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MICROBIOLOGICAL IMPACT OF TREATMENT LAGOONS ON THE ECONOMICS OF WATER FOR REUSE IN AGRICULTURE A CASE STUDY IN MOROCCO (SETTAT AND SOUALEM REGIONS)

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Abstract

This study was undertaken to enumerate pathogens: fecal coliforms, *Escherichia coli*, fecal enterococci and *Salmonella* in the areas irrigated with treated wastewater. The samples were isolated from Settat (33°00'N, 7°37'W) and Soualem regions (34°26'N, 5°53'W). A total of (n= 48) raw water, (n=48) treated water, (n=71) of vegetables samples irrigated by treated water taken from Waste Water Treatment Plant Settat; A total of (n=24) raw water, (n=24) treated water, (n=97) of vegetables samples irrigated by treated water taken from Waste Water Treatment Plant Soualem. The results show the total average in the two stations of raw water 7.9, 6.1 log MPN 100 ml^{-1} for respectively fecal coliforms and *E. coli*, 5.4 log CFU 100 ml^{-1} for fecal enterococci and 5.2 log MPN L^{-1} for Salmonella; for treated water 4.6, 3.1 log MPN 100 ml^{-1} for respectively fecal coliforms and *E.coli* and 3.5 log CFU 100ml^{-1} for fecal enterococci. Regarding plants, four types of crops were harvested and analyzed (forage, herbs, cereals and vegetables), the germs charges were found with fecal coliforms, *E.coli* and fecal enterococci respectively 3.2, 2.8 and 4.1 log $CFUg^{-1}$. *Salmonella* was never detected in both treated water and crops samples.

Key words: Waste Water Treatment Plant, *Escherichia coli*, fecal coliforms, fecal enterococci and *Salmonella*.

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Abbreviations: MPN: Most Probable number; **CFU**: Colony Forming Unit; **UV**: ultraviolet; **WHO**: World Health Organization; **NF**: Norme Française; **EN**: Norme Européenne; **ISO**: International Organization for Standardization; **API20E**: Analytical Profile Index for *Enterobacteriaceae*; **API20strept**: Analytical Profile Index for *Streptococci*.

INTRODUCTION

Recycling water is an important aspect of water resource and environment management being a real alternative to high water consume, mainly in agricultural activity providing reducing environmental pollution and achievement sustainable form of development. Related advantages to wastewater reuse are: economic way to dispose of wastewater, preventing environmental pollution and sanitary problems; efficient use of valuable nutrients to crop demands and provide additional treatment before reaching water's body (23). Agriculture is often attributed as the source of water quality impairments, with both nutrients and bacteria cited as the pollutant (2). On the other hand the use of wastewater in agriculture is often associated with significant health risks because of the presence of high concentrations of human pathogens, enteric in origin, such as bacteria, viruses, protozoa and helminthes (30).

The treatment lagoons can result in a significant reduction of fecal agents in the effluent through biological processes, but with considerable reductions of treatment cost. This is typical application for Mediterranean countries with moderate treatment facilities (12). The schemes used were designed to produce water for irrigation reuse with higher levels of organic matter, nitrogen and phosphorus, all being important for soil fertility and plant metabolic activities and usually eliminated during the sewage treatment (14). Wastewater treatment technologies suitable for meeting the revised microbiological guidelines for agriculture include the use of Waste Stabilisation Ponds (WSP), Wastewater Storage and Treatment Reservoirs (WSTR), or conventional treatment processes. When using WSP, the revised guidelines usually require the use of one or more maturation ponds after the anaerobic and facultative ponds (4).

Some sanitary quality indicators are used as fecal coliforms and *Escherichia coli*, because the presence of this bacterium proves the possible existence of pathogens (20); faecal coliforms are defined by their ability to grow at an elevated temperature (44.5°C), most coliforms are not harmful; some strains, including *E. coli* 0157: H7 are pathogenic (1). Epidemiological research in Israel and New Zealand demonstrated strong relationships between *Enterococcus* densities and the incidence of illness among swimmers in marine waters receiving raw sewage and treated sewage discharges (24).

