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Evaluation of the microbiological quality of the leaves of *Solanum macrocarpum L.* cultivated with the chicken's droppings and water of marsh in Cotonou (Republic of Benin)

Victorien T. Dougnon[1,3,*], Honoré S. Bankolé[3], Patrick A. Edorh[1,2], Jacques T. Dougnon[4], Modeste Gouissi[1], Armelle Hounkpatin[1], Sabine Montcho[1], Hervé Azonhè[5], Jean-Robert Klotoé[3], Michel Boko[1]

[1]*Interfaculty Center of Formation and Research in Environment for the Sustainable Development, Laboratory of Toxicology and Environmental Health, University of Abomey-Calavi (UAC), 01 BP 1463 Cotonou, Benin*

[2]*Faculty of Science and Technology, Department of Biochemistry and Cellular Biology, University of Abomey-Calavi (UAC), 01 BP 526 Cotonou, Benin*

[3]*Polytechnic School of Abomey-Calavi, Department of Human Biology, University of Abomey-Calavi, 01 BP 2009 Cotonou, Benin*

[4]*Polytechnic School of Abomey-Calavi, Department of Animal Production and Health, Research Laboratory in Applied Biology , University of Abomey-Calavi, 01 BP 2009 Cotonou, Benin*

[5]*Faculty of Arts and Social Sciences, Department of Geography, University of Abomey-Calavi, Benin*

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Abstract

This study has evaluated the microbiological quality of the leaves of *Solanum macrocarpum L.* cultivated with chicken's droppings and water of watering. A pilot site at Glo and market-gardening sites of Houeyiho, Fidjrosse and Agongbomey were used as sites of study. The salmonellas and fecal coliforms whose *Escherichia coli* were required in the environment of culture of *Solanum macrocarpum L.* Eight samples resulting respectively from water of watering; ground and leaves were analyzed. No salmonella was detected on these samples. Water of watering of the pilot site of Glo is not contaminated by *Escherichia coli* compared to those of the sites of Houeyiho ($1.8.10^4 \pm 28.42$ UFC/100 ml) ; Fidjrosse ($0.95.10^4 \pm 70.10$ UFC/100 ml) ; Agongbomey (5545 ± 77.53 UFC/100 ml) ($p<0.05$). The leaves of *Solanum macrocarpum L.* were contaminated by *Escherichia coli* with different degrees according to the site: Glo ($35.65.10^4 \pm 457.08$ UFC/g); Houeyiho ($0,95.10^3 \pm 70.71$ UFC/g); Fidjrosse ($1.10^3 \pm 0$ UFC/g) ; Agongbomey ($0.9.10^3 \pm 0$ UFC/g) ($p<0.05$). In addition, *Escherichia coli* is strongly developed on the leaves of *Solanum macrocarpum L.* from the pilot site of Glo with a neutral pH (7.305 ± 0.064) compared to the other sites: 6.550 at Houeyiho; 6.480 at Fidjrosse and 6.445 at Agongbomey ($p<0.05$). The study has shown that the leaves of *Solanum macrocarpum L.* are contaminated by the enterobacteria and their consumption may expose the populations to some risks of enteric diseases.

*Corresponding Author: Dougnon Tamègnon Victorien ✉ victorien88@hotmail.com

Introduction

Urban agriculture became throughout the whole world and specifically in the developing countries, one of the activities necessary to ensure the food safety of the townsmen and to get financial resources with the unemployed persons of the cities. Mougeot (2006) reported that between 2015 and 2020, more half of the world population will live in urban or perish-urban zone. The role of urban agriculture is fully justified when taking into consideration this demographic explosion on a worldwide scale. Its contribution with the food production on a world level which was at 15 % in 1993 can have exceeded 33 % as from the year 2005 (Niang, 1996).

Smit *et al.* (1996) revealed that this activity is practiced in at least 90 towns of 31 countries of the Southeast Asia, of the Middle East, of Europa, of Sub-Saharan Africa, Antilles and North, Center, South America. In addition, 800 million people practice this activity on a worldwide scale (Koc *et al.*, 2006). The problems of urban agriculture and in West Africa were the subject of several publications which show well its importance in cities (Akinbamijo *et al.*, 2002). Urban agriculture is thus socially very significant. The activities of production, transformation and marketing offer employment opportunities for a significant mass of the urban population in situation of chronic unemployment and the rural ones in seasonal migration.

