



## Comparison of the efficiency of extended aeration activated sludge system and stabilization ponds in real scale in the removal of protozoan cysts and parasite ova from domestic wastewater using Bailenger method: a case study, Kermanshah, Iran

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### ABSTRACT

One of the most significant qualitative characteristics that is associated with reusing wastewater in agriculture is microbial quality. The present study is aimed to determine the efficiency of extended aeration activated sludge system and stabilization ponds in real scale in removing protozoan cysts and parasite ova from domestic wastewater in Kermanshah. Within 6 months, influents and effluents of four wastewater treatment plants (WTPs) were collected weekly. A total of 192 samples were collected and examined microscopically (McMaster Counting Slide), applying modified Bailenger method. The results revealed that mean removal efficiencies of protozoan cysts and parasite ova for both stabilization pond systems were 100% and 100%, respectively. The mean efficiencies of removing these elements in the extended aeration activated sludge in Sarpol-e-Zahab wastewater plant was 99–100% and 100%, respectively. Also, in the extended aeration activated sludge in Paveh, these mean removal efficiencies were 97.5–100% and 100%, respectively. The results indicated that the efficiency of the stabilization pond system was more honorable than the extended aeration activated sludge system. Moreover, the efficiency of all three WTPs was desirable. Thereafter, the effluent quality of all three WTPs was consistent with Engelberg standard indicator (number of Nematode eggs:  $1 \geq$  per liter).

*Keywords:* Extended aeration activated sludge; Stabilization ponds; Protozoan cysts; Parasite ova; Kermanshah

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## 1. Introduction

Reusing treated domestic wastewater, as a valuable source of water for various uses, including agriculture and irrigation of landscaping, 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 primary benefits (earning profits from selling treated effluent, reducing the amount of dust through water spray, utilizing nutrients such as phosphorus and nitrogen in wastewater and thus reducing the use of chemical fertilizers, reducing costs, and consumption of fresh water), secondary benefits (subsequent impacts of wastewater reuse projects), and public interest (protecting the environment and improving its quality and beauty) [4–6]. In this regard, the suitability of wastewater quality, particularly in terms of microbial quality and its compliance with valid national and international standards, is significant [7,8].

If microbial quality and health aspects are neglected in the reuse of wastewater, serious risks to human health and the environment will arise. The issue is more significant when the effluent is intended to be used for irrigation of landscapes and parks, food products including vegetables and summer crops [9–11].

Parasites ova and protozoan cysts are the most important wastewater pollutants that are resistant to unfavorable environmental conditions; hence, conventional disinfection methods are not useful for removing them. Therefore, various mechanisms need to be applied through wastewater treatment processes to remove these pollutants [1]. The mechanisms of parasite removal during wastewater treatment processes are different. The most significant mechanisms are the sediment and deposition through high-density and due to weight force, filtration, absorption by plant roots, trapping in biological activated sludge clots, and deactivation due to unfavorable environmental conditions [2,12,13].

Research has indicated that the percentage of parasite ova removal in trickling filters, aerated lagoons, activated sludge, stabilization ponds (due to high retention time), and artificial reed beds with subsurface flow are 99, 99.9, 99, and 100%, respectively. It appears that the removal efficiency of each of these processes is a function of characteristics and design criteria of the WTP and, therefore, it may highly fluctuate [13–15].

Since few studies have been conducted in Iran to evaluate the efficiency of wastewater treatment systems in removing protozoan cysts and parasite ova;

considering that no study ever is conducted to evaluate the efficiency of several natural and mechanical wastewater treatment systems on the field and in a relatively similar weather condition; and regarding the fact that sewage treatment systems of Kermanshah province are newly built; the aim of the present research is to compare the efficiency of wastewater treatment systems of Gilan-e-Gharb and Islamabad-e-Gharb (stabilization ponds), Sarpol-e-Zahab and Paveh (extended aeration activated sludge) in the removal of protozoan cysts and parasite ova. Moreover, the proportion of wastewater produced for agricultural irrigation was explained scientifically.

## 2. Materials and methods

The operation units and process of all treatment systems were investigated and shown in Table 1, and also the location of all treatment plants in Kermanshah province are revealed in Fig. 1.

