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Bacteriological Quality of Crops Irrigated with Wastewater in the Xochimilco Plots, Mexico City, Mexico

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Xochimilco county plots (Mexico City), one of the most fertile agricultural areas in the Valley of Mexico, produce a large portion of the fresh vegetables consumed in the city. These plots are generally irrigated with domestic wastewater, and for this reason, it was deemed important to examine and evaluate the bacteriological quality of the water, soil, and vegetables from these plots that are harvested and marketed. The soils were also examined for the classical parameters such as nitrates, ammonia, etc., and organic matter and texture. The crops selected for this study were radishes, spinach, lettuce, parsley, and celery because they are usually consumed raw. The highest bacterial counts were encountered in leafy vegetables, i.e., spinach (8,700 for total coliform and 2,400 for fecal coliform) and lettuce (37,000 for total coliform and 3,600 for fecal coliform). Statistically significant differences in bacterial counts between rinsed and unrinsed edible portions of the crops were observed even in rinsed vegetables, and high densities of fecal coliform were detected, indicating that their consumption represents a potential health hazard. The total coliform values found in irrigation water ranged from 4×10^4 to 29×10^4, and for fecal coliform the values ranged from 5×10^2 to 30×10^2.

Agricultural employment of wastewater for irrigation is based on the value of its water content and its constituents which are used as fertilizers (12). In addition, wastewater also contains salts, toxic metallic compounds, and pathogenic organisms; thus, its content can also be harmful to the soil, crops, grazing animals, and public health (22, 27). Although more acreage is being developed for irrigation, the demand for water is greater, but its quality tends to be degraded as it is reused.

It has been well established that bacteria, viruses, protozoa, nematodes, and fungi capable of causing diseases can be found in foods contaminated with sewage water (3, 9, 12, 24, 29). Therefore, for more satisfactory results, wastewater should be treated to remove harmful substances and microorganisms before it is used for irrigation.

Different authors have proved that vegetables are contaminated with microorganisms when they are irrigated with sewage water and when the soil is fertilized with manure because both usually contain great amounts of pathogenic organisms (6, 9, 13). Special attention has been paid to those vegetables that are eaten raw since the microorganisms that settle over them are able to survive for several weeks, and when these vegetables are consumed, they could produce diarrhea, salmonellosis, shigellosis, etc. (5, 12, 18–20).

A large amount of the vegetables consumed by Mexico City inhabitants comes mainly from the Xochimilco county plots and agricultural areas near the city, and since these areas are generally irrigated with raw municipal wastewater, the vegetables represent a threat to the public health.

This study deals with the bacteriological contamination of crops grown in Xochimilco county plots and was undertaken to demonstrate that the consumption of these vegetables represents a potential hazard to public health, especially when the vegetables are eaten raw.

This study was performed in the village of San Gregorio (21 km southeast of Mexico City) (Fig. 1). The village is a section of Xochimilco county and is built on a system of channels and land parcels as part of the Xochimilco-Chalco lake basin.

The Xochimilco-Chalco lake basin is a closed basin in which the water level of the lake is maintained by a series of springs. At present most of the lake is dessicated, and there are only 82 km of channels left, with plots between them which the natives call "chinampas."

The chinampas or plots are some of the most fertile and productive agricultural areas in the Valley of Mexico. The fertility of the soil in the plots is due mainly to the existence of sufficient water for irrigation and to the lime extracted from the channels that is used as a soil conditioner (G. E. Cerrántes and E. R. Tórres, M.S. thesis, Universidad Nacional Autónoma de México, Mexico, D.F., 1981).

The water supply for domestic and industrial use in Mexico City, one of the most populated cities in the world, has become one of the major problems faced by the government. As a result of the continuous water shortage, the government initiated a program to satisfy the increasing demand for water.

One of the solutions to this problem proposed by the program was the drilling of water wells in the Xochimilco area. As a consequence of the continuous extraction of underground water, however, the water levels decreased drastically, which caused the springs to dry and the lake to almost disappear.

