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Quantitative Microbial Risk Assessment of Urban Wastewater Pertaining to Irrigation of Agricultural Products in Qom, Iran, in 2020

Ahmad Reza Yari [1], Yadollah Ghafuri [1*], Rahim Aali [1]

[1] Research Center for Environmental Pollutants, Qom University of Medical Sciences, Qom, Iran.

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***Corresponding Author:**
Yadollah.Ghafuri

Email:
yadollahghafuri@yahoo.com

Tel:
+98 25 37833362

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ABSTRACT

Introduction: The present study aims to evaluate adverse health effects caused by the use of wastewater for the irrigation of fields in Qom province, Iran.

Materials and Methods: An environmental monitoring program was designed for 3 pathogens-*Escherichia coli*, *Vibrio cholerae*, and *E. coli* O157 and carried out on 120 samples from raw wastewater, effluent, and irrigated products with wastewater. In the next phase, exposure assessment and microbial risk assessment were performed using a questionnaire and interviewing 200 participants.

Results: Concentrations of *E. coli*, *V. Cholerae*, and *E. coli* O157:H7 in raw wastewater were determined to be $3.4 \times 10^3 \pm 500$ cfu/100ml, $2.1 \times 10^3 \pm 100$ cfu/100ml, and 312 cfu/100ml, respectively. Concentrations of *E. coli*, *V. Cholerae*, and *E. coli* O157:H7 in effluent were determined to be $2.1 \times 10^3 \pm 100$ cfu/100ml, $0.8 \times 10^3 \pm 100$ cfu/100ml, and 176 cfu/100ml, respectively. The conventional wastewater treatment system was effective in removing *E. coli*, *V. Cholerae*, and *E. coli* O157: H7 by 50%, 59%, and 43%, respectively. Crops irrigated with effluent contained 400 ± 250 cfu/100ml, $0.1 \times 10^3 \pm 0.019$ cfu/100ml, and 52 cfu/100ml of *E. coli*, *V. Cholerae*, and *E. coli* O157:H7, respectively. According to the exposure scenarios, the total annual probability of infection in the studied population for *E. coli*, *V. Cholerae*, and *E. coli* O157:H7 was determined to be 8×10^{-2}, 8×10^{-4}, and 17×10^{-2}, respectively.

Conclusions: In irrigating agricultural crops with wastewater implementing wastewater safety plans (WWSP) is crucial.

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Introduction

Lack of water resources for agriculture and its aggravation due to drought, population growth, and increasing fresh water needs in the drinking water sector and irrigation for food production are the main factors that have had an impact on the expansion of wastewater use[1]. Availability of wastewater for farmers due to insufficient development of wastewater collection and treatment facilities and the presence of nutrients in wastewater have also led to the use of treated wastewater [2]. Due to the presence of organic matter and nutrients required by the plant, the use of treated municipal wastewater can increase crop yield; however, due to a wide range of pathogens such as coliform bacteria and heavy metals, it is dangerous to people's health [3,4]. Numerous studies have been conducted on the health effects of the use of wastewater in irrigation of agricultural products. According to the 2010 report of the international development research center on various risks related to wastewater use and agricultural effluent in developed countries, spreading fecal pathogens such as bacteria (*E. coli*, Vibrio cholerea, Salmonella, and Shigella), worms (soil-borne ascaris and tapeworm and water sources including schistosomia), parasites (Giardia, Cryptosporidium and Entamoeba), and viruses (hepatitis A and E, adenovirus and norovirus) has been warned [5]. The use of the microbial risk assessment to make decisions about the use of treated wastewater has been recently expanded. Shi, Wang, and Jiang studied public health risks for using gray water by means of quantitative microbial risk assessment (QMRA). They reported that gray water could be safely used after the treatment of gray water using a microfiltration. One of the important functions of microbial risk assessment is to generate data for the investigation of fat and transport of bacterial pathogens and other microbial indicators used for health effects in wastewater safety plans (WWSP). Smeets et al. reported that quantitative microbial risk assessment (QMRA) helps to prepare adequate corrective action in the stages of WWSP [6-8]

