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Microbial Contamination of Vegetables Produced at Smallholdings in the Urban and Peri-Urban Area of Meknes City, Morocco

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ABSTRACT

The use of wastewater for irrigation is common in urban and peri-urban farms in Meknes city (Morocco). However, this water may contain waterborne pathogens that cause serious infections, including gastroenteritis, typhoid, cholera, and more severe pathologies. The consumption of contaminated vegetables could be a significant public health problem. This study aims to determine the bacterial and parasitic quality of vegetables produced in wastewater-irrigated farms in urban and peri urban areas of Meknes. The results showed a high bacterial load of vegetables. Total coliforms ranged from $1.2.10^2$ CFU/g to 10^5 CFU/g, The consumption of contaminated vegetables could be a significant public health problem. This study aims to determine the bacterial and parasitic quality of vegetables produced in wastewater-irrigated farms in urban and peri urban areas of Meknes. The results showed a high bacterial load of vegetables. Total coliforms ranged from $1.2.10^2$ CFU/g to 10^5 CFU/g, and fecal coliforms ranged from 10 CFU/g to $3.18.10^4$ CFU/g. Many bacterial pathogenic species have been isolated, including *Escherichia Coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Clostridium spp*. For parasite contamination, helminths eggs were the most prevalent, followed by protozoan cysts, *Cryptosporidium parvum* and *Giardia Lamblia*. Of all the vegetables examined, lettuce was the most contaminated. Therefore, the handling and consumption of irrigated vegetables can present a high risk of infection among farmers and consumers.

Key words: Contamination, Waterborne Pathogens, Parasitic Contamination, Irrigation, Wastewater, Vegetables.

INTRODUCTION

Rapid population growth, improved living conditions, and rapid economic and industrial development have led to a striking increase in water demand. With the pollutant releases and the intermittent droughts, conventional water resources have been severely depleted. To overcome this lack of water, farmers in arid areas have resorted to uncontrolled irrigation by untreated wastewater. This practice supports livelihoods and can help to generate an income by providing the essential nutrients and organic matter, therefore, saving water and nutrients (Asano & Levine, 1996). However, health problems associated with the consumption of crops irrigated with wastewater remain the most concerning as this water contains a wide range of pathogens that can cause multiple diseases (Scott et al., 2000). As a result of this practice, fresh vegetables present a potential risk of microbial contamination (Taban & Halkman, 2011). It may occur before, during, or after irrigation. Or through the inhalation of aerosols in wastewater (Feachem et al., 1983). It can also happen before, during, or after harvest, transport, and distribution (Beuchat, 2006; Maffei et al., 2016). Which can be a threat to the health of farmworkers, crop handlers and consumers (Feenstra et al., 2000). Foodborne diseases outbreaks associated to fresh vegetables have led to concerns about contamination of vegetables with fecal pathogens in the agricultural environment (Tauxe et al., 1997).

In Morocco, the use of polluted water whether it is surface water or untreated wastewater for irrigation is common in many regions. Some studies have shown that

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wastewater irrigation has contributed in vegetables contamination with a wide range of pathogens (Ibenyassine et al., 2007; Hajjami et al., 2013).

In Meknes, wastewater is used directly from a sewage outlet or mixed with surface water (Dugué et al., 2015). All types of produce can be found in those farms. Horticulture crops are the most produced in those farms, and easily sold at the local markets. Therefore, this study aim to study the bacteriological and parasitic quality of some fresh vegetables produced in urban and periurban farms of Meknes.

MATERIALS AND METHODS

Presentation of Study Area

The city of Meknes is located in the northwest of Morocco, 140 km east of the administrative capital Rabat and 60 km west of the spiritual capital Fez. Its geographical coordinates are: Longitude: 5° 33', Latitude: 33° 52' and Altitude: 530 m (Figure 1). Meknes prefecture counts a 835 695 inhabitants about 19.7% of Fez-Meknes region and 2.5% of the total population of Morocco.

Meknes prefecture is characterized by a semi-continental Mediterranean climate. Meknes has an average annual temperature of 17.2°C. Precipitation averages 576 mm/year. December is the rainiest month with average precipitation of 65.2 mm, while the driest month is July with an average of 1.6 mm.