To replenish water in the lake, an activated sludge treatment plant was built. The plant (consisting of grit removal, primary sedimentation, conventional diffused aeration tank, final clarifier, and chlorine disinfection) began to operate in 1959 with a capacity of 400 liter s^{-1} that could be increased up to 1,250 liter s^{-1}. The continuous expansion of the plant was considered adequate for maintaining the water level in the remaining channels and in the lake.

It is important to point out that at the time the plant began to operate, water quality regulations for agricultural and

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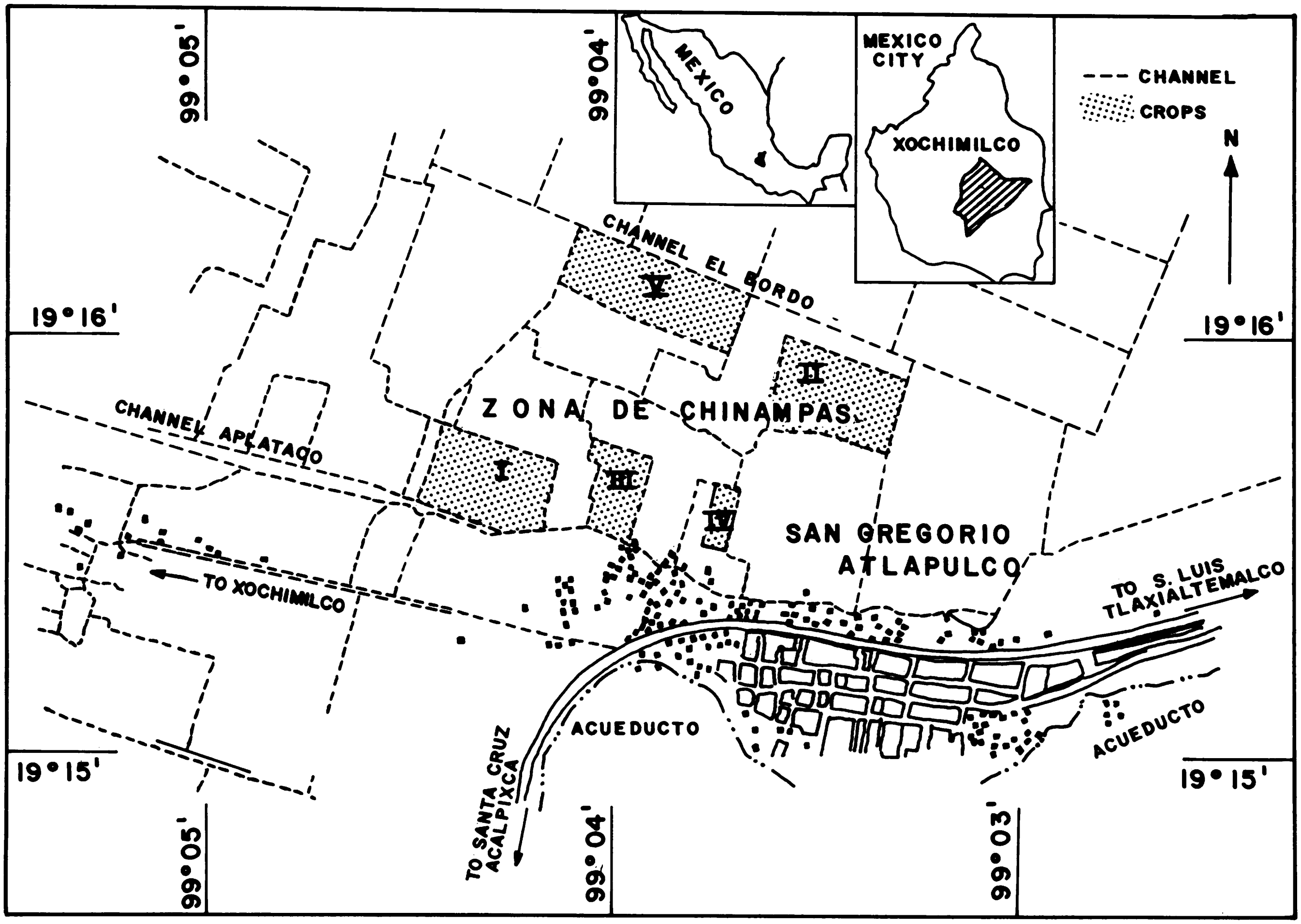