The use of the indicator bacterial group *Enterococci* is frequently suggested as an alternative to coliforms, their advantage over *E. coli* lies in their greater resistance and their inability to grow in any environment, such as soil, water and others (32, 25).

For *Salmonella* genus, it's a complex bacterial group widely distributed in nature, they are often isolated from the intestinal tract of warm- and cold-blooded animals, as well as from environmental samples contaminated with fecal discharges (5). The genus *Salmonella* includes pathogens affecting humans and animals, and the vast majority is considered zoonotic (33); Infection with *S. typhi* or *S. paratyphi*, which are exclusively human pathogens, results in enteric fever (17). Clinical symptoms of enteric fever include diarrhea, abdominal pain, fever, and sometimes a maculopapular rash, the pathological sign of enteric fever is mononuclear cell infiltration and hypertrophy of the reticulo endothelial system, mortality from *S. typhi* and *S. paratyphi* is estimated to be between 10 to 15 percent without treatment (17).

Pathogens have to be present in sufficient concentrations to initiate infection and to develop the diseases or the susceptible host has to come into contact with pathogens at minimum critical dose i.e. infective dose level; Generally enteric viruses and protozoa have low infectious dose in comparison to bacterial pathogens (16), infection and development of clinical symptoms depend on a number of specific and non-specific host factors such as age, immunity status, gastric acidity, nutritional status, vitamin A deficiency and possibly genetic predisposition; For example, the infectious dose is between 10^6-10^{10} and 10^4-10^7 Number per liter for respectively *E. coli* enteropathogenic and *Salmonella typhi* (16).

A different approach was adopted by the World Health Organization (WHO) which recommends the more liberal threshold of 1000 CFU 100 ml^{-1} of fecal coliforms for unrestricted irrigation of crops to be eaten uncooked, sports fields and public parks (33); Although there are no hygienic standards concerning restricted irrigation of cereals crops, industrial and fodder crops, pasture and trees (33), some authors have suggested a threshold from $\leq 10^3$ to $\leq 10^5$ fecal coliform bacteria 100 ml^{-1} on treated wastewater depending on the exposed groups and irrigation techniques (19). The classification of treated wastewater in microbiological categories and its use for irrigation, should take into account crops types (edible or not) and their human consumption (without or after processing), health hazards for risk groups (young, old, pregnant or immunocompromised consumers and operators such as farmers), water application technologies and the duration of the irrigation season; All this could allow a wider use of the treated wastewater for irrigation associated with minimal health and environmental risk (18, 26 and 7).

The aims of this study were: (i) Enumeration of fecal coliforms, *Escherichia coli*, fecal enterococci and *Salmonella* in raw, purified waters and crops irrigated with treated wastewater (iii) Assess associations between the prevalence of *Salmonella* and the levels of fecal indicators: *Escherichia coli* and intestinal enterococci, to evaluate the importance for public health of the analysis of this specific pathogen in raw, treated wastewater and crops irrigated by treated water.

MATERIALS AND METHODS

Hydro-climatological general context of the two cities Settat and Soualem that both belong to the region of Chaouia is semi-arid; The average annual rainfall is

400 mm and the average temperature is 25° C (40 ° C in summer and 10 ° C in winter) (34).

Description of WWTP (WasteWater Treatment Plant) Settat

The treatment plant is located 8km north entrance of the Settat city (33°00'N, 7°37'W) (http://www.tageo.com/index-e-mo-v-26-d-m71695.htm), it covers an area of 80 hectares, it will allow the treatment of flow of 13500 m3 per day, the treated wastewater will be used to irrigate some 300 hectares in the vicinity of that city, a population of around 175 000 people will benefit (21).

Description of WWTP Soualem

Soualem city is located west of Morocco (34°26'N, 5°53'W) (http://toolserver.org/~geohack/geohack.php?pagename=Soualem&language=fr&params=34.43333_N_5.88333_E_type:city), his station covers an area of 13 hectares; it will treat $1860m^3$/day; The treated water will irrigate 50 hectares; a population of around 25.000 people will benefit (22).

