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Vertical Transfer of Bacteriological and Parasitological Pollutants from Irrigation Water to Soil and Crops

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ABSTRACT

In response to food needs and the growing desire to exploit local food, urban and peri-urban agriculture is meeting these needs by producing vegetables, fruits and other foods in cities and their suburbs. In addition to the increasing need for water due to droughts, this agriculture provides wastewater (WW) and treated wastewater (TWW) that is used for irrigation. This study was conducted to compare urban irrigation water: water from Oued Fez upstream and well water. As well as peri-urban irrigation water: water from Oued Fez downstream considered as WW and TWW from the treatment plant of the city of Fez. These in comparison with the rural irrigation waters: waters of Oued Bitit. The microorganisms investigated are total and thermotolerant coliforms, helminth eggs, Salmonella and cholera vibrio. The study took into account the transfer of these pathogenic bacteria at the level of soils and cultivated plants, cardoon and eggplant. The results showed a contamination out of national and international standards of the two types of coliforms that it is in winter or in summer in the TWW, WW, the water of Oued Fez and the water of wells located upstream of the city. This fecal contamination was found in soils and crops irrigated by urban and peri-urban water. The same was true for helminth eggs, but the number of eggs was greater in winter than in summer for soils. Cholera Vibrio was present in the different types of irrigation water in summer. But still in winter in WW and TWW. This bacterium was also present in soils, cardoons and eggplants irrigated by WW, TWW and Oued Fez waters upstream. Salmonella was present only in the TWW in summer. Only the plot irrigated with water from Oued Bitit in the rural zone was within the norms on the three levels of irrigated water, soil and plants and in both periods.

Keywords: micobiological contamination, wastewater, treated wastewater, soil, crops

INTRODUCTION

The current global drought and water unavailability necessitate the reuse of wastewater in agriculture, a practice that has gained momentum in arid and semi-arid countries (Ensink et al., 2002; Rejebet al., 2002; Scott et al., 2004). In addition to the advantages of this water (high yields, less use of organic or mineral fertilizers), the reuse of raw wastewater can be harmful to humans and the environment. Wastewater contains heavy metals (Faouzi et al., 2022) and all the microorganisms excreted with the feces. Pathogenic bacteria coexist with normal enteric flora. Bacteria, viruses, protozoa and helminths are the four main types of microorganisms present in wastewater (Belaid, 2010). According to Pescod (1992), viruses, bacteria, and parasites can survive at high concentrations in raw wastewater for long periods of time up to several months. The application of wastewater to crops, especially raw crops, can result in microbiological contamination and the introduction

of pathogenic organisms into the food chain. In addition, groundwater can be contaminated with chemicals such as nitrogen and pathogens as a result of this practice. Several studies have shown that wastewater has a negative impact on health (Cissé, 1997; Feenstra et al., 2000; Bahri et al., 2009; Becerra-Castro et al., 2015). The main health problem associated with wastewater pollution is diarrheal diseases, such as rotavirus, cholera, and typhoid, which killed 1.6 million people in 2017 (Dadonaite, Ritchie, & Roser, 2018). The Fez-Meknes region is a semi-arid region with a temperate winter. This type of climate is also compounded by the water stress that has been taking hold in Morocco for several years. Urban and peri-urban agriculture, located on the outskirts of the city and in contact with rural areas, uses surface water. The permanent presence of water in the wadis has allowed the intensification of agriculture through irrigation (market gardening, arboriculture, summer fodder and associated cattle and sheep breeding). Our objectives in this work, is to evaluate the contamination by microorganisms in urban waters used in irrigation which are the Oued Fez upstream and downstream of the STEP and well waters located in the urban area in comparison with the waters of the Oued Bitit located outside the urban and industrial areas. We also studied the transmission of contaminants in irrigated soil, cardoon and eggplant. The microorganisms studied were total and thermotolerant coliforms, vibrio cholera, salmonella and helminths. In addition, the effect of seasonality was studied in irrigation water and soil.

