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ORIGINAL ARTICLE

Microbiological determinations of some vegetables from the Xochimilco zone in Mexico City, Mexico

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ABSTRACT. Vegetables intake is widely recommended because of its high content of vitamins, minerals and fiber. However, the irrigation of these vegetables, using wastewaters that have received inadequate treatment often carries unseen microbial pollution that becomes a high risk potential for humans. In the present research, two of the most consumed fresh vegetables cultivated in Mexico City were analyzed, lettuce *(Lactuca sativa)* and Mexican coriander *(Eryngium foetidum)*. These vegetables are commonly consumed raw. The vegetable choice and the disinfection's method were carried out by the application of two tests to two hundred people in an aleatory form. Similarly, vegetable sampling was carried out by means of a random sampling from the cultivated areas in a chosen *"chinampa"* (from Náhuatl or Aztec, *chinamitl, b*ulrush or cattail stalks lattice for hydroponics cultivation). Vegetable samples were transferred, in dark plastic bags and in cool boxes at 4 ± 1.5ºC, to the laboratory. Microbiological analysis for *Salmonella typhi*, mesophilic microorganisms, and fecal coliforms were done according to the "NOM-093SSA1-1994" (Mexico). Results obtained demonstrated that samples treated with the most preferred disinfectant, a colloidal silver based one, had a partial elimination of pathogenic microorganisms found in both vegetables lettuce *(Lactuca sativa)* and coriander *(Eryngium foetidum)* samples (mesophyllic microorganisms from 200,000 to 96,500 UFC/g and from 175,000 to 125,000 UFC/g and fecal coliforms from 75 to 0.43 NMP/g and from 150 to 2.10 NMP/g, respectively). *Salmonella typhi* for all samples gave a positive result. Therefore, it was recommended to the cultivators of the Xochimilco (Náhuatl or Aztec name that means "place where flowers bloom") zone, either stop using contaminated water for irrigation or to use more efficient methods in order to eliminate pathogenic microorganisms, such as diluted chlorine solutions made with commercial cotton clothing bleachers.

Key words: Mesophilic microorganisms, fecal coliforms, *Salmonella typhi*, Mexican coriander (*Eryngium foetidum*), *lettuc*e (*Lactuca sativa),* colloidal silver.

RESUMEN. El consumo de vegetales es ampliamente recomendado por su alto contenido de vitaminas minerales y fibra. Sin embargo el riego con aguas negras o inadecuadamente tratadas acarrea contaminación microbiana que se convierte en un riesgo potencial para los humanos. En este estudio se analizaron 2 de los vegetales frescos más consumidos y cultivados en México, lechuga *(Lactuca sativa)* y cilantro mexicano *(Eryngium foetidum)*. Estos vegetales son comúnmente consumidos crudos. Se eligió elección al vegetal y el método de desinfección entre 200 personas en forma aleatoria mediante la aplicación de 2 pruebas. Se tomaron muestras de los vegetales al azar del área cultivada "chinampa".
Las muestras de los vegetales fueron transportadas al laboratorio en bolsas de plástico obscuras a 4±1.5°C.
Se realizó un análisis microbiológico para *Salmonella typhi*, microorganismos mesofílicos y coliformes fecales de acuerdo a la norma NOM-093SSA-1 1994 (México). Los resultados obtenidos demostraron que las muestras tratadas con un desinfectante de base de plata coloidal tenían una eliminación parcial de microorganismos patogénicos encontrados en ambos vegetales. (microorganismos mesofílicos de 200,000 a 96,500 UFC/g y de 175,000 a 125,000 UFC/g y coliformes fecales de 75 a 0.43 NMP/g y de 150 a 2.10 NMP/g respectivamente.
Todas las muestras fueron positivas para *Salmonella typhi*. Se recomendó a los campesinos de la zona de Xochimilco (del Náhuatl o Azteca "lugar donde las flores florecen") ya sea evitar el uso de agua contaminada para riego o usar métodos más eficientes de desinfección, tales como el uso de soluciones cloradas.

Palabras clave: Microorganismos mesofílicos, coliformes fecales, *Salmonella typhi*, *Eryngium foetidum*, lechuga *(Lactuca sativa)*, plata coloidal.

