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## Comparative evaluation of parasitic removal in municipal wastewater using constructed wetland and extended aeration-activated sludge system in full scale: Kermanshah province, Iran

Kiomars Sharafi<sup>a,b</sup>, Masoud Moradi<sup>a</sup>, Ali Azari<sup>a,b</sup>, Hooshmand Sharafi<sup>a</sup>, Meghdad Pirsaeheb<sup>a</sup>

<sup>a</sup> Research Center for Environmental Determinants of Health (RCEDH), Kermanshah University of Medical Sciences, Kermanshah, Iran

<sup>b</sup> Department of Environmental Health Engineering, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

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#### Corresponding author

Meghdad Pirsaeheb

Email: [mpirsaeheb@yahoo.com](mailto:mpirsaeheb@yahoo.com)

Tel: +98-9123446880

### ABSTRACT

One of the most significant factors when reusing wastewater in agriculture is microbial quality. The present study assessed the efficiency of the Sarpol-e-Zahab and Paveh treatment plants, an extended aeration-activated sludge system and that of Qaser-e-Shirn, a constructed wetland, in removing protozoan cysts and parasite ova from municipal wastewater for the purposes of reuse in agricultural irrigation. In this six-month study, samples were collected weekly from influents and effluents of three wastewater treatment plants (WTPs). A total of 144 samples were examined by applying a modified Baileger method using the McMaster counting slide with a pore volume of 3.0 ml. Data were analyzed using SPSS version 16. The results revealed that the mean removal efficacies of parasite ova and protozoan cysts for the constructed wetland were 99.7–100% and 100%, treatment plant of Paveh 97.5–100% and 100%, treatment plant of Sarpol-e-Zahab 99–100% and 100% respectively. There WTPs showed significant differences in their removal parameters ( $P < 0.001$ ). The results showed both extended aeration-activated sludge systems had adequate effectiveness for parasitic removal, but the constructed wetland was significantly more effective than the extended aeration-activated sludge system. The effluent quality of all WTPs was consistent with Engelberg standards ( $\geq 1$  nematode egg per liter).

## Introduction

Treated domestic wastewater is a valuable water source for many uses, including agriculture and landscape irrigation. So, wastewater reusing is one of the most important goals of wastewater treatment plants (WTPs) and water conservation, especially in dry areas [1–3]. Wastewater reuse, particularly in agriculture, has numerous benefits, including the direct benefits of selling treated effluent; reducing the amount of dust through water spray; delivering nutrients like phosphorus and nitrogen in wastewater and thereby reducing the use of chemical fertilizers; and reducing the cost and consumption of fresh water. Reuse also delivers indirect benefits from the subsequent

agricultural impacts of wastewater reuse projects, and promotes public interest by protecting the environment and improving its quality and beauty [4–6]. Therefore, wastewater quality, particularly microbial content and compliance with valid national and international standards, is important [7,8].

If microbial quality and health aspects are neglected in the reuse of wastewater, serious risks to human health and the environment will result. Water quality is even more crucial when the effluent is used to irrigate landscapes, parks, and food crops [9–11]. Wastewater must be treated to remove contaminants such as toxic organic compounds and pathogens. Different treatment processes include utilizing activated sludge,

stabilization ponds, constructed wetlands, aerated lagoons, and trickling filters [4]. There are multiple mechanisms of parasite removal that occur in different wastewater treatment processes. The most significant mechanisms include sedimentation and deposition through high densities due to weight, filtration, absorption by plant roots, sequestration in biologically activated sludge clots, and deactivation due to unfavorable environmental conditions [2,12,13]. Last researches had indicated that the percentages of parasite ova removal in trickling filters, aerated lagoons, activated sludge, stabilization ponds, and constructed wetlands with subsurface flow are 99%, 99.9%, 99%, and 100%, respectively. The effectiveness of each of these processes is a function of the characteristics and design criteria of the WTP and, therefore, may fluctuate considerably [13-15]. Patricia et al. [13] reported that parasitic removal effectiveness in constructed wetlands was 100% and Reinoso et al. [16] stated that the efficiency of constructed wetland in the removal of the *Giardia* cyst was higher than stabilization ponds with about 97%. Feachem et al. [17] showed that the efficiency of activated sludge in the removal of the parasite ova was 99%. Miranzadeh and Mahmodi [12] showed that the efficiency of nematode egg removal using extended aeration activated sludge process was 100%. This study was developed to address the lack of Iranian studies that evaluate the effectiveness of wastewater treatment systems in removing protozoan cysts and parasite ova, and the absence of past studies that have evaluated the effectiveness of several natural and mechanical wastewater treatment systems in the field and in similar weather conditions. This study also provided an opportunity to assess the newly wastewater treatment systems in Kermanshah province. The aim of the present research was to compare the effectiveness in removing protozoan cysts and parasite ova for the constructed wetland wastewater treatment system of Qasr-e-Shirin, and the extended aeration-activated sludge treatment systems of Sarpol-e-Zahab and Paveh.