In addition to the significance of the explanations offered in regard to the issue of microbial risk assessment, one of the crucial aspects of this study is the focus on emerging waterborne bacterial pathogens, including pathogenic *E. coli* (O157:H7) and *V. Cholerae*. Accordingly, this study was designed and implemented due to the need to evaluate the consequences of adverse health effects caused by the use of wastewater for the irrigation of fields in Qom province. This research also aims to develop a plan for continuous monitoring of microbial pathogens in wastewater used for the irrigation of agricultural crops.

Materials and Methods

Study site

In Qom province, which is located in the arid regions of the central desert of Iran, the annual rainfall is much lower than the national average. The reception of wastewater treatment plants in irrigation of products has become inevitable due to uncontrolled abstraction of water and declining water levels of wells and reduced water resources, the growth of urban margins due to migration in recent years, and decrease of surface water resources [7]. The entry of municipal and industrial wastewater into surface water canals and its application in irrigation of agricultural products has significant health and environmental consequences in the province. The study was carried out in Qanavat region of Qom province and also in the rural district of Qomrud in 2020. According to the 2019 census, its population was 10922 in 1443 families, making it the most populated village in Qom province. People in Qomrud are close to the outlet of the largest municipal wastewater drainage canal. The wastewater from this canal runs for almost 15 km from the wastewater treatment plant, and raw urban wastewater is discharged in the open canal (Figure 1). Two general steps have been predicted for monitoring and the microbial risk assessment related to the use of raw wastewater and treated wastewater in the study area. The first stage involved setting up and performing microbial diagnostic tests based on sampling and environmental monitoring program for three pathogens including *Escherichia coli (E. coli)*, *V. Cholerae*, and *E. coli* O157 in raw sewage, treated wastewater, and crops irrigated with wastewater. In the second phase of the study, the process of microbial risk assessment for three pathogens of *E. coli*, *V. Cholerae*, and *E. coli* O157 in raw wastewater and treated wastewater was completed.

Figure 1: Location of the study area

Sampling, preparation, and bacterial analysis

The study samples included raw and treated wastewater, as well as barley, cucumber, and corn crops irrigated by raw and treated wastewater. A total of 54 samples for raw wastewater, 36 samples for effluent based on a 24-h composite sampling, and 30 samples of crops were collected and analyzed.

Methods of diagnostic test for V. Cholerae in crop samples irrigated with sewage

About 500 g of mud-free vegetables was gathered in a clean bag and sent to the laboratory on an ice pack. The vegetables were washed with water in a suitable container and the suspended particles were allowed to settle. The supernatant was filtered using a 0.45 µm cellulose acetate filter, which was then immersed in sterile alkaline peptone water (APW) with a pH of 4.8-8.6 and a salt concentration of 10 g /L at 35°C for 6 to 48 h (enrichment stage). The surface of the turbid layer was transferred to the selected thiosulfate-citrate-bile salts-sucrose (TCBS) medium for culture within 24 h at 35°C. The emergence of glossy yellow colonies might indicate the presence of *Vibrio* colonies, which should be tested. We used biochemical assays to differentiate *Vibrio* from other bacteria [7].

Methods of diagnostic test for V. Cholerae in wastewater samples

For sampling of raw wastewater, one liter in a sterile container was collected and after settling of coarse particles and its suspension, the supernatant was filtered. For concentrated wastewater, the amount of 100 ml was mixed well with 900 ml of sterile physiological saline, and after settling large particles, the suspended supernatant was passed through a 0.45 µm filter. The filter paper collected in sterile conditions in APW medium was incubated for 6-18 h at 35 °C and the surface of the turbid layer was transferred for culture with selective TCBS within 24 h at 35 °C. The presence of glossy yellow colonies was suspected of *Vibrio* colonies and then a confirmatory test was performed.