INTRODUCTION

The Dictionary of the Royal Spanish Language Academy defines vegetables as "the edible plants that are cultivated in the orchards". As well, it defines the orchard as "the site of short extension, generally surrounded of walls, where vegetables and fruit trees are planted (Real Academia Española).[10] Another definition is: Herbaceous plants, of annual or biennial cycle (exceptionally perennial), of intensive agronomic practices, whose products displaying a high water content (greater to 70%), a low energetic content (less than 100 cal/100 g) and one short life utility in post harvest (variable from few days to a year at the most) are used for food purposes either in its natural state or processed.[7]

Vegetables in general, are rich in vitamins, minerals and dietetic fiber, mainly if they are ingested raw; since most of vitamins are altered or destroyed by the action of the heat. The presented green coloration, is due to the chlorophyll, that confers to the green leaves several virtues or properties for the nutrition of the man: 1) Contribute to sanguineous and red globules regeneration, 2) Help to the assimilation

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of proteins, 3) Present a regulating action of the nutrition and the arterial voltage and, finally, 4) Influence the organism acid-base balance (Acevedo, 1998).

Vegetables intake is widely spread in Mexico. However, the irrigation of these vegetables, using wastewaters that have received inadequate treatment or surface water that have been polluted with human sewage such as the ones coming from the waste water treatment plants named "Cerro de la Estrella" and "San Luis Tlalxialtemalco", in the Southeastern and South zone of Mexico City, and the channels of the former lake of Xochimilco (Náhuatl or Aztec name that means "place where flowers bloom") makes them potentially dangerous. They become vehicles of pathogenic microorganisms and other polluting agents, almost all of them of chemical origin (from wastewaters from the industrial area located in the Southeastern part of Mexico City (Iztapalapa Delegation, another Náhuatl or Aztec name that means "place with white houses"). Besides, the disinfection methods used by the cultivators and the consumers seem to be not very reliable, since the majority of the population in Mexico City has serious problems with gastrointestinal diseases.[6,8]

Remembering that Mexico basin was formed by several lakes and lagoons, some of them located in the Southern part of what it is now Mexico City, particularly the Chalco (Náhuatl or Aztec name that means "place where fine sand is") and Xochimilco lakes, this surface water was used to plant with a sophisticated hydroponic system known as chinampa (from Náhuatl or Aztec, *chinamitl*, bulrush or cattail stalks lattice covered with a thin layer of earth and moss), a name given to the structures of pre-Hispanic origin that supported the growth of vegetables taking advantage of the high content of available water. This system has been maintained by the Mexican culture settled there by these almost five centuries after the Spaniards came and conquered Mexico, although its operation is not exactly the same as before the Spanish conquest. Still around 1930, the channels reached downtown Mexico City and Xochimilcan peasants used to bring their produce in acalli (wooden long boats, "water houses" in Náhuatl or Aztec) to the main market in downtown, la Merced, through the La Viga channel.[5]

With the anarchical growth that has lived the Mexico City basin, from the Spanish conquest up to date, the lakes are practically dried and only a small fraction of Xochimilco lake survives, where it is received for its recharge the treated wastewater from two constructed plants in the southeastern and southern part of the city, mentioned paragraphs above, "Cerro de la Estrella" and "San Luis Tlalxialtemalco".

As Mexico City has single sewage lines, rainwater, domestic type of waste waters, and residual waters of industries, commerce, hospitals, businesses, etc., are all mixed, and the composition of the residual waters that arrive to all wastewater treatment plants is highly variable. Most of the time, it does not receive and adequate processing, since the plants only use a biological treatment (activated sludge), and the recalcitrant or slowly degradable substances remain in the "treated" water.

On the other hand, the inhabitants of the Xochimilco *chinampas* zone have lost the ancestral technologies used by the lakes pre-Columbian inhabitants for efficient water use and for suitable residual or wastewaters, and the majority spills their own waste waters without any treatment to the channels (that are the only remains of the old lake of Xochimilco now).

This situation entails enormous problems for the consumers of vegetables cultivated in *chinampas*. "*Chinamperos"* or people that live around and cultivate the *chinampas* have looked for palliating this problem by adding to their fresh produce disinfectants to sell them in better sanitary conditions.