This city is known for its agricultural wealth. The urban and peri-urban agriculture covers a total area of 31897 ha, of which 3557 ha are in urban area. The importance of this activity stems from its multiple economic, social, and environmental benefits. But this practice is well known for its polluting practices, including irrigation with wastewater. This is facilitated by the degradation of conventional water resources.

Sampling & Analysis

The study area is located at urban and peri-urban agricultural area of Meknes city. Irrigation is mainly based on surface water, and wastewater of various origins (Dugué et al. 2015). The sampling was carried out randomly at various urban and peri-urban farms of the city of Meknes. Samples include irrigation water: surface water (10 samples) and wastewater (7 samples), irrigated vegetables (48 samples) and crop soil samples (11 samples) that were taken from pits 1.2 m and 1 m deep from the same location in the field as the vegetable samples. Three types of vegetables were chosen for this study lettuce (*Lactuca sativa*), beet (*Beta vulgaris*) and radish (*Raphanus sativus*). Only the edible part of the plant was examined. Samples were collected aseptically and transported to the laboratory for analysis within 48 hours.

Bacteriological Testing

Water Analyses

Bacteriological analyses were carried out using the membrane filtration method (cellulosic membrane 0.45 µm in diameter) using selective media based on standard methods described in (Rodier et al., 2009). All water samples had undergone serial dilutions prior to filtration. Total and fecal coliforms were determined using Lactose TTC Agar with Tergitol-7 media, one incubated for 48h at 37 °C and the other at 44 °C. Confirmation was done using an oxidase test and gram stain. For the identification of the *Escherichia Coli*, indole test was performed. Clostridium spp was isolated using Tryptone Sulfite

