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Evaluating the Efficiency of Aerated Lagoon System regarding the Removal of Indicator Bacteria from Municipal Wastewater

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ABSTRACT

Introduction: Treatment of municipal wastewater is essential to remove bacteria. This study is designed to evaluate the efficacy of a wastewater treatment plant (WWTP) for the removal of bacteria and using for irrigation or discharge in the Caspian Sea according to the World Health Organization (WHO) regulations.

Materials and Methods: A total of 105 samples were collected from 7 stations, including the inlet and the outlet of the WWTP in Bandargaz City (Iran), the intersection point of wastewater effluent with Caspian Sea (Gorgan Bay), and a radius of 200 meters in three directions east, west, and north of the intersection point of wastewater in Gorgan Bay. The multiple-tube fermentation technique was used to enumerate bacteria, and results were expressed as the Most Probable Number (MPN) per 100 ml.

Results: Bacteriological analysis exhibited that the concentration of *total coliform*, *fecal coliform*, *fecal streptococci*, and *Clostridium perfringens* were 1.38×10^{10}, 5.57×10^{7}, 5.53×10^{9}, 1.26×10^{9} in inlet, and 1.38×10^{10} , 5.57×10^{7}, 5.53×10^{9} , 1.26 × 109 in outlet of WWTP, respectively.

Conclusion: The aeration lagoon has a low performance in bacteria population removal, which may be due to the climate condition of this region (few sunny days and many cloudy and rainy days). This effluent was not generally acceptable for discharge in the environment and reuse. Therefore, it is essential to modify the disinfection process to keep the concentration of bacteria under control. Additionally, continuous monitoring is necessary to control the quality of wastewater before discharge into the environment or reuse.

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Introduction

Municipal wastewater refers to any water used domestically, which is of no further value regarding the primary purpose [1]. Wastewater contains a wide range of contaminants, including organic constitutes like carbohydrates, proteins, fats, detergents, lignin, and synthetic chemicals; inorganic solids such as arsenic, cadmium, chromium, copper, lead, mercury, and zinc, etc.; and microorganism as *Vibrio cholera*, *Shigella* spp., *Salmonella* spp., *Enteroviruses*, *Entamoeba histolytica*, *Taenia saginata Trichuris*

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trichiura, *Schistosoma mansoni*, and Hookworms are known as the most important contaminants [1]. Among these constituents, microorganisms are more significant because they increase the risk of infectious disease, particularly when they are discarded in the environment without treatment or insufficient treatment [2]. The most prevalent infections transmitted by untreated wastewater are *gastroenteric*, *cholera*, *typhoid fever*, *bacillary dysentery*, *tuberculosis*, *Poliomyelitis*, *Hepatitis A* and *E*, *Cryptosporidiosis*, *Giardiasis*, *Amebiasis*, *Taeniasis*, *Ascariasis*, *Ancylostomiasis*, *Balantidiasis*, and *Trichuriasis* [3-5]. These diseases are commonly transmitted through consuming some raw foods, like fruits and vegetables irrigated by untreated wastewater, eating fish, and birds present in water resources contaminated with wastewater [2].

In recent years, wastewater use has drawn more attention due to global climate change, water quality deterioration, rising water shortage, and the need for food during population growth [6, 7]. The irrigation of agricultural fields is the main reason for the use of municipal wastewater for water scarcity solutions, particularly since municipal wastewater contains a significant amount of nutrients such as nitrogen, phosphorus, sulfur, and potassium which reduces the use of fertilizers, increases crop productivity, and improves soil fertility [8, 9]. Hence, safe disposal of municipal wastewater is essential to ensure that wastewater quality matches WHO standard limits for use in irrigation [10-12], or discharge in water resources like the Caspian Sea as a valuable habitat of animals, birds, and plants [13].