The present paper is a descriptive cross-sectional study. In this study, which lasted 6 months, samples were weekly collected from influents (screening unit) with the volume of 1 L and effluents (after chlorination unit) with the volume of 10 L (44 equal samples were collected from each plant). The composed samples were collected over a 24-h period in random days of a week according to standard methods. A total of 192 samples was analyzed in this study. Thereafter, collected samples were analyzed to examine parasitic ova according to the modified Bailenjer method using McMaster counting Slide (total volume = 3.0 mL) [16]. In the beginning, samples were deposited for more than 2 h. Afterwards, 90% of the supernatant liquid was extracted off using a siphon and the rest was transferred to different centrifuge tubes; then, the tubes were centrifuged at 1,000 g for 15 min. Then, the total sediment in centrifuge tubes was transferred to a single centrifuge tube and re-centrifuged at 1,000 g for 15 min. Afterwards, in the second phase, an equal volume of sediment, stokes buffer (pH 4.5), and ethyl acetate, twice its volume, was added to the centrifuge tube. Samples are mixed entirely by stirring method. The samples were then centrifuged for 15 min at 1,000 g. By doing this, three layers were composed in the centrifuge tubes from which the black top layer and the opaque center layer were drained. Then, the final sediment (the lower layer) was suspended in five volumes of 33% zinc sulfate (specific gravity of 1.18) and was mixed by a stirrer. The volume of the solution (sediment + zinc sulfate) was considered and recorded as the volume of the final product. In the following stage, the last product was transferred to three 0.3 mL

Table 1  
Characteristic of treatment plants

Treatment plants location	Capacity (m <sup>3</sup> /d)	Current population (person)	Operating year	Process type	Different units of treatment plants	Land area (ha)	Effluent application	Collection network and transmission line	Climates condition
Eastern of Islamabad-e-Gharb	13,500	90,000	2005	Stabilization pond	Screening, anaerobic ponds, primary facultative ponds, secondary facultative ponds, chlorination unit	63	Irrigation green areas around the treatment plant, fruit and un-fruit trees(limited)	164 km (network line), 5.3 km (transmission line)	Winter (cold), summer (moderate)
Western of Sarpol-e-Zahab	7,200	54,000	2008	Extended aeration activated sludge	Screening, gritting removal unit, primary sedimentation, aeration tank, secondary sedimentation, chlorination unit	23.5	Irrigation green areas around the treatment plant, fruit and un-fruit trees(limited)	107 km (network line), 5.6 km transmission line	Winter (moderate), summer (dry and hot)
Western of Gilan-e-Gharb	3,400	22,000	2005	Stabilization pond	Screening, anaerobic ponds, primary facultative ponds, secondary facultative ponds, chlorination unit	14	Irrigation green areas around the treatment plant, fruit and un-fruit trees(limited)	83 km (network line), 6 km transmission line	Winter (moderate), summer (dry and hot)
Paveh down town center	4,700	18,000	2005	Extended aeration activated sludge	Screening, gritting removal unit, primary sedimentation, aeration tank, secondary sedimentation, chlorination unit	2	Irrigation green areas around the treatment plant, fruit and un-fruit trees (limited)	49 km (network line), 5.1 km transmission line	Winter (cold), summer (moderate)

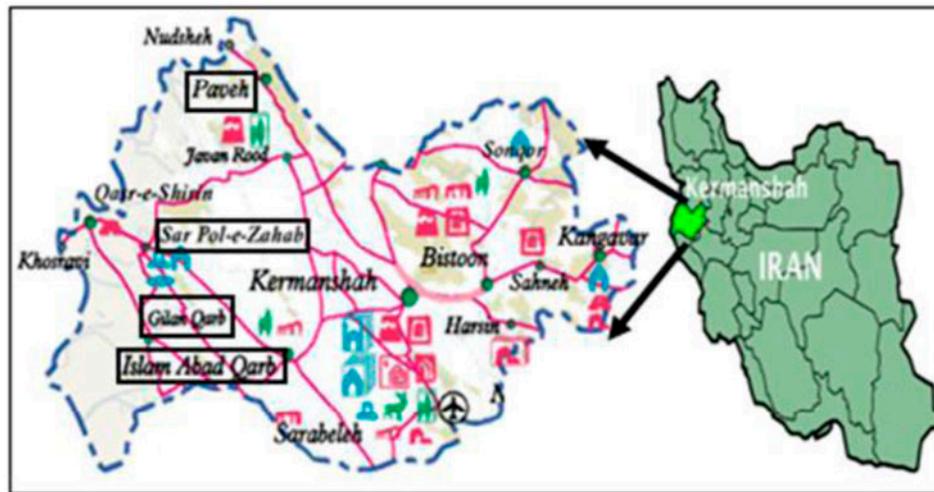


Fig. 1. Map of Kermanshah province and treatment plant areas.

McMaster slides using a Pasteur pipette. Before moving the slides under the microscope, they were staying fixed in 5 min. Then, cysts and parasite ova were identified and counted under the microscope with a magnification of 100 and then the number of cysts and parasites in one liter was obtained using the following (Eq. 1).

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

where  $N$ , 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).

