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Quantitative microbial risk assessment of *Giardia* cyst and *Ascaris* egg in effluent of wastewater treatment plants used for agriculture irrigation – a case study

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ABSTRACT

The aim of this study was to determine the concentration of *Giardia* cyst and *Ascaris* egg in influent and effluent of six wastewater treatment plants (WWTPs) in Kermanshah province, Iran, and to assess human health risk imposed by these organisms. Samples were taken from influent and effluent at weekly interval during 6 months. Samples were analyzed for *Giardia* cyst and *Ascaris* egg using McMaster egg counting technique according to Bailenger method. The Monte Carlo simulation method was used to calculate daily and annual infection risks. The efficiencies of all WWTPs to remove *Giardia* cyst and *Ascaris* egg from raw wastewater were more than 95%. However, maximum concentrations of these organisms were higher than acceptable level in some WWTPs effluents. Maximum concentration of *Giardia* cyst (2 counts/L) and *Ascaris* egg (4 counts/L) in effluent were observed in Kermanshah WWTP. The results of risk assessment indicated that annual infection risk related to both organisms were much more than acceptable level (10^{-4} pppy). Also, the imposed risk by *Ascaris* was higher than *Giardia*. There is a need for more precautions to be considered by farmers and other susceptible groups in contact to reclaimed wastewater in agricultural land, landscape and parks. Also, responsible organization should conduct more vigorous safety plans in site where WWTPs effluent is used for irrigation.

Keywords: Risk assessment; *Giardia* cyst; *Ascaris* egg; Wastewater treatment plants; Agriculture irrigation

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1. Introduction

One of the most important aims considered for wastewater treatment plants (WWTPs), especially in dry regions, is wastewater reuse for various applications, including agricultural and landscape irrigation [1–3]. The wastewater reuse has many benefits, such as providing an additional source of water, decreasing wastewater discharge and preventing pollution, energy saving, dust reducing through speckling, nutrient recovery, providing treatment costs through the sale of effluent, and finally improvement in the quality of the environment [4–6]. The advantages of wastewater reuse may be limited by public health problems related to the transmission of pathogenic organisms if the treatment system does not work properly [7]. This is an important issue, especially when the effluent is used for the irrigation of public parks and agricultural products [8–10]. Therefore, appropriate quality of the effluent in terms of organisms and their consistency with regulations is very important [6,7,10,11]. Among the reclaimed waterborne microorganisms, protozoa and helminths are of great importance in terms of public health concern because of cyst and ova production which can promote their resistant to environmental conditions and disinfectants [12–15]. These organisms are also resistant to disinfectants at the concentration commonly used in water and WWTPs.

Ascariasis is the most common helminthic disease which is caused by the parasitic roundworm *Ascaris lumbricoides* [16,17]. There are 1.3 billion ascariasis infections worldwide. Although ascariasis has a low mortality rate, affected people who are mostly children under 15 years can experience some problems such as faltering growth and/or decreased physical fitness [13].

Giardia is one of the most common protozoan pathogens which can lead to some disorders such as weight loss, dehydration from diarrhea, lactose intolerance, and physical and mental implications, called giardiasis [18].

Irrigation is the largest wastewater reuse worldwide because of less quality requirements [19]. Therefore, the most important ways by which parasitic organisms can be transmitted to humans are by crops, plants, soil, and water in agricultural land, parks, and landscape which are irrigated using reused wastewater [20]. Although all the human populations may be at risk of being affected by transmitted organisms, farmers and irrigators are at higher risk because they are more in contact with reused wastewater [21].

While some research has been carried out on wastewater reuse risk assessment [22], to our knowledge, too little attention has been paid to the risk of *Giardia* cyst and *Ascaris* egg for farmers as the most important group in contact with reused wastewater. The main aim of this study, therefore, was microbial risk assessment of WWTPs effluent in terms of these organisms for farmers. Besides, the removal efficiency of WWTPs to remove these organisms was investigated.