The treatment of raw water adopted for the two stations is natural lagoons; the sojourn time for each station is between 40 to 46 days for WWTP Settat and 22 days for WWTP Soualem, the first step is screening, then the ponds are arranged and operated in series, with anaerobic ponds preceding the facultative ponds which then feed into several maturation ponds.

Raw water, purified and vegetable crops and fodder sampling

Sampling was done monthly over a period of two years from July 2007 to August 2009. Water samples were collected in sterile flasks 1000 ml and stored at 4-8°C before microbiological analysis; Samples of cultures were directly irrigated by treated wastewater as they are grown on farmland alongside around sewage treatment plants, sampling was done according to the availability of such crops; Quantities more than 100g were collected from vegetable crops and fodder in sterile bags at 4°C and transported to the laboratory before microbiological analysis; Samples were collected on time between 11h and 13h and analyzed within six hours after collection.

Microbiological analyses

The detection and enumeration of fecal coliforms and *Escherichia coli* in water were done by MPN (Most Probable Numeration) according to standard NF T 90-413. Buffered Peptone Water -BPW- (Oxoid) is used as a presumptive medium with incubation at 37 ° C for 24 hours and the broth lactose bile brilliant green -BLBVB-(Oxoid) as a confirmation medium with incubation at 42°C for 24h, a 0.1 ml aliquot was taken from tubes showing gas production (considered a positive reaction) and placed in a tube of peptone water free of indole –EPPI- (Oxoid) to perform the indol test. After 48 h at 45°C, several drops of Kovac's reagent were added to the broths agitating slightly: a cherry red colour visible at the surface of the broth was considered positive for indol confirming the presence of *E. coli*. In parallel, *E. coli* was performed using Violet Red Bile Lactose Agar -VRBL- (Biorad) , Plates were incubated for 24 h at 44°C. The density is reported as log MPN 100 ml^{-1} for both fecal coliforms and *E. coli*.

The detection and enumeration of fecal enterococci in water was done by a membrane filter procedure according to standard NF-EN ISO 7899-2:2000. Water samples were subjected to suitable dilutions and filtered through cellulose nitrate membrane filters with 0.45 mm pore size; The fecal enterococci were enumerated on a Slanetz & Bartley medium (Oxoid), after plate incubation (for 48 h at 37°C), membranes were placed on Bile Esculin Azide Agar (Merck) and incubated for 24 h at 37°C; When any blackening of the medium occurred colonies were counted as enterococci. The authors have chosen to lower the temperature at 37 ° C and prolonging the incubation time up to 24 hours to ensure better growth of enterococci and ease of counting, because the laboratory experiments showed that after 2 hours at 44 ° C, there are sometimes difficult to count colonies that are not well developed. The density is reported as log CFU 100 ml^{-1}.

Table 1. Microbiological hygienic quality of raw sewage and treated in Wastewater Treatment Plants Settat and Soualem

		WWTP Settat				WWTP Soualem			
		1st year		2nd year		1st year		2nd year	
Germ searched	Sample	Cold season	Hot season	Cold season	Hot season	Cold season	Hot season	Cold season	Hot season
Fecal coliforms logMPN100 ml^{-1}	Raw water	6.8±0.3	9.3±0.3	6.7±0.4	9.9±0.3	6.8±0.5	8.1±0.5	6.8±0.5	9.3±0.5
	Treated water	2.3±0.4	6.0±0.4	3.7±0.4	6.1±0.3	2.3±0.4	6.3±0.4	3.7±0.4	6.9±0.4
	Rate abattement	4	3	3	3	4	2	3	3
Escherichia coli logMPN100 ml^{-1}	Raw water	4.6±0.3	8.0±0.3	4.8±0.4	8.6±0.3	2.9±0.4	8.0±0.4	2.6±0.4	9.3±0.4
	Treated water	2.1±0.3	5.3±0.3	1.0±0.4	4.7±0.3	1.6±0.3	4.6±0.3	1.3±0.3	4.7±0.3
Fecal Enterococci logCFU100 ml^{-1}	Raw water	4.6±0.3	6.6±0.3	4.3±0.4	6.4±0.3	5.1±0.4	6.0±0.3	4.6±0.4	6.0±0.3
	Treated water	2.3±0.3	4.9±0.3	2.9±0.4	4.8±0.3	2.0±0.4	3.9±0.3	2.9±0.4	4.6±0.3
Salmonella logMPN l^{-1}	Raw water	5.3±0.4	5.9±0.3	3.4±0.4	5.6±0.3	5.4±0.5	6.5±0.4	3.3±0.4	6.5±0.4
	Treated water	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Note. Mean log MPN