Methods of diagnostic test for E. coli in Wastewater samples

One liter of composite samples of wastewater was transferred to the laboratory by maintaining the sampling conditions and adjacent to the ice pack. Three dilutions of 2, 5, and 10 EC broth medium were prepared and used. Produced gas at 44.5 °C can indicate the presence of *E. coli*.

Methods of E. coli diagnostic test in samples of irrigated crops by wastewater

Using the enrichment and culture method, 25 g of each sample was supplemented by 225 mg of tryptone agar medium along with nobiocin and incubated at 37 °C for 18-24 h. Then colorless colonies (sorbitol negative) were cultured on nutrient agar medium and after 20 h of incubation at 36 °C the colonies were transferred to TSI and SIM medium to ensure *E. coli* presence. It was incubated again for 36 h at 36 ° C. The presence of *E. coli* bacterium was confirmed by adding coaxial reagent to the tube containing SIM medium (endolysis test). The presence of *E. coli* O157 in isolated *E. coli* positive samples was investigated by using microbial culture method with sorbitol Mc-conkey medium and an incubation period of 24 h [5, 9].

Development of microbial exposure risk assessment

In this study, quantitative microbial risk assessment (QMRA) was used, which is a probabilistic modeling technique, to determine the health risks from raw wastewater and effluents of wastewater. It was designed in four steps including hazard identification, identification of dose-response model, exposure assessment, and risk characterization[10].

Hazard identification

The first step in any microbial risk assessment is to identify the pathogen hazards. *E. coli* is a gram negative bacterium that is commonly present in the intestines of humans and animals. However, some types of *E. coli*, particularly *E. coli* O157:H7, can cause intestinal infection and are always present in sewage and readily inactivated by disinfection. *E. coli* O157:H7 and other strains that cause intestinal sickness are called Shiga toxin–producing *E. coli* (STEC). Most *E. coli* strains are harmless, but some serotypes (EPEC, ETEC etc.) can cause serious food poisoning in their hosts, and are occasionally responsible for food contamination incidents that prompt product recalls. Considering that only 8% of the total *E. coli* population is pathogenic, the average result from the laboratory analysis was multiplied by 0.08 in order to obtain the risk assessment from agent [11, 12].

V. Cholerae is a comma-shaped, Gram-negative, facultative anaerobe. These bacteria naturally live in brackish or saltwater, where they attach themselves easily to the chitin-containing shells of crabs, shrimps, and other shellfish. Some strains of *V. Cholerae* are pathogenic to humans and cause the deadly cholera disease, which can be derived from the consumption of undercooked or raw marine life species [4, 13].

Dose-response model

Dose-response parameters for risk assessment used in this study are shown in Table 1. The quantity value risk was estimated by using dose-response equations of β-Poisson model [14].

Table 1: Dose-response parameters for risk assessment

Parameter	Dose unit	Distribution and fit parameter	Response	Reference
E. coli concentration in raw wastewater, reclaimed waste water and crop	cfu/100ml	Beta-Poisson, α = 1.55E-01 , N50 = 2.11E + 06	Infection	Haas, Rose, & Gerba, 1999
E. coli O157:H7 concentration in raw wastewater, effluent and crop	cfu/100ml	beta-Poisson, *N*50 = 5.96E + 05, α = 0.49	Infection	Haas, Rose, & Gerba, 1999
V. colera concentration in raw wastewater, effluent and crop	cfu/100ml	Beta-Poisson, α = 2.50E-01, N50 = 2.43E + 02	Infection	Hornick et al., (1971)

Beta-Poisson model:

$$P_{inf} = 1 - \left[1 + N/N50\left(2^{\frac{1}{\alpha}} - 1\right)\right]^{-\alpha} \qquad Eq(1)$$

Where, P_{inf} is the risk of infection by ingesting pathogens in drinking water, N is the dose of microorganisms ingested, N50 is the microbial dose resulting in 50% infection, and α is a slope parameter [15].