MATERIALS AND METHODS

Study area

Urban and pre-urban agriculture is located in the extremities of the city of Fez along the Oueds. For this study (Table 1 and Figure 1), we selected five plots, two of which are urban upstream of the Doukkarat industrial zone: a plot irrigated by well water and a plot irrigated by Oued Fes upstream. Two other pre-urban plots downstream of the industrial zone Ain Nokbi and the city of Fez: A plot irrigated by Oued Fez downstream before the meeting of Oued Fez with Oued Sebou and a plot irrigated by the TWW at the exit of the Water Treatment Plant (STEP). The fifth plot located in the rural area irrigated by surface water Ain Bitit. It is located in the province of El Hajeb at about

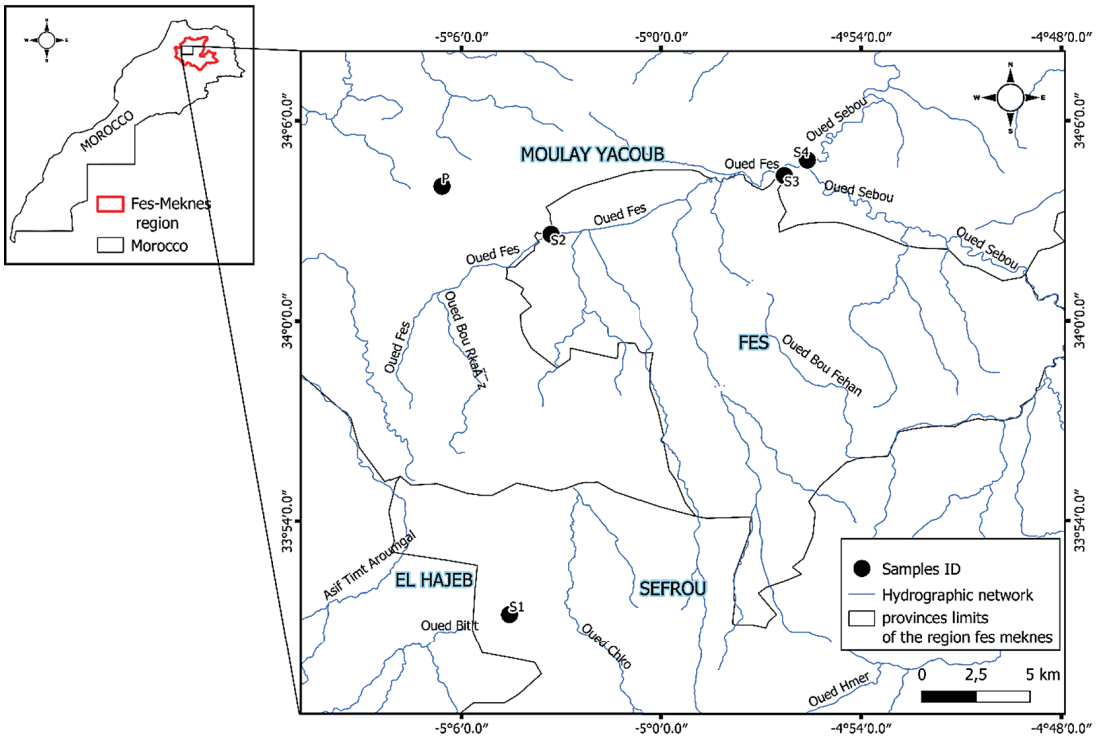

Figure 1. Location of study points on map, P: plot irrigated by well water; S1: plot irrigated by Oued Bitit water; S2: plot irrigated by Oued Fez water; S3: plot irrigated by Oued Fez downstream wastewater; S4: plot irrigated by treated wastewater

Table 1. Descriptions and location of sampling sites

Location	Name and address of stations	Geographical coordinates		Definition of the zones
		Latitude N	Longitude W	
P	Plot near Oued Fez and irrigated by well water	34°04'02.18"	-5°06'35.41"	Urban
S1	Plot located in the province of Sefrou irrigated by surface water from Ain Bitit	33°51'11.9"	5°04'33.9"	Agricultural/rural
S2	Plot irrigated by Oued Fez water located before the Doukkarat industrial zone	34°02'35.9"	5°03'19.3"	Urban
S3	Plot irrigated by Oued Fez water located before the industrial zone Ain Nokbi	34°04'21.1"	4°56'18.4"	Suburban/ agricultural
S4	Plot irrigated by TWW located after the WWTP before the Oued Sebou meeting	34°04'49.0"	4°55'37.0"	Suburban/ agricultural

24 km from the city of Fez. This plot is far from the city of Fez as well as urban and industrial pollution, unlike the above mentioned plots.

All five plots have the same irrigation system, which is surface gravity irrigation using a furrow system. The farmers irrigate their plots every 10 days when there is no rain.

Sampling and enumeration of bacteria and parasites in irrigation water, soil and plants

The biological and parasitological parameters studied are the following: Total coliforms, thermotolerant coliforms, salmonella, cholera vibrio and helminth eggs. We studied these parameters at three levels, irrigation water, soil and also on cardoon and eggplant. The analyses were carried out in the laboratory of microbiology and biotechnology of the Agronomic and Veterinary Institute (IAV) in Rabat.