The detection and enumeration for *Salmonella* was done by MPN (Most Probable Numeration) according to standard NF ISO 6579: 2002, Buffered Peptone Water -BPW- (Oxoid) is used as a presumptive medium with incubation at 37 ° C for 24 h, after wards a 0.1ml aliquot of culture was transferred to 10 ml Rappaport Vassiliadis (Oxoid) and incubated for 24 h at 44°C; When the incubation is at 41.5°C, the result was not always satisfactory because the *Salmonella* strain appeared only in the first three tubes of MPN series, sometimes in a single tube, this is due to the heavy load and the competition of microorganisms present in raw water, by cons, for treated water samples that are moderately loaded microorganisms, the incubation was done to 41.5 ° C. The broth was then streaked onto XLD Agar (Merck) plates and Edel Kampel Macher Agar (Difco), after 24 h at 37°C, 3 colonies with typical morphology were inoculated in tubes with Triple Sugar Iron Agar (Oxoid).The density is reported as log MPN 1000 ml^{-1}.

Regarding the microbiological analysis of vegetable and fodder, a weight of 25g homogenized with 225ml of BPW and then pummeled with a MIX I mixer (AES Laboratory, Combourg, France), one milliliter of this suspension and decimal dilution were streaked onto VRBL and incubated for 24 h at 44°C for fecal coliforms, and one milliliter and decimal dilution were streaked onto Slanetz & Bartley medium for fecal enterococci and incubated for 48 h at 37°C. For the isolation of *Salmonella* the homogenate was pre-enriched at 37°C for 24h, afterwards a 0.1ml aliquot of culture was transferred to 10 ml Rappaport Vassiliadis Broth (Oxoid) and incubated for 24 h at 44°C, the broth was then streaked onto XLD Agar (Merck) plates and Edel Kampel Macher Agar (Difco), after 24 h at 37°C, 3 colonies with typical morphology were inoculated in tubes with Triple Sugar Iron Agar (Oxoid). For all germs, the density is reported as log CFU per g.

The isolates were confirmed *E. coli* and *Salmonella* using the *Enterobacteriaceae* API 20E commercial kit (Biomerieux, Marcy l'Etoile, France), for fecal enterococci the confirmation is sent by the API 20 strep commercial kit (Biomerieux, Marcy l'Etoile, France).

Statistical analysis

For each organism, duplicate plates were enumerated and the means calculated; the mean log *(X)* value and standard deviation *(SD)* were calculated on the assumption of a log normal distribution. Statistical treatment of data is based on analysis of the correlation; performing calculations were performed using *XLSTAT* Software version 2011.1.05. Pearson's correlation coefficient (*r*) was used to show correlation between microbiological data on the one hand and rainfall and temperature on the other.

RESULTS

Microbiological quality and effect of season of water samples

The table 1 summarizes the results of the bacterial load and the cumulative rate reduction at the entrance and exit of the two stations during the 48 months of sampling.

The rate abatement varies between 3 and 4 log units for WWTP Settat, and between 2 and 4 log units for WWTP Soualem. With regard to *Salmonella*, it was detected only in raw water for both WWTP Settat and Soualem.