2. Materials and methods

2.1. Study area

Kermanshah province is located in western Iran and has generally dry and warm climate (Fig. 1). Kermanshah is one of the most important Iranian province point of view agricultural activities and a large number of population. However, drought and water shortage have caused concerns about water supply. The WWTP effluent is the most probable substitute for surface and groundwater that can be used for irrigation of agricultural product and landscape. This province has 14 cities; 6 out of 14 have a WWTP. These cities were indicated in Fig. 1 and the characteristics of their WWTPs were presented in Table 1. Activated sludge, stabilization pond, and wetland are wastewater treatment systems in different cities.

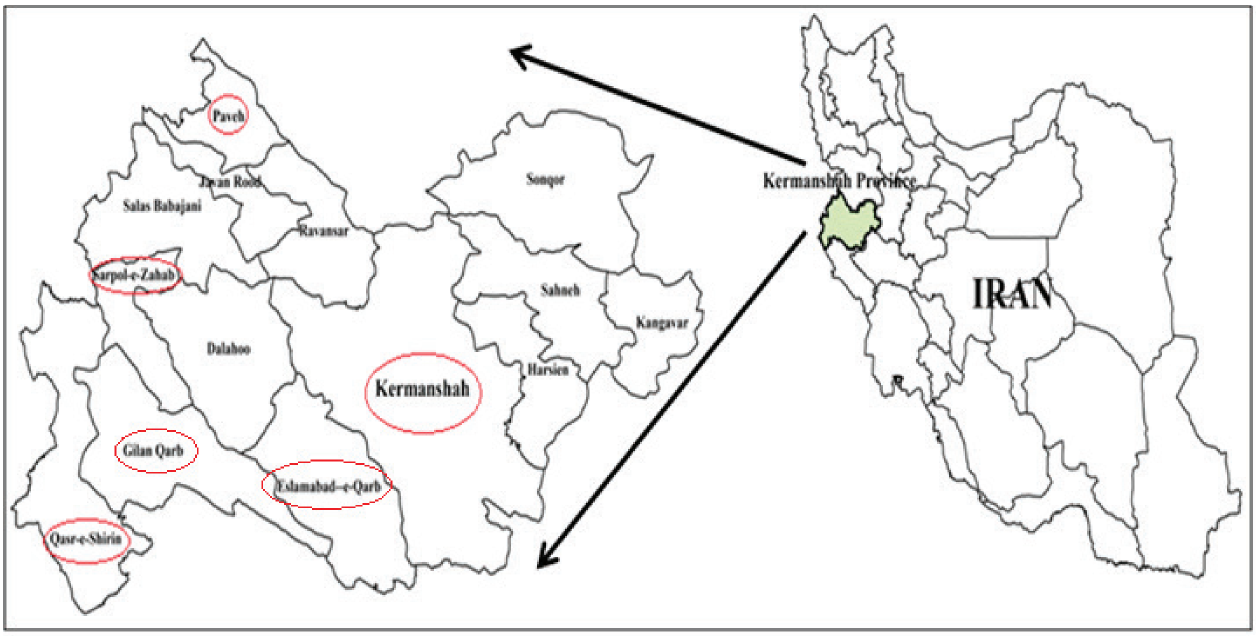

Fig. 1. Map of Kermanshah province and its cities, Iran.

2.2. Sampling and laboratory analysis

This cross-sectional study was performed within 6 months during the spring and summer. Samples were collected weekly from raw wastewater (before screening unit) with 1 L volume, and final treated effluents (after chlorination unit) with 10 L volume. In total, 288 samples were collected and analyzed (i.e., 48 samples from each plant). Sampling days during the week were randomly selected. Parasitological analysis was conducted based on the modified Bailenger method with McMaster counting slides (with volume held under the grid equal to 0.3 mL) [26].

3. Quantitative microbial risk assessment

3.1. Exposure assessment

This step assessed how individuals or populations are exposed to target organism and how much organism is ingested by exposed cases [23]. For a farmer who irrigates using treated wastewater, it is assumed that he ingests accidentally 1–2 mL effluent [24]. The person is assumed to ingest effluent 0.25–46 time (day) per year [25]. More details about exposure scenario were shown in Tables 2 and 3. The exposure or daily ingested organism (d) can be calculated using the following equation:

$$d = \text{IV.OC.ED} \quad (1)$$

where IV is ingested volume of effluent (mL/d), OC is a concentration of organism (number/mL), and ED is days of exposure (day). This equation is reformed equation which was introduced by Lim et al. [26].