## Materials and Methods

This study was performed by collecting weekly samples from three WTPs over the course of six months. Each weekly sample included 1 L of

influent from water in the screening units, and 10 L of effluents taken after water had left the chlorination unit. Therefore, each plant provided 24 samples of influent and 24 samples of effluent. A total of 144 samples were analyzed. The day that each plant was sampled during the week was randomly selected and both influent and effluent samples taken on a given day from a given plant. After collection, the samples were delivered to a microbiological laboratory at the Public Health School of Kermanshah University of Medical Sciences for evaluation. Parasitological analysis was conducted based on a modified Baillenger method with McMaster counting slides, using 0.3 ml volume held under the grid [18].

Influent and effluent raw samples were deposited for over 2 h. Then, 90% of the supernatant was extracted using a siphon and the remainder was transferred to different centrifuge tubes. The tubes were centrifuged at 1,000 g for 15 min. Then, the total sediment in the centrifuge tubes was transferred to a single centrifuge tube and re-centrifuged at 1,000 g for 15 min. In the second phase, an equal volume of sediment and Stokes buffer (pH 4.5), and twice the volume of ethyl acetate were added to the centrifuge tube. Samples were mixed entirely by stirring, and then centrifuged for 15 min at 1,000 g. By doing this, three layers developed in the centrifuge tubes, and the upper black layer and the opaque center layer were drained. The remaining lower sediment was suspended in a 1:5 ratio of sediment and 33% zinc sulfate and mixed with a stirrer. The solution volume was measured and recorded as the volume of the final product. The final product was transferred to three 0.3 ml McMaster slides using a Pasteur pipette. Slides were fixed for 5 min before placing them under the microscope. The cysts and parasite ova were identified and counted under the microscope using 100x magnification, and the numbers of cysts and parasites in one L of solution was calculated using the following equation (Eq. 1).

$$N = \frac{AX}{PV} \quad (1)$$

N, number of ova or cysts in 1 L sample; A, mean number of counting ova or cysts on three slides; X, final product volume (mL); P, volume of McMaster slide (0.3 mL); V, initial sample volume (L).

Finally, to compare treatment plant effectiveness in removing protozoan cysts and parasite ova, and to compare the levels of parasite ova and protozoan cysts in the raw wastewater of investigated cities, the Kruskal-Wallis H statistical test with a significance level of  $\alpha=0.05$  was used. The Mann-Whitney U test with a significance level of  $\alpha=0.05$  was used to compare total levels of parasite ova and protozoan cysts present in raw sewage in spring and summer, and to compare the effectiveness of parasite removal for constructed wetland and extended aeration-activated sludge systems. All analyses were conducted using SPSS version 11.5.

## Results

The outcomes show that the mean effectiveness rates for removing parasite ova and protozoan cysts in constructed wetland were 99.7–100% and 100%, respectively. At the Paveh treatment plant, the mean effectiveness rates of removing parasitic ova and protozoan cysts were 97.5–100% and 100%, respectively, and 99–100% and 100%, respectively, for the WTP at Sarpol-e-Zahab. Mann-Whitney U test results showed a significant difference between the mean effectiveness of parasite removal for constructed wetland and extended aeration-activated sludge systems ( $p<0.001$ ).

The results of the Kruskal-Wallis H statistical tests indicated significant differences for the mean effectiveness of the two WTPs with extended aeration-activated sludge systems and the constructed wetland WTP ( $p<0.001$ ), but the two sludge system WTPs did not show significant differences in effectiveness from one another ( $p>0.05$ ). The one-sample Kolmogorov-Smirnov test results revealed that the mean number of nematode parasite ova in the effluents of the three treatment plants were significantly lower than the standard rate ( $p<0.001$ ).

Table 1 depicts minimum, maximum and mean numbers of parasites ova and protozoan cysts in the raw sewage and effluents of the three treatment plants.

## Discussion

The results of the statistical tests showed that the mean of nematode eggs in the effluents of all three treatment systems were significantly lower than the recommended standards for wastewater reuse in agriculture and irrigation ( $\leq 1$  egg per liter;  $p$ -value  $< 0.05$ ). Additionally, there was a significant difference in the effectiveness of constructed wetland and extended aeration-activated sludge systems in removing protozoan cysts and parasite ova ( $p$ -value  $< 0.05$ ). The mean efficiency of constructed wetland in the removal of protozoan cysts and parasite was 99.7–100% and 100% respectively. Long retention times and thus, deposition, are the predominant mechanisms for removing parasites and protozoan cysts [13,19]. Therefore, the 12 canebreaks along with the two parallel anaerobic ponds at the beginning of the system at the in Qasr-e-Shirin WTP may interfere with the length of the retention time needed for removing parasites. This interference may occur in the stabilization ponds, as most parasite ova are removed in the anaerobic ponds [20]. In addition, other factors in the removing parasite ova and protozoan cysts in constructed wetland systems is filtration and absorption by plant roots [21, 22].