Benin, like many African countries, experienced these last years a development of urban agriculture. In Cotonou, the market-gardening production and more precisely that of the vegetable-leaves is a reality impossible to circumvent. It contributes to the provisioning of the markets of the city in food products. The modernization and the intensification of the systems of production induce a stronger use of manures and comparable. The producers answer this request by the recycling of various types of waste (Akinbamijo *et al.*, 2002).

Thus, inside the city, the population developed a local strategy of market-gardening production which resulted in the use of the chicken's droppings and water of the marshes to amend and sprinkle the market-gardening products. These poultry's droppings, as the many waste used in agriculture, contains organic matter and biogenic salts which constitute a contribution interesting for the grounds of culture (Legret *et al.*, 1988). But the presence of pathogenic in the droppings represents a major constraint with its use in agriculture. Indeed, in the event of use of animals waste on the market gardenings (whose leaves are consumed), some pathogenic micro-organisms could pass in the excreta of the people infected to find itself in water of the marshes being used for watering of the cultures. The enterobacteria could be contained in these droppings and contaminate the plants which they are used to amend (Métras, 2003). This situation could induce medical problems with the fresh vegetable consumers.

Therefore, the conditions of production of the vegetable-leaves on the market-gardening grounds at Cotonou do not guarantee their sanitary quality. As remarked that more and more market-gardeners prefer to use the droppings of chickens and water of marsh to amend and sprinkle their cultures, the study evaluated that practice's impact on the microbiological quality of *Solanum macrocarpum L.*, a vegetable highly appreciated at Cotonou (MVAD, 2003). It is then about a contribution in favor of food safety in Republic of Benin.

Materials and methods

Localization of the sites of study

Our survey took in account a witness site at Glo (6° 56 ' Northern latitude and 2°30' Eastern longitude) and market-gardening sites of Houeyiho (6° 21' 20" Northern latitude and 2° 21' 35" Eastern longitude), of Fidjrosse (6° 22' Northern latitude and 2° 24' Eastern longitude); of Agongbomey (6° 21' Northern latitude and 2° 24' 45" Eastern longitude) (Fig. 1).

Fig. 1. Localization of sites included in the survey.

Sampling

It has been used among others of the white sachets labeled to collect samples, of sterile gloves for withdrawals, an icebox for the transportation of samples. For exams to the laboratory, we used surroundings of culture as Rapid *E.coli*, some serological pipettes, drying ovens, the crushers.

On each site, we carried out in two different market-gardeners, some samples of ground; water of watering; leaves of *Solanum macrocarpum L*. 500 grams of fresh leaves were collected on the vegetable seedlings then introduced into hermetically tied plastic sachets. 500 grams of ground were taken with a depth ranging between 0 and 15 centimeters and put in plastic sachets. The samples of the water of watering were directly taken in sterile sachets at a rate of 0.5 liter. All the samples were transported in a refrigerator towards the laboratory in a one hour interval after the taking away where they were preserved at once at a temperature of 4° C. The microbiological analyses were carried out in the 24 hours following the test sample selections. The research for pH was made by the two weeks.

Methods of analysis

Microbiological analyses

The microbiological analyses were carried out in the Hygiene Section of Water and Food at the Service of the Biomedical Analyses Laboratories (Benin). The purpose of the microbiological analyses are to highlight the presence or not of fecal coliforms, specifically *Escherichia coli (E.coli)* and salmonellas in the samples of water, ground and leaves of *Solanum macrocarpum L*. The fecal coliforms and especially *Escherichia coli* were required because they are pilot presence of pathogenic microorganisms.

Research of fecal coliforms and *E.coli* on the leaves of *Solanum macrocarpum L.*

After having weighed 25 grams of the samples, we add Peptoned Plug Water (EPT) until the limit of 250 grams. That's what we call pre-enrichment. Then we carry out successive dilutions from 10^{-2} to 10^{-4} starting from pre-enrichment before sow by incorporation 1 ml of the dilutions of 10^{-3} and 10^{-4} on the medium Rapid *E.coli*. It has been incubated with 44°C during 24 hours ± 2 hours. All the colonies *of E.coli* appeared as violet and those of the fecal coliforms were blue.

Research of fecal coliforms and *E.coli* on the samples of water of marsh

It has been taken 1 ml of the sample and carried out successive dilutions with 10^{-1} and 10^{-2}. Then it has been taken 1ml of dilution with 10^{-2} and sow by incorporation on the medium Rapid *E.coli*. After incubating with 44°C during 24 hours ± 2 hours, the colonies *of E.coli* appeared as violets and those of the fecal coliforms were blue.

