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A Survey of Parasitic Contamination in Qasr-e-Shirin Raw Wastewater: the Role of a Constructed Wetland in Removing Contamination

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ABSTRACT

The use of raw wastewater or effluent from inefficient wastewater treatment plants can result in an outbreak of parasitic diseases in a community. The aim of this study was to measure the parasite contamination of raw wastewater in Qasr-e-Shirin and evaluate the performance of a constructed wetland system in removing this contamination. This descriptive cross-sectional study was conducted over the course of 6 months (24 weeks), with a total of 48 samples selected to be tested. Every week a sample was taken from the raw wastewater of the influent and effluent. To identify parasites on the basis of a modified Bailenger method, parasitic analysis was conducted using a McMaster counting slide. The results showed that the minimum, average, and maximum number of all parasite eggs in the raw wastewater of Qasr-e Shirin was 0.08, 35.85, and 167.8 per liter, respectively. The minimum, average, and maximum number of protozoan cysts was 0, 19.95, and 75 per liter, respectively. In the wetland system of Qasr-e-Shirin, removal levels of protozoan cysts and parasite eggs were found to be 99.7 ± 0.23% and 100%, respectively. Because of parasitic wastewater contamination in Qasr-e-Shirin, outbreaks of parasitic diseases in this city are important issues. On the basis of the results of this study, constructed wetlands are very effective in removing parasitic contaminants. In addition, in term of parasite nematode eggs, the final effluent was consistent with the standards for reusing agriculture irrigation (1≥ number per L).

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Introduction

Reusing treated domestic wastewater for various uses, including agriculture and landscape irrigation, is one of the most important goals of wastewater treatment in protecting water resources, especially in dry regions [1,2]. The reuse of treated wastewater, especially in agriculture, has many benefits including profits from the sale of effluent, reducing dust through sprinkling, recovery of nutrients such as phosphorus and nitrogen for use as fertilizers, and lowering the costs and consumption of fresh water. Water reuse also has secondary benefits including the subsequent impacts from wastewater reuse projects and the improvement the quality and beauty of the environment [3-5].

In this regard, having an appropriate quality of the used effluent, in terms of a lack of microbial contamination and its consistency with national and international standards is very important [6,7]. Microbial contamination in wastewater effluent may endanger human and environment health. This is an especially pressing issue when the effluent is used for the irrigation of public parks and agricultural products [8,10].

In order to remove contaminants including organic materials and pathogens, wastewater must be treated. There are many different wastewater treatment processes, for example activated sludge, stabilization ponds, constructed wetlands, aerated lagoons, and trickling filters [1].

The mechanism of parasite removal is different in each of these processes. The most effective ways are settling and sedimentation due to the high density and the weight force, filtration, absorption by plant roots, adhering to the activated sludge biological clot, and being disabled due to unfavorable environmental conditions [2,11,12].

Studies and surveys show that the level of parasite removal in trickling filters, aerated lagoons, and activated sludge reaches to 99%, 99.9%, and 99%, respectively. In stabilization ponds (due to the high retention time) and constructed wetlands, through subsurface flow, this level increases to 100%. In each of these processes, removal efficiency is a function of the wastewater characteristics and the criteria of treatment plant design, which may vary greatly.

In Iran, few studies have been carried out in connection with the performance of wastewater treatment systems to remove protozoan cysts and parasites [12-14]. In addition, no studies have been performed to examine the performance of natural wastewater treatment systems in climates identical to those of Iran.

Another reason for conducting this research is to evaluate the condition of newly built natural wastewater treatment systems (constructed wetlands), in Kermanshah, including available straws. In this study, in addition to determining the parasitic contamination status of raw sewage in Qasr-e Shirin, the performance of the wastewater treatment system (a constructed wetland) in removing protozoan cysts and parasites and the effluent’s potential for agricultural irrigation are also evaluated and discussed.

Materials and Methods

Sampling site

Qasr-e Shirin is a city in the Kermanshah province of Iran, 167 km from Kermanshah city. It has a semi-arid climate and is situated 400 m above sea level. Qasr-e Shirin is located between 45°35’east and 34°31’north, bordering the country of Iraq to the West, Ilam province to the South, and the cities of Gilan Gharb and Sarpol-e Zahab to the East.

The wastewater treatment system used is constructed wetlands (including screening, grit chamber, anaerobic ponds, 12 constructed wetland beds, and chlorination), and it has been in operation since 2008. It currently serves 15,000 individuals, with projected growth up to 30,000 in the future. The current rate of wastewater discharge is 2200 cubic meters per day.

Treated effluent is used for different purposes, including landscaping irrigation, watering of fruit trees, and restricted farming. The lengths of the wastewater collection system and the pipeline transmitting effluent to the treatment plant are 117 and 11.5 km, respectively.