Reinoso et al. [16] stated that the effectiveness of constructed wetland systems in removing *Giardia* cysts was greater than that of than stabilization ponds, with values of around 97%. This study's finding of greater effectiveness (99.7%) could be due to several factors, including treatment plant design, operation and maintenance of the treatment plant, and weather conditions [2]. Patricia et al. [22] reported that parasitic removal effectiveness in constructed wetland systems was 100%, which is similar to this study's results.

The mean effectiveness of the Paveh and Sarpol-e-Zahab WTPs in the removing parasite ova and protozoan cysts were (97.5%–100% and 100%) and (99–100% and 100%) respectively. The results of this study align with previous studies, as Feachem et al. [17] reported that the maximum effectiveness of activated sludge in removing parasite ova removal is 99%. Miranzadeh and Mahmodi [12] showed that the effectiveness of nematode egg removal using extended aeration

activated sludge process was 100%. Donald and Rowe [8] showed primary precipitation unit of conventional activated sludge has 99% efficiency in parasites ova removal, While Shuval [23] reported that removing of parasites ova is 90%.

Caccio et al. [24] reported that the number of cysts removed, when secondary treatment consisted of active oxidation with O<sub>2</sub> and sedimentation, was higher (94.5%) than when secondary treatment consisted of the activated sludge and sedimentation (72.1–88%). Casson et

al. [25] showed that activated sludge systems are able to eliminate over 99% of Giardia cysts. In Wiandt's study [26], the efficiency was 99.8–99.5%. The study that conducted by Sharafi et al. [27] results revealed that mean removal efficiencies of protozoan cysts and parasite ova for both stabilization pond systems were 100% and 100%, respectively. Also, Sharafi et al. [28] showed the conventional activated sludge of Kermanshah removed 97–99% and 99–100% of parasite eggs and protozoan cysts.

**Table 1**

Mean, minimum, and maximum number of parasite eggs and protozoa cysts in raw and treated wastewater of Pavah, Sarpol-e-Zahab and Qasr-e-Shirintreatment plants (per liter)

Plant name	Sampling location	Parameter	Ascaris lumbricoide eggs	nana eggs	Hymenolepis trichuris eggs	Giardia cyst	Amoeba cyst	Total amount of parasite egg	Nematode parasite egg	Protozoan cysts
Pavah	Influent	Min	6.7	0	0	0	0	6.7	6.7	0
		Max	80	33.3	0	82	65	83.3	80	82
		Mean	38.88	6.84	0	15.55	9.87	45.72	38.88	25.42
	Effluent	Min	0	0	0	0	0	0	0	0
		Max	3	2	0	2	0	3	3	2
		Mean	0.2	0.56	0.67	0	0.2	0	0.11	0.56
Sarpol-e-Zahab	Influent	Max	6	0	0	0	0	6	6	0
		Min	120	33.2	18.3	46.7	90	120	120	120
		Mean	21.93	45.85	5.07	2.49	14.44	7.49	53.41	48.34
	Effluent	Max	0	0	0	0	0	0	0	0
		Min	2.7	0	0	1	0	2.7	2.7	2.3
		Mean	0.45	0	0	0.24	0	0.45	0.45	0.24
Qasr-e-Shirin	Influent	Max	0	0	0	0	0	0	0	0
		Min	100	67.7	0	30.7	50	167.8	100	75
		Mean	30.43	5.42	0	6.85	13.1	35.85	30.43	19.95
	Effluent	Max	0	0	0	0	0	0	0	0
		Min	0.8	0.67	0	0	0	0.8	0.8	0
		Mean	0.08	0	0	0	0	0.08	19.95	0

## Conclusions

According to the results, it can be concluded that the effectiveness of constructed wetland systems is higher than that of extended aeration-activated sludge systems in removing parasites ova and protozoan cysts. Constructed wetland systems can therefore offer safer effluents for reuse in agricultural irrigation. However, the effectiveness of all of the WTPs considered in this study were adequate, producing effluent with parasite ova levels that were lower than the Engelberg

indicator of 1≥ nematode egg per liter of water. Consequently, it can be argued that the current conditions, operation, and maintenance of the studied WTPs easily meet the standards required for reusing wastewater in agricultural irrigation.

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