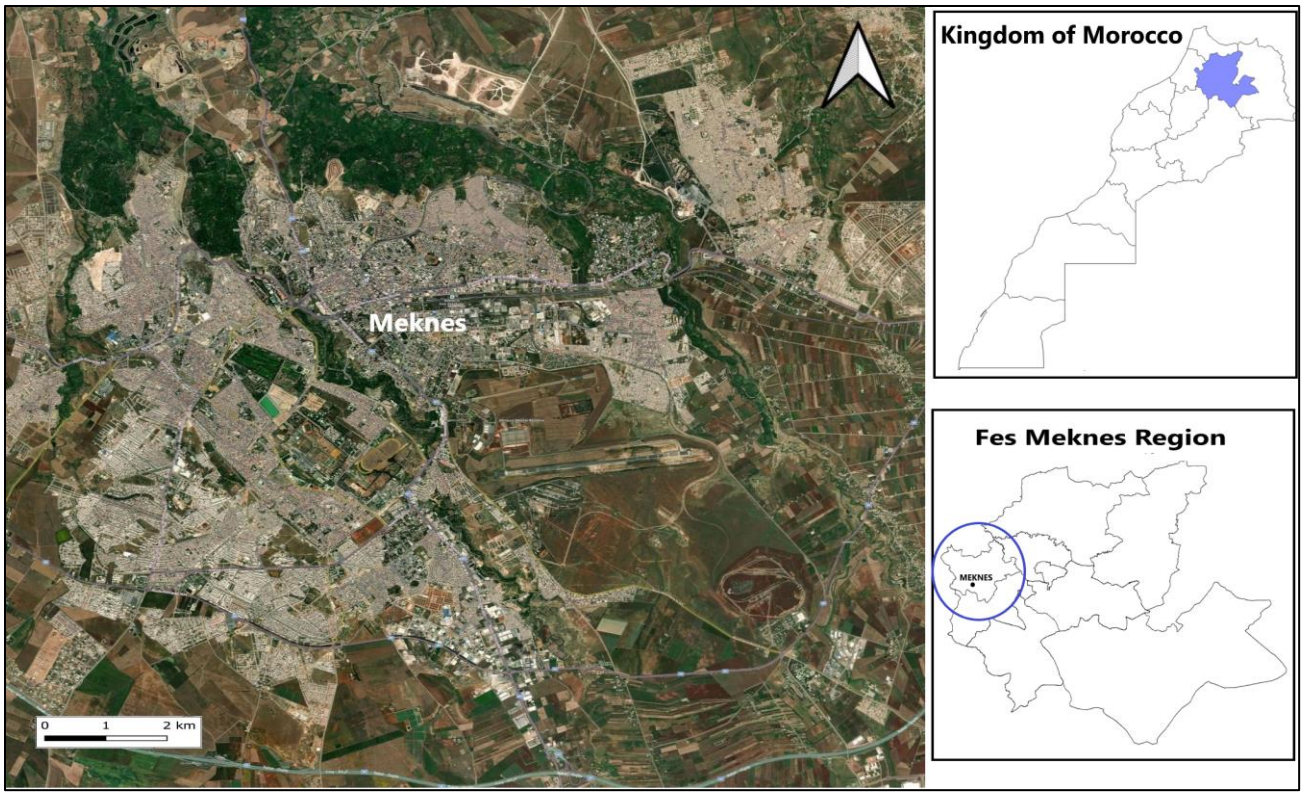

Fig. 1: Map of satellite view of the study area (city of Meknes).

Table 1: Standard Water Analysis Method

Microorganism	Standard Methods
Coliforms	ISO 9308-1
Escherichia Coli	ISO 9308-1
Staphylococcu aureus	NM 03.7.036
Pseudomonas aeruginosa	ISO 16266
Clostridium spp.	ISO 6461-2

Table 2: Standard Food Analyses Method

Microorganism	Moroccan standard method	International Standards Reference Method
Total coliform	NM 08.0.1.142	NFV 08-050
Fecal coliform	NM 08.0.124	NFV 08-060
Escherichia Coli	NM 08.0.164	ISO 16649-2
Staphylococcus aureus	NM 08.0.112	NF V 08-057-1
Pseudomonas aeruginosa	NM 08.6.104	ISO 13720
Clostridium spp	NM 08.0.105	ISO 7937

Cycloserine Agar plus 1ml D-cycloserine incubated at 37 °C in an anaerobic jar for 24h. Pseudomonas aeruginosa was identified using medium with cetrimide and nalidixic acid for 48h at 36°C. The confirmation was done through gram stain, motility test, and the search of Pyocyanin in King A medium at 30 °C for 24-48h. The search for *Staphylococcu aureus* was performed using CHAPMAN - Mannitol Salt Agar medium, incubated for 24h at 37°C. Gram stain and catalase test were performed for confirmation.

Vegetables and Soil Analyses

Vegetables and soil were analyzed based on the standards methods described in (Da Silva et al., 2018). Twenty five gram of each sample added in 225 ml of Buffered Peptone Water and homogenized with a stomacher. Three decimal dilutions homogenate for each sample were prepared. Total and fecal coliforms were counted using Red Bile Lactose Agar Violet and then incubated respectively at 30°C and 44°C for 24h. *Escherichia Coli* was isolated using Tryptone Bile X-Glucuronide medium and incubated at 44°C for 21h. The search for *Staphylococcus aureus* was done by incorporation in a Baird-Parker medium and incubation at 37°C for 24h-48h. The suspect colonies (a typical black with a clear halo) were transplanted into the brain heart infusion broth tubes at 37 °C for 24h. The coagulase test was performed for the confirmation with coagulase plasma with EDTA for 37 °C. *Pseudomonas aeruginosa* were identified using cetrimide agar and incubated at 25°C for 44h. Gram stain, as well as motility, oxidase, and catalase test were performed for confirmation. *Clostridium spp* are isolated by incorporation into the Tryptone Sulfite Cyclo-serine medium, incubated at 37°C for 24h in an anaerobic jar.

Parasitic Testing

The parasitic examination was done as described in a paper of Ayres et al. (1996). Vegetables and soil samples were put in physiological saline solution (0.95% NaCl) in a plastic bag and allowed to sediment for 12 hours. Irrigation Water samples were also left for about 12 hours for sedimentation. After sedimentation, supernatant was removed using a siphon. The sediment was centrifuged at 1000 g for 15 min. After removing the supernatant, the pellet was suspended in an equal volume of acetoacetic buffer pH 4.5. Samples were centrifuged at 1000g for 15 min again. Two volumes of ether are added, and the sample mixed for 10 min before being again centrifuged for 15 minutes at 1000g. After obtaining three distinct phases. Resuspend the pellet in zinc sulfate solution. The sample was mixed thoroughly. The aliquot was transferred in the slide for the final examination under microscope (magnification×100, ×400).