### 2.1. Data analysis

Finally, due to the lack of normal total results ( $p$ -value < 0.05), comparison of data gathered from the effluent quality of investigating treatment plants with standards was conducted using statistical test of One-Sample Kolmogorov Smirnov at significance level of  $\alpha = 0.05$ . Moreover, to compare the obtained data from treatment plant efficiency in the removal of protozoan cysts and parasite ova and the date of parasite ova rates and protozoan cysts in raw sewage of various cities of the mentioned province, the Kruskal-Wallis H statistical tests at the significance level of  $\alpha = 0.05$ , were applied. Likewise, to compare the data connected to the efficiency of stabilization ponds and extended aeration activated sludge systems in the removal of cysts and parasites ova and data related to the entire amount of parasite ova and protozoan cysts in raw sewage

produced in the spring and summer, the Mann-Whitney U test at the significance level of  $\alpha = 0.05$  was used, using SPSS-Version 11.5.

### 3. Results

The outcomes show that the mean removal efficiencies of parasites ova and protozoan cysts in both stabilization pond systems are 100% and 100%, respectively. Moreover, the mean efficiencies of removing parasite ova and protozoan cysts in the extended aeration activated sludge of Sarpol-e-Zahab is 99–100% and 100%, respectively. These mean efficiencies in extended aeration activated sludge in Paveh are 97.5–100% and 100%, respectively.

Results of Mann-Whitney U test shows that there is a significant difference between the mean efficiency of the two treatment systems (stabilization pond and extended aeration activated sludge) in terms of removal of parameters of the study ( $p < 0.001$ ). The results of the Kruskal-Wallis H statistical tests indicates that there is a significant difference between the mean efficiency of these three WTPs ( $p < 0.001$ ). This difference is related to WTP with the stabilization pond system and other two plants with extended aeration activated sludge wastewater treatment system. Nevertheless, no significant difference is found between two WTPs of Paveh and Sarpol-e-Zahab and between the two treatment plants of Gilan-e-Gharb and Islamabad which have the same filtration system ( $p > 0.05$ ). According to the results, using the One-Sample Kolmogorov Smirnov, the mean number of Nematode parasite ova in the effluents of four treatment plants is significantly lower than the standard rate ( $p < 0.001$ ).

Table 2  
Mean concentration of parasitic contamination in raw and treated wastewater in all four WTP (per liter)

Treatment plant	Sampling location	<i>Ascaris lumbricoides</i> egg	<i>Hymenolepis nana</i> egg	<i>Trichuris trichiura</i> egg	Giardia cyst	Amoeba cyst	Total amount of parasite egg	Nematode parasite egg	Protozoan cysts
Islam Abad-e-Gharb	Influent	29.98	9.96	0	7.6	10.5	39.94	28.98	18.1
	Effluent	0	0	0	0	0	0	0	0
Sarpol-e-Zahab	Influent	45.85	5.07	2.49	14.44	7.49	53.41	48.34	21.93
	Effluent	0.45	0	0	0.24	0	0.45	0.45	0.24
Paveh	Influent	3.88	6.84	0	15.55	9.87	45.72	38.88	25.42
	Effluent	0.56	0.11	0	0.2	0	0.67	0.56	0.2
Gilan-e-Gharb	Influent	37.99	6.81	2.53	9.11	6.5	44.8	39.99	15.61
	Effluent	0	0	0	0	0	0	0	0

Table 3  
Minimum and maximum number of parasite eggs and protozoa cysts in raw and treated wastewater in all four WTP (per liter)

Treatment plant	Sampling location	Min/Max*		<i>Ascaris lumbricoides</i> egg	<i>Hymenolepis nana</i> egg	<i>Trichuris trichiura</i> egg	Giardia cyst	Amoeba cyst	Total amount of parasite egg	Nematode parasite egg	Protozoan cysts
		Min	Max								
Islam Abad-e-Gharb	Influent	0	80	0	41.7	0	0	0	0	0	0
	Effluent	0	0	0	0	0	50	75	106.7	80	73.7
Sarpol-e-Zahab	Influent	6	120	0	0	0	0	0	6	6	0
	Effluent	0	2.7	18.3	33.25	0	46.7	90	120	120	120
Paveh	Influent	2.7	6.7	0	0	0	1	0	2.7	2.7	2.7
	Effluent	0	80	0	33.3	0	82	65	6.7	6.7	0
Gilan-e-Gharb	Influent	0	3	0	0	0	0	0	83.3	80	82
	Effluent	7.3	88	25.7	44.4	0	106.7	20.7	158	113.7	106.7
		0	0	0	0	0	0	0	0	0	0

\*Min/Max, minimum and maximum number of parasite eggs and protozoa cysts in raw and treated wastewater.

Table 1 depicts an overview of mean concentration rate of parasitic contamination in raw sewage and effluents of the treatment plants. Table 2 demonstrates the minimum and maximum number of parasites ova and protozoan cysts in the raw sewage and effluents of the treatment plant (Table 3).