FIG. 1. Location of sampling sites in Xochimilco lake plots. (I) Parsley crop; (II) lettuce crop; (III) radish crop; (IV) spinach crop; (V) celery crop.

recreational use did not exist in Mexico, and several mistakes were made. (i) The transport of the treated wastewater was made through an open channel (10 km long) that was promptly used by the inhabitants as a dumping site for their solid and liquid wastes, invalidating in great part the function of the treatment plant. (ii) The biological oxygen demand values, as reported by Báez et al. (2), of the secondary effluents averaged 47 mg liter^{-1}. These values fluctuated between a minimum of 18 mg liter^{-1} and a maximum of 88 mg liter^{-1}, with an average of 80 mg liter^{-1}, at the point of discharge to the channels. It is important to note that according to the Water Quality Criteria established by the U.S. Environmental Protection Agency (27), the maximum permissible biological oxygen demand concentration for domestic or industrial wastewater effluent is 30 mg liter^{-1}.

The discharge of the secondary effluent from the sewage treatment plant into the lake and canals altered completely their water quality and characteristics, i.e., they went from oligotrophic to eutrophic and from mesosaprobic to polysaprobic as reported by Báez et al. (2) and Rosas et al. (17), who additionally reported that the bacterial densities in water (coliform per 100 ml) varied from 4.5×10^4 to 1.1×10^7 in some of the Xochimilco canals.

MATERIALS AND METHODS

Source of samples. Samples of irrigation water, soil, and vegetables (parsley, lettuce, radish, spinach, celery) were collected from San Gregorio village agricultural plots (Fig. 1).

The sampling period extended from October 1980 through December 1981, with one vegetable specimen of each type collected at each sampling time.

Sampling was done at intervals of 15 days until 18 samples of each specimen were obtained, except parsley, for which only 12 samples were collected. Vegetable specimens were selected at random from the corners and center of each plot. Water and soil specimens were obtained at each sampling time.

Water from the irrigating canals was sampled with sterilized biological oxygen demand bottles. Soil core samples were obtained with the aid of a polyvinyl chloride tube (diameter, 5 cm; length, 31 cm) at depths of 0 to 3 and 12 to 15 cm.

Handling of samples. Vegetable samples were placed in sterile containers and transported to the laboratory (1, 28), where roots, leaves, and stalks from each vegetable were separated. Two sets of the edible portion of each crop were prepared and set aside. One set was rinsed in tap water before bacteriological analysis, whereas the second set was analyzed without previous rinsing.

Tap water was analyzed weekly, and the bacterial counts were always less than 2 total coliform (TC) per 100 ml with an absence of fecal coliform (FC). Besides this, water was disinfected by chlorine addition.

TABLE 1. Bacterial numbers (TC and FC) in irrigation water, vegetables, and soil from Xochimilco plots

Type of crop	Geometric mean of the following types of bacteria in the following samples:					
	TC			FC		
	Irrigation water[a] (n^b = 15)	Vegetable[c] (n = 18)	Soil[c] (n = 36)	Irrigation water[a] (n = 15)	Vegetable[c] (n = 18)	Soil[c,d] (n = 36)
Celery	41,000	4,300	91,000	1,500	1,000	3,000
Spinach	200,000	1,800,000	232,000	700	40,000	9,000
Lettuce	37,000	291,000	223,000	800	13,000	3,000
Parsley[e]	90,000	43,000	144,000	550	7,000	3,000
Radish	290,000	8,600	310,000	3,000	700	8,000
MPC[f]	5,000			1,000		

[a] Bacteria per 100 ml.
[b] Number of observations for each vegetable, soil, and water sample.
[c] Most probable number per 100 g (wet weight).
[d] Average of the combined bacterial count of 0- to 3-cm and 12- to 15-cm soil depth samples.
[e] Only 12 samples were collected for this vegetable.
[f] Maximum permissible concentration (coliforms per 100 ml) (Federal Water Pollution Control Administration [26]).