3.1.1. Dose–response assessment

This stage determined the quantitative relation between dose and the incidence of adverse health effect. For estimation

Table 1
Characteristic of wastewater treatment plant in different cities

Treatment plant location (city)	Capacity (m^3/d)	Population (person)	Operating year	Process type	Climatic condition
Kermanshah	60,000	400,000	2004	Conventional activated sludge	Winter (cold), summer (moderate)
Ghare-e-Shirin	15,000	30,000	2003	Constructed wetland	Winter (moderate), summer (dry and very hot)
Eslamabad-e Gharb	13,500	90,000	2005	Stabilization pond	Winter (cold), summer (moderate)
Sarpol-e Zahab	7,200	54,000	2008	Extended aeration activated sludge	Winter (moderate), summer (dry and hot)
Gilan-e Gharb	3,400	22,000	2005	Stabilization pond	Winter (moderate), summer (dry and hot)
Paveh	4,700	18,000	2005	Extended aeration activated sludge	Winter (cold), summer (moderate)

Table 2
Parameters and their probability distribution for assessment of the infection incidence by *Giardia* cyst

City	Ingested volume	Exposure day	Organism concentration (count/L)			r
			Distribution	Minimum	Maximum	
Kermanshah	Distribution: uniform	Distribution: uniform	Uniform	0	2	Distribution: PERT
Sarpol-e Zahab	Minimum = 1	Minimum = 0.25		0	1	Minimum = 0.014925
Paveh	Maximum = 2	Maximum = 46		0	2	Maximum = 0.024875
						Mode = 0.0199

Table 3
Parameters and their probability distribution for assessment of the infection incidence by *Ascaris* egg

City	Ingested volume	Exposure day	Organism concentration (count/L)			Alpha	N_{50}
			Distribution	Minimum	Maximum		
Kermanshah	Distribution:	Distribution:	Uniform	0	4	Distribution: PERT	Distribution: PERT
Sarpol-e Zahab	uniform	uniform		0	2.7	Minimum = 0.078	Minimum = 644
Ghasr-e Shirin	Minimum = 1	Minimum = 0.25		0	0.8	Maximum = 0.13	Maximum = 1073.75
	Maximum = 2	Maximum = 46				Mode = 0.104	Mode = 0.859
Paveh				0	3		

of risk originated from *Ascaris*, The β-Poisson model was used [26]. The equation for β-Poisson model is as follows:

$$P_{id} = 1 - \left[(1 + (d/N_{50})(2^{1/\alpha} - 1) \right]^{-\alpha} \quad (2)$$

where P_{id} is the probability of the daily risk of infection, N_{50} is the median infective dose, α is the pathogen infectivity, and d is the ingested organism per day. The annual infection risk related to *Giardia* cyst was calculated by exponential dose–response model. The equation for exponential model is as follows [26,27]:

$$P_{id} = 1 - \exp(-rd) \quad (3)$$

where P_{id} is the probability daily risk of infection, r is the probability survival of the organism and initiation of infection, and d is the exposure.

The probability annual infection risk (P_{ia}) was calculated using the following equation:

$$P_{ia} = 1 - (1 - P_{id})^n \quad (4)$$

where n is the exposure time (day) per year [27–29].

3.1.2. Two-dimensional Monte Carlo simulation

Because of uncertainty and variability related to a fixed value of parameters in exponential and β-Poisson model, the two-dimensional Monte Carlo simulation with 1,001 iterations for the uncertainty and variability was performed for calculation of daily and annual infection risk using mc2d package in R software version 3.1.3 [30]. The selected ranges for parameters of exponential and β-Poisson model are shown in Tables 2 and 3, respectively. The Monte Carlo program selects the value of the parameter in the determined range and then calculates infection risk. This process is repeated a large number of times and produce median risk as final result. These repetitions remove uncertainty and variability of parameters. Therefore, the obtained results are more robust and more precious than calculated results by fixed values [31].

