Sjournals
Scientific Journals
Scientific Journal of Microbiology (2013) 2(9) 166-173
ISSN 2322-2948
doi: 10.14196/sjm.v2i9.950

Contents lists available at Sjournals
Scientific Journal of Microbiology
Journal homepage: www.Sjournals.com

Original article

Evaluation of the effectiveness of bleach on microbial population of lettuce

S. Traoré[a,]*, F. Samaké[a], A.H. Babana[a], D. Traoré[a], Y. Sanogo[b], K. Maiga[a]

[a]*Research Laboratory of Microbiology and Microbial Biotechnology, Faculty of Science and Technology, University of Science, Technique and Technology Bamako, BPE. Bamako-Mali 3206.*

[b]*Laboratory of Applied Molecular Biology, Faculty of Science and Technology, University of Science, Technique and Technology Bamako, BPE. Bamako-Mali 3206.*

*Corresponding author; Research Laboratory of Microbiology and Microbial Biotechnology, Faculty of Science and Technology, University of Science, Technique and Technology Bamako, BPE. Bamako-Mali 3206.

ARTICLE INFO

Article history:
Received 09 September 2013
Accepted 18 September 2013
Available online 29 September 2013

Keywords:
Lettuce
Bleach
Efficiency
Disinfection
Concentration of microbial population

ABSTRACT

The effectiveness of bleach on the microbial population of lettuce was evaluated. Microorganisms on lettuce before and after washing with bleach were searched and counted and a new dose for cleaning to eliminate a high concentration of bacteria on the lettuce has been proposed to minimize the health risk linked to its consumption. The lettuce samples were collected from farms municipalities V and VI of the District of Bamako. Ten lettuces were collected from different plots and mixed to form a composite sample. The concentration of microbial population was determined by finding and counting the total microflora in 100g of lettuce composite sample before and after washing with bleach different chlorine concentrations for different contact times. Lettuces grown in Bamako are heavily contaminated with microbial population average between 25 to 1363UFC / g of lettuce. The application of the recommended dose of chlorine (133.10-5mg / l) in Mali for the disinfection of fruits and vegetables for 15, 30 and 60 min resulted in a drawdown respectively 94%, 97% and 99% of the initial microbial population lettuce. The recommended dose and double dose of this leading to high rates of drawdown of 97% and 99% of the initial microbial population of lettuce. 1% of the concentration of microbial population remains on the treated leaves. Dose which totally

eliminates the microflora is 2.6 mg / l of chlorine applied for fifteen minutes. The new dose we can offer is the application of 2.6 mg / l of active chlorine.

© 2013 Sjournals. All rights reserved.

1. Introduction

Lettuce (BOTANICA, 2012) is an annual plant of the Asteraceae family. Cultivated for its tender leaves eaten as a vegetable, usually raw in salads, lettuce is rich in water (95%), minerals, vitamins (Nadou, 2006) and is also important in terms of health. The annual consumption of vegetables is 32.94 kg per capita in the world (Unctad, 2005). Among the vegetables eaten raw in Mali, lettuce is one of the most cultivated with a proportion of 77.7% of the acreage for leafy vegetables (Dra, 2010), a production of 20,325 tons, or 30.38% of the all vegetables with an area of 1350 hectares or 37.12% of the total acreage for gardening (Dra, 2011). It is one of the most consumed raw vegetables with an average annual consumption of 2 kg per person (Dnsi, 2003). The short duration of the production cycle (30-40 days) and permanent income it provides, make the lettuce is grown by the majority of vegetable growers.

Despite the importance and the high consumption of lettuce, it has some limitations in its production including the limited availability of cultivable land in urban and peri-urban Bamako. Strong demand involves the use of water of questionable quality and organic manures unconventional production.