Today, activated sludge, trickling filters, and stabilization ponds are commonly used for urban wastewater treatment. Aeration lagoon is similar to stabilization ponds in terms of structure and activated sludge in biosynthetic relationships [1]. This procedure is a simple, low-cost, easy-to-use method for tropical regions, especially for communities with limited populations [11, 14]. It is estimated that aeration lagoon has an 80 to 95 % efficiency in removing BOD_5 [15] . The result of Ellouze et al.'s research showed that aeration lagoons had an efficiency of 1.65 $\log_{10}$, 1.42 $\log_{10}$, 1.23 $\log_{10}$, and 0.9 $\log_{10}$ in removing *total coliform*, *fecal coliform*, *fecal streptococci*, and *salmonella* spp., respectively [16].

To evaluate sanitation processes and the impact of untreated or treated wastewater on water pollution, it is essential to monitor the presence of pathogens and microbes that indicate fecal contamination in wastewater and treated water as well as their behavior after being released into the environment. As explained below, the levels of bacteria typically used as indicators of fecal contamination, such as *Escherichia coli*, *enterococci*, and sulfite-reducing *clostridia* are significantly reduced (more than 99.99%) [17]. This study is designed to determine (1) the concentration levels of indicator bacteria in inlet and outlet of WWTP, the confluence point of the outlet of WWTP with Caspian Sea (Gorgan Bay) and sea waters in three directions of west, east, and north of Gorgan Bay. (2) the efficiency of aerated lagoon regarding the removal of indicator bacteria from municipal wastewater for use in irrigation or discharge in the Caspian Sea according to the WHO regulation, (3) and the association between indicator bacteria.

Material and Methods

Study site and sample collection

The study was carried out in Bandargaz, located in the north of Iran and south-eastern fringes of the Gorgan Bay, with geographical co-ordinates 36° 46′ 27″ North, 53° 56′ 53″ East (Figure.1). Bandargaz has a mild, hot, and humid climate with an average annual rainfall of around 600 to 800 mm and temperature 12 to 18 °C. The WWTP of this city has a nominal capacity of 1.1 million meter cubic per year. The daily wastewater volume arriving at WWTP is 3500 $m^3/_d$ .The wastewater enters the plant by a pressure primary through a PVC-gated pipe. After processing through the bar screen, the wastewater flows into the grit chamber, complete-mix lagoon aeration, mixed aerobic reactor, clarification tank and chlorine contact tanks. Finally, treated influent discharged into Gorgan Bay at the south-eastern shore of the

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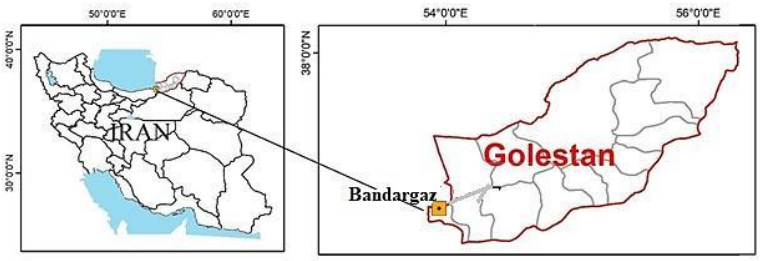
Caspian Sea through a concreted closed drain.



Station	Geographical location	
1) Inlet to WWTP	36° 46' 34.686" N	53° 58' 57.281" E
2) outlet of WWTP	36° 46' 59.787" N	53° 58' 39.663" E
3) confluence point of the Outlet with Gorgan Bay	36° 46' 59.783" N	53° 58' 39.663" E
4) 200 meters in east direction of site 3	36° 47' 31.417" N	53° 56' 25.484" E
5) 200 meters in West direction of site 3	36° 47' 30.994" N	53° 56' 25.733" E
6) 200 meters in North direction of site 3	36° 47' 37.605" N	53° 56' 23.450" E

Figure 1: Locations of sampling stations in Bandargaz city, north of Iran

A total of 105 samples were collected from 7 sites (each site 15 times) comprising of (1) inlet to WWTP, (2) outlet of WWTP, (3) confluence point of outlet with Gorgan Bay, (4) 200 meters in the direction of east of site 3 in Gorgan Bay (5) 200 meters in the direction of west of site 3 in Gorgan Bay, and (6) 200 meters in the direction of north of site 3 in Gorgan Bay (Figure .1) Sampling was done in the morning around 7 a.m. within six months from Apr to Jul 2022 fortnightly from each site. The wastewater and Sea water were separately collected in sterile glass vessels of 500 ml. Before examination, all the samples were stored in an insulated cooler (temperature less than 4°C). Samples were analyzed in a microbiological laboratory in less than two hours, as described in the standard method for examining water and wastewater [18].