Sample collection

This cross- sectional study was conducted over a six month period. 24 identical samples were collected once a week from the inlet (screened, 1 l) and outlet (after the chlorination unit, 10 l) of treatment plant, resulting in a total of 48 samples tested. Sampling days were selected randomly during a week. The samples were transferred to specialized laboratories for microbiological analysis of parasites. Parasitic analysis was conducted based on Bailenger methods using McMaster counting slides (with a hole size of 0.3 mL) [15].

Identification and counting of helminth eggs and protozoan cysts

After two hours of sedimentation of the samples, 90% of the supernatant was removed using a siphon, and the remaining sediment was transferred to a centrifuge tube and centrifuged at 1000g for 15 minutes. Then, all of the sediment in the centrifuge tubes were transferred to a single tube and centrifuged at 1000 g for another 15 min. Following that stage, an equal volume of Acetoacetic acid (pH=4.5) was added to the sediments and in the second stage of centrifugation, a double volume of ethyl acetate was added to the centrifuge tubes. After complete mixing, the sediment was centrifuged for 15 min at 1000g.

By doing this, three layers were formed in the centrifuge tube, and the black upper layer and turbid middle layer were evacuated. The final precipitate (bottom layer) was then suspended in five volumes of zinc sulfate 33% (SG = 1.18) and thoroughly mixed by stirring.

The volume of this solution (precipitate + zinc sulfate) was considered to be the final product volume. 0.3 mL of the final product was transferred with a Pasteur pipette to three McMaster slides and allowed to rest for 5 minutes. Identification and counting of cysts and parasites were performed by microscope with a magnification level of 100 and 40, respectively. The number of cysts and parasites per liter were found using the following formula.

$$N = {}^{AX}/_{PV}$$

Where
N is the number of eggs or cysts per liter,
A represents the average number of counted eggs or cysts on three slides
X is the final product volume (ml)
P is the McMaster slide size (3/0 ml)
V is the volume of the initial sample (ml)

Statistical Analysis

Due to abnormality of all results ($P < 0.05$), data representing the quality of the effluent of two systems were compared using the One-Sample Kolmogorov Smirnov method at a significance level of 0.05.

In order to analyze the total amount of parasite eggs and protozoan cysts of raw wastewater produced in spring and summer, a Mann-Whitney U test was performed with SPSS software with a significance level of 0.05. Obtained results of the studied effluent quality of the aforementioned wastewater treatment plants were compared with the existing standards.

Results

The average, minimum, and maximum number of observed parasite eggs and protozoan cysts found in the raw wastewater and the effluent from the treatment plant of Qasr-e Shirin are shown in Tables 1 and 2, respectively.

The eggs of the detected parasites in the Qasr-e Shirin raw wastewater, highest and lowest parasites were *Ascaris lumbricoides* and *Trichuris Trichuria* respectively (**Table 1**)

Table 1. Average number of parasite eggs and protozoan cysts found in raw wastewater and effluent of the treatment plant of Qasr-e Shirin, respectively (number/liter).

Name of the treatment plant	**Sampling location**	*Ascaris lumbricoides*	*Hymenolepis Nana*	*Trichuris Trichuria*	*Giardia cysts*	*Amoeba cysts*	*The total number of parasites*	*Nematode larvae*	*Number of protozoan cysts*	**Percent removal efficiency (assuming normality)**	
										Parasite eggs	**cysts**
Qasr-e Shirin	**Influent**	30.43	5.42	0	6.85	13.1	35.85	30.43	19.95	99.7±0.23	100
	Effluent	0.08	0	0	0	0	0.08	0.08	0		

Table 2. Minimum and maximum number of parasite eggs and protozoan cysts found in raw wastewater and effluent of the treatment plant of Qasr-e Shirin, respectively (number/liter).

Place of sampling	Number of minimum/maximum	*Ascaris lumbricoides*	*Hymenolepis Nana*	*Trichuris Trichuria*	*Giardia cysts*	*Amoeba cysts*	*The total number of parasites*	*Nematode larvae*	*Number of protozoan cysts*
Influent	Minimum	0	0	0	0	0	0	0	0
	Maximum	100	67.7	0	30.7	50	167.8	100	75
Effluent	Minimum	0	0	0	0	0	0	0	0
	Maximum	0.8	0.67	0	0	0	0.8	0.8	0

In some raw wastewater samples there were no protozoan parasite eggs or cysts, but in some samples concentrations of parasite eggs and protozoan cysts reached levels of 167.8 and 19.95 per liter, respectively (**Table 2**). Averages of nematode parasites analyzed with the Engelberg index were significantly different ($p < 0.05$), and the mean protozoan parasite eggs and cysts in raw wastewater in the spring and summer were significantly different ($p < 0.05$).