Research of fecal coliforms and *E.coli* on the samples of water of tap

We had to filter the sample and to deposit the filtrated membrane on the medium Rapid *E.coli* and incubate it with 44°C during 24 hours ± 2. The colonies *of E.coli* appeared as violets and those of the fecal coliforms were blue.

Research of salmonellas on the samples of leaves, grounds and water

Either it is a sample of ground, water or leaf, the procedure is the same one. Indeed, it is necessary to weigh 25 grams of the sample, to supplement to 250 grams with Peptoned Plug Water (EPT), to incubate with 37°C during 18 hours, to sow 0,1 ml of this pre-

enrichment in 10 ml of medium Rappaport Vasiliadis and 2 ml of the same pre-enrichment in 20 ml of medium Kaufman, to incubate with 37°C during 24 hours ± 4, to insulate the colonies resulting from this enrichment on mediums Xylose Lysine Decarboxylase (XLD) and Hektoen, to incubate with 37°C during 24 hours ± 4, to select the characteristic colonies of salmonellas, to carry out the test of discrimination by sowing the urea medium and waiting for two hours. The salmonellas don't have urease.

Physicochemical analyses

The physicochemical analyses were carried out in the Analysis laboratory of the Ground of the Agronomic Faculty of Science, in Republic of the Benin. It was about the determination of the pH. The measurement of the pH of the ground and the droppings informs about their degree of acidity or of alkalinity. With this intention, and in accordance with the standard of reference NF ISO 10390: 1994, the samples were filtered with a sieve of mesh 0,2 mm. Twenty grams of each sample were weighed in bechers and we add it 50 ml of distilled water which and agitated during 15 minutes using an agitator. The solution obtained was left at rest during thirty minutes. The reading of the pH was made using a pH-meter of straw mattress CG 825. For the water samples, the determination was done directly using the pH-meter of straw mattress CG 825 of manner in conformity with the standard of reference NF T 90-008: 2001.

Statistical analyses

It was calculated the averages and the standard deviations. Multiple comparisons consisting in comparing the averages using the test of Student p (T>t) = 0.05 were made. The softwares used are Microsoft Excel 2010 and XL Stat 2011.

Results

Presence of fecal coliforms in the culture's environment of Solanum macrocarpum L.

Water of watering

Water of watering of the witness site of Glo is not contaminated by fecal coliforms (0 UFC/100ml) whereas those of Houeyiho, Fidjrosse and Agongbomey are it respectively with $14.5.10^4 \pm 70.06$ UFC/100 ml; $8.5.10^4 \pm 106.01$ UFC/100 ml and $1.05.10^5 \pm 70.06$ UFC/100 ml. *Escherichia coli* misses on the witness site of Glo contrary with Houeyiho ($1.8.10^4 \pm 28.42$ UFC/100 ml), with Fidjrosse ($0.95.10^4 \pm 70.10$ UFC/100 ml) and with Agongbomey (5545 ± 77.53 UFC/100 ml) (Fig. 2). These differences prove to be significant since it is about total absence on the site of Glo and presence of coliforms on the other sites ($p < 0.05$).

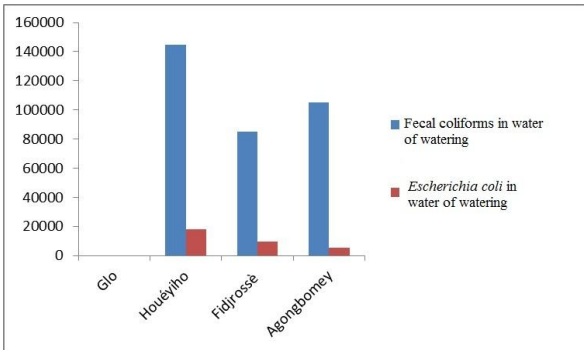

Fig. 2. Presence of fecal coliforms in water of watering.

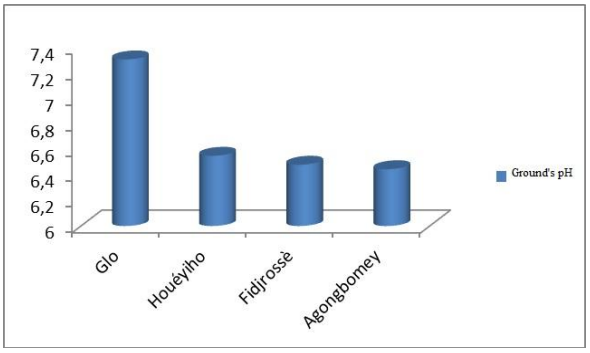

Fig.3. Variation of the grounds pH according to site.