Sampling method

In the winter season in February and the summer season in August 2020, water and soil samples were taken in the five plots at the same time and at the same period. For the plants (cardoon and eggplant) the samples were taken in summer in parallel with the water and soil. All samples were taken three times and all samples were carefully labeled and placed in a cooler with ice and transported immediately to the laboratory. Irrigation water – the volume of water used is about 500 ml per sample for bacteriological analysis. And about one liter for parasitological analysis. Using borosilicate glass vials, carefully washed with distilled water. The cleaned and rinsed vials were sterilized in an autoclave at 120°C and subjected to a pressure of 120 kg/cm^2 for 30 minutes. Irrigated soils – soil sampling consisted of systematic stainless steel auger sampling between 0 and 10 cm depth. We took 500 g for each sample in sterile bags. Each sample was a composite of three samples taken from three different locations spaced apart. Cardoon and eggplant irrigated – the sampling was done in such a way as to recover the majority of the leaves in contact with the soil and also the roots of both crops. These samples were taken using sterile gloves and randomly placed in cleaned bottles rinsed and sterilized in an autoclave at 120°C and subjected to a pressure of 120 kg/cm^2 for 30 minutes.

Parasite research – eggs of helminths

Collection and parasitological analysis of irrigation water

Samples collected were decanted for eight hours. Suction was used to remove the supernatant and the sediment was centrifuged at 4500 rpm for 5 minutes. The resulting pellet was submitted for analysis. The identification of helminth eggs is performed according to the experimental protocol of the Rodier technique (1996). The enumeration of the eggs is done via the Mac Master slide under a photonic microscope.

Soil sampling and parasitological analysis

A 100g sample of soil in sterile polyethylene bags was taken three times for each plot. In the laboratory, the soils were sieved to remove coarse particles. To 50g of sieved soil was added 300 ml of NaOH (0.01N). This mixture was stirred for one minute and then allowed to stand for five minutes. After these five minutes of rest, the mixture (soil+NaOH) was stirred again for one minute and then filtered through a tea strainer. To the recovered filtrate, 210 ml NaOH (0.01N) was added then centrifuged at 2000 rpm for 2 minutes. The supernatant was removed and the pellet was used for parasitological analysis according to the experimental protocol of Arther's technique (Arther et al., 1981). Using the Mac Master slide under a light microscope.

Collection and parasitological analysis of plants

The recovered plant parts are washed abundantly with distilled water. For the roots, the underlying soil was removed. This washing water is sieved and left to decant. After removal of the supernatant, the residues are subjected to centrifugation at 3500 rpm for 15 minutes. The pellet obtained is analyzed in the presence of an enrichment liquid which is sucrose at 1560g/l using the same technique mentioned above.

Total coliforms and thermo-tolerant coliforms

Analysis of TC and TTC in irrigation water

TC and TTC are marker microorganisms of fecal contamination. For the enumeration, we suspended 10 ml of sample in 90 ml of sterile 2% (v/v) trisodium citrate solution at 45°C for 3 min after homogenization with a Stomacher (Stomacher 400 lab blender, UK). Also we performed decimal dilutions in peptone water up to 10-7. Seeding was done in two petri dishes for each dilution and each culture medium. For enumeration we used Violet Red Bile Lactose Agar (VRBL, Oxoid CM0107). It contains 7g peptone; 3g yeast extract; 10g lactose; 5g sodium chloride; 1.5g bile salt mixture; 0.002g crystal violet; 0.03g neutral red; 15g agar and the pH is adjusted to 7.4. The TC medium is seeded and incubated at 37°C for 24 hours. For CTT the medium is placed in the dishes at 44°C for 24 and 48 hours.

Analysis of TC and CTT in irrigated soils and crops

A test sample of 10g of soil and 10g of the mixture of the two parts of the plant. These two elements are crushed with a grinder, is introduced into a sterile dilution bag (plastic bag) by adding aseptically 90 ml of buffered peptone water. Decimal dilutions (from 10-1 to 10-3) are then made from the stock solution, depending on the contamination status of the sample. Determination of the degree of contamination of cardoon and eggplant by irrigation water is done by the deep culture method, where 1 ml of the stock suspension and decimal dilutions are placed in sterile petri dishes (or tubes for RSC enumeration). Then, 15 ml of the previously melted culture medium is poured and mixed well with the inoculum in a Deoxycholate lactose agar (DLA) culture medium. Incubation of CT is performed at 37°C for 24 hours. For CTT the incubation is performed at 44°C for 24 and 48 hours. Coliforms are dark red colonies.