3.1.3. Sensitivity analysis

Sensitivity analysis assessed the effect of variability and uncertainty related to independent parameters without best fixed value on annual infection risk [31]. In this study, the Spearman's rank order correlation was used to determine relationship between dependent and independent parameters and to assess the relative contribution of input parameters to uncertainty and variability of annual infection risk.

4. Results and discussion

4.1. Organism concentration in influent and effluent

The results of *Giardia* cyst and *Ascaris* egg concentration in influent and effluent of different WWTPs were shown in Figs. 2 and 3. As can be seen from these figures, constructed wetland (Ghare-e-Shirin) and stabilization ponds (Eslamabad-e Gharb and Gilan-e Gharb) can remove more than 99% of parasite eggs and protozoan cysts. The efficiencies for extended aeration activated sludge in Paveh were more than 97.5% and 99%, respectively. The conventional activated sludge system in Kermanshah removed 97%–99% and 99% of *Ascaris* eggs and *Giardia* cysts, respectively. No *Giardia* cyst and *Ascaris* egg were observed in the effluents of stabilization pond systems.

The study findings can be confirmed by the results of similar studies. The efficiencies as high as 100% for nematode egg removal in stabilization ponds have been reported by Arbabi and Zahedi [32], and Amahmid et al. [33]. In a study conducted by Grimason et al. [34] removal efficiencies less than 100% were reported for *Giardia* cysts in stabilization ponds. It was because of poor design and inadequate retention time. Similarly, Ellis et al. [35] showed that stabilization pond could not eliminate parasite eggs completely. A study based upon five stabilization ponds in Tunisia revealed that three plants effectively removed 100% of parasites, while two plants did not have such a situation due to insufficient retention time. None of the five plants, however, removed protozoan cysts completely [36]. Research performed by Reinoso et al. [37] showed better performance for

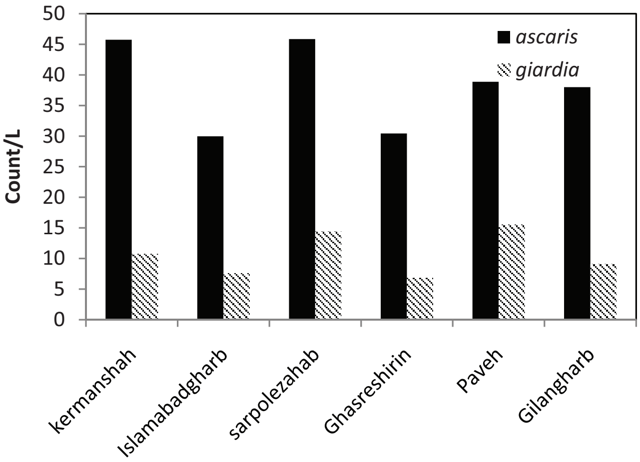

Fig. 2. The mean of parasitic contamination level in influent.

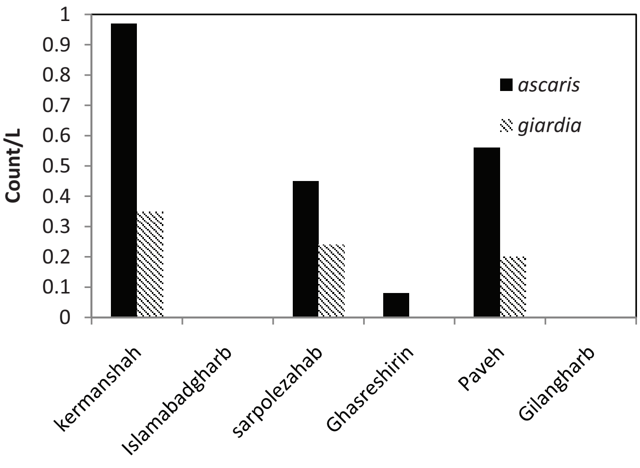

Fig. 3. The mean of parasitic contamination level in effluent.

constructed wetland (i.e., 97%) in *Giardia* cysts removal than stabilization pond. Molleda et al. [38] also showed removal rates of equal to 100% for constructed wetland. Miranzadeh and Mahmoudi [39] found that the extended aeration activated sludge can remove 100% of nematode eggs [39]. Research conducted by Donald and Rowe [40] showed that the primary sedimentation unit of a conventional activated sludge process eliminates about 99% of parasite eggs. Caccio et al. [41] conducted an investigation in four WWTPs in Italy and revealed that the removal efficiency in the number of cysts is significantly higher when the secondary treatment consisted of active oxidation with O_2 and sedimentation instead of activated sludge and sedimentation (94.5% vs. 72.1%–88%). Casson et al. [42] showed that activated sludge system can remove more than 99% of *Giardia* cysts and the removal efficiencies reported by Wiandt et al. [43] ranged from 99.5% to 99.8%.