In West Africa, many studies have shown high levels of contamination by pathogens in irrigation water and vegetables to both the firm and the market (Cisse, 1997; Olayemi, 1997; Armar- Klemesu et al., 1998; Niang., 1999; Faruqui et Al., 2004; Amoah et Al., 2005, 2006), which far exceeds the standards of the ICMSF (ICMSF 1974) such as Salmonella thyphirium and Escherichia coli. The quality of the lettuce depends on the quality of the soil and of the quality of water used for the irrigation. Indeed, it is established that the use of wastewater and waste in urban gardening is a source of microbiological contamination (Hamadoun A., 1995). Samake et al. (2011) found a relatively high number of Salmonella and coliforms in water used by gardeners and horticultural products in the periurban area of Bamako. The use of marginal waters in industrialized countries is also a source of contamination (Altekruse et al., 1997; Blumenthal et al., 2000). The health risks associated with consumption of raw products without prior disinfection are high, particularly those of lettuce. These risks are either due to its culture in inappropriate locations in gardening, the hygienic conditions of the gardener or the use of inputs such as organic manure and use of irrigation water of poor quality. Cleaning methods practiced by consumers can not often be effective. Lettuce is often contaminated with pathogenic bacteria or parasites from animals and the environment as a result of production practices (Taura and Habibu, 2009). Consumption of contaminated lettuce is mostly in charge of food poisoning, gastroenteritis and food poisoning due to certain bacterial genera that easily fasten at its leaves (Ethelberg et al., 2010).

To minimize the health risk associated with consumption of lettuce, different control methods such as other bleach, potassium permanganate, salt, etc ... are used to eliminate pathogenic bacteria or reduce the bacterial population. Amoah et al. (2007) have shown that the most commonly used disinfectants to wash vegetables in restaurants were bleach (55%) and potassium permanganate (31%), followed by the salt / lemon or soap (7% each) in the household, the common method of disinfection of vegetables was the use of bleach (50%), followed by potassium permanganate (22%), salt (14%) and water only (12%). Bleach, thanks to its ease of use, its very low cost, its availability and its broad spectrum of activity, is a product that contributes to the health worldwide.

The present study aims to evaluate the effectiveness of washing lettuce with bleach by determining the microbial population of lettuce before and after washing with bleach, and the chlorine dose effective for the removal of the microflora of lettuce to minimize the health risks associated with its use.

4. Materials and methods

The study was conducted in the parcels in the communes V and VI of the District of Bamako. These sites were selected based on their accessibility and the presence of active gardeners. The lettuce samples were collected

randomly. Farm 10 feet of lettuce were collected from different plots placed in a sterile plastic bag and placed in a cooler with fresh ice and transported to the laboratory. Total of thirty samples were collected in thirty farms. For various laboratory analyzes these feet were thoroughly mixed and cut for a composite sample. Laboratory for a composite sample, 10 feet were mixed. The composite sample was cut using sterile scissors and then mixed for a uniform batch. To determine the microbial populations of lettuce before and after treatment with the bleach 100g samples were formed from this lot for different analyzes. Analysis of samples of lettuce was to find and count the total flora. To evaluate the initial population, the method used is AMOAH et al. (2004). 100 µl of the suspension collected using micropipette were seeded in mass in Petri dishes containing medium PCA (Plate Count Agar) with two replicates incubated in an oven at 37 ° C for 48 hours. The number of Colony Forming Unit (CFU) of aerobic mesophilic bacteria (TVC) was counted (Face 2).

To determine the load of lettuce after treatment depending on the contact time three batches of 100g of lettuce were made and put in chlorinated water (350 µl of 12 ° bleach in 1 liter of water, or 133.10-5 mg of active chlorine) for 15 min, 30 min and 60 min. These batches of 100g were then placed in 900 ml of physiological saline and then stirred vigorously for two minutes; 100 µl of different suspensions were plated onto PCA medium with two replicates and incubated at 37 ° C for 48 hours. The number of CFU of aerobic mesophilic bacteria was determined (Face 3).

To estimate the effect of concentration on the microbial population of lettuce depending on the concentration of chlorine, three batches of 100g of lettuce were made and put in chlorinated water at different concentrations: 175 µl of water bleach 12 ° in 1 liter of water, or chlorine 665.10-6 mg, 350 µl of 12 ° bleach in 1 liter of water, or chlorine 133.10-5 mg, 700 µl of bleach 12 ° in 1 liter of water or mg 266.10-5 chlorine. Then 100g were each in 900ml buffer solution, stirred vigorously for two minutes and 100 µl of different suspensions were seeded onto PCA medium with two replicates and incubated at 37 ° C for 48 hours. The number of total viable CFU was counted (Face 4).