Ten grams of each crop and soil sample were weighed, diluted to 100 ml with sterile phosphate buffer, and homogenized for 1 min in a Waring blender (21).

Bacteriological examination. (i) Water. TC and FC were enumerated by the membrane filter technique (Millipore Corp.) (14, 28). Portions of the dilute sample (1.0, 0.1, and 0.01 ml) were filtered through sterile membrane filters (pore size, 0.45 μm; diameter, 47 mm). M-Endo broth was used as the culture medium for TC counts.

Sample dilutions of 1.0 and 0.1 ml were filtered through (pore size, 0.70 μm; diameter, 47 mm) sterile membrane filters, and FC broth was used for FC enumeration.

(ii) Vegetables and soils. Bacteriological examination was performed by the three-tube fermentation technique, as specified in *Standard Methods for the Examination of Water and Wastewater* (1, 28), in which lactose and brilliant green bile broths were used for the presumptive and confirmative test of TC, whereas lactose broth and EC broth were used for the presumptive and confirmative test of FC.

From the homogenized solutions of stalks and leaves, dilutions of 1.0, 0.1, and 0.01 ml were taken for the TC analysis, whereas dilutions of 10, 1.0, and 0.1 ml were taken for FC analysis.

Dilutions of 0.1, 0.01, and 0.001 ml were taken from the homogenized solutions of soil and root core samples for TC analysis, and dilutions of 1.0, 0.1 and 0.01 ml were taken for FC analysis.

Physical and chemical examination of soils. Soil samples were dried and sieved (no. 10 sieve) before analysis.

Color was determined by the Bouyoucos method. Solid particle density was determined by weighing 10 ml of soil, and the bulk density was determined with a picnometer. The percentage of pore space was calculated by considering bulk and particle densities. The percentage of organic matter was determined by the method of Walkley and Black and from the pH of the soil solution.

Cation exchange capacity was determined by titrating a 1.0 N NaCl soil solution with 0.02 N EDTA solution, and the Morgan technique was used for the determination of $N-NO_3$ and $P-PO_4$ (11).

RESULTS AND DISCUSSION

Bacterial numbers in irrigation water. The bacteriological analysis results of the water used for irrigation are given in Table 1.

The values obtained were compared with the maximum permissible concentration (MPC) established by the U.S. Department of Interior Federal Water Pollution Control Administration for Water in Agricultural Use (26).