Cholera vibrio detection

Cholera vibrios are detected in three steps by enrichment on EPA medium (alkaline peptone water) and isolation on GNAB agar (Lebres, 2002). The reading is limited to the presence or absence of specific colonies, taking into account that vibrios are most often presented as smooth and transparent colonies. The search for cholera vibrios in irrigation water and soil and crop test 8g each crushed, consists of three steps (Moroccan Standard: No. 03.7.051): Enrichment, isolation and identification.

Research of Salmonella

The search for Salmonella is carried out in 4 steps by enrichment and isolation on Hektoen agar. The Hektoen agar plates will be read for the presence or absence of specific colonies, taking into account that Salmonella are most often found as blue-gray, blue-green colonies with or without a black center of very small size. The research of Salmonella for a test sample of 200 ml of irrigation water was carried out in accordance with the Moroccan standard: NM 03.7.050 of 1995. For the soil and plants, a test sample of 25 g of sole and 25 g of the mixture of cardoon and eggplant roots and leaves was tested for Salmonella according to the Moroccan standard NM 08.0.116.

Statistical analysis

The experiments used a completely randomized style with three replicates for each evaluation. The results were presented as the mean SE. Each analysis of variance was used to determine significant values (ANOVA). Fisher's minimum significant difference (LSD) test was used as a post hoc test for multiple comparisons at = 0.05.

RESULTS AND DISCUSSIONS

Evolution of pollution indicator germs in irrigation waters

Coliform and helminth egg contamination in irrigation water

According to WHO (1989) and Moroccan standards of water quality for irrigation, the required concentration of Coliforms must be less than or equal to 1000 CFU/100 ml. The average annual concentration of TC in irrigation water is

out of norms (Table 2) and this at the level of well water, water of Oued Fez upstream and downstream WW and also TME, the values are respectively 1.3×10^4; 6.9×10^5; 1×10^6; 11×10^6 CFU/100 ml Regarding the CTT, the values are less important compared to the CT and they are out of standards for irrigation waters mentioned above (Well waters, waters of Oued Fez in front and downstream and TWW) the average concentrations are respectively 6.5×10^3; 3.6×10^4; 31.5×10^4; 16.8×10^5. However, the water of oued Bitit used in irrigation is within the standards.

The results of the TWW show the highest concentrations of TC and TLC. This difference can be explained by the large quantity of water collected in the city of Fez which contains large quantities of faeces. The samples are more loaded with coliforms in dry periods than in rainy periods. This characteristic has also been noted by other authors (Coulibaly-Kalpy et al.,2017). Indeed, the contamination of these reservoirs was superimposable to that of urban WW (El Ouali Lalami et al., 2014). Thus, the source of contamination in the reservoir was driven by that of the wastewater.

Whether eggs or cysts of parasites according to the quality standards of irrigation water they must be absent. This requirement is met in winter and in the irrigation waters of Oued Bitit and Oued Fez upstream. On the other hand, the average annual number of helminth eggs in well water, Oued Fez downstream and TWW used for irrigation are largely higher than the WHO guidelines and Moroccan standards. Indeed, the WHO requires a concentration lower or equal to 1 egg/l. The highest numbers of helminth eggs are recorded in the TWW with an average of 254 eggs/l and the waters of Oued Fez downstream with a value of 41 eggs/l. These results are in line with the findings of El Ouali (2014). Parasitic helminth eggs in urban wastewater were associated with the number of inhabitants connected to the WWTP. In winter, the waters upstream of Oued Fez did not contain helminth eggs than in summer (Table 2). Due to their resistance and persistence in the environment, the health risk posed by helminth eggs when reusing wastewater in agriculture was significant (Derwich et al., 2008; Hamaidi-Cherguiet al., 2016). Water from upstream of the Fez city discharges partially met the criteria for water quality standards for irrigation. However, water from the areas downstream of the city and the confluence of Oued Fez and TWW was the most polluted with microbes and parasites. Consequently, farmers in the Oued Sebou watershed would be exposed to bacterial and parasitic diseases.

Pathogenic microorganisms in irrigation water

To ensure human health, no Salmonella should be detectable in 5 liters and no cholera vibrio contamination in 450 ml of irrigation water. In Table 3, in both summer and winter, Vibrio cholera was present in both TME sites and in the downstream Oued Fez water. During winter, C. vibrio was absent in the upstream Oued Fez WTs, but present downstream in the WW and TWA. These results are not consistent with those found in 2010, 2011 and 2012 in Oued Fez by El Ouali Lalami et al. (2014).