Exposure assessment

In the process of exposure assessment, four parameters including pathogen concentration in raw wastewater and effluent, the number of individuals exposed, frequency of exposure, consumption of crops irrigated with raw wastewater and effluent, and volume of wastewater ingested during accidental exposure with effluent were considered. In addition, three scenarios associated with the worst-case exposures were evaluated, including food crop consumption, dermal contact with raw and effluent, and accidental drinking. Key assumptions for this QMRA included the following:

- Average doses estimated as the number of microbial cells expressed as bacterial colony-forming units (CFU) or virus plaque-forming units (PFU)/cm^2. They were used to calculate exposure without taking into account the spatial distribution of the microbe in the indoor environment, the number of individuals in the environment, and the likelihood that an individual would touch the areas where the microbes had been deposited.

- Transfer efficiencies from the fingertips to the eyes, nose, and mouth were assumed to be equal, and the amount transferred at that point was considered to be the dose. Maximum transfer rates were used.

- Pathogen die-off over time was not taken into account, which seems reasonable since adults touch their hand to their nose, mouth, or eye once every 3.75 min on average.

A questionnaire was used to gather information regarding irrigation characteristics, health practices such as hand-washing techniques and unintentional hand-washing with effluent, the consumption pattern of crops irrigated with effluent, the number of individuals exposed, and the frequency of exposure.

In this study, 200 participants were interviewed. Equation 2 was used to determine average doses (cfu/day) from the presented scenarios.

$$Dose = C\ I\ 10^{-w} e^{-kt} \qquad Eq(2)$$

In this equation, C is the concentration of bacteria in the wastewater (cfu/mL), I is the average amount of produce consumed by the study population exposed per person per day (g/day), or the volume of wastewater which might cling to the hand and ingested (mL), w is the log10 reduction in bacterial concentration from washing the product, k is the kinetic decay constant (per day), and t is the withholding period (days). It seems reasonable that for the worst-case scenarios and no protective measures for irrigation activities, the assumed value for w and t in Equation 2 be considered zero. The chosen parameters and frequencies are based on literature, and questionnaire results in the study area was estimated in Table 2 [16, 17].

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Table 2: Parameters for exposure scenario according to potential worst-case

Exposure scenario	Exposure route	Volume(ml)/ consumption (gr/d)	Frequency (per year)	Exposed population	Comments	Reference
Accidental drinking	Accidental consumption of effluent and raw wastewater	100	2	10000	Due to the openness of the sewerage canal, recreational uses and interviews with people, the number of exposed population has been extracted.	15, 20
Food crop consumption	Ingestion of crops irrigated with effluent	400 [a]	10	2500	According to the results of the questionnaire, 25% of households used products irrigated with wastewater	15, 16
Dermal contact by effluent	Hands contacting with effluent	0.1	20	10000	Hand to mouth transmission of wastewater , based on the days that people work on the farms as well as other recreational uses, it was considered for the entire exposed population.	9, 15, 20
Dermal contact by raw wastewater	Hands contacting with raw wastewater	0.1	10	10000	Hand to mouth transmission of wastewater, based on the days that people work on the farms as well as other recreational uses, it was considered for the entire exposed population.	15,9,20

a: gr/0.1 ml

Risk characterization

In this section, the probability of infection and illness in the exposed population is assessed.

The risk characterization consists of calculating the annual infection probability. It is linked to multiple exposures per person and was calculated as

$$P = 1 - (1 - P_{inf})^{n} \qquad \text{Eq(3)}$$

Where, P is the annual probability of infection, P_{inf} is the probability of infection for a single exposure to a dose of organisms and n is the frequency of exposure, n is the number of days per year during which a person is exposed to a dose of pathogenic agents.

A target of a 1 in 1 million (10^{-6}) risk of infection per touch was set as the safety goal, and a target pathogen concentration needed to reach this risk was then calculated using the dose response functions. This target is comparable to a daily risk acceptable for drinking water [13].