Enumeration of bacteria

All of the indicator bacteria including *total coliform*, *fecal coliform*, *Escherichia coli*, and *Clostridium perfringens* were analyzed according to standard methods [18]. Multiple-tube Fermentation Technique (9221) was used to determine microbiological parameters, including *total coliform* (9221-B), *fecal coliform* (9221-E), and *fecal streptococci* (9230-B). Fluorogenic method was employed for isolation and identification of *Escherichia coli* (9221-F). The media used included lactose broth and brilliant-green lactose bile broth for *total coliform*, EC broth for *fecal coliform*, and Azide dextrose broth and Pfizer Selective Enterococcus (PSE) agar for *fecal streptococci* [18].

For detection of *Clostridium perfringens*, 10 mL of samples were transferred to 15.0 test tubes containing 10 mL double-strength thioglycollate broth medium incubated at 12% carbon dioxide at 35 ± 0.5 °C for 48 h under microaerophilic condition in CO_2 incubator. The positive sample was subcultured anaerobically on Tryptose Sulphite Cycloserine (TSC) agar. The formation of yellow-brown, grey, or black colonies on TSC was positive. The confirmed test was then performed using gram staining, motility, and nitrate reduction [19]. The final results were reported as the Most Probable Number (MPN)/100.0 mL of a sample.

Peptone water medium was used to prepare a dilution of 0.10 from the original sample (10 ml of original with 90 ml 0.5 peptone water medium). Six dilutions, including 0.1, 0.01, 0.001, 0001, 00001, and 0.000001 were further prepared from 1 ml of the diluted sample. Finally, serial dilutions 0.001 and 0.000001 were used for Gorgan Bay water and wastewater microbiological analysis, respectively. In each stage of microbial tests, a 10 ml tube of peptone water was used as control.

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Quality control and quality assessment were carefully conducted according to the standard method at all stages of the study regarding sampling, preservation, and examination [18].

Statistical analysis

Data were analyzed using SPSS version 23.0 (IBM Statistical Package) for analysis with a p-value of less than 0.05 as the significance level. The normality of data was checked by Shapiro-Wilk test before analysis. Chi-square tests were applied to find the relationship between microbiological parameters at different locations and months of sampling.

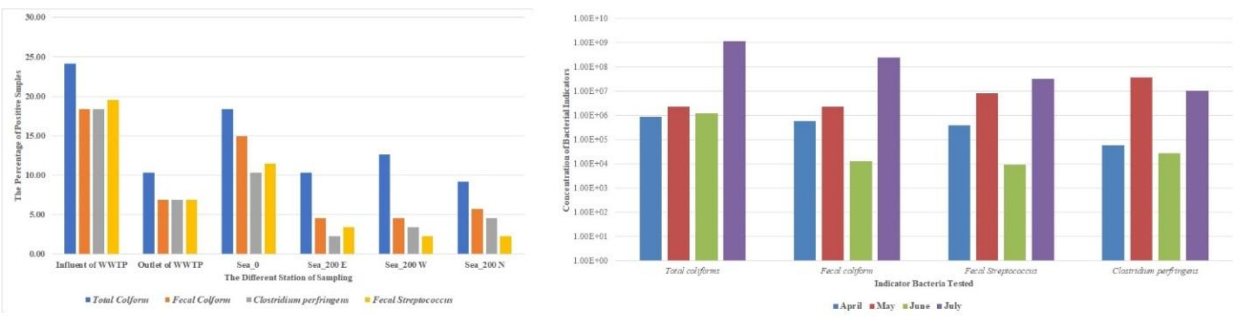
Results

From 105 samples analyzed, *total coliform* (85.1%) was the most frequently detected bacteria, followed by *fecal coliform* (55.2%), *fecal streptococci* (46.0%) and *Clostridium perfringens* (46.0%). The mean and standard deviation of bacterial concentration and the percentage of positive samples are presented in Table 1.