For a point-total elimination of microbial population, including other 266.10-5 concentrations; 0.26 mg; 1 mg and 2.6 mg of active chlorine in a liter of water for 15 minutes were applied. Then 100g were each in 900ml buffer solution, stirred vigorously for two minutes and 100 µl of different suspensions were seeded onto PCA medium with two replicates and incubated at 37 °C for 48 hours. The number of total viable CFU was counted (Face 5).

The chlorine content was determined by titration of bleach used in washing solution before and after treatment in lettuce after treatment. Using a pipette, 10 ml of 1N H_2SO_4 normality and 10ml of bleach solution were introduced into a beaker 250ml, and then a spoonful of potassium iodide was added to the contents of the beaker. This solution was titrated with a solution of sodium thiosulfate normality 0.1 N. The volume was noted after turning the white of the solution. Chlorine concentration in g / l was determined by multiplying the volume of thiosulfate used by 17.75. The chlorine residual was determined in lettuce after treatment with different concentrations of bleach. 10g treated lettuce were ground in 100 ml of distilled water to give a suspension. This suspension was filtered. Chlorine was determined in the filtrate as before.
The mean, standard deviation and percentage drawdown of microbial population of lettuce samples were determined using Excel. Analysis of variance determined microbial populations were analyzed by SAS software.

5. Results

The microbial population of lettuce was determined before and after application of 133.10-5 mg/ l of chlorine for 15 minutes, 30 minutes and 60 minutes. The results are shown in Table 1.

We note that with the same concentration of the percentage increases as drawdown as the contact time increases. By act 133.10-5 mg / l of chlorine for 15 minutes total flora, we observe a drawdown rate of 94%. After increasing the contact time of 15 min with the same concentration of chlorine percentage drawdown increased by 3% and 5% after increasing the contact time of 45 minutes (Table 1). The contact time has a positive impact on the destruction of microorganisms during washing lettuce. The increase in the rate of inactivation is not proportional to the increase in contact time, but any increase in contact time results in a slight increase in the rate of drawdown. Varying the speed of destruction of the bacteria of lettuce according to the concentration of chlorine.

The bacterial population of the lettuce was evaluated based on the concentration of chlorine. Different concentrations of chlorine 665.10-6 mg / l; 133.10-5 mg / l and 266.10-5 mg / l were applied for 30 minutes. The results are shown in Table 2.

Table 1
Rate of drawdown per gram of lettuce.

Number Samples	amount of chlorine in mg / l	Bacterial count before treatment in CFU/ g of lettuce	Time in minutes	Number of bacteria after treatment in CFU / g of lettuce	Number of dead bacteria in CFU / g lettuce	% drawdown
			0	281	0	0
			15	12.47	268.53	94
30	133.10-5	281	30	3.93	277.07	97
			60	1.35	279.65	99

Table 2
Rate of drawdown per gram of lettuce according to the concentration of chlorine.

Number of samples	Time in minutes	Bacterial count before treatment in CFU / g of lettuce	Amount of chlorine in mg / L	Number of bacteria after treatment in CFU / g of lettuce	Number of dead bacteria in CFU / g lettuce	Percentage drawdown
			0	281	0	0
			665.10-6	37.06	243.94	83
30	30	281	133.10-5	3.93	277.07	97
			266.10-5	1.53	279.47	99

We note that with the same contact time (30 min) the rate of folding depends on the amount of active chlorine. An increase in the concentration of chlorine 665.10-6 mg / l results in an increase in the rate of drawdown of 13% but a three-fold increase 665.10-6 mg / l leads to an increase of 16% (Table 2). Chlorine has a positive impact on microorganisms in cleaning with bleach. Speed of destruction of microorganisms is not proportional to the concentration of chlorine. It is up to a threshold concentration but decreases as the concentration increases.