Ethical issues

The research was found to be in accordance to the ethical principles and the national norms and standards for conducting Medical Research in Iran and Approval ID: IR.MUQ.REC.1401.079. In addition, this research was performed in accordance with the declaration of Helsinki. All participants were interviewed in this study after obtaining informed consent form.

Results

Monitoring and microbiological assessment of raw wastewater, effluent, and irrigated crops

Statistical analysis and concentration values of *E. coli* and *V. Cholerae* detected in samples of raw wastewater and effluent and crops irrigated with wastewater are presented in Figures 2 and 3.

The concentrations of *E. coli*, *V. Cholerae*, and *E. coli* O157:H7, and their other physic-chemical parameters are presented in Table 3.

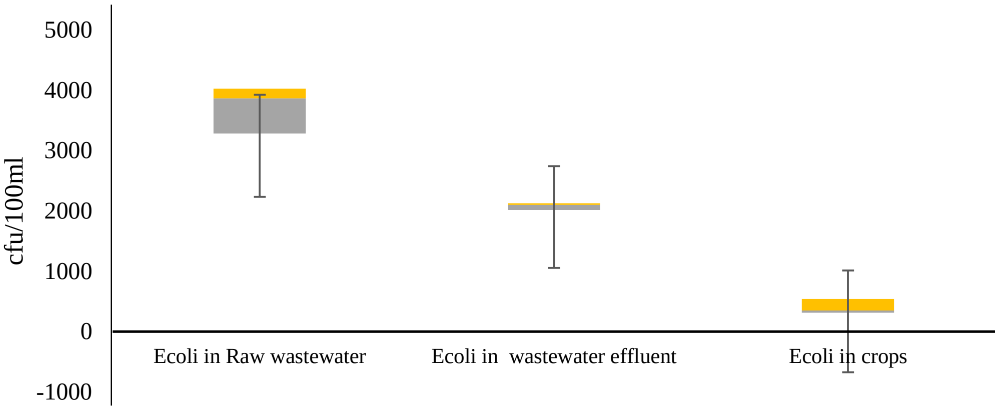

Figure 2: Box plot representing *E. coli* counts (cfu/100ml) in the sample. Bar labelled indicated significant difference among the samples ($p < 0.05$).

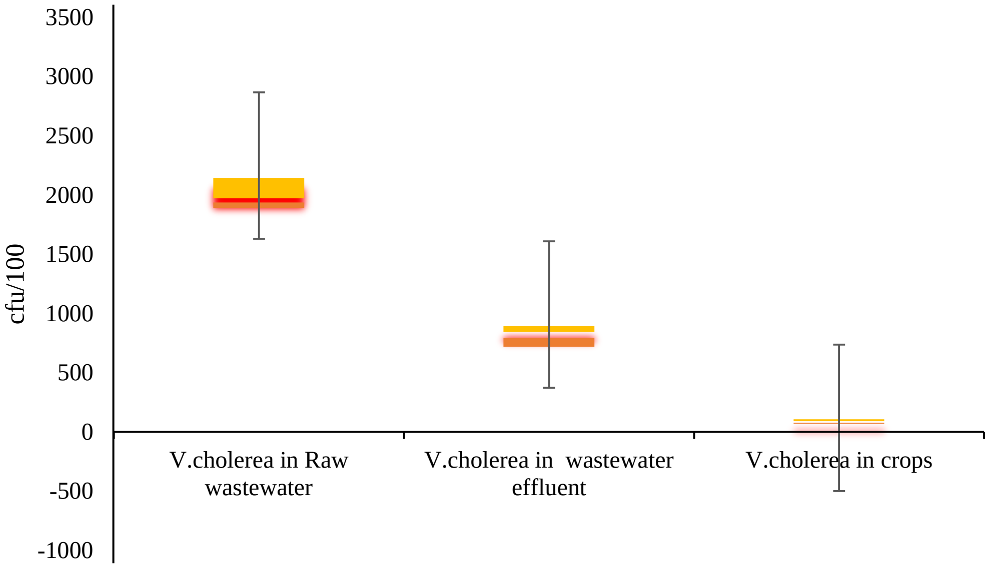

Figure 3: Box plot representing *V. Cholerae* counts (cfu/100ml) in the sample. Bar labelled indicated significant difference among the samples ($p < 0.05$).