As can be seen from the figures, natural systems (constructed wetland and stabilization ponds) have more removal efficiency than mechanical systems (activated sludge systems). The most probable reasons for this are long retention time, solar radiation, high pH (due to algal biomass), hunter microorganisms, and filtration and adsorption by plant roots in natural systems [10,44–46].

WHO guideline for microbiological quality of treated wastewaters used in agriculture in terms of parasite for restricted and unrestricted irrigation is ≤1 nematode egg per liter [22]. As can be seen from Fig. 3, the mean concentration of *Ascaris* and *Giardia* in all of the effluents is lower than the determined guideline.

4.2. Daily and annual infection risk

Since *Giardia* cyst and *Ascaris* egg were not found in the effluent of Eslamabad-e Gharb and Gilan-e Gharb WWTPs, the risk assessment for these organisms was not evaluated. For the same reason, no risk assessment was done for *Giardia* cysts in Ghasr-e Shirin WWTP.

The mean and standard deviation of daily and annual infection risk that was imposed by *Giardia* cyst and *Ascaris* egg for different WWTPs are presented in Tables 4 and 5. The obtained risk was compared with WHO and EPA standard. EPA and WHO have determined 10^{-4} per person per year (pppy) as an acceptable annual infection risk. The mean annual infection risk due to *Giardia* in all WWTPs is 2–5 times more than standard levels determined by WHO and EPA. The infection risk of *Giardia* in the effluent of Kermanshah and Paveh WWTPs is same and is higher than Sarpol-e Zahab WWTP. Based on mean annual infection risk, the order of *Ascaris* annual infection risk from high to low is as Kermanshah > Sarpol-e Zahab > Ghasr-e Shirin > Paveh. The higher infection risk in Kermanshah WWTP is due to higher concentration of *Ascaris* egg than that of other WWTPs. The infection risk produced by *Ascaris* is higher than *Giardia*. The annual infection risk due to *Ascaris* is about 4–20 times more than permissible limit assigned by WHO and EPA (for every WWTP). There are some suggestions in order to reduce risk for farmers which are wastewater filtering using sand filtration, protecting cloth wearing, cessation of irrigation before harvesting, and more precaution when raw agricultural products are used [24].

4.3. Annual symptomatic risk

The maximum annual infection risk related to public population was used to determine annual symptomatic case. The results of annual symptomatic case were shown in Tables 6 and 7. For both organisms, the highest cases with symptom were attributed to Kermanshah WWTP effluent. The lowest

Table 4
Daily and annual infection risk imposed by *Giardia* cyst for different WWTPs

City	Daily infection risk		Annual infection risk	
	Mean	SD	Mean	SD
Kermanshah	2.95e–05	0.000651	0.000506	0.0118
Sarpol-e Zahab	1.51e–05	0.000389	0.000223	0.00609
Paveh	2.86e–05	0.000639	0.00053	0.0123

Table 5
Daily and annual infection risk imposed by *Ascaris* egg for different WWTPs

City	Daily infection risk		Annual infection risk	
	Mean	SD	Mean	SD
Kermanshah	0.000211	0.00377	0.00205	0.0371
Sarpol-e Zahab	0.000144	0.00293	0.00143	0.0295
Ghasr-e Shirin	4.04e–05	0.00110	0.000406	0.0111
Paveh	0.000154	0.00308	0.00153	0.0310

Table 6
Annual symptomatic cases per pathogen per pathways for *Giardia*

WWTP	Annual infection risk	Exposed population (person)	Annual symptomatic cases[a]
Kermanshah	0.000506	425	0.21505
Sarpol-e Zahab	0.000223	172	0.038356
Paveh	0.00053	118	0.06254

[a]Annual symptomatic cases = annual infection risk × exposed population.