To find the concentration that completely eliminates the microbial population of lettuce. the effect of different concentrations of chlorine was evaluated. Concentrations of chlorine 266.10-5 mg / l 0.26 mg / l; 1 mg / l and 2.6 mg / l were applied to lettuce for 15 minutes. The results are shown in Table 3.

Table 3
Determination of chlorine which completely eliminates the microbial population of lettuce.

Number of samples	Time in minutes	Bacterial count before treatment CFU / g of lettuce	Amount of chlorine in mg/ L	Number of bacteria after treatment in CFU / g of lettuce	Number of dead bacteria in CFU / g lettuce	Percentage drawdown
			0	281	0	0
			266.10-5	5.40	275.6	93
30	15	281	0.26	0.8	280.2	99
			1	0.14	280.86	99.9
			2.6	0	281	100

These results also show an increase in percentage of destruction of microorganisms when the contact time was maintained at 15 minutes with an increase in the concentration of chlorine. The drawdown rate increases by 6% when varying the concentration of active chlorine of 266.10-5 mg / l to 0.26 mg / l; 0.9% 0.26 mg / l 1 mg / l

and 0.1% of 1mg / l to 2.6 mg / l. The total destruction of the flora of lettuce (drawdown rate 100%) was obtained with a concentration of 2.6 mg / l of active chlorine (Table 3).

For the chlorine behavior during processing the amount of residual chlorine was measured in the washing water and on lettuce after treatment dosage.

Table 4

Concentration of chlorine in the wash water before and after washing the lettuce and residual chlorine concentration in lettuce washed with different concentrations of chlorine.

Chlorine concentration in the cleaning water before treatment (mg / l)	Chlorine concentration in the cleaning water after treatment (mg / l)	Chlorine per gram of lettuce after treatment (mg / l)
133.10-5	665.10-6	0
266.10-5	665.10-6	0
0.26	166.25.10-3	0
1	904.10.10-3	0
2.6	168.10-2	887.10-5

After treatment of lettuce with different concentrations of chlorine (133.10-5 mg / l; 266.10-5 mg / l; 266.10-3 mg / l 1 mg / l), no residual chlorine was detected in lettuce. But lettuce washed with a solution containing 266.10-2 mg / l of active chlorine contained 887.10-5mg / l residual chlorine per gram of lettuce (Table 4). The concentration obtained in lettuce is less than the maximum acceptable value of chlorine in drinking water, which is 5 mg / l chlorine (Grondin. 2005). This means that the lettuce washed at a concentration of 266.10-2 mg / l of active chlorine kills all bacteria and can be ingested without risk to humans.

6. Discussion

The effectiveness of the method used to reduce microbial populations is generally dependent on the type of treatment, type and physiology of target microorganisms, the surface characteristics of the product, the time of exposure and the concentration of product disinfectant, pH and temperature (Parish and al. 2003).

Our study was to verify the effectiveness of washing lettuce with bleach at different contact times and different concentrations of chlorine.

The microbial population of lettuce was determined before and after application of 133.10-5 mg / l of chlorine for different contact times, When chlorine is fixed, an increase in contact time of 15 min resulted in an increase in the percentage drawdown of 3%, an increase of contact time of 45 min resulted in an increase in the percentage of 5% drawdown. The increase in the rate of inactivation is not proportional to the increase in contact time, but any increase in contact time results in a slight increase in the rate of drawdown. It has been established that the process of inactivation of microorganisms is often not uniform and does not follow necessarily an exponential (Montgomery. 1985; Hass. 1990). The rate of inactivation may be modified during the process. It can increase or decrease over time under the influence of many factors such as the decrease in the concentration of disinfectant, the conditions of his transfer to the water, reducing its effectiveness and changing environmental conditions. The concentration of chlorine (133.10-5 mg / l), typically used for disinfecting lettuce, applied for 15 min eliminates 94% of bacteria encountered on lettuce leaves. These results are in agreement with other studies on lettuce (Dns. 2012). In general, the residual bacterial population after treatment is composed of bacteria that tolerate the bactericidal activity of chlorine. Studies have shown that after treatment of lettuce for 15 min with a concentration of 200 ppm (0.2 mg / l) of sodium hypochlorite there is a decimal reduction of the population of mesophilic aerobic microorganisms, mold and yeasts, total coliforms and E. coli, respectively, 2.63. 2.75. 1.91 and 0.26 log 10 CFU / g. whereas the average initial of these organisms in the samples were 6.94 log 10 CFU / g for mesophilic aerobic microorganisms . 5.62 log 10 CFU / g for molds and yeasts, and 3.25 log 10 CFU / g for total coliforms (Nascimento et al.. 2003). 1% of the initial microbial population survives the application of the recommended dose (133.10-5 mg / l) for one hour. Dose commonly applied in Mali for the disinfection of fruits and vegetables is inefficient for complete destruction of the microbial flora of lettuce. This inefficiency can be