Table 3: Characteristics and microbial concentrations (mg/kg) in the examined samples

Sample	*E. coli* (cfu/100ml)	*V. Cholerae* (cfu/ml)	*E. coli* O157:H7 (cfu/100ml)	pH	T(°C)	O_2 (mg/L)
Raw wastewater	$3.4 \times 10^3 \pm 500$	$2.1 \times 10^3 \pm 100$	312	7.43 ± 0.12	27.5 ± 2.2	2.4 ± 0.85
Wastewater effluent	$2.1 \times 10^3 \pm 100$	$0.8 \times 10^3 \pm 100$	176	7.1 ± 0.18	28.15 ± 0.12	3.4 ± 0.42
Irrigated crops	400 ± 250	$0.1 \times 10^3 \pm 0.019$	52	6.8 ± 0.09	-	-

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Exposure and health risk assessment

Table 4 displays the exposed dose, probability, and annual probability of infection in the studied population according to the exposure scenario parameters.

Table 4: Exposed dose, probability and annual probability of infection in the studied population

Pathway and scenario exposure	Exposed dose	Probability of infection	Annual probability of infection
E. coli			
Accidental drinking	3400	2×10^{-2}	4×10^{-2}
Food crop consumption	650	39×10^{-4}	38×10^{-3}
Dermal contact by effluent	2.2	1×10^{-4}	2×10^{-3}
Dermal contact by raw wastewater	3.9	1×10^{-4}	9.9×10^{-4}
E. coli O157:H7			
Accidental drinking	312	3×10^{-4}	6×10^{-4}
Food crop consumption	12	2×10^{-5}	2×10^{-4}
Dermal contact by effluent	176×10^{-3}	3×10^{-7}	3×10^{-6}
Dermal contact by raw wastewater	312×10^{-3}	3×10^{-7}	3×10^{-6}
V. Cholerea			
Accidental drinking	22×10^{4}	71×10^{-2}	91×10^{-2}
Food crop consumption	11	13×10^{-2}	76×10^{-2}
Dermal contact by effluent	9.5	3×10^{-3}	6×10^{-2}
Dermal contact by raw wastewater	210	5×10^{-3}	5×10^{-2}

Discussion

In order to evaluate the health risk associated with wastewater consumption, a static model for evaluating microbial risk and prediction the probability of infection were used without considering secondary transmission and immunity.

The main finding of this study was the high concentrations of *E. coli* and *Cholerae* in the sewage. This investigation demonstrated the risk of infectious gastroenteritis attributable to the wastewater treatment. Table 3 indicates that in the raw wastewater, concentrations of *E. coli*, *V. cholera*, and *E. coli* O157:H7 were $3.4 \times 10^{3} \pm 500$, $2.1 \times 10^{3} \pm 100$, and 312, respectively. Moreover, concentrations of *E. coli*, *V. Cholerae*, and *E. coli* O157:H7 in effluent were $2.1 \times 10^{3} \pm 100$, $0.8 \times 10^{3} \pm 100$, and 176, respectively. Based on these findings, it is determined that the conventional wastewater treatment system has been effective in removing *E. coli*, *V. Cholerae*, and *E. coli* O157: H7 by 50%, 59%, and 43%, respectively. According to the results, the crops irrigated with effluent contained *E. coli*, *V. Cholerae*, and *E. coli* O157:H7 at concentrations of 400 ± 250, $0.1 \times 10^{3} \pm 0.019$, and 52, respectively. In this study, according to the exposure scenarios including accidental drinking, food crop consumption, dermal contact with effluent, and dermal contact with raw wastewater, the total annual probability of infection with *E. coli*, *V. Cholerae*, and *E. coli* O157:H7 in the studied population was determined to be 8×10^{-2}, 8×10^{-4}, and 17×10^{-2}, respectively.