Vegetable leaves

The leaves of *Solanum macrocarpum L.* of the witness site of Glo are contaminated by fecal coliforms

($6.05.10^5 \pm 219.10$ UFC/g) whereas those of Houeyiho, Fidjrosse and Agongbomey are it respectively at a rate of $0.95.10^3 \pm 70.71$ UFC/g; $3.5.10^3 \pm 21.32$ UFC/g and $182.10^3 \pm 25.01$ UFC/g (Table 1).

Table 1. Presence of fecal coliforms in the leaves of *Solanum macrocarpum L.* according to sites.

Sites	G	H	F	A
Samples of *S.macrocarpum* leaves	$4.5.10^5$	1.10^3	5.10^3	4.10^3
	$7.6.10^5$	$0.9.10^3$	2.10^3	$3.6.10^5$
Average	**$6.05.10^5$**	**$0.95.10^3$**	**$3,5.10^3$**	**182.10^3**
±Standard deviation	219.10a	70.71a	21.32a	25.01a

G, H, F and A are respectively the initials of Glo, Houeyiho, Fidjrosse and Agongbomey. The averages carrying the same letters are not significantly different with the threshold from significance $\alpha = 0.05$.

Table 2. Presence of *Escherichia coli* in the leaves of *Solanum macrocarpum L.* according to sites.

	Escherichia coli (UFC/100 ml)			
Sites	G	H	F	A
Samples of leaves	$3.3.10^4$	1.10^3	1.10^3	$0.9.10^3$
	$6.8.10^5$	$0.9.10^3$	1.10^3	$0.9.10^3$
Average	**$35.65.10^4$**a	**$0.95.10^3$**a	**1.10^3**b	**$0.9.10^3$**b
± Standard deviation	457,08	70,71	0	0

G, H, F and A are respectively the initials of Glo, Houeyiho, Fidjrosse and Agongbomey. The averages carrying the same letters are not significantly different with the threshold from significance $\alpha = 0.05$.

The contamination of the leaves of *Solanum macrocarpum L.* by *Escherichia coli* is of $35.65.10^4 \pm 457.08$ UFC/g on the witness site of Glo whereas it is respectively of $0.95.10^3 \pm 70.71$ UFC/g; $1.10^3 \pm 0$ UFC/g and $0.9.10^3 \pm 0$ UFC/g on the sites of Houeyiho, Fidjrosse and Agongbomey. The statistical analyses revealed that the difference between the averages of Glo and Houeyiho is not significant with the threshold of significance $\alpha = 0.05$. On the other hand, it is it with the same threshold between the averages of Glo and Fidjrosse then Glo and Agongbomey (Table 2).

Contamination by the salmonellas of the environment of culture of Solanum macrocarpum L.

The salmonellas were not detected in the environment of culture of *Solanum macrocarpum L.* Indeed, it was observed 0 UFC/100 ml in all the samples of grounds, of water of watering and the leaves of *Solanum macrocarpum L.* resulting from the sites of study.

Variation of the grounds pH

With regard to the pH, the averages obtained are as follows: Glo (7.305 ± 0.064); Houeyiho (6.550 ± 0.014); Agongbomey (6.445 ± 0.120) ($p < 0.05$); Fidjrosse (6.480 ± 0.311) (Fig. 3).

Discussion

Presence of fecal coliforms in the environment of culture of Solanum macrocarpum L.

It was required the fecal coliforms whose *Escherichia coli* in water of watering and the leaves of *Solanum macrocarpum L.* because these bacteria are produced in the intestine of the animals. The ground also constitutes according to Pilet (1981) their natural habitat. But their presence in vegetables is abnormal and accounts for the sanitary quality of this food. The presence of fecal coliforms brought by our results can be an indication of the presence of micro-organisms like one notified Zmirou *et al.* (1987). Moreover, the presence of *Escherichia coli* confirms the effective presence of the fecal coliforms. Also, Habtesealami *et al.* (2010) stress that *Escherichia coli* develops close to the roots of the plants and can contaminate the culture of the young growths. It can live during weeks around the roots of the plants and be transferred towards the edible parts.