Microbiological quality and effect of season of crops samples

In our study, four types of crops were harvested and analyzed (forage, herbs, cereals and vegetables), a total of (n=168) of crops samples irrigated by treated water taken from both Waste Water Treatment Plant Settat and Soualem and a number of (n=24) crops irrigated with freshwater were analyzed; Table2 summarizes the results of the bacterial load of crops analyzed and control. With regard to the vegetables, no germ was detected in both seasons.

The relationship between the log removal of micro-organisms (fecal coliforms, *E. coli*, fecal enterococci and *Salmonella*), the temperatures and precipitation in both sites are shown in tables 3 and 4, for WWTP Settat and Soualem respectively.

Pearson's correlation coefficients showed that bacterial counts have a positive correlation with water temperature (*r*=+0.552, +0.767, +0.510 and +0.283 for respectively fecal coliforms, *E. coli*, fecal enterococci and *Salmonella*) and negative correlation with rainfall (*r*= -0.483, -0.736, -0.480 and -0.317 for respectively fecal coliforms, *E. coli*, fecal enterococci and *Salmonella*).

Similar analysis for WWTP Soualem showed that bacterial counts have a positive correlation with water temperature (*r*=+0.618, +0.822, +0.288 and +0.231 for respectively fecal coliforms, *E. coli*, fecal enterococci and *Salmonella*) and negative correlation with rainfall (*r*= -0.574, -0.767, -0.245 and -0.285 for respectively fecal coliforms, *E. coli*, fecal enterococci and *Salmonella*).

DISCUSSION

The presence and removal of the indicator micro-organisms were monitored over two different sampling periods, there were significant differences between the two seasons hot (April to September) and cold (October to March) for raw and treated water, the correlation between surface water temperature, rainfall and bacterial counts observed in this study are significant ($p<0.05$) and are strongly supported by other studies (6, 28), similar correlations to those obtained in our study were reported by other authors which showed that temperature, UV-light and rainfall

Table 2. Microbiological quality of crops analyzed and control

	For (*n*=30)		C.For	Cer(*n*=64)		C.Cer	Her(*n*=56)		C.Her	Veg (*n*=18)		C.Veg
	H.S. *n*=13	C.S. *n*=17	*n*=4	H.S. *n*=29	C.S. *n*=35	*n*=4	H.S. *n*=26	C.S. *n*=30	*n*=10	H.S. *n*=9	C.S. *n*=9	*n*=6
No. of samples positive F.C. (%)	9(69%)	6(35%)	2(50%)	13(45%)	7(20%)	1(25%)	14(54%)	6(20%)	3(30%)	0(0%)	0(0%)	0(0%)
Average load F.C. (log CFU g-1)	4.2±0.3	2.7±0.3	3.3±0.4	4.3±0.3	2.6±0.4	3.7±0.3	3.7±0.3	2.0±0.4	3.0±0.4	0.0	0.0	0.0
No. of samples positive E.C. (%)	6(46%)	3(17%)	0(0%)	8(27%)	4(11%)	1(25%)	9(34%)	2(6%)	1(10%)	0(0%)	0(0%)	0(0%)
Average load E.C. (log CFU g^{-1})	3.6±0.3	2.0±0.3	0.0	3.4±0.3	2.4±0.3	2.4±0.3	3.6±0.3	1.9±0.3	1.2±0.3	0.0	0.0	0.0
No. of samples positive F.E. (%)	6(46%)	3(17%)	2(50%)	8(27%)	4(11%)	2(50%)	24(92%)	2(6%)	6(60%)	0(0%)	0(0%)	0(0%)
Average load F.E. (log CFU g^{-1})	5.8±0.4	4.1±0.4	7.3±0.3	4.4±0.3	2.7±0.4	2.1±0.3	5.5±0.4	2.2±0.4	3.6±0.3	0.0	0.0	0.0

Note. Mean log CFU 100 g^{-1} ± standard deviation
H.S.: Hot Season, C.S.: Cold Season
For: Forage, C.For: Control Forage, Her: Herbs, C.Her: Control Herbs, Cer: Cereals, C.Cer: Control Cereals, Veg: Vegetables, C.Veg: Control Vegetables
F.C.: *Fecal Coliforms*, E.C.: *Escherichia coli*, F.E.: *Fecal enterococci*

