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ORIGINAL ARTICLE

Investigating Nitrate and Nitrite Concentrations in Drinking Water of Five Districts in Tehran and Assessing the Presence of Nitrate Reducing Bacteria

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KEYWORDS

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ABSTRACT: High levels of nitrate and nitrite in groundwater lead into environmental issues and nitrate poisoning causes unpleasant consequences in human bodies. The present study was conducted to evaluate the concentration of nitrate, nitrite, and nitrate-reducing bacteria in treated drinking water in five areas in Tehran using optical spectroscopy. The results of one-way ANOVA showed that the average concentrations of nitrate in summer and autumn are significantly higher than those of nitrite. Moreover, the highest mean of concentration of nitrate and nitrate was observed in the west of Tehran in autumn 2016 (1.2±0.01 and 4.6±0.02 mg L^{-1}). On the other hand, the lowest mean was found in the south of Tehran (0.06±0.02 and 0.001±0.01 mg L^{-1}). However, only in the southern region of Tehran, signs of *Pasteurella* sp. and *Acinetobacter* sp. were observed. Additionally, the results of the Duncan test indicated no significant differences concerning nitrate concentration in the morning, noon, and at night in the southern, eastern, and central regions in autumn and summer. The samples of the north, east, and center had the lowest risk and, in the west, and south of Tehran, the highest risk and adverse effects of nitrite and nitrate were observed. Although the average concentration of nitrate and nitrite in all these areas, except for the west of Tehran, was less than the standard of the WHO and the latest national standard, protection of groundwater resources against the entry of pollutants treatment networks to prevent the increase of these ions in water seems to be necessary.

INTRODUCTION

Water is a vital fluid that passes through different places during its natural cycle and carries several different substances with it self. It consequently becomes contaminated through contact with surface and subsurface pollutants. Nitrate and nitrite anions are among the water pollutants. Over the last few decades, due to excessive use of chemical fertilizers, pesticides, and industrial substances, their average amount in groundwater has increased and

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caused pollution of water resources [1]. In addition, high rainfall in the water basin increases the presence of nitrate in surface runoff and has a negative impact on water quality [2]. To maintain the health of the community, the water used for various purposes must comply with the national and international standards. According to the studies conducted by the World Health Organization and the National Water Hygiene Standard of Iran, the permitted levels of nitrate and nitrite in drinking water are 50 and 3 mg L^{-1}, respectively. Furthermore, due to the possibility of the simultaneous presence of nitrate and nitrite in drinking water, the total concentration ratios of each to the standard values must be less than 1. On a global scale, agricultural activities are known to be the most important factor in increasing nitrate concentrations in groundwater, due to the land-use change and widespread use of chemical fertilizers and animal manures that contain high levels of nitrogen compounds. Nonetheless, studies in Iran have recognized continuous and unregulated discharge of municipal and industrial wastewater into underground aquifers as the main factor in increasing the nitrate content of groundwater, particularly in drinking water wells located within the cities in Iran [3]. In fact, when an excessive amount of nitrate enters the human body, it causes adverse effects on human health, especially children, the elderly and pregnant women; for example, in children, due to the low acidity of the gastrointestinal tract, Entero bacteriaceae bacteria are able to reduce nitrate to nitrite due to the enzyme nitrate reductase [3]. The nitrite ion produced by hemoglobin forms a highly stable complex called methemoglobin, which causes hemoglobin to leave the important life cycle of oxygen-carbon dioxide transport between the lungs and cells and can lead to suffocation or death. In subsequent reactions, nitrite with other molecules, especially type II amines and amides, leads to the formation of dangerous compounds of nitrous amine and nitrous amide with carcinogenic potential [4]. Therefore, paying attention to the quality of healthy drinking water is essential for maintaining the health of human societies. Despite extensive research on the level of water pollution in terms of nitrate and nitrite around the world, no research has to date been done on drinking water in the five regions of Tehran province, specifically on the identification of nitrate-reducing bacteria [5-24]. The inhabitants of Tehran, with a population of more than eight million people, currently use the water from old dams and wells. On the one hand, since the volume of surface water is decreasing and due to the air and soil pollution and lack of a widespread sewerage system in the metropolitan area of Tehran, the possibility of contamination of these drinking-water supplying wells and dams is high. This study was conducted for the first time with the aim of investigating the status of treated water resources and measuring the concentration of nitrate and nitrite in drinking water in five areas of Tehran via spectrophotometry. In addition, the possibility of the presence of nitrate-reducing bacteria in water samples together with the thermic and temporal impacts (the two seasons of autumn and summer, and three sampling shifts in the morning, at noon, and night) were studied.