explained by the low concentration. Indeed, this concentration is ten times less than the European standard for bactericidal bleach. This residual charge (1%) may be a potential risk to consumers of lettuce.

The application of increasing amounts of chlorine for a contact time of 30 minutes fixed resulting in drawdown rates which vary according to the amount of active chlorine. Indeed. an increase in the concentration of chlorine 665.10-6 mg / l results in an increase in the rate of drawdown 13% but an increase of three times 665.10-6 mg / l results in an increase of 16 %, Similarly, for a given contact time, increasing the concentration of chlorine leads to destruction decimal, but the rate of destruction decreases rapidly despite the multiplication twice this concentration. The dose of chlorine found in treated wastewater 100ml with 1 mg / l of active chlorine is 0.09 mg / l for 10 min. 0.1 mg / l for 20 min. 0.1 mg / l and 0.09 mg for 30 minutes / l for 40 minutes (Shayeb et al.. 1998), whereas in our study the dose of chlorine in the wash water lettuce decreased by 0.096 mg / l for 15 minutes. This is explained by the fact that chlorine reacts with organic matter contained in wastewater. We used tap water which contains substantially no organic matter, the waste water contains against organic materials which interact with the chlorine. It was established that the effectiveness of the chlorine treatment varies depending on the microorganisms. Order of increasing resistance to chlorine, we have: viruses (hepatitis A virus. poliovirus. and rotavirus), bacteria (Salmonella. fecal coliforms (E. coli). fecal streptococci), protozoa (Giardia lamblia cysts. oocysts Cryptosporidium) and Cryptosporidium cysts are the most resistant (Costigan. 1937). The same author had noticed that when applying a concentration-contact time (Ct. mg.min in. / L) determined using the Chick and Watson Ct = k * log (N0 / N) / λ (where N0 and N represent respectively the initial and final concentration after treatment microorganism. k is a parameter to reflect patch of pH and free chlorine (Cl) and λ reflects the sensitivity to chlorine micro-organism). and allowing a drawdown given Giardia lamblia, we eliminate the advantage of micro-organisms with a sensitivity to chlorine higher than Giardia lamblia is 0.05. Eg bacteria with λ equal to 0.133 will be eliminated much faster compared to Giardia lamblia.

In this study, working in conditions of neutrality, laboratory temperature (25 ° C) the value of the dose-contact time expressed as free chlorine concentration (C. in mg / L) and contact time (t) minimum to ensure the bacteria off lettuce leaves with a concentration of 1 mg / l. 19.84 ct is calculated mg.min / L for a drawdown rate of 99.9% is log (No / N) = 3. This result is consistent with those of other researchers who have worked. not on the bacteria, but protozoan cysts.

Costigan (1937) found that at pH equal to 7 and 20 ° C the concentration-minimum contact time (ct) expressed in mg.min / L with concentrations of 0.5. 1. 1.5 and 2 mg was respectively 50. 53. 56. 59 mg min / L for a log (No / N) equal to 3 for inactivation of Giardia lamblia cysts by free chlorine.