Statistical Analyses

The data were processed using Microsoft Excel Office 2010. The t-test was performed to determine the significant difference mean loads of total coliform and fecal coliform between different type of irrigation water and vegetables. The chi-square analysis ($\chi 2$) was used to determine the association between the pathogens contamination rate and vegetables samples. $p<0.05$ was considered statistically significant.

RESULTS

Bacterial Contamination

Total Coliform and Fecal Coliform Count

The analysis of coliform count of irrigation water showed a high contamination (Table 3). The total and fecal coliform loads for wastewater were $2.24.10^8$ CFU/100ml and 6.10^6 CFU/100ml respectively. For stream waters, total and fecal coliform load averaged $3.23.10^7$ CFU/100ml and $2.7.10^6$ CFU/100ml, respectively. There was no significant difference between wastewater and stream waters in terms of coliform load ($P>0.05$).

Table 3: Coliform Count of Irrigation Water and Soil

Samples		Total coliform	Fecal coliform
Stream water	Mean	$3.23.10^7$	2.1910^6
(CFU/100ml)	Max	$6.70.10^7$	$7.10.10^6$
	Min	$3.20.10^6$	$6.10.10^4$
Wastewater	Mean	$2.24.10^8$	$6.06.10^6$
(CFU/100ml)	Max	$6.7.10^7$	$7.1.10^6$
	Min	$3.2.10^6$	$6.1.10^4$
Soil (CFU/g)	Mean	$5.99.10^3$	$6.82.10^2$
	Max	2.10^4	3.10^3
	Min	3.10	1.10
Lettuce	Mean	$4.99.10^4$	$5.78.10^3$
(CFU/g)	Max	$1.00.10^5$	$3.18.10^4$
	Min	$1.14.10^3$	$1.71.10^2$
Radish (CFU/g)	Mean	$8.10.10^3$	$1.40.10^3$
	Max	$5.13.10^4$	$8.44.10^3$
	Min	$1.2.10^2$	10
Beets (CFU/g)	Mean	$8.86.10^3$	$1.53.10^3$
	Max	$5.14.10^4$	$7.50.10^3$
	Min	7.10	3.9.10

Vegetables produced in wastewater irrigated farms (Figure 2) showed a high microbial load (Table 4). Lettuce had the highest value of total coliforms with an average of $4,99.10^4$cfu/g, followed by Beets with an average of $8,86.10^3$ CFU/g and finally radish with an average of $8,10.10^3$ CFU/g. For fecal coliform, lettuce had the highest value with an average of $2.78.10^4$ CFU/g, followed by radishes with an average of $3.6.10^2$ and finally beets with an average of $3.11.10^3$ CFU/g. The coliform count of lettuce was significantly higher than

radish and beets (P>0.05). However they were no significant differences between the coliform total count of beets and radish (P<0.05).

Bacteria Isolated

Four bacteria were examined: *Pseudomonas aeruginosa*, *Clostridium spp*, *Escherichia coli*, and *Staphylococcus aureus* (Table 5).

Fig. 2: Irrigation of market garden crops with raw wastewater in the city of Meknes.

Irrigation water was the most contaminated of all samples. *Escherichia coli*, *Clostridium spp*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were found in all samples (stream water and wastewater).

For soil samples, *Staphylococcus aureus* was the least detected bacteria (45%). *Pseudomonas aeruginosa*, *Escherichia coli*, and *Clostridium spp* were found in almost all soil samples.

For vegetables, *Pseudomonas aeruginosa* was detected in all samples, followed by *Escherichia coli* (67%) and *Clostridium spp* (58%). Staphylococcus was the least detected (31%) of all samples.