MATERIALS AND METHODS

Sampling

We carried out the sampling of urban drinking water in summer and autumn of 2016 from five geographical areas of Tehran, including north, south, east, west, and center at different times of the day (three times a day) in triplicate. An average of 10 to 15 neighborhoods was selected from each area for the sampling. Laboratory sterile containers were used to store the samples. The samples were stored at 4°C. After 24 hours, they were transferred to Zakaria Razi Laboratory located in Islamic Azad University, Tehran Science and Research Branch, and kept at 4 ° C until the experiment started.

Measurement of nitrate and nitrite in water using DR5000 spectrophotometer

To measure the amount of nitrate and nitrite in the samples, the spectrophotometer program No. 355 with a wavelength of 500 nm and powder containing nitrate and program No. 371 with a wavelength of 507 nm and powder containing nitrite were used [9].

Evaluation of harmful amount of nitrate and nitrite in drinking water samples from the five districts of Tehran

In order to achieve the standard values of nitrate and nitrite of drinking water based on standard 1053, the following formula was employed [25].

Microbial examination of samples

Nitrate reduction test was performed to determine the presence of bacteria that can convert nitrate to nitrite. To perform this test, the suspect water sample was added to broth nitrate medium (Sigma) and kept in an incubator at 35 °C for 24 to 48 hours. After the desired time, the samples created in the Durham gas tube were tested for nitrate. For the nitrate test, 500 µL of nitrate (I) reagent (1 g of sulfanilic acid and 125 cc of 5 N acetic acid) and 500 µL of nitrate (II) reagent (625 mg of alpha naphthylamine with 125 cc of 5 N acetic acid) were added to the test tube with a positive sample. To determine whether the nitrate test was positive or negative, the color change of the culture medium was examined. Subsequently, to identify the type of bacteria, hot staining, ability to grow in McConkey agar medium, and oxidase test were performed. Motion and indole testing and the ability to produce hydrogen sulfide gas in the SIM environment were also investigated. The chemicals and culture media used were prepared by the German company Merck.

Statistical analysis

The results of each representative experiment were analyzed with one-way analysis of variance (ANOVA) using the statistical software SPSS version 24 and the difference between the groups were detected with Duncan's grouping tests. P values smaller than 0.05 were considered to be significant. The means and the standard deviation were calculated based on the data obtained from three replicates. Moreover, data distribution was examined via Kolmogorov–Smirnov test and based on whether the data distribution was normal or not, the relevant tests (Kruskal-Wallis, Mann-Whitney and Tamhane tests) were performed.

RESULTS

Studies have shown that the distribution of data in these experiments do not follow the normal distribution. Therefore, since there were more than two data groups in this experiment and the objective was to investigate the statistical parametric differences in these groups, the Kruskal-Wallis test was used. Nitrite and nitrate concentrations in the studied areas of downtown Tehran in summer and autumn were significantly different ($p < 0.05$); accordingly, in all the areas, the amount of nitrate with a significant difference was more than nitrite in the two seasons of autumn and summer.

The results of one-way analysis of variance of nitrite and nitrate averages in summer and autumn in the five studied areas showed a significant difference. In the south of Tehran, the highest rate of nitrite concentration belonged to Rajaei St., with an average of 0.36±0.005 mg L^{-1} in summer and the lowest rate was observed in Dolatabad's 1st square in summer with an average of 0.00107±0.07 mg L^{-1}. Nitrate concentration in Shahrvand-E-Rey with an average of 0.856±0.04 mg L^{-1} had the highest rate in summer whereas Fadaiyan-e-Islam had the lowest rate in summer with an average of 0.06±0.02 mg L^{-1}. In the north of Tehran, the highest rate of nitrite concentration belonged to in Zaferaniyeh with an average of 0.044±0.02 mg L^{-1} in summer and the lowest rate belonged to Bahonar and the north of Tehran in autumn with an average of 0.002±0.01 mg L^{-1}. Nitrate concentration in Zaferaniyeh with an average of 1.4±0.03 mg L^{-1} in summer had the highest and Vanak in summer with an average of 0.5±0.01 mg L^{-1} had the lowest rate. In western Tehran, nitrite concentration in circulation with an average of 1.2±0.01 mg L^{-1} had the highest rate in autumn and Ariashahr and Kashani in summer with an average of 0.003±0.01 mg L^{-1} had the lowest rate. The highest rate of nitrate concentration was found to be in Kashani with an average of 4.6±0.02 mg L^{-1} in summer and the lowest rate was observed in Hyper in autumn with an average of 0.7±0.01 mg L^{-1}. In eastern Tehran, nitrite concentration in