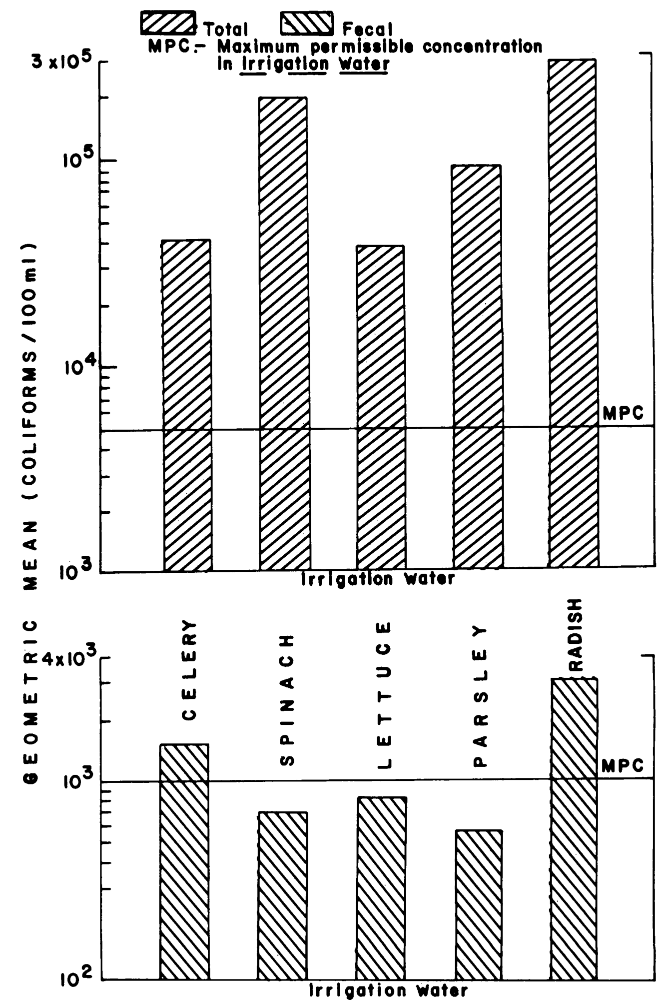

FIG. 2. TC and FC counts in water used for crop irrigation and a comparison with the MPC (Federal Water Pollution Control Administration [26]).

TABLE 2. Physical and chemical characteristics of soil from San Gregorio Village, Xochimilco

Parameter	Parameter characteristic at the following depths (cm)	
	0–3	12–15
Color of soil		
Dry	Gray	Gray
Humid	Black	Black
Texture of soil (%)		
Clay	20	28.4
Lime	34.4	32.0
Sand	45.6	39.6
Solid particle density (g cm^{-3})	0.59	0.54
Bulk density (g cm^{-3})	2.1	2.0
Pore space (%)	71.7	73.1
pH	6.2	7
Exchange capacity (meq 100 g^{-1})	52.4	50.0
Organic matter (%)	19.1	17.3
NO_3 (ppm[a])	40	40
NH_4 (ppm)	150	150
T-PO_4 (ppm)	100	100
K (ppm)	250	180
Ca (ppm)	1,600	1,600
Al (ppm)	0	0
Mn (ppm)	5	5
SO_4 (ppm)	250	250
CO_3 (HCOmeq 100 g^{-1})	0	0
HCO_3 (HCOmeq 100 g^{-1})	0.34	0.46

[a] ppm, Parts per million equivalent to micrograms per milliliter.

The results indicate that the TC values found are considerably higher (7 to 50 times) than the MPC in 85% of channels sampled. However, it is also noted that the FC values exceed the MPC in 90% of the samples taken from two of the channels (Fig. 2) near the poultry and porcine farm, from which solid and liquid wastes were usually discharged into the channels. The water from these channels was used to irrigate the radish parcels. The results further indicate that this water is unsuitable for irrigating the parcels.

Bacterial content of soil. The role that the soil plays in the bacteriological contamination of vegetables, due to the direct contact between soil and plants, is well known, especially when they are irrigated with wastewater containing 4×10^4 to 2×10^5 bacteria per 100 ml as in this case.

There is a high probability for soil contamination due mainly to the protection from solar radiation that leaves and other parts of the plants give to bacteria and other microorganisms (12) (Table 1). Soil samples taken from plots in which celery and parsley, which have smaller foliar structures, were grown exhibited lower TC concentrations.