#### 4. Discussion

According to the results of the One-Sample Kolmogorov Smirnov statistical test, significance at the level of  $\alpha=0.05$ , it could be argued that the mean value for the amount of Nematode eggs in effluents of all four treatment systems was significantly lower ( $p$ -value  $< 0.05$ ) than the recommended amount in the relevant standards for wastewater reuse in agriculture and irrigation ( $\leq 1$  per liter). Additionally, according to the results of the Mann–Whitney U test, there was a significant difference in the efficiency rate of stabilization pond systems (WTPs of Islamabad-e-Gharb and Gilan-e-Gharb) and extended aeration activated sludge systems (treatment plants of Sarpol-e-Zahab and Paveh) in the removal of protozoan cysts and parasite ova ( $p$ -value  $< 0.05$ ). The results showed that the general efficiency of removal of cysts and parasite ova in the natural system of stabilization pond was higher than other processes such as extended aeration activated sludge system.

The efficiency of both natural systems (stabilization ponds) for the removal of parameters was obtained the same as equal as 100%. Long retention time (and thus deposition) is the predominant mechanism for parasites and protozoan cysts removal; therefore, appropriately planned and administered, stabilization ponds can demonstrate the highest efficiency. Additionally, other factors could be effective in removing protozoan cysts and parasite ova in this system, including solar ray and high pH value, due to algal biomass and the presence of micro-organisms [13,17]. According to the retention time factor in anaerobic ponds, compared to facultative and complementary ponds, it could be stated that the highest removal rates of parasitic elements in the stabilization pond system occurred in anaerobic ponds. In facultative ponds, algae growth rises during the daytime due to the sun. Consequently, the alkalinity of wastewater increases since it consumes  $\text{CO}_2$ . Therefore, nitrate and phosphorous compounds settle and some parasite ova and protozoan cysts are trapped and deposited along with them. Reducing the nutrient requirements of algae leads to death and deposition of the algae, which can trap the parasites or protozoan cysts effectively [18–20].

The results of the current study are consistent with the results of similar studies. Amahmid et al. [21] as

well as Arbabi and Zahedi [18] reported that the stabilization ponds can remove 100% of Nematode eggs. In addition, Grimason et al. [22], based on a study carried out in Kenya and France, argued that the removal efficiency of Giardia cysts by stabilization ponds is less than 100%. This may be due to the effect of poor design and insufficient retention time [22]. In another research conducted by Ellis et al. [23] in England, it was revealed that parasite ova removal by stabilization ponds would not reach 100%. Ben Ayed et al. [24] conducted a study in Tunisia and indicated that among wastewater stabilization pond systems three plants had 100% efficiency in parasite ova removal, while two other plants did not have such efficiency due to insufficient retention time. Reinoso et al. [25] stated that the efficiency of artificial reed bed in the removal of the Giardia cyst was higher than stabilization ponds with about 97%. However, Patricia et al. [13] reported that parasites removal efficiency was 100%.

According to the results, the mean efficiency of the treatment plants in Paveh and Sarpol-e-Zahab was not significantly different which could be due to the same retention time in both activated sludge systems. Moreover, high efficiency of both extended aeration activated sludge systems which met the Engelberg indicator (number of Nematode egg  $\leq 1$  per liter) could be due to different reasons such as physical settlement in primary settling tank and important mechanisms of trapping parasite ova and protozoan cysts in settlement of biological solids in secondary settling tank [12].

These findings are in line with other studies' results. Mara et al. [26] reported that the activated sludge process can remove up to 99% of parasite ova. Miranzadeh and Mahmodi [12] showed that the efficiency of Nematode egg removal using extended aeration activated sludge process was 100%. In another study conducted by Rows and Abdel-Magid [2] and in a review carried out by Goosen and Shayya [14], it had been argued that the initial sediment unit of conventional activated sludge process eliminated about 99% of the parasites [2]. Caccio et al. [27] reported that the number of cysts removed, when secondary treatment consisted of active oxidation with  $\text{O}_2$  and sedimentation, was higher (94.5%) than when secondary treatment consisted of the activated sludge and sedimentation (72.1–88%). Casson et al. [28] showed that activated sludge systems are able to eliminate over 99% of Giardia cysts. In Wiandt's study [29], the efficiency was 99.8–99.5%.

#### 5. Conclusions

According to the results, it can be concluded that the efficiency of all WTPs, especially stabilization pond

systems, in removing parasites ova and protozoan cysts have been desirable. Consequently, it can be argued that the current conditions, operation, and maintenance can easily meet the standards required for reusing wastewater in agricultural irrigation (Engelberg indicator: number of Nematode eggs:  $1 \geq$  per liter).

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