The assessment of bacterial contamination rate (Figure 3) among vegetables showed that all lettuce samples were contaminated with *Pseudomonas aeruginosa*, (87.5%) of lettuce samples were contaminated with *Escherichia coli*, (81.0%) were contaminated with *Clostridium spp.* and (50.0%) were contaminated with *Staphylococcus aureus*. For radish (56.0%) of samples were contaminated with *Escherichia coli*. (37.5%) were contaminated with *Clostridium spp*, (25.0%) were contaminated with *Staphylococcus aureus* and all samples were positive for *Pseudomonas aeruginosa*. For beets, 56.0% of samples were contaminated with *Escherichia coli* and *Clostridium spp*, 19.0 % were contaminated with *Staphylococcus aureus* and *Pseudomonas aeruginosa* was detected in all samples. The prevalence of *Escherichia coli* and *Staphylococcus aureus* did not show any significant difference between types of vegetables (X^2= 4.68; p=0.09) and (X^2= 4.07; p=0.13) respectively. Yet, the prevalence of *Clostridium spp.* differs significantly between the different types of vegetables (X^2= 6, 31; p= 0,042).

Table 4: Prevalence of Pathogen Bacteria in Urban and Periurban Farms

Samples	*Escherichia coli*	*Clostridium spp*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
Irrigation water n= 17	17	17	17	17
Soil n= 11	10	10	11	5
Vegetables n= 48	32	28	48	15

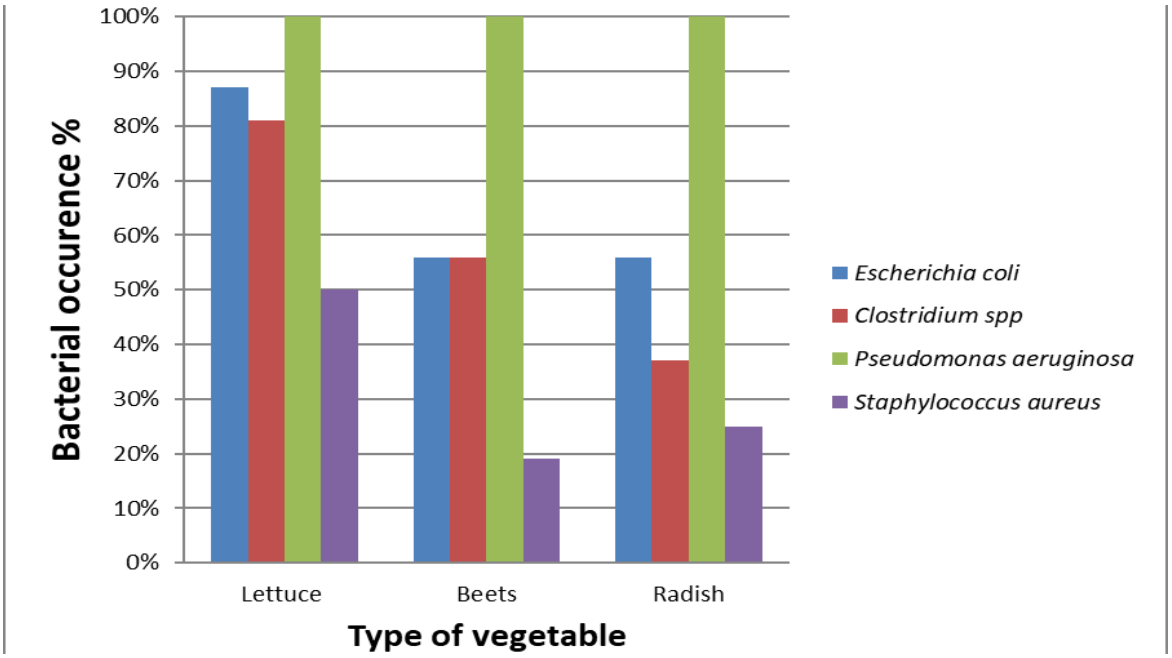

Fig. 3: The rate of bacteriological contamination of each vegetable.