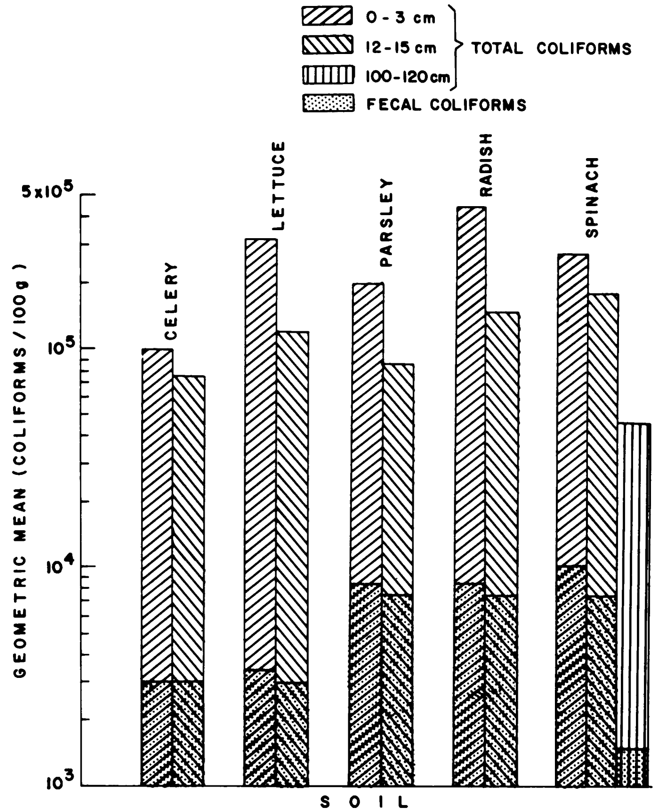

FIG. 3. Soil distribution of TC and FC counts at different depths in the plots from which the studied crops were harvested.

When FC counts between water and soil were compared, a higher bacterial density was found in the latter, which can be attributed to: (i) the use of sewage as fertilizer and (ii) physical and chemical characteristics of the soil (Table 2), such as permanent humidity, richness in organic matter, and a pH between 6 and 7, which are features that favor the reproduction of microorganisms (2, 25; Cervántes and Tórres, M.S. thesis).

Vertical distribution of bacteria in soil. As expected, the highest bacterial densities were found on the soil surface. However, a significant quantity was found at a depth of 20 cm, and even in samples taken at a depth of 1.20 m from ditches dug on the plots, the numbers of bacteria were still significant, due mainly to soil porosity, runoff, and infiltration of polluted water (Fig. 3).

Bacterial distribution in vegetables. Table 3 shows the TC and FC distribution on different parts of the crops sampled.

TABLE 3. Percent distribution of bacteria in leaves, stems, and roots

Type of crop	% of the following types of bacteria in the following samples: TC				FC			
	BC[a]	Leaf	Stem	Root	BC[a]	Leaf	Stem	Root
Celery	4,300	13.0	30.0	57.0	1,000	22.0	30.0	48.0
Spinach	1,800,000	0.5		99.5	40,000	6.0		94.0
Lettuce	291,000	13.0		87.0	13,000	28.0		72.0
Parsley	43,000	7.0		93.0	7,000	9.0		91.0
Radish	8,600	69.4		30.6	700	47.7		52.3

[a] BC, Bacterial counts in whole vegetable (geometric mean, most probable number per 100 g).

TABLE 4. Bacterial numbers in the edible part of rinsed and unrinsed sampled crops

Type of crop	Geometric mean[a] of the following samples of the following types of bacteria:			
	Rinsed		Unrinsed	
	TC	FC	TC	FC
Celery	300	30	1,300	300
Spinach	2,400	1,700	8,700	2,400
Lettuce	700	570	37,000	3,600
Parsley	370	300	3,100	660
Radish	650	300	2,600	360

[a] Most probable number per 100 g.

As expected, the higher concentrations of bacteria were found in the roots due to their direct contact with the contaminated soils as mentioned above. The results indicate that the concentration in roots range from 57 to 99% for TC and from 48 to 91% for FC.

It was also observed that the highest concentrations corresponded to those in lettuce and spinach. These high concentrations could be attributed to the fibrous roots of these vegetables and to their surface area which is larger than that of celery and radishes.

In the leaves, the values varied from 0.5 to 69% for TC and from 6 to 47% for FC.

A bacterial density relationship was noted among crops, soil, and irrigation water (Table 1) with one exception: the roots of radishes, which exhibited the lowest bacterial counts, possibly due to their content of iodine which has a bactericidal effect.