Table 1: Mean and standard deviation of bacterial value in different stations of sampling (MPN/100 ml)

Station	*Total coliform*	*Fecal coliform*	*Fecal Streptococci*	*Clostridium perfringens*
1	$2.55 \times 10^{14} \pm 1.01 \times 10^{15}$	$1.94 \times 10^{15} \pm 7.25 \times 10^{15}$	$8.25 \times 10^{13} \pm 2.41 \times 10^{14}$	$9.36 \times 10^{13} \pm 2.54 \times 10^{14}$
2	$1.38 \times 10^{10} \pm 2.03 \times 10^{09}$	$5.57 \times 10^{7} \pm 1.22 \times 10^{08}$	$5.53 \times 10^{9} \pm 1.21 \times 1010$	$1.26 \times 10^{9} \pm 2.9 \times 10^{9}$
3	$1.49 \times 10^{12} \pm 5.79 \times 10^{12}$	$2.44 \times 10^{10} \pm 4.82 \times 10^{10}$	$2.48 \times 10^{10} \pm 5.95 \times 10^{10}$	$1.29 \times 10^{10} \pm 3.66 \times 10^{10}$
4	$1.68 \times 10^{9} \pm 4.93 \times 10^{09}$	$9.05 \times 10^{9} \pm 1.79 \times 10^{10}$	$1.22 \times 10^{8} \pm 2.05 \times 10^{8}$	$2.71 \times 10^{6} \pm 3.79 \times 10^{6}$
5	$3.34 \times 10^{9} \pm 1.08 \times 10^{10}$	$8.98 \times 10^{9} \pm 1.79 \times 10^{10}$	$1.60 \times 10^{6} \pm 1.99 \times 10^{6}$	$1.26 \times 10^{6} \pm 2.02 \times 10^{6}$
6	$7.64 \times 10^{10} \pm 2.16 \times 10^{11}$	$8.56 \times 10^{5} \pm 1.28 \times 10^{6}$	$1.55 \times 10^{6} \pm 2.06 \times 10^{6}$	$3.78 \times 10^{6} \pm 7.38 \times 10^{6}$

The percentage of isolated bacteria from different parts of sampling (A) and the average concentration of bacterial indicators in all the examined sampling months (B) are depicted in Figure 2. The number of bacteria in inlet of WWTP was high in all of the examined sampling sites. This is because domestic wastewater and various types of macro and micronutrient constitute more bacteria growth [20]. The result showed that the highest concentrations of bacteria were detected in July, with an average concentration of 1.16×10^{9}, 2.25×10^{8}, and 33.18×10^{6} MPN/100 ml for *total coliform*, *fecal coliform*, and *fecal streptococci*, respectively.



A B

Figure 2: Illustration of isolated bacteria: (A) the samples tested were considered positive for bacteria in all the examined sampling sites. (B) the average concentration of bacterial in all the examined sampling months

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To identify possible associations between the measured parameters regardless of dependence between observations, a multivariate analysis of variance was performed, the results of which are presented in Figure 3. The results of this study showed that there was a significant difference between the average concentration of *fecal coliform* in the inlet of WWTP (station 1) and the outlet of WWTP (station 2), station 3 (entrance to the treatment plant), and station 6 (200 meters north of the sea); and station 3 (The intersection of wastewater in the sea) and station 6 (200 meters north of the sea). Additionally, there was a significant difference between the average concentration of *Clostridium perfringens* in inlet wastewater (station 1) and the outlet of the treatment plant (station 2), and the west point of the sea and the north point of the sea, but between the east station and the confluence of the sea, it was not significant. The only statistically significant difference for *Streptococci* was related to the influent wastewater (station 1) and station 6 (200 meters north of the sea). The statistical analysis showed no significant relationship between bacterial concentration in the outlet of wastewater treatment plant and samples taken from station 3 entrance to the treatment plant of Gorgan Bay.