Furthermore, Salmonella was absent from all four sites, except TWA, during the winter (Table 3). Pathogenic bacteria of the genus Salmonella were not detected in the studies of Hamaidi-Chergui et al. (2016). While the results of Ndiaye, Mamadou Lamine, et al (2011) showed that 35% of irrigation water was contaminated with Salmonella spp between the two types of water used for irrigation (groundwater and wastewater). This absence has been reported in other wastewater studies, despite the presence of a high fecal bacterial load. Studies on the effect of irrigation methods on the level of contamination of vegetables showed that even for highly contaminated irrigation water, drip or furrow methods significantly reduced the risk of crop contamination (Hamaidi-Chergui et al., 2016; Ndiaye et al., 2011).

BACTERIOLOGICAL AND PARASITIC CONTAMINATION OF IRRIGATED SOILS

Coliform and helminth egg contamination in irrigated soils

The contribution of irrigation water polluted with microorganisms in the soil is a risk of contamination of the groundwater of irrigated crops and thus a threat to human health. The retention of bacteria in soils is done by attachment and filtration mechanisms. In many experiments, it has been observed that attachment is an important mechanism influencing bacterial retention in porous media. The constraint mechanism involves the physical blockage of movement through smaller pores than the bacteria (Stevik et al., 2004).

Table 2. Indicator microorganisms, total coliforms, thermo-tolerant and parasitic coliforms, helminth eggs in irrigation water

Location	Season	Total coliforms (UFC/100ml)	Thermo tolerant coliforms (UFC/100ml)	Helmintheggs (Œuf/l)
P	Wetseason	6971±105 [a]	3587±97 [a]	22±2.3 [a]
	Dry period	18487±321 [b]	9457±124 [b]	15±1.4 [b]
Annual M.V.		12729±289	6522±141	18.5±4.2
S1	Wetseason	284±24 [a]	108±25 [a]	0 [a]
	Dry period	710.33±87 [b]	558±57 [b]	13±2.1 [b]
Annual M.V.		497,17±125	333±68	6.5±0.05
S2	Wetseason	70063.33±457 [a]	2224.67±111 [a]	0 [a]
	Dry period	1300156.66±3052 [b]	71030±207 [b]	24.67±1.2 [b]
Annual M.V.		685110±808	36800±185	12.33±0.7
S3	Wetseason	1100110±645 [a]	480046.67±905 [a]	50±4.2 [a]
	Dry period	880083.33±709 [b]	150036.6667±754 [b]	32±1.8 [b]
Annual M.V.		990055±978	315041.67±987	41±2.3
S4	Wetseason	20000156.67±1059 [a]	2000046.67±2865 [a]	479.67±17.2 [a]
	Dry period	2400060±2584 [b]	1360066.67±1825 [b]	76±10.6 [b]
Annual M.V.		11200108.33±3587	1680056.67±10214	254.83±25.3

Note: Each number represents the average standard error of three replicates. Means in a column (within each group) followed by the same letter are not significantly different and means followed by different letters are significant at $P < 0.01$ according to the LSD test ($\alpha = 0.05$).

Table 3. Presence or absence of pathogenic microorganisms, *Salmonella* and *Vibrio cholerae* in irrigation water

Location	Season	*Cholera vibrio*	*Salmonella*
P	Wetseason	Absent	Absent
	Dry period	Presence	Absent
S1	Wetseason	Absent	Absent
	Dry period	Presence	Absent
S2	Wetseason	Absent	Absent
	Dry period	Presence	Absent
S3	Wetseason	Presence	Absent
	Dry period	Presence	Absent
S4	Wetseason	Presence	Presence
	Dry period	Presence	Absent

Our soil samples from the five irrigated plots showed a large difference in coliform levels in winter versus summer. Indeed, in summer, the maximum content of Coliforms found is 24×10^6 CFU/100 ml, while in winter, the maximum value is 15×10^2 CFU/100 ml. These two values are recorded in soils irrigated by TWW (Table 4). These results are also found in TWW in Isfahan, Iron (Farhadkhani et al., 2018)and Dakar, Senegal (Ndiayeet al., 2011) and also in Bangar El-Sokkar, Egipte (Elsokkary and Abukila, 2014). In decreasing order, the TME is the most loaded with fecal bacteria followed by Oued Fez water downstream then well water and the least polluted irrigation water is Oued Bitit water since it is not in the vicinity of the urban and industrial area. Most of the fecal coliform bacteria were retained in the first centimeters below the column inlet and the profile decreases exponentially with increasing depth (Farhadkhani et al., 2018). Regardless of the different microbial load of the irrigation water, the microbial count in all soils in this study was always above the recommended range (<1000 CFU/100 ml)(Mara and Cairncross, 1991). With the exception of irrigated soils not Oued Bitit.