Table 6. Prevalence of intestinal parasite in urban and periurban farms

Samples	Helminths	*Giardia spp*	*Crystoporidum spp*
Irrigation water n= 17	17	17	17
Soil n= 11	11	6	6
Vegetables n= 48	37	18	13

Parasitic Contamination

Intestinal parasites were prevalent in urban and periurban farms in Meknes (Table 6). All water samples (stream water and wastewater) were contaminated with Helminthes eggs, *Giardia cysts*, and *Cryptosporidium cysts*. Helminthes eggs were found in all soil samples and 77% of vegetable samples. *Giardia spp* was detected in 54% of soil samples and 18% of vegetable samples. *Cryptosporidium spp* was detected in 54% of soil samples and 27% of vegetable samples.

The assessment of the parasitic contamination rate among vegetables showed (Figure 4) that lettuce is the most contaminated; Helminthes eggs (87.5%) were the most prevalent group, followed by *Giardia* cysts (56.0%) and *Cryptosporidium* cysts (45.0%). For beets, 75.0 % of samples were contaminated with helminthes eggs, and 25.0% were contaminated with *Giardia* cysts and *Cryptosporidium* cysts. As for Radish, 68.0 % of samples were contaminated with helminthes eggs, 31.0% were contaminated with *Giardia* cysts, and 12.5% were contaminated with *Cryptosporidium* cysts. The parasitic prevalence did not show any significant difference between vegetable groups (P>0.05).

DISCUSSION

Using polluted water for irrigation can be a vector for pathogen transmission. It can be transferred to plants by irrigation water and survive for several days on the external and internal parts of the plant (Islam et al., 2004). In our study, the irrigation water analysis showed significant contamination, and total and fecal coliform was very high, exceeding the recommended limits set by the World Health Organization and Moroccan irrigation standards (CF>10^3 CFU/100ml). *Pseudomonas aeruginous*, *Escherichia coli*, *Clostridium spp*, and *Staphylococcus aureus*, add to this, intestinal parasites, namely helminthes eggs, *Giardia sp*, and *Cryptosporidium* were detected in all irrigation water samples. The quality of irrigation water and the type of irrigation system affect the microbial quality of fresh products (Brackett, 1999). Several studies have shown the ability of irrigation water to be the source of microbial contamination of vegetables (Solomon et al., 2002; Anh et al., 2007; Kayombo & Mayo, 2018; Al-Gamal et al., 2019). This contamination can also pose an infection risk for farmers, crop handlers due to prolonged contact and overall public health. Several studies (Cifuentes et al., 2000; Blumenthal et al., 2001; Ensink et al., 2005; Ensink et al., 2008), have shown that farm workers and their families associated with wastewater are more likely to get intestinal parasitic infections and diarrheal diseases. Also in Morocco, many studies (Melloul et al., 2002; Amahmid & Bouhoum, 2005; El Kettani & Azzouzi, 2006; El Kettani et al., 2008) have shown that people living near wastewater irrigated areas are more exposed to pathogens. In fact a study carried out in 2006 (El Kettani et al., 2008), has shown that the prevalence of intestinal parasitosis was high among people exposed to wastewater irrigation.

Assessment of soil showed a high prevalence of pathogenic bacteria and parasites. This could be due to direct transfer of pathogens from wastewater to soil which may eventually transferred into the vegetables (Weldezgina & Muleta, 2016).

The analysis of coliform loads and the prevalence of pathogenic bacteria and parasites among vegetables showed that lettuce is the most contaminated. These results are consistent with those of Gupta et al. (2009) and Akoachere et al. (2018). Lettuce leaves have a large surface area that is more exposed to irrigation water and soil making it more susceptible to contamination than other vegetables (Akoachere et al., 2018). However, the study carried out by Hajjami et al. (2013) in Morocco showed that radish was the most contaminated by helminthes. Lettuce and radish are the most concerning since they are eaten raw. Consumption of these contaminated vegetables can be considered a public health threat.

Conclusion

Our results show that water used for irrigation in urban and periurban farms of Meknes is unsuitable for agricultural use, as it contains many pathogenic bacteria and parasites. Pathogens accumulate in soil and can be transferred to the crops. The vegetables produced in those farms are contaminated as well, which can be a potential cause of disease transmission. Unfortunately, this practice can be a threat to public health. The authorities, farmers, retailers, and consumers must work together to reduce the negative effects and consolidate the positive aspects of urban farming, elaborating wastewater guidelines that balance health protection and farmers' livelihoods, and a food supply.

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