flies, etc.) are also responsible for the high levels of microorganisms on these plant products [22,23,24].

The highest level of contamination in total coliforms is around $5.33±8.47.10^6$ CFU/g and in faecal coliforms around $2.14±1.19.10^6$ CFU/g obtained for site1. There was no significant difference between these sites and their contamination levels (P>.05). Lettuce samples are highly contaminated with coliforms (total and faecal). The latter are good indicators of hygiene.

This can be explained by the use of organic fertilizers (manure), which are made up of animal faeces. Some market gardeners use watering cans to water their vegetables, and in order to collect water, they step into the pool, sometimes barefoot. In Côte d'Ivoire, for example, [25] reports that the use of excreta contributes to the contamination of lettuce produced on Abidjan's market garden sites. In addition, the presence of animals such as reptiles, migratory birds and domestic pets (dogs, etc.) can contribute to the faecal contamination of production sites. The excreta of these animals are carried by run-off water during the rainy season to sources of water for irrigating vegetables [26,27]. This may explain the presence of these indicators in lettuce samples. According to [28], irrigation techniques play an important role in the contamination of lettuce. The sprinkling of polluted water on lettuce with a watering can thought to be the root cause of *Salmonella* contamination. A similar finding has been made by other authors [22, 25]. The presence of enteric bacteria in lettuces suggests a lack of good hygiene practices, and faecal contamination could be due to the inappropriate processing undergone by these raw edible vegetables. Indeed, enterobacteria are normal hosts of the digestive tract of humans and animals, capable of proliferating in abundance in the environment (soil and water) and thus participating in the major cycles of organic matter degradation [27,29]. These are indicators of faecal contamination that provide a more complete picture of potentially pathogenic germs [22,27,28,30].

The highest level of faecal Streptococcus contamination is observed at site2 ($6.57\pm9.40.10^5$ CFU/g). A high level of faecal streptococcus contamination has been reported by other authors in other countries Obiri-Danso; Amoah; Wognin) [25,26,31]. Also noteworthy is the high *E. coli* load of $1.95\pm1.17.10^5$ CFU/g at site3. This can be explained by the proximity of the *Gounti yéna* valley (site3) to metallurgical activity centers, main roads and uncontrolled dumps, but also by the use of untreated raw wastewater for irrigation of market garden crops [32]. Indeed, these results corroborate those obtained in Nigeria [33,34], Egypt [35], Ghana [36] and Côte d'Ivoire [37].

On the other hand, faecal contamination of irrigation water and lettuce samples is almost entirely of human origin at almost all sites. Only faecal contamination of lettuce samples from site 2 was of mixed origin, predominantly human. This situation may be due to the state of the market garden environment in the urban community of Niamey. The results of the survey carried out on these sites showed a lack of sanitary facilities both in the fields and in the vicinity of the fields. Some 85% to 90% of fields have no latrines, and 80% to 90% have none in their immediate vicinity. The lack of latrines in or near fields leads market gardeners to defecate in the open air in or near fields [38]. This defecation in the vicinity of fields, constitutes another additional source of contamination for vegetables [37]. In addition, sites2 and 3 receive wastewater, which is most often used for watering (generally on site 3). A similar situation has been observed in Côte d'Ivoire [2]. They reported that the source of faecal contamination of well water at site 2 was its location on the edge of the Ebrié Lagoon, the main receptacle for wastewater from the city of Abidjan. It is also a place of defecation for local populations [2,39,40].

However, a wide diversity of *Enterobacteriaceae* species has been isolated from irrigation water and lettuce samples. This diversity is represented by twenty-three (23) species, belonging to nine (9) genera of *Enterobacteriaceae*. It generally consists of *Salmonella spp, Escherichia coli1, Citrobacter freundii, Enterobacter cloacae, Enterobacter sakazakii, Serratia liquefaciens*. The presence of *Enterobacteriaceae* in lettuce samples could be due to the precarious hygienic conditions under which lettuce is grown, as previously reported by Koffi-Nevry et al.; Amoah et al. [2,41]. The presence of these germs indicates the presence of enteropathogenic bacteria in lettuce. Thus, Maïwore et al. report the presence of enterobacteria in irrigation water and lettuce samples [42]. Many of these enteric bacteria are incriminated in a growing number of collective food poisoning outbreaks [42,43, 44]. *Salmonella enterica* serovars are among the pathogenic bacteria causing serious health problems in humans [2,45].

4. CONCLUSION

The aim of this study was to assess the impact of irrigation water on the bacteriological quality of lettuce produced in the market-garden areas of the urban community of Niamey, and to identify a number of enterobacteria species. The results show that irrigation water has a negative impact on the bacteriological quality of lettuce produced in these areas. Microbiological analysis results

show that all lettuce contamination indicators are above French standards for fresh vegetables. In addition to the numerous species of *Enterobacteria* isolated from the irrigation water, these were also found in the lettuce samples. The worrying potential for the spread of food-borne infectious diseases therefore exists in the consumption of lettuce. If consumers fail to observe hygiene rules, eating raw lettuce may increase the risk of carrying infectious diseases such as cholera, typhoid fever, gastroenteritis, etc. It would be important to establish the antibiotic resistance profile of these *Enterobacteria* in order to better control infectious diseases.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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