Table 4 shows the load of Helminth Eggs in soils in winter is much higher than in summer. This was observed in Oujda by Dsouli (2006), the peak of helminth eggs values is in spring and

Table 4. Indicator microorganisms, total coliforms, thermo-tolerant and parasitic coliforms, helminth eggs in soil

Location	Season	Total coliforms (UFC/100 ml)	Thermo tolerant coliforms (UFC/100 ml)	Helmintheggs (Œuf/l)
P	Wetseason	420±64 [a]	146±24 [a]	440±47 [a]
	Dry period	9000000±3087 [b]	1400000±2187 [b]	64±11.2 [b]
Annual M.V.		4500210±2108	700073±1087	252±87
S1	Wetseason	200±70 [a]	78±27 [a]	52±3.8 [a]
	Dry period	723±67 [b]	573±97 [b]	6±2.9 [b]
Annual M.V.		461,5±97	325,5±38	29±19
S2	Wetseason	100±27 [a]	80±14 [a]	250±39 [a]
	Dry period	9950000±5887 [b]	654000±1094 [b]	4±3.8 [b]
Annual M.V.		4975050±998	327040±2554	127±57.2
S3	Wetseason	600±87 [a]	100±30 [a]	580±97.2 [a]
	Dry period	17000000±4588 [b]	5600000±12458 [b]	12±8.9 [b]
Annual M.V.		8500300±3254	2800050±8187	296±103
S4	Wetseason	1500±154 [a]	110±37 [a]	416±201 [a]
	Dry period	24000000±23087 [b]	3300000±10554 [b]	38±8.7 [b]
Annual M.V.		12000750±34145	1650055±3057	227±97.6

Note: Each number represents the average standard error of three replicates. Means in a column (within each group) followed by the same letter are not significantly different and means followed by different letters are significant at $P < 0.01$ according to the LSD test ($\alpha = 0.05$).

autumn while the minimum value was in summer. The maximum average value is 296 Eggs/l found in Oued Fez downstream (WW) followed by well water with a value of 252 eggs/l. This high content of helminth eggs in the well water can be explained by the contamination of the groundwater in the vicinity of Oued Fez upstream and also in the urban area.

Pathogenic microorganisms in irrigated soils

In Table 5, the soils irrigated with different types of water from the five plots shows that no Salmonella bacteria were detected. This is in agreement with the studies done by Farhadkhani (2018). In contrast, Cholera Vibrio was well present in the summer period in the soils irrigated by TWW and WW (Oued Fez downstream). This contamination is also detected by this pathogenic bacteria in soils according to the study made by Cui (2019). Several factors can influence the microbial load of soils and crops irrigated by wastewater and the associated health risks. Ambient temperature and humidity, ultraviolet radiation rate, soil moisture and pH, antagonism with native soil microorganisms, irrigation method, and finally the type of plant could impact the fate and population of microorganisms in the soil and on crop surfaces (Becerra-Castro et al., 2015; WHO, 2006).

Table 5. Presence or absence of pathogenic microorganisms, *Salmonella* and *Vibrio cholerae* in soil

Location	Season	*Cholera vibrio*	*Salmonella*
P	Wetseason	Absent	Absent
	Dry period	Absent	Absent
S1	Wetseason	Absent	Absent
	Dry period	Absent	Absent
S2	Wetseason	Absent	Absent
	Dry period	Presence	Absent
S3	Wetseason	Absent	Absent
	Dry period	Presence	Absent
S4	Wetseason	Absent	Absent
	Dry period	Presence	Absent