Table 7
Annual symptomatic cases per pathogen per pathways for *Ascaris*

WWTP	Annual infection risk	Exposed population (person)	Annual symptomatic cases[a]
Kermanshah	0.00205	425	0.82125
Sarpol-e Zahab	0.00143	172	0.24596
Ghasr-e Shirin	0.000406	121	0.049126
Paveh	0.00153	118	0.18054

[a]Annual symptomatic cases = annual infection risk × exposed population.

cases with symptom for *Giardia* cyst and *Ascaris* egg were related to Paveh and Ghasr-e Shirin WWTPs, respectively.

4.4. Sensitivity analysis

The results of sensitivity analysis for annual infection risk due to *Giardia* cyst and *Ascaris* egg in different effluent of WWTPs are shown in Figs. 4 and 5, respectively. The relative contribution of each input parameter to uncertainty and variability of annual infection risk is assessed by Spearman's correlation coefficient. The higher coefficient shows higher relation to uncertainty and variability of annual infection risk and vice versa. As seen from Figs. 3 and 4, for *Giardia* cyst and *Ascaris* egg in all WWTPs, the organism concentration in effluent has the highest contribution to uncertainty and variability of annual infection risk. The parameters of both exponential and β-Poisson model have a relatively small share to uncertainty and variability of annual infection risk.

5. Conclusion

The present study was conducted to measure the efficiency of WWTPs to remove *Giardia* cyst and *Ascaris* egg and to determine the infection risk for farmers imposed by them. The concentration of these organisms was lower than acceptable level for agricultural use. However, the levels of obtained annual infection risk due to these pathogen organisms were higher than determined acceptable levels by WHO and EPA in all WWTPs. The high infection risk may be attributed to designed scenarios and assumed parameters for them such as exposure times (days) and ingested volume of wastewater. However, these results can be an alert for population to consider more precaution against exposure to raw wastewaters and treated effluents. Because of very high exposure, there is a need for more protection measures to be followed by farmers.

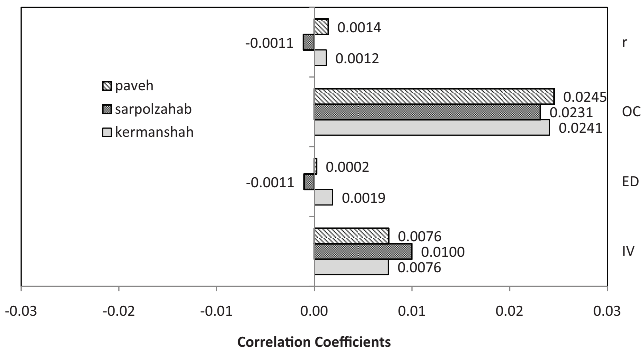

Fig. 4. Sensitivity analysis for the annual infection risk due to *Giardia* cyst in effluent of different WWTPs. IV, Ingested volume of effluent; ED, exposure day; OC, organism concentration; *r*, exponential model parameter.

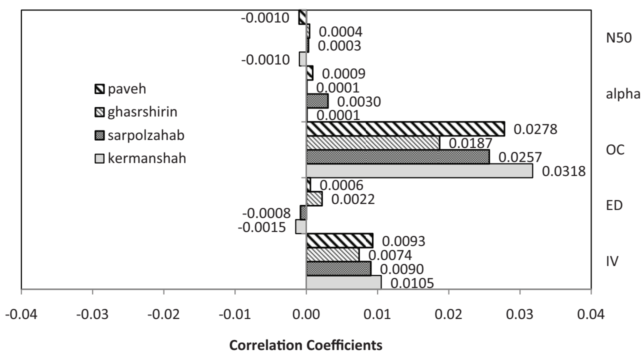

Fig. 5. Sensitivity analysis for the annual infection risk due to *Ascaris* egg in effluent of different WWTPs. IV, Ingested volume of effluent; ED, exposure day; OC, organism concentration; alpha and N_{50}, β-Poisson model parameters.

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