The minimum concentration of chlorine which completely eliminates the microbial population of lettuce leaves was investigated using different concentrations of chlorine for 15 minutes.
The total destruction of the flora of lettuce (drawdown rate 100%) was obtained with a concentration of 2.6 mg / l of active chlorine. This concentration is well below the bactericidal concentration of the bleach according to European standards (EN 1040. EN 1276). Chlorine concentration determined by EN 1040 (36mg / l) reduces to 105 the number of bacteria in 5 minutes and 20 °, whereas in our study the percentage drawdown is 94% with 133.10-5 mg / l chlorine in 15 minutes. We can say that the contact time plays an important role in the disinfection of microorganisms and chlorine is a function of contact time. After treatment of lettuce with different concentrations of chlorine (133.10-5 mg / l; 266.10-5 mg / l; 1 mg / l; 266.10-3 mg/l), no residual chlorine was detected in lettuce. But lettuce washed with a solution containing 2.6 mg / l of active chlorine contained 887.10-5 mg / l per gram of lettuce. This concentration does not reach the maximum acceptable value of chlorine in drinking water according to WHO, which is 5 mg / L chlorine (Grondin. 2005). This means that the lettuce washed at a concentration of 2.6 mg / l of active chlorine kills all bacteria and can be ingested without risk to humans provided there is no formation of toxic chlorinated derivatives.

Conclusion

Lettuce grown in Bamako are heavily contaminated with initial microbial population average between 25 to 1363UFC / g lettuce compared to the standard which is ICMSF 10UFC / g of food. The application of the recommended dose of chlorine (133.10-5 mg / l) in Mali for the disinfection of fruits and vegetables for 15, 30 and 60 min did not eliminate the microflora of lettuce. The application of the recommended dose of chlorine (133.10-5 mg / l) in Mali for the disinfection of fruits and vegetables for 15, 30 and 60 min respectively leads to a drawdown of 94%, 97% and 99% of the initial microbial load of lettuce. The application of the recommended dose of chlorine

for 60 min and twice this dose for 30 minutes kills 99% of microorganisms; bacteria survive 1% in all cases. Lettuce washed at a concentration of 2.6 mg/l (70 ml bleach 12 °) of chlorine for 15 minutes eliminates all bacteria and can be ingested without risk to the consumer provided that there is no training toxic chlorinated derivatives.

Acknowledgements

The authors are the people who participated in the data collection and the anonymous who participated in the reading of the article.We thank the German Federal Ministry for Economic Co-operation and Development (BMZ) for funding the cashew project, the International Centre of Insect Pathology and Ecology (ICIPE). Ministry of Agriculture and Food Security in Tanzania through Naliendele Agricultural Research Institute (NARI). We are grateful to CABI, UK, for providing a reference specimen.

References

Altekruse, S.F., Cohen, M.L., Swerdlow, D.L., 1997. Emerging foodborne diseases. Emerg. Infect. Dis., 3, 285-293.

Amoah, P., Drechsel, P., Abaidoo, R.C., 2005. Irrigated urban vegetable production in Ghana: sources of pathogen contamination and health risk elimination. Irrigat. Drain., 54, 49-61.

Amoah, P., Drechsel, P., Abaidoo, R.C., Ntow, W.J., 2006. Pesticide and pathogen contamination of vegetables in Ghana's urban markets. Arch. Env. Contam. Toxicol., 50, 1-6.

Amoah, P., Drechsel, P., Abaidoo, R.C., Klutse, A., 2007. Effectiveness of common and Improved sanitary washing methods in selected cities of West Africa for the reduction of coliform bacteria and helminth eggs on vegetables. Trop. Med. Int. Health., 12, 40-50. Doi, 10.1111/j.1365-3156.2007.01940.x

Armar-Klemesu, M., Akpedonu, P., EGBI, G., Maxwell, D., 1998. Food Contamination in Urban Agriculture: Vegetable Production using wastewater. In: Armar-Klemesu, M. and Maxwell, D. (Eds) Urban Agriculture in Greater Accra Metropolitan Area. Final Report to IDRC (project 003149). Noguchi Memorial Institute for Med. Res., University of Ghana.