BACTERIOLOGICAL AND PARASITIC CONTAMINATION OF CARDOON AND EGGPLANT

Coliform and helminth egg contamination in crops

Foodborne diseases caused by consumption of contaminated products are a major concern when using irrigation by WW (Bahri et al., 2009; WHO, 2006; Becerra-Castro et al., 2015). The results of fecal and parasitic contamination are shown in Table 6. Cardoon culture (Figure 2) – our results showed a fecal contamination of cardoon by TCs and CTTs under WW irrigation (Oued Fez downstream): for TCs: 2.8×10^6 CFU/100 ml and CTTs of 2×10^6 CFU/100 ml. The cardoon irrigated by the waters of Oued Fez upstream a contamination by CT of 4×10^4 UFC/100 ml and the CTT of 3.7×103 UFC/100 ml. As for the cardoon irrigated by well water, the CT are 7.6×10^3 CFU/100 ml and the CTT of 4.8×10^4 CFU/100 ml. This same plant irrigated by TWW contamination by TC is 1.5×10^3 CFU/100 ml and by CTT is 7.5×10^2 CFU/100 ml and this plant under the irrigation of Oued Bitit waters with the lowest concentrations of TC: 7.1×10^2 CFU/100 ml and CTT: 5.4×10^2 CFU/100 ml. These data explain that the most contaminated cardoon is irrigated by the waters of Oued Fes downstream and upstream. In comparison with studies done on lettuce in Senegal (Ndiaye et al., 2011) the lettuce was also contaminated following WW irrigation. Environmental conditions, as well as contact with the type of irrigation water and irrigated soil, have a significant impact on the level of fecal contamination of irrigated crops (Forslund et al., 2012).

Eggplant crop (Figure 3) – the concentrations of CT, CTT are less important compared to cardoon. The maximum concentration is recorded in eggplants irrigated by WW with values of 3.6×10^5 CFU/100 ml for CT and 2.2×10^5 CFU/100 ml for CTT. In decreasing order of contamination of eggplants after those irrigated by WW, there are then eggplants irrigated by water from Oued Fez upstream, then those irrigated by well water, then those irrigated by TWW and finally those irrigated by water from Oued Bitit. The same observation was made in comparison with the study by Orlofsky et al (2016) which showed no significant difference in the detection of fecal indicator bacteria in tomatoes irrigated with treated wastewater compared to tomatoes irrigated with drinking water.

As far as helminth eggs are concerned, they are absent in the roots and leaves of eggplant. Only the cardoon which has a very low number under the irrigation of WW and TWW 3 and 6 Œuf/l respectively. This low content and even absence of helminth eggs can be explained on the one hand by the fact that the leaves of cardoon and especially eggplant grow far from the soil and on the other hand by the exposure to the effects of solar radiation and desiccation and wind. These effects are also mentioned by Dsouli (2006).

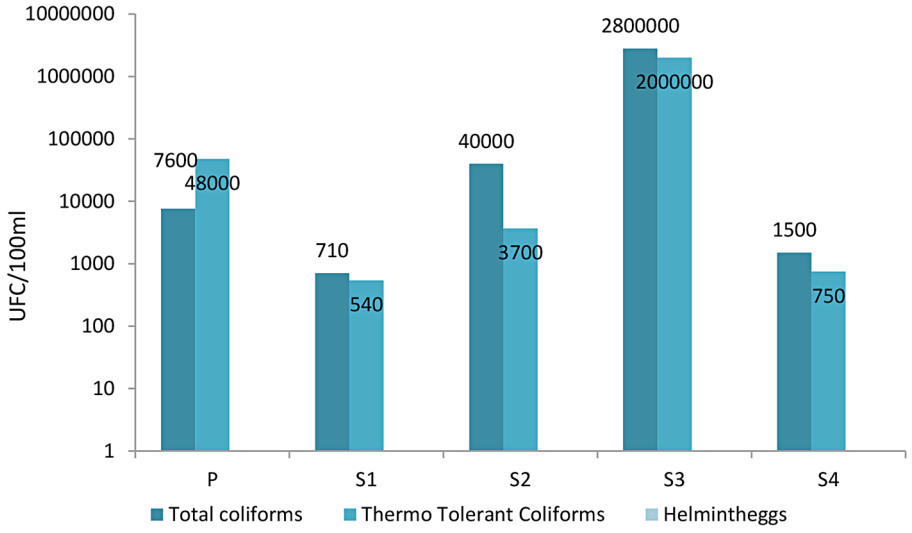

Figure 2. Concentration of Total and Termotolerous coliforms in CFU/100ml found in the cardoon crop grown in the five plots: P – plot irrigated by well water; S1 – plot irrigated by Oued Bitit water; S2 – plot irrigated by Oued Fez water; S3 – plot irrigated by Oued Fez downstream wastewater; S4 – plot irrigated by treated wastewater