Table 3 Pearson's correlation co-efficient between removal of micro-organisms and the temperatures, precipitation, in WWTP Settat

Variables	T(°C)	PP(mm)	FC	*E. coli*	FE	S
T(°C)	**1**	**-0.794**	**0.552**	**0.767**	**0.510**	0.283
PP(mm)	**-0.794**	**1**	**-0.483**	**-0.736**	**-0.483**	**-0.317**
FC	**0.552**	**-0.483**	**1**	**0.841**	**0.874**	**0.781**
E. coli	**0.767**	**-0.736**	**0.841**	**1**	**0.837**	**0.680**
FE	**0.510**	**-0.483**	**0.874**	**0.837**	**1**	**0.767**
S	0.283	**-0.317**	**0.781**	**0.680**	**0.767**	**1**

Bold values are different from 0 at a significance level of alpha = 0.05
FC, fecal coliforms; *E.coli*; FE, fecal enterococci; S, *Salmonella.*

Table 4. Pearson's correlation co-efficient between removal of micro-organisms and the temperatures, precipitation, in WWTP Soualem

Variables	T(°C)	PP (mm)	FC	*E. coli*	FE	S
T(°C)	**1**	**-0.788**	**0.618**	**0.822**	0.288	0.231
PP(mm)	**-0.788**	**1**	**-0.574**	**-0.767**	-0.245	-0.285
FC	**0.618**	**-0.574**	**1**	**0.870**	**0.759**	**0.734**
E. coli	**0.822**	**-0.767**	**0.870**	**1**	**0.586**	**0.604**
FE	0.288	-0.245	**0.759**	**0.586**	**1**	**0.801**
S	0.231	-0.285	**0.734**	**0.604**	**0.801**	**1**

Bold values are different from 0 at a significance level of alpha = 0.05
FC, fecal coliforms; *E. coli*; FE, fecal enterococci; S, *Salmonella.*

are key factors of bacterial growth in water (9). As shown in table 1, the abatement of fecal coliforms and *E. coli* is in the same order of magnitude, identical results were obtained in a study in India on the performance of stabilization pond with respect to the reduction of indicator bacteria (16).

Fecal coliforms still remain the major organisms used to indicate fecal pollution and the performance of treatment processes as reported in the literature in a number of countries around the world (16); According to the results obtained, the rate of reduction varies between 3 and 4 log units at the station Settat, contrary to the station which has been Soualem a fall in the rate of reduction equal to 2 log units during the first year to stabilize at a rate of 3 log units, this can be explain for two reasons, first: the residence time applied in the wastewater treatment plant Settat ranging from 40 to 46 days, against 22 days for the station Soualem, indeed several studies have shown the effectiveness of the storage and prolonged solar radiation in decontamination (31), during daylight, lethal solar radiation greatly accelerates the bacterial die-off; the ultraviolet light is the most lethal (31); The second reason is the large number of ponds, 12 units which are all functional in the resort of Settat , while for station Soualem containing 6 units, one of the maturation ponds is non-functional; The barrier efficacy is thus determined by its 'weakest link', because when a treatment step consists of several parallel treatment units, the poorest performing unit will dominate the pathogen removal (27); The floating macrophytes such as *Eichhornia crassipes* (water hyacinth) and *Lemna* sp. may be responsible for the poor sewage treatment (15), but in the case of our study there was no presence of floating macrophytes.

Several studies showed that the die-off rate of fecal enterococci was low compared to that of the fecal coliforms, some authors are found that fecal enterococci is better indicators than fecal coliforms (15). Considering the results of this study, it is reasonable to conclude that the die-off rate of fecal coliforms is low compared to the fecal enterococci and correlate better with the pathogenic microorganisms, their advantage over *E. coli* lies in their greater resistance and their inability to grow in any environment, such as soil, water and others (32, 25).