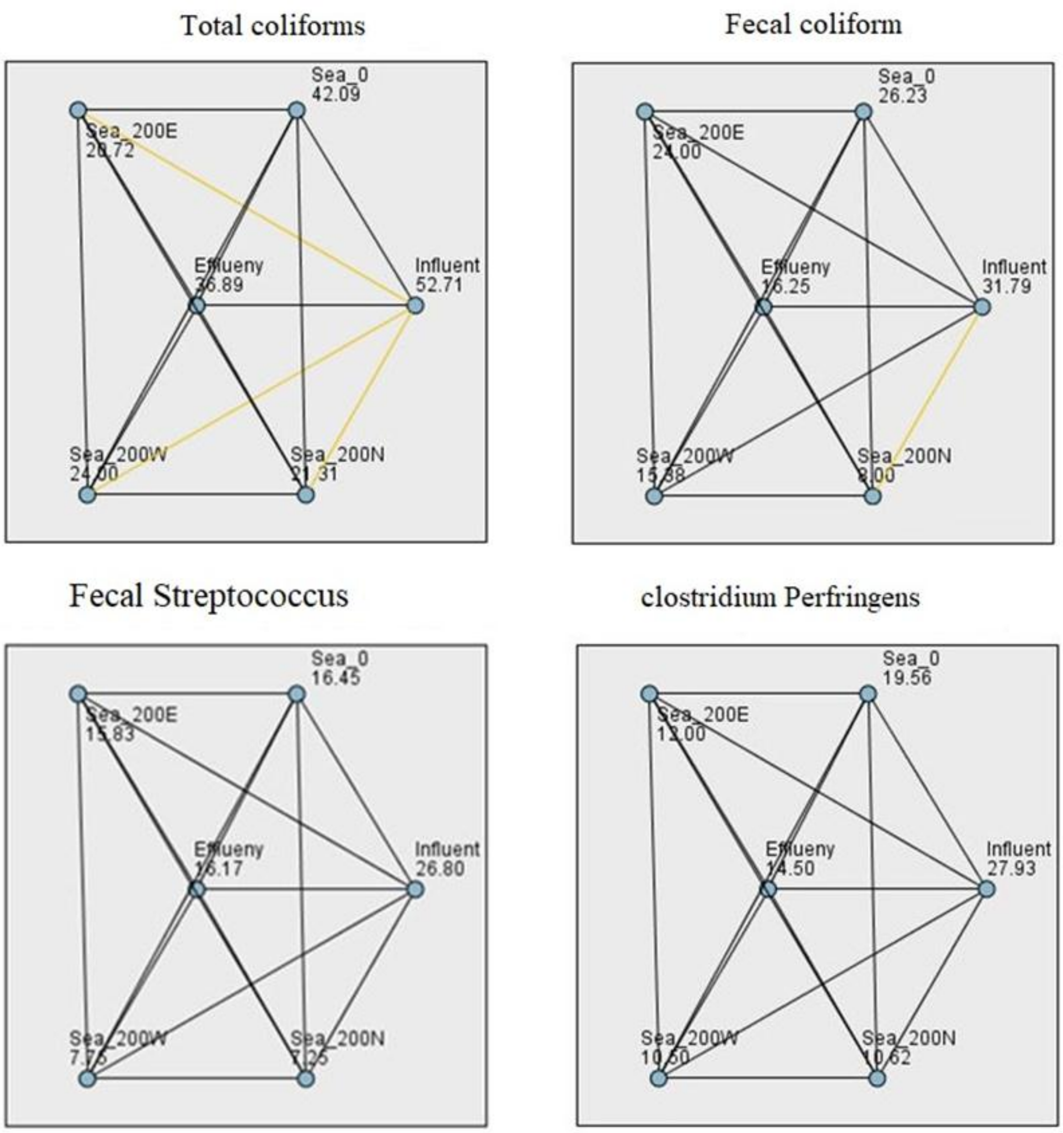

Figure 3: Comparison between groups, regardless of dependence between observations