Generally, the water of watering used on the market-gardening sites is contaminated by the fecal coliforms except for the witness site of Glo. This difference could be explained by the system of watering used. Indeed, on the site of Glo, the market-gardeners use the tap of the National Company of Water of Benin whereas on the other sites, the ground systems of marsh dug

(Houeyiho and Agongbomey) and of drilling (Fidjrosse) are adopted. Water of watering of Houeyiho is polluted in fecal coliforms than those of Agongbomey and Fidjrosse. The results of this study are in agreement with those of Akodogbo (2005) which stress that the water of drilling is polluted than that of the wells (grounds dug of marsh). This author remarked in addition that the majority of the well rivers and drilling of Cotonou are invaded by the coliforms.

It is the same for *Escherichia coli* absent on the site of Glo but present on the other market-gardening sites. That confirms well this water of watering knew a recent fecal contamination. This is possible insofar as the majority of the aforesaid sites have a considerable insalubrity (human excrements and other waste). The depth of the wells is also a factor very significant because more water infiltrates, more it gets rid of its impurities; however the majority of the sources of watering of the market-gardening sites are located in hollows and are not deep. None water of watering, put besides those of the site of Glo meets the standards varying between 100 and 200 UFC/100 ml for the coliforms and *Escherichia coli*, proposed by Santé Canada (1991).

The leaves of *Solanum macrocarpum L.*, indeed, are contaminated by the fecal coliforms on all the sites of study. This contamination would be certainly due either to the microbiological quality of the water of watering used on these sites, or with the chicken dropping being used for the amendment. The presence of coliforms on the witness site of Glo, amended by the chicken dropping and where water of watering is those of tap could accuse the droppings of chickens like principal source of contamination. Indeed, the vegetable leaves resulting from the pilot site showed a strong rate of contamination by the fecal coliforms because the droppings used did not know any composting before the amendment of the seedlings. It confirms the results of Florin *et al.* (2009) which affirmed that the fecal rate of coliforms in vegetables decreases considerably if the composting of the droppings used to amend them is adequate. In addition, the leaves coming from all the sites present values higher than those imposed by the provincial Committee of Canada on the standardization and the interpretation of the microbiological criteria in the food which is of $1,0.10^2$ UFC/g (Santé Canada, 1991).

Presence of the salmonellas in the culture's environment of Solanum macrocarpum L.

Generally, no salmonella was found in the environment of culture of *Solanum macrocarpum*. The absence of these germs in the water of watering, the ground and the leaves of *Solanum macrocarpum L.* could mean there's no contamination of the droppings used for the amendment of this culture. Similar results were reported by Araba *et al.* (2000) which sought the salmonellas in nine samples of chicken droppings. Indeed, the enterobacteria are naturally present in the small intestine of the animals; their presence in a medium always has a fecal origin (Pilet, 1981).

However, Rose *et al.* (1999) identified factors of risk relating to the contamination at 70% by Salmonella in the chicken droppings. This difference could be related to the taking away of droppings coldly collected by these authors on the level of the hen houses contrary to the method of collection adopted during the present study. Indeed, the chicken droppings were obtained after transport and storage during several weeks before their use. Storage aims in particular to cleanse the manure, by the heating which produced there. Kwak *et al.* (2005) have for this purpose, evaluated the effects of an in core treatment deep of the chicken litter. They thus suggested that the correctly piled up poultry litter, with or without ventilation, ensures the elimination of the pathogenic enterobacteria in 8 days. Moreover, Araba *et al.* (2000) bound the absence of salmonellas in their study with storage carried out at the level of the farms preceding the microbiological examinations.

However, it could be that the droppings used for the present study results from farms which were free of salmonella's contamination.

The pH and Escherichia coli

Within the framework of this study, the ground of the witness site of Glo has a pH of 7,305, very near to neutrality. It is noticed that *Escherichia coli* strongly developed on the leaves resulting from this site contrary to the other sites whose pH is basic. Enterobacteria such that *Escherichia coli* develop with an optimum pH near to the neutrality which varies between 7, 2 and 7, 4 like remarked by Delahaye (2009). In addition, the alkalinity of the grounds of Houeyiho and Agongbomey compared to that of the other sites could be explained by the seniority of the site; exploitation of the sites of Glo and Fidjrosse being more recent.