The difference of *Salmonella* load between cold and hot season is not significant during the first year while she is during the 2nd year, this may be due to the low rainfall experienced by this region during the 1st year of levy 246mm in the cold season against 400mm for the same season for the 2nd year and 51mm in the hot season for the 1st year against 13mm for the 2nd year during the same season (http://www.tutiempo.net/clima/Nouasseur/601560.htm). Water temperature has been proposed as playing an important role in the long-term survival of *Salmonella* in the environment, the presence of cold waters may reduce the survival of *Salmonella* in the marine environment, while warm waters together with high levels of organic matter, typical conditions prevailing in tropical areas may contribute to a more appropriate habitat for an increased survival of bacteria, as reflected in the disparate incidence of *Salmonella* described in diverse studies in temperate and tropical regions (10). International organizations recommend to their member countries microbiological studies correlating the density of bacterial indicator organisms with the presence and density of pathogens such as *Salmonella* (13, http://www.cdc.gov; http://www.hpa.org.uk).

However, surveillance of *Salmonella* is only performed by a few countries, the reliability of the indicator based standards to predict the presence of this pathogen is still a matter of debate (8).

Regarding the control of crops and fodder, the charge of fecal enterococci is significantly higher that recorded by the fecal coliform, this is due to resistance of fecal enterococci and their inability to grow in any environment as mentioned earlier (32, 25), while *Salmonella* was never detected in crops. On the whole, higher rates were obtained during the hot season ranging from 3.4 ± 0.3 and 5.8 ±0.4 log CFU g^{-1}, while during the cold season charges range from 1.9 ± 0.3 and 4.1 ± 0.4 log CFU g^{1}, this variation is due to the high temperature that promotes the growth of bacteria, the values obtained for control cultures ranged from 1.2 ± 0.3 at 7.3 ± 0.3 log CFU g^{-1}, bacteria detected in these samples can be explained by the fact that they are from the ground, in this sense, studies have shown that *E. coli* O157: H7 can be transmitted from a contaminated soil with compost or irrigation water contaminated with to crop plants such as lettuce (29).

Analysis of samples of vegetables showed the complete absence of germ; According to another study, organic acids naturally present in fruits and vegetables or accumulated as a result of fermentation are relied upon to retard the growth of some microorganisms and prevent the growth of others (3); Some organic acids naturally found in or applied to fruits and vegetables behave primarily as fungistats, while others are more effective at inhibiting bacterial growth; Acetic, citric, succinic, malic, tartaric, benzoic and sorbic acids are the major organic acids that occur naturally in many fruits and vegetables, the mode of action of these acids is attributed to direct pH reduction, depression of the internal pH of microbial cells by ionization of the undissociated acid molecule, or disruption of substrate transport by alteration of cell membrane permeability(3).

A study comparing vegetable production using freshwater and untreated wastewater in Haroonabad, Pakistan, found that the gross margins were significantly higher for wastewater (US$150 per hectare), because farmers spent less on chemical fertilizer and achieved higher yields (11).

The new guidelines have been controversial, particularly relaxation of the guideline for unrestricted irrigation to 1000 fecal coliform per 100ml (geometric mean). Criticisms have included the use of 'partial' epidemiological studies in developing countries, ignoring the acquired immunity of the population involved, and ignoring the health risk assessment methodology used as a foundation for developing drinking water quality standards, concern has been expressed over the lack of sensitivity of epidemiological methods to detect disease transmission that may not lead to apparent infection in exposed individuals but to secondary transmission from them to cause illness in susceptible individuals (4). Indeed, various authors have attempted to provide typologies for wastewater recycling and use, but none of these has been taken up universally or been standardized (11).

As presented here, the efficiency of this treatment system in removing the various bacteria is clear when the results obtained from the series of samples are considered, so it's possible to examine the potential risks of infection associated with the consumption of food crops that are irrigated with treated wastewater using a quantitative microbial analysis; it can be a tool to test the usefulness of international guidelines and standards for acceptable levels of pathogens in treated wastewater.

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