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Discussion

According to WHO guidelines, aeration lagoons have an efficiency of above 95% for *fecal coliform* reducing to ≤ 10^3/100 ml and nematode eggs ≤ 1/L [21]. In this study, the removal efficiency of the aeration lagoon was 31.78%, 32.87%, 26.03%, and 21.15% for *total coliform*, *fecal coliform*, *fecal streptococci* and *Clostridium perfringens*, respectively. In another word, the performance of the aeration lagoon was significantly lower than the expected limit for *fecal coliform* removal ($p<0.001$). In Malhas et al. Study, a reduced concentration of 99.31% and 99.52% were obtained for *total coliform* and *fecal coliform*, respectively [22]. The weak performance of the aeration lagoon may be related to faulty technology, lack of operator skill, and environmental variables. Bandargaz city has a Mediterranean climate with few sunny days and many cloudy and rainy days, while this method is mostly proposed for tropical areas with more sunny and less rainy days. Hence, intervention for an operator training program and investments in sewerage may be necessary to improve the effectiveness of aeration lagoon at removing indicator bacteria in the effluent of WWTP. The study by Blanco et al. also indicated that wastewater treatment plants should be improved to protect fish health in small Mediterranean rivers [23]. The finding of Petri et al. study revealed a remarkable decrease in microbial population of recreational waters after the sewerage system and wastewater treatment in Albania [24]. The results of Chatterjee et al. study revealed that faulty technology and inexperienced operators affected WWTP's performance [25]. For this reason, it is recommended to perform a comprehensive research to identify the reasons for improper performance of treatment process and not reducing the population of bacteria regarding the permissible limit. However, using filtration followed by storing effluent in a final tank can be proposed to raise aeration lagoon performance for bacteria reduction. Based on the research by Malhas et al., applying an ultrafiltration membrane followed by an activated carbon filter can reduce 99.99% of coliform, *fecal coliform*, and *salmonella* spp. [22]. The comparison of *fecal coliform* concentration with permissible limit of WHO showed that the effluent of wastewater treatment plants was not suited for irrigation, and farmers were most likely at risk through flood irrigation. Additionally, irrigation with this treated wastewater could transmit infectious diseases to consumers of crops [26].

Based on the findings, with increase of distance from the confluence of the outlet with Gorgan Bay, *fecal coliform* concentration decreased. This study was in accordance with Owili's study considering the fact that *fecal coliform* count of Hafnarfjordur beach, Iceland decreased with increasing distance from wastewater outlet; however, the number of fecal coliforms was higher than EU limit for swimming [27]. Statistical analysis showed that coliform concentration in 200 meters in the direction east of site 4 in Gorgan Bay (0.002), 200 meters in the direction West of site 4 in Gorgan Bay (0.004), and 200 meters in the direction north of site 4 (0.006) in Gorgan Bay was significantly lower than the concentration of total coliform at the intersection of wastewater with the sea.

The results of microbial analysis showed that the bacterial concentration varied during the months of the study. At the present study, the lowest number of *total coliform*, *fecal coliform*, and *fecal streptococci* were recorded in April, June, and June with an average concentration of 87×10^4, 13.07×10^3 and 90.74×10^2 MPN/100 ml, respectively. In addition, the highest and lowest average count of *Clostridium perfringens* were recorded in May and June with an average concentration of 36.06×10^6 and 27.80×10^3 MPN/100 ml, respectively. A statistically significant difference was found between the number of *total coliforms* ($p < 0.001$) and *fecal streptococci* ($p = 0.01$) in different months of sampling; however, the authors didn't find any associations between fecal coliform ($p = 0.07$) and *Clostridium perfringens* ($p = 0.09$). Chi-square tests showed that there was a significant difference between the level of *total coliform* ($p < 0.001$) and *fecal streptococci* ($p = 0.011$) in different months of sampling. In contrast, statistical analysis showed

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no association between the number of total coliforms (p = 0.066) and *Clostridium perfringens* (p = 0.089) in different months of sampling. Similarly, Karbasdehi et al. reported no statistically significant correlation between temperature and indicator microorganisms [28]. On the contrary, Placha et al. found that indicator's *total coliform*, *fecal coliform*, and *fecal streptococci* were significantly affected by temperature [29]. It is a fact that environmental temperature has an important role in the biological treatment of wastewater; however, it is difficult to determine optimal temperature for treating wastewater in regions with different climates [30].