Conclusion

The results of this study indicate that no risk of contamination of the leaves of *Solanum macrocarpum L.* by the salmonellas was detected on the sites of production. On the other hand, some risks of contamination of water of watering by the fecal coliforms whose *Escherichia coli* were identified on the market-gardening sites except that of Glo on the one hand and some risks of contamination of the leaves of *Solanum macrocarpum L.* by the fecal coliforms and *Escherichia coli* were detected on all the studied sites on the other hand. The presence of pathogenic micro-organisms on vegetables of the various sites can be at the origin of food poisonings; this forecasts new prospects for research in order to cleanse vegetables consumed by the populations of Cotonou.

References

Akinbamijo OO., Fall ST., Smith OB. 2002. Advances in crop-livestock integration in West African cities. Dakar: ITC/ISRA/CRDI, p. 213.

Akodogbo H. 2005. Contribution à l'amélioration de la qualité de l'eau à usage domestique dans le 5ème arrondissement de la commune de Porto-Novo au Bénin, Mémoire de Maîtrise Professionnelle en Environnement et Santé, FLASH/UAC, p. 65.

Araba A, Hachana Y. 2000. Caractérisation chimique et microbiologique des fientes de volaille en vue de leur utilisation en alimentation des ruminants, Actes Institut Agronomique et Vétérinaire du Maroc, **20(2),** 83-88

Chidikofan G. 2010. Contribution à l'amélioration de la qualité des cultures maraîchères du site de Houéyiho à Cotonou au BENIN : cas de la laitue (*Lactuca sativa L.*), Mémoire de Master, 2IE Ouagadougou, p. 56.

Delahaye A. 2009. La famille des entérobactéries, disponible sur http://www.arnobio.com, Consulté le 18/07/2011

Florin NH., Maddocks AR., Wood S., Harris AT. 2009. High-temperature thermal destruction of poultry derived wastes for energy recovery in Australia. Waste Management **29(4),** 1399-1408

Habteselassie-Mussie Y, Bischoff M, Applegate B, Reuhs B, Turco Ronald F. 2010. Understanding the role of Agricultural Practices in the Potential Colonization and Contamination by Escherichia coli in the rhizospheres of Fresh Produce. Journal of Food Protection **73,** 2001-2009.

Koc M, Macrae R, Mougeot JAL, Welsh J. 2000. Armer les villes contre la faim: systèmes alimentaires urbains durables. CRDI p. 243.

Kwak WS, Huh JW, Maccaskey TA. 2005. Effect of processing time on enteric bacteria survival and on temperature and chemical composition of broiler

poultry litter processed by two methods. Bioresource Technology **96(14),** 1529-1536.

Legret M, Divet L, Juste C. 1988. Migration et spéciation des métaux lourds dans un sol soumis à des épandages boues de station d'épuration à très forte charge en Cd et Ni. Wat. Res. **22(8),** 953-959.

Métras R. 2003. Utilisations et dangers sanitaires microbiologiques liés aux effluents d'élevage, Thèse de doctorat unique, Université Claude Bernard p. 150.

Mougeot J. 2006. Focus: cultiver de meilleures villes. Agriculture urbaine et développement durable, CRDI, 136p.

MVAD. 2003. Les matières organiques produites à la Réunion, chapitre 5, p. 63.

Niang S. 1996. Utilisation des eaux usées domestiques en maraîchage périurbain à Dakar (Sénégal). Sécheresse **3,** 217-23

Pilet C, Bourdon L, Toma B, Marchal N, Balbastre C. 1981. Bactériologie médicale et vétérinaire, systématique bactérienne, Doin éditeurs-Paris ; 2ème édition, 2ème tirage, p. 431.

Rose N, Beaudreau F, Drouin P, Toux JY, Colin RP. 1999. Risk factors for Salmonella enterica subsp. enterica contamination in French broiler-chicken flocks at the end of the rearing period. Preventive Veterinary Medicine **39(4),** 265-277.

Santé C. 1991. La qualité bactériologique. Document de support aux « recommandations pour la qualité de l'eau potable au Canada ». Accessible à : www.hc-sc.gc.ca/ehp/dhm/dpc_eau_qualite/eauguide.htm

Smit J, Ratta A, Bernstein J. 1996. Urban agriculture: an opportunity for environmentally sustainable development in sub-Saharan Africa, Washington (DC, E.-U.), Banque mondiale, Environmentally Sustainable Division, African Technical Department. Post-UNCED Series, Building Blocks for Africa 2025, Paper No. 11.

Zmirou D, Ferley JP, Collin JF, Charrel M, Berlin J. 1987. A follow-up study of gastro-intestinal diseases related to bacteriologically substandard drinking water. American Journal of Public Health **77,** 582-584.