The Official Mexican Norm[12] has an informative appendix on microbial counts in which recommendations are settled down for specific types of foods and ready to eat products. Among these recommendations are the corresponding ones to raw produce, such as green salads, coriander, etc., establishing the following limits:

a) Aerobic mesophilic microorganisms: 150,000 FCU/g and
b) Fecal coliforms: 100 MPN/g.

Indicators are microorganisms that have been generally used to determine some objectionable microbiological conditions referring to foods, related with fecal origin contamination. Besides, they indicate the presence of potentially pathogenic organisms or the potential proliferation of microorganisms in foods and the sanitary situation during processing, storage, or transport of foods.[1]

The number of "plate count" aerobic mesophilic microorganisms found in food has been one of the microbiological indicators of foods quality more commonly used. Aerobic mesophilic organisms reflect the exposure of the sample to any contamination, and in general, to the existence of favorable conditions for the multiplication of pathogenic microorganism and the presence of organic matter. For diverse reasons, the aerobic mesophilic flora contents is useful to indicate if the cleaning, disinfection, and temperature control during industrial processing, transportation, and storage, have been made in a satisfactory form.[19]

The coliforms microorganisms are the facultative aerobic or anaerobic bacteria, Gram negative, non forming of spores that ferment the lactose with gas formation within 48 hours to have placed in lactosade broth to 35ºC. The coliforms are commonly inhabitants of mammalian ani-

Rev Latinoam Microbiol 2002; 44 (1): 24-30

mals' guts (including the man). Nevertheless, the bacteria of this group can come from, as much of non fecal or fecal matter, reason why these organisms can be in great amounts in the contaminated ground with fecal matter.[19]

Unfortunately, as some organisms do not have fecal origin, the presence of those organisms in foods can not be determined by fecal contamination or potential enteric pathogens determinations. In spite of this, it is possible to orient these results saying that the non-fecal coliforms give an extra safety margin.[18] The detection of fecal contamination in foods has always been a discussion subject. The coliforms that are able to grow at 44.5ºC have been considered as indicators. Of these, *Escherichia coli* is still the species more associated to fecal matter (intestinal remaining matter). Therefore, its presence in foods is an indication of fecal contamination. Nevertheless, there is no evidence that allows to know the origin of the contamination, since it can be human, environmental, or animal[2]. Fecal coliforms are the indicating organisms commonly used to determine the microbiological quality of water and human population effluents or residual waters. The Environmental Protection Agency of the United States of America (USEPA) has specified the dilution method using multiple tubes (known as most probable number, MPN) for the determination of fecal coliforms in water and waste water.[11]

The fecal coliforms are more closely related with the fecal contamination than the total coliforms. The organisms can be present in inadequately sanitized working surfaces in a food processing plant. In this case, its presence could reflect the quality of the aseptic processes and not the direct contamination of the product.[18]

Given this problematics, in this research, that is a part of a comprehensive rescue project of the Xochimilco zone, the microbiological characteristics of two representative samples of raw consumable vegetables of great demand by the population were studied, using fecal coliforms organisms and *Salmonella typhi* as indicators. Tables 1 and 2 display the nutritional value (bromatological analysis, vitamins contents, and caloric value) of the lettuce, one of the most popular vegetables, with respect to others of common ingestion.

The vegetables choice and the disinfection method preferences among the cultivators and consumers were made by means of a representative questionnaire. In the same way, the sampling of the two most popular vegetables was randomly made for each one of them in *chinampas* where they were cultivated.

The information of the microbiological quality of these two vegetables will be of utility to design the strategies concerning this part of the rescue program for the zone and

Table 1. Caloric value and composition of some vegetables. (Composition given by 100 grams of food) (Acevedo, 1998)

Vegetable	Water %	Fat %	Proteins %	Carbohydrates %	Minerals %	Caloric value cal/kg
Artichokes	84	3	0.2	12	1.7	550
Cabbage	92	1.6	0.1	5.8	0.5	250
Lettuce	95	1.2	0.2	3	0.9	180
Onion	89	1.4	0.2	9	0.6	400
Pepper	93	1.2	0.2	5	1.5	240
Potatoes	78	2	0.1	19	1	800
Pumpkin	95	0.8	0.1	3.5	0.5	150
Spinach	92	2.2	0.3	4	1.8	230