Blumenthal, U.J., Peasey, A., Ruiz-Palacios, G., Mara, D.D., 2000. Guidelines for Wastewater Reuse in Agriculture and Aquaculture: Recommended Revisions Based on New Research Evidence (Well Study Task No: 68 Part 1). Water and Environmental Health at London and Loughborough, London School of Hyg. Trop. Med., London.

Cisse, G., 1997. Health impact of the use of polluted water in urban agriculture. The case of gardening in Ouagadougou. PhD thesis, EPFL, Lausanne., p. 267.

Costigan, M.S., 1937. Comparison of the Germicidal Efficiency of Hypochlorites of High and Low Alkalinity. J. Bacteriol., 34, 1-7.

National Department of Health. Health and Safety Division of Mali., 2012. Fact Sheet on the disinfection of fruits and vegetables in Mali.

National Directorate of Statistics and Informatics (DNSI)., 2003. Malian Survey on poverty assessment. Ministry of Planning. Bamako. Mali.

Regional District of Bamako: DRA / DB., 2010. Crop Assess. Rep.

Regional District of Bamako: DRA / DB., 2011. Crop Assess. Rep.

Ethelberg, S., Lisby, M., Böttiger, B., Schultz, C.A., Villif, A., Jensen, T., Olsen, K.E., Scheutz, F., Kjelsø, C., Müller, L., 2010. Outbreaks of gastroenteritis linked to lettuce, Denmark. Eurosurveillance., Volume 15, Issue 6.

Faruqui NI., Niang, S., Redwood, M., 2004. Untreated wastewater use in market gardens: a case study of Dakar, Senegal. In: Wastewater Use in Irrigated Agriculture: Confronting the Livelihood and Environmental Realities (eds CScott, NIFaruqui & LRaschid) IWMI-IDRC-CABI, Wallingford., p. 113-125.

Hamadoun, M., 1995. The industrial development in Mali: the problem of waste in the industrial zone of Bamako. In: Small Grants Program for Research in Population and Development. Report Synthesis 12p.

Hass, C.N., 1990. Disinfection <<. Water quality and treatment >>. American water works association, Mac Graw-Hill, Inc.., P. 877-932.

ICMSF., 1974. Microorganisms in foods. Sampling for microbiological analysis: Principles and specific applications. The International Commission on Microbiological Specifications for Food. University of Toronto Press. Toronto.

Montgomery, J.M., 1985. Water Treatment-Principles << and >> Design, John Wiley & Sons. Stanford Univ. Tech. Rep., 251, October., p. 262-281.

Paul Nadu Sanogo., 2006. The vegetable crops, course material IPR / IFRA Katibougou, 47p. - S. Niang (1999). Use of raw wastewater in urban agriculture in Senegal: results and perspectives. In: Urban Agriculture in West Africa (ed OSmith) IDRC Books. Ottawa., p. 101-115.

Olayemi, A.B., 1997. Microbiological hazards associated with agricultural utilization of urban polluted river water. Int. J. Env. Health Res., 7, 49-154.

Parish, M.E., Beuchat, L.R., Suslow, T.V., et al., 2003. Methods to Reduce / Eliminate pathogens from fresh and fresh-cut produce. Compr. Rev.Food Sci.Food Saf., 2 (Suppl), 161-173.

Samaké, F., Babana, A.H., Yaro, F., Cissé, D., Traoré, I., Kante, F., Kone, S., Diallo, S., Toure, O., Sako, M., Iknane, A.G. 2011. Health risks related to the consumption of fresh produce grown in urban and peri-urban Bamako, Mali Health., page 33-40.

Taura, D.W., Habibu, A.U., 2009. Bacterial contamination of Lactuca sativa, Brassica and Spinacia olerencea olerencea in Kano Metropolis, Int. J. Biomed. Health Sci., No1, volume 5.

Tela, B., 2012. Nomenclatural Database of the Flora of France by Benedict Bock BDNFF v4.02.http:// www.tela-botanica.org

United Nations Conference On Trade and Development: UNCTAD. 2005. Report on vegetables.