Discussion

The comparison of raw wastewater parasitic contamination between Qasr-e Shirin and different countries revealed that the average number of parasite eggs in the observed location is lower than those of developing countries (70–300 eggs per liter); Brazil (166–202 eggs per liter), Morocco (214–840 eggs per liter), Jordan (300 eggs per liter), Pakistan (144 eggs per liter), Russia (≤2000 eggs per liter), and Ukraine (60 eggs per liter). However, parasitic contamination found in the present study is higher than that reported for more developed countries, such as the United States (1–8 eggs per liter), France (9–10 eggs per liter), and Germany (≤ 40 eggs per liter) [16]. Zamo et al. reported that the average number of total parasite eggs found in the raw wastewater of Kinetra, Morocco is 31 per liter [17].

Due to the high resistance of *Ascaris lumbricoides* eggs in comparison with other parasitic eggs such as *Trichuris Trichuria* and hookworm against unfavorable environmental conditions, *Ascaris lumbricoides*, like Himnulipos Nana, are the most common parasite eggs found in the raw wastewater of all countries [7,17]. These result showed that, currently, contamination by *Ascaris lumbricoides* is higher than by other parasite eggs throughout Iran. Other studies carried out by Miranzadeh and Mahmoudi, Mahvi and Kia, Arbabi and Zahedi in Tehran, Esfahan and Shahre Kord, prove the results obtained in this study [16-18]. Jimenez conducted a review to study parasitic contamination of raw wastewater in different countries and reported similar results [19]. While, in another study, Zamo showed that the Toxocara parasite is the dominant parasite egg. Therefore, other conditions such as climate, geography, culture, public health behavior, etc. can have an effect in determining societies' infection levels to a dominant parasite egg [20].

Considering the obtained results and the Mann-Whitney U statistical tests at a significance level of a = 0.05, it becomes evident that the levels of raw wastewater contamination from parasites at Qasr-e Shirin are higher in the spring than in the summer. Note that, in this study, all samples of raw wastewater have been taken in the non-rainy days and that water consumption in summer is more than in spring. With regard to the lower volume of wastewater in spring, the level of raw wastewater parasite contamination produced in the spring is more than in the summer. According to the above results, particular attention must paid to the parasitic contamination status of raw wastewater of Qasr-e Shirin to further study the sources of its contamination.

To do this, the wastewater treatment plant must be active to consider its optimal operation. Also, in order to reduce parasitic infection in humans, irrigation of agriculture production using raw wastewater should be avoided. Increasing public awareness of health problems, making major changes in attitudes and behaviors of families, and promoting principles of producing healthy vegetables and other food crops can be important solutions to decreasing the levels of parasitic contamination. On the basis of the presented results, the constructed wetland system's efficiency in removing protozoan cysts and parasites was found to be 99.7 ± 0.23 and 100 %, respectively. Because of the long retention time (and thus deposition) of the dominate mechanisms of the parasite egg and protozoan cyst removal system, proper design and operation of anaerobic ponds can show the highest performance prior to a constructed wetland.

In addition to the high retention time, solar rays, pH, and the presence of microorganisms' predators may be other factors affecting parasite egg and protozoan cyst removal [18]. In 12 ponds of constructed wetlands, filtration and absorption by plant roots creates suitable conditions for the removal of cysts and parasites [7,12]. The results of this study were confirmed by similar studies. Amahmid et al. and Arbabi et al. reported that the efficiency of nematode egg removal in stabilization ponds is 100% in which anaerobic ponds have played a significant role [18,21]. Grimason et al. have reported that because of the improper design and insufficient retention time, the efficiency of a Giardia cyst removal system in stabilization ponds in Kenya and France was less than 100% [22]. Ellis et al. revealed that a parasite egg removal system in stabilization ponds of England is not 100 % [23]. A Ben Ayed et al. study showed that in five wastewater stabilization pond systems of Tunisia, the efficiency of parasite egg removal systems in three treatment plants was 100% while in other treatment plants the rate was less. Moreover, none of the five treatment plants had complete protozoa removal (100%) [24]. Reinoso et al. reported that the efficiency of constructed wetlands in Giardia cyst removal (97%) has been more than stabilization ponds [25]. In the study of Patricia et al., it was reported that the efficiency of a parasites egg removal system was 100 % [12].

Conclusion

The results showed that the average number of nematode eggs in the effluent of the constructed wetland was less than one per liter. Thus, it can be concluded that the efficiency of natural wastewater treatment systems (such as constructed wetlands) in removing nematode egg is very high and is sufficient for achieving the required standards (Anglbrg indicator: number of nematode eggs: ≤ 1).

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