Bacteria in vegetables. The largest number of TC and FC were found in lettuce and spinach. This great contamination can be attributed to the size of these crops (7). The average size of lettuce in this study was approximately 20 cm in height and for spinach it was near 50 cm; for this reason, bacteria from the soil could easily be deposited by rain splash or other mechanisms. Besides, lettuce and spinach also have foliar surfaces with many folds and fissures that provide good shelter for microorganisms (12), and the leaves are fragile and thus allow the penetration and reproduction of bacteria in their inner tissues (7, 15).

Celery exhibited the lowest degree of contamination on leaves and stalks. This can be attributed to the presence of more resistant tissues and less-extended surface areas when compared with those from lettuce and spinach.

Removal of bacteria by tap water rinse. One of the general methods used to clean vegetables is tap water rinse (8). Part of this study focused on determining the number of TC and FC remaining after the edible part of the vegetables was rinsed.

The results (Table 4) indicate that the TC and FC numbers decreased in variable quantities depending on the kind of crop.

A TC and FC removal of 75 to 95% and 17 to 90%, respectively, was observed, notwithstanding the fact that leaves and stems were rinsed for 30 s. The low removal of TC and FC observed in the leaves of the spinach could be attributed to the structure of the leaves, which does not allow an effective rinse. With respect to the radishes, the rinse procedure alone was not sufficient to remove soil particles attached to the tubercle surface, and a brush washing had to be used. The variability in bacterial removal was considered to be due to the fact that the same rinse method was applied for all vegetables, in spite of the different structures of leaves and roots of each vegetable.

A distribution-free rank sum test (Wilcoxon) (10, 23) was applied to the results for significant differences in the TC and FC numbers between the edible parts of rinsed and unrinsed crops, indicating that there was a significant difference in TC counts in only three of the sampled crops: lettuce, parsley, and celery (Table 5). The results also indicate that the tap water rinse was not effective in the removal of FC and proved the presence of TC and FC in the vegetables when they were irrigated with domestic raw wastewater.

Generally, the consumption of raw vegetables that have not been properly cleaned implies a health hazard, because they are a reservoir for bacteria capable of producing infection in susceptible patients in hospitals (16, 30).

In Mexico, the bacteriological contamination of crops by the use of raw wastewater in irrigation increases the gastrointestinal diseases which are at present one of the most significant causes of death, mainly in children (4).

In Mexico, vegetables are harvested and marketed with roots that contain soil residues which contribute to the microbiological contamination of the edible portions of the crops (8, 9, 13, 18).

To reduce and/or eliminate the microbiological contamination of vegetables after harvest, some recommendations can be suggested: the use of contaminated water for the bulk-soil removal and for the first wash should be avoided; instead, drinking water should be used for bulk-soil removal and first cleaning. The vegetables should be transported to the market in proper containers.

Removal of roots and nonedible parts of the vegetables before storing is recommended so as to avoid the proliferation of microorganisms (15). The vegetables should be carefully rinsed for at least 30 s. Finally, the rinsed vegetables should be soaked in a disinfectant solution before consumption.

TABLE 5. Statistical analysis of the bacterial results obtained from rinsed and unrinsed vegetables

Type of crop	TC			FC		
	Us[a]	$U_{0.05}$ (n_1,n_2)[b]	P	Us[a]	$U_{0.05}$ (n_1,n_2)[b]	P
Celery	230.0	215 (18,18)	<0.05	88.0	215 (18,18)	>0.05
Spinach	151.5	215 (18,18)	>0.05	141.5	215 (18,18)	>0.05
Lettuce	245.5	215 (18,18)	<0.05	212.5	215 (18,18)	>0.05
Parsley	107.0	102 (12,12)	<0.05	100.0	102 (12,12)	>0.05
Radish	151.5	215 (18,18)	>0.05	148.0	215 (18,18)	>0.05

[a] Us, Experimental statistical test value.

[b] $U_{0.05}(n_1,n_2)$, Theoretical statistical test value; n_1,n_2, sample size.

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