The present study results show a high risk of different infections. The key finding is that, based on the epidemiological evidence, Qom Province is one of the endemic foci of cholera outbreaks. Moreover, the use of effluent in irrigation is a risk factor for the spread of cholera in the region[17].

The violation of WHO guidelines for using wastewater in irrigation was observed, since the recommended limit in the guideline for faecal coliform bacteria in unrestricted irrigation is ≤ 1000 faecal coliform bacteria per 100 mL. In the present study, *E. coli* concentrations of $3.4 \times 10^{3} \pm 500$ cfu/100 in raw wastewater and $2.1 \times 10^{3} \pm 100$ cfu/100 in effluent indicate a significant risk of infection for the exposed adult farmworkers and children[18].

According to the findings of the present study, there are similarities with other studies related to the microbial quality of wastewater and its hazards. Shuval et al. reported that the Cholerea concentration in the products irrigated by effluent (lettuce and cucumber) was 10^5-10^6/100mL and the annual risk of illness compared to the USEPA benchmark was < 10^{-4} infections per year [19].

According to the study by Hussni et al. on the risk of illness from the consumption of mutton contaminated with *E. coli* O157:H7 prepared at a restaurant in Qatar, the probability of infection for a healthy female ranged from 7 × 10^{-3} to 28 × 10^{-2}, which clearly depends on the volume and quantity of the food consumed. Table 3 shows that the risk of O157 outbreak through the consumption of irrigated crops is significant[20]. These results emphasize the importance of *E. coli* O157:H7 pathogenicity. The findings of the study by Rock CM indicated that irrigation with reclaimed water containing 126 CFU/100 mL *E. coli* can lead to a risk of gastrointestinal (GI) illness (diarrhea) in 9 cases per 100,000,000 people (0.000009% risk) for subsurface irrigation, 1.1 cases per 100,000 people (a 0.0011% risk) for furrow irrigation, and 1.1 cases per 1,000 people (0.11% risk) for sprinkler irrigation of lettuce [21]. The study by Yapo et al. on the QMRA of urban wastewater and lagoon water reuse in Abidjan showed that a high concentration of *E. coli* (12.8 CFU/100 mL to 2.97 × 10^4 CFU/100 mL) in wastewater can result in an annual infection risk for *E. coli* (90.07–99.90%, assuming that 8% of *E. coli* is *E. coli* O157:H7), which is significantly higher than the acceptable risk (10^{-4}) [22]. According to the study by Truchado, when using wastewater for the irrigation of products, Spanish legislation specifies permissible *E. coli* levels based on the crop and mode of water application. When reclaimed water comes into direct contact with the crops and the crops are consumed raw, the maximum authorized level for *E. coli* is 10^2 CFU/100 mL. Based on the findings of the present study and according to the obtained *E. coli* concentrations in raw wastewater and effluent, we should impose serious restrictions on the consumption of products that are irrigated by wastewater [23].

Conclusion

Findings of this study, while limited to a one-time exposure event of agricultural products irrigated with wastewater, highlight the need for additional assessments to determine if the scientific-basis of this study is protective of public health. These findings show that the issue of wastewater use safety in Qom is serious and presence of indicator organisms in wastewater does not provide sufficient guarantee for microbial safety. Therefore, implementing WWSP to safeguard wastewater quality, raising awareness in the population in contact with urban wastewater and upgrading wastewater treatment plants is inevitable.

Abbreviations

APW: Alkaline Peptone Water
QMRA: Quantification Microbial Risk Assessment
PFU: Plaque Forming Unit
USEPA: United States Environmental Protection Agency
WWSP: Wastewater Safety Plans.

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Conflict of interests

The authors declare that there is no conflict of interest.

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