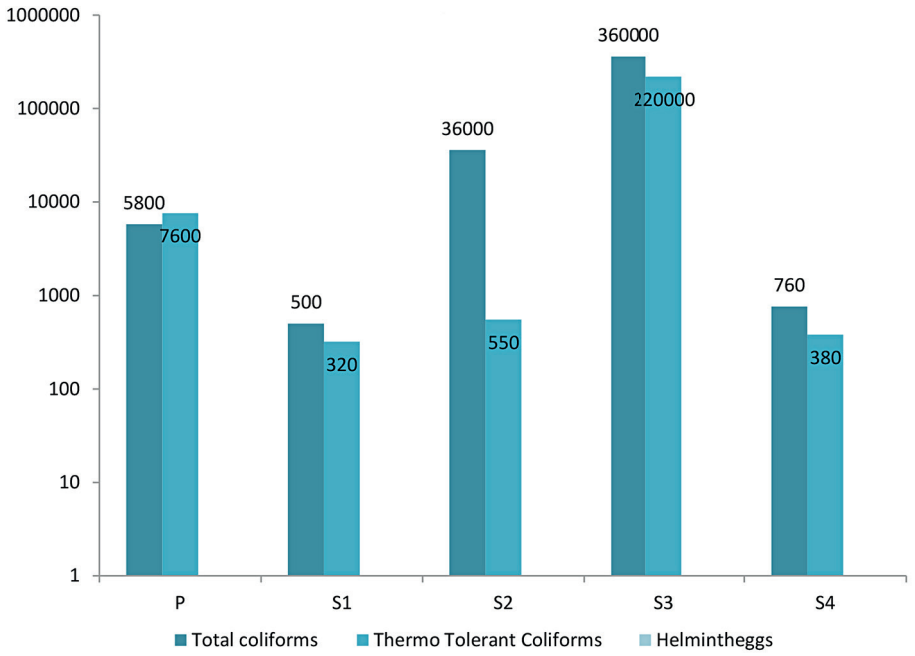

Figure 3. Concentration of total and thermotolerant coliforms in CFU/100ml found in the eggplant crop grown in the five plots: P – plot irrigated by well water; S1 – plot irrigated by Oued Bitit water; S2 – plot irrigated by Oued Fez water; S3 – plot irrigated by Oued Fez wastewater downstream; S4 – plot irrigated by treated wastewater

Table 6. Presence or absence of pathogenic microorganisms, Salmonella and Vibrio cholerae in cardoon

Location		*Cholera vibrio*	*Salmonella*
P	Aubergine	Absent	Absent
	Cardon	Absent	Absent
S1	Aubergine	Absent	Absent
	Cardon	Absent	Absent
S2	Aubergine	Presence	Absent
	Cardon	Presence	Absent
S3	Aubergine	Presence	Absent
	Cardon	Presence	Absent
S4	Aubergine	Presence	Absent
	Cardon	Presence	Absent

Pathogenic microorganisms in cardoon and eggplant

The last three plots located downstream of the city of Fez which are irrigated by water from Oued Fez upstream and downstream WW and TWW, the crops are contaminated by Vibrio cholerae (Table 6). Like the soil, the crops studied also show no Salmonella contamination in the five plots (Table 6). In contrast to the studies done on lettuce, the level of contamination in lettuce was higher, which may be related to the higher holding capacity of the irrigation water and thus a higher potential for contamination (WHO, 2006; Mok et al., 2014). According to Christouet al., 2014, 2016; Cirelli et al., 2012; Urbano et al., 2017 TWW do not pose any microbial risk to consumers. However, the climatic conditions, irrigation method, and type of plant are crucial to ensure healthy consumption (Becerra Castro et al., 2015; Fonseca et al., 2011; Farhadkhani et al., 2018).

CONCLUSIONS

The microbial quality of WW and TWW as well as the water of Oued Fes upstream and well water used in irrigation can cause a public health problem for crops consumed raw and especially in summer period. Indeed, our results showed a

contamination by coliforms outside the WHO standards in the studied irrigation waters except for the Oued Bitit waters affecting the irrigated soils and the cardoons cultivated in the four plots.

Regarding helminth eggs, certainly in winter, the number of parasitic eggs is more numerous in the soils compared to the irrigation waters following the accumulation effect of these parasites but the cardoons did not retain these eggs. For Salmonella, this pathogenic bacterium was indeed present only in the TWW in summer but our results confirm its absence in the soil and plants. Contrary to Cholera Vibrio, in addition to its presence in irrigation waters studied except for Oued Bitit waters, it is also present in the soils irrigated by Oued Fes waters upstream and downstream WW and TWW and it was transmitted to cardoons and eggplants irrigated by these waters.

For a better protection of public health and for a reduction of potential risks it is therefore necessary to apply control measures in the field and after harvest to reduce microorganisms, including drip irrigation which is a more efficient method to reduce microorganisms in the soil and on the surface of crops as well as washing, disinfection and peeling of raw consumed products.

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