Table 2. Vitamin content of some vegetables. (Composition given by 100 grams of food) (Acevedo, 1998)

Vegetable	Retinol (µg)	Ascorbic acid (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Pyridoxine (mg)	Folic acid (µg)	Cobalamine (µg)
Celery	10	8	0.02	0.04	0.4	0.03	12	0
Coriander	384	11	0.12	0.06	1	*	*	*
Cress	161	51	0.13	0.2	1.5	0.13	200	0
Lettuce	44	6	0.14	0.05	0.3	0.16	34	*
(Roman) Lettuce	44	7	0.05	0.03	0.3	*	136	0
Spinach	320	40	0.1	0.16	0.5	0.18	140	0

* Unknown values.

Rev Latinoam Microbiol 2002; 44 (1): 24-30

to improve the life quality of Xochimilco inhabitants and their vegetables consumers. This is the contribution of this phase of the investigation.

MATERIAL AND METHODS

Vegetables choice

Vegetables were selected by the accomplishment of a survey applied in a determined zone of Xochimilco (known as the Central Market of Xochimilco), to a population sector of 200 people randomly taken. The survey included two specific questions, one the preferred raw vegetable consumed, and the other one, the disinfection method used before its presentation to public (fot he cultivators/sellers) and before its consumption (to the customers):

To consumers:

a) Which are the fresh raw vegetables that you consume most frequently? And
b) How do you clean or disinfect them before consuming them?

To cultivators/sellers:

c) Which are the fresh raw vegetables that you cultivate and sell most? And
d) How do you clean or disinfect them before putting them for sale?

These answers allowed to know, on the one hand, the vegetables of greater consumption in that area and, by the other one, the type of disinfection used by that sector of population (Chávez Espinosa, 2002).

Sampling of produce and lab preparation

The selected vegetables, previous application of the questionnaire, lettuce (Lactuca sativa) and Mexican coriander *(Eryngium foetidum)*, were acquired in two *chinampas* near of the market of Xochimilco, by a random sampling. The samples were transferred to the laboratory, in dark plastic bags previously sterilized to avoid the contamination by other microorganisms, within cool boxes, to maintain its temperature to 4 ± 1.5ºC, and later, in the lab were stored in a cool room at the same temperature, and taken for the microbiological analysis, according to the procedures established by the official Mexican norms.[13-17]

Sample preparation consisted on the homogenization of each type of vegetable, doing a single composed sample of the four collected samples in each *chinampa*. The homogeneous sample was divided in two parts for the accomplishment of the most preferred disinfection process. One of the homogenized samples did not receive any disinfection process and they only were washed with tap water. The second sample, once washed with tap water, they were submerged in a basin that contained the preferred disinfectant dissolved in tap water (commercial name, "Microdyn", and active principle is colloidal silver), in according to the instructions provided by the manufacturer (amount per liter of water and soaking time). The efficiency of these small amounts of the argentiferous metal in colloidal form to eliminate microorganisms is based in the great affinity that have certain cellular proteins by these ions and replacing other metals in its coordination compounds, being accumulated in considerable amounts in the cells from the diluted solutions[9] (Pelczar, 1990).

Microbiological analyses

In both samples (with and without disinfection) for each one of the two selected vegetables, fecal coliforms and aerobic mesophilic microorganisms were quantified. As it was already mentioned, the proposed methodologies shown in NOM 092, 093, 109, 110, and 112 (SSa, 1994a-e) were used. Additionally to the established procedure in these norms, the determination of a potentially dangerous pathogenic microorganism, *Salmonella typhi* was also carried out, according to the proposed NOM (SSa, 1994f).

RESULTS

As a result of the survey, in Figures 1 and 2 are the raw freshly eaten vegetables that were preferred by the cultivators/sellers and consumers in the zone of Xochimilco's Central Market and the method of cleaning and/or disinfection most commonly used by them. When disinfectant is mentioned, most of the people who use it refer to is by a particular trade name. Mexican coriander or cilantro *(Eryngium foetidum)* and lettuce *(Lactuca sativa)* were those of greater consumption by population (28% and 30%, respectively), followed by spinach, beets, and parsley (20, 12.5, and 9.5%, respectively).