According to this study, a high level of bacterial pollution was found in all the sampling sites in Gorgan Bay (Table 2). A similar result was reported by Moazeni et al., who found a high level of pathogenic fungi (75.1% of investigated samples) in the Caspian coastline[31]. In recent years, many studies reported about microbial pollution of the Caspian Sea and Gorgan Bay and public health concerns [13, 31-33]. The authors cannot clearly describe the lack of correlation between bacteria concentrations in different sample stations of Gorgan Bay because they did not investigate all the pollution sources in the study area. Yang et al. reported that due to the complexity of aquatic ecosystems, it is difficult to determine how water resources respond to pollutants [34]. However, the Caspian Sea has organic carbon that provides a primary nutrient for shaping microbial communities [35]. In other words, the adverse effect of effluent discharge into water resources remains poorly understood [36]. Ziegler et al. reported that anthropogenic impacts were found on the coral near Jeddah, even seemingly healthy corals [37]. Due to these risks, it is better to consider Gorgan Bay ecosystems by related authorities. A comprehensive study is recommended to provide accurate information about the point or non-point pollution source affecting the quality of the Caspian Sea in Gorgan Bay.

This study showed a significant relationship between *fecal coliform* and *fecal streptococci* (p = 0.003, R = 0.317), and no significant relationship was observed for other investigated microorganisms. Bonadonna et al. found no statistically significant relationship between *Clostridium perfringens* and *Cryptosporidium*, *Giardia*, *and enteroviruses* [38]. The lack of association between indicator bacteria and pathogens is an important challenge for public health. Hemati et al. reported that monitoring methods for parasites e.g., *Cryptosporidium* and *Giardia* were insufficient, and an effective measure should be considered to determine the presence of these parasites in water and wastewater treatment plants [39].

Limitations of the study

One of the limitations of this study was that a short period was selected, and it was better to increase the study's duration and sample size by at least one year to increase the precision of the results. Unfortunately, environmental parameters like air temperature, rainfall, condition of sunny or cloudy, and the physical and chemical characteristics of wastewater and seawater were not surveyed. It is, therefore, impossible to determine their effect on the performance of aerated lagoons.

Conclusions

The results of this study showed that aeration lagoon performance was below the permissible limit set by WHO for discharge into water resources, agricultural irrigation, and recreational activities. Consequently, an infection risk likely existed for humans through consuming fish, crops, and recreational activities. The use of filtration and the disinfection process may be required to improve the quality of WWTP to protect human health from risks of bacterial contamination. Comprehensive monitoring efforts are suggested to investigate all the environmental factors such as air temperature, sunlight intensity, cloudy and rainy days, and physicochemical characteristics of wastewater like dissolved oxygen, pH, and chloride to achieve accurate results about the performance of aeration lagoon in the Caspian Sea region. Additionally, continuous monitoring is essential to control the quality of wastewater

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before discharge into the environment or reuse.

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Conflict of interest

The authors declared no conflict of interests.

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Ethical considerations

Code of ethics

This research was approved by the Ethics Committee of Golestan University of Medical Sciences under ID No: IR.GOUMS.REC.1401.443.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

Ali Shahryari was the supervisor of the study in all the steps and edited the final version of the manuscript. Navisa Sadat Seyed Ghasemi performed the statistical analysis. Hasan Safari, Seyed Abedin Moosavi, and Ebrahim Moghadar collected the samples. Maryam Shafipour, Reza Ebrahimnia, Fatimah Eftekhari, and Sara Salar conducted the experiments on the sample. All the authors wrote the first draft of the manuscript and approved the submitted version of the manuscript.

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