In the same way, in Figure 2 it is appraised that the most popular method for cleaning and/or disinfecting, is the use of tap water with the commercial disinfectant already mentioned in the previous section (30%), followed by simple tap water (28%), and finally, water with sodium hypochlorite (25%). The other methods were not taken into account in this phase of the investigation.

Table 3 shows the counts of microorganisms in the samples of both vegetables, visualizing the reduction of the microbial count (aerobic mesophilic organisms) when the dis-

Rev Latinoam Microbiol 2002; 44 (1): 24-30

infectant is applied (from 200,000 UFC/g lettuce sample washed only with tap water to 96,500 UFC/g lettuce sample washed and disinfected with colloidal silver and from 175,000 UFC/g coriander sample washed only with tap water to 125,000 UFC/g coriander sample washed and disinfected with colloidal silver), which gives a reduction of 52% for lettuce and of 28.6% for coriander.

For fecal coliforms in the same samples, there is a reduction of 99.4% for lettuce and 98.6% for Mexican coriander (of 75 to 0.43 NMP/g lettuce sample washed to washed and disinfected and of 150 and 2,10 NMP/g of sample of coriander, washed and disinfected, respectively). In the same table it can be appraised that neither the washing with tap running water nor the disinfection with colloidal silver are able to eliminate the presence of the indicator microorganism, *Salmonella typhi*, since all samples gave positive results.

DISCUSSION

As Xochimilco is one of the most important Mexico City's source of these vegetables (well, more for the Federal District or D.F. for its name in Spanish, that comprises Mexico City and other neighboring areas), through its commercialization in popular markets or within the Mexico City Central Market (Central de Abastos), which is the main center of food storage and retail within the city, these results are quite worrisome.

It can be seen from data gathered, for both lettuce and Mexican coriander washed with tap water (without chemical addition), values exceeded the permissible ones by the NOM.093-SSA1.1994, and although the values of the samples treated with colloidal silver diminished, the reduction is not considerable and remain in the limit or even exceed it too. This raises the necessity to lead consumers towards the use of more effective substances to disinfect its fresh foods and, at the same time, to look for appropriate solutions to avoid the sending of contaminated waters to the Xochimilco channels.

According to the identification of the proposed indicator *(Salmonella)* it was observed that the corresponding biochemical tests and the confirmation of serological tests, the species present was typhi, a potentially hazardous pathogenic microorganism, and cause of the typhoid fever in men.

These results seem to indicate, on one hand, that the vegetables that are cultivated in the lacustrine zone of Xochimilco exceed the limits established by the Mexican law norms indicating the presence of organisms with health hazardous potential, particularly those causing typhoid fever. The origin of this contamination definitively comes from spilled residual waters to the Xochimilco channels, both by the wastewater treatment plants and by the inhabitants of the zone who do not possess sanitary installations appropriately run for the disposal of domestic waste waters and domestic animals wastes.

As the climate of the ex-basin of Mexico is very benign and promote the proliferation of potentially pathogenic microorganisms that survive after the waste water treatment processes, on one side, it is important to initiate massive awareness campaigns both for the Xochimilco *chinampa*

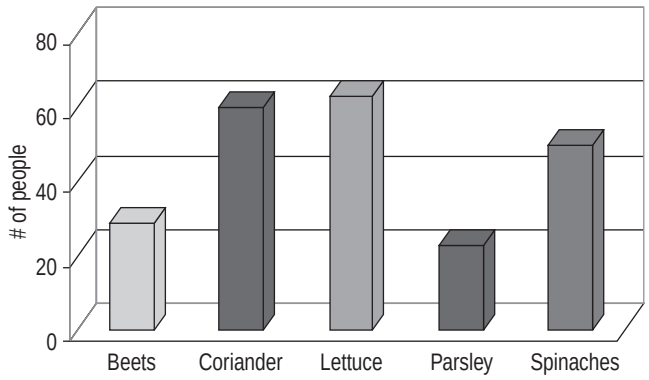

Figure 1. *Preferred raw freshly eaten vegetables in the zone of Xochimilco, D.F., Mexico.*

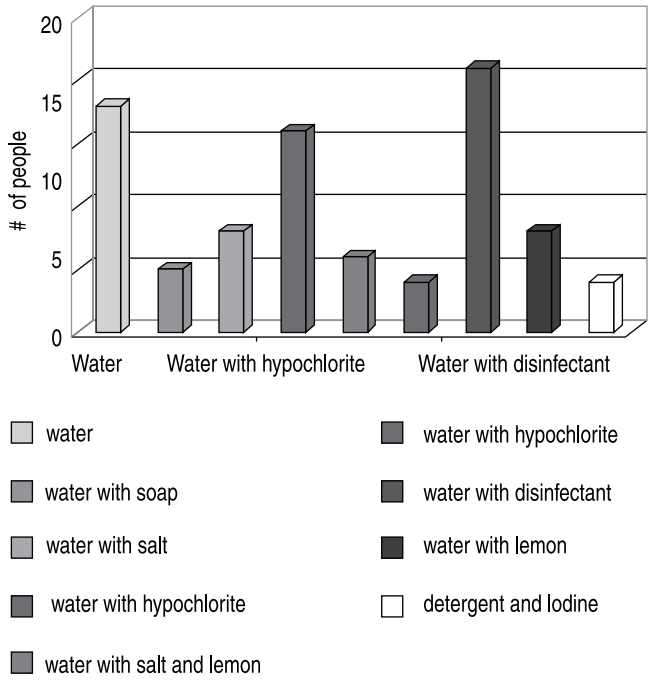

Figure 2. *Methods commonly used by the inhabitants of Xochimilco (cultivators/sellers) and their customers for cleaning and disinfection of raw freshly eaten vegetables.*

Table 3. Results of the microbiological determinations.

	LETTUCE *(Lactuca sativa)*		MEXICAN CORIANDER *(Eryngium foetidum)*	
	With no disinfection treatment	With treatment	With no disinfection treatment	With treatment
Aerobic mesophilic * UFC/g	** 200,000	* 96,500	** 175,000	* 125,000
Fecal coliforms * NMP/g	* 75	* 0.43	** 150	* 2.10
Salmonella typhi	Present	Present	Present	Present

* Normative limits: Aerobic mesophilic: 150,000 UFC/g and fecal coliforms: 100 NMP/g.
** Exceeds the limits.

inhabitants as well as for the employees and engineers of the City Government treatment plants to improve the existing treatment systems and to support the installation of household processing systems *("in situ")* for the Xochimilco and neighboring zones. The confinement and treatment of their corporal fluids and other wastes before sending them to the environment and from there go to the channels would reduce the vectors for the contamination of their own food products but also for their agricultural products improving their living standards by reducing the health problems associated with poverty.

CONCLUSIONS

According with the results obtained in this investigation it is necessary to corroborate that the processes of wastewater treatment applied in the plants "Cerro de la Estrella " and "San Luis Tlalxialtemalco", located in the "Iztapalapa" and "Xochimilco" Delegations of the Federal District are adequate and that the treated waters that these plants dispose in the Xochimilco channels comply with sanitary and chemical quality standards that guarantee a better life quality for the inhabitants of the chinampa zone.

Also, it is important that the sanitary authorities of the Government of the Federal District, GDF in Spanish, start looking for creative solutions to treat residual waters for the inhabitants' households of the *chinampas* and neighboring zones and to teach young, adult, and old people in the Aztecs' ancestral customs who were able to maintain a paradise in these zones before the arrival of the Spaniards, in which sanitary quality was observed by the conquerors.[4]

The disinfectant agent commonly used by consumers and *"chinamperos"* displayed positive effects diminishing the microbial load. Nevertheless, it these effects were not as expected, since *Salmonella typhi* tests showed positive results. In the lab, the tests using only the organisms in tap water samples with hypochlorite solutions proved to be very effective and with a lower cost than the preferred disinfectant. This suggests the necessity that the same sanitary authorities not only verify the goodness of these disinfectant substances for the socalled normative organisms. In fact, the organisms that can cause endemics as the typhoid fever should be of public health priority in Mexico City.

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Rev Latinoam Microbiol 2002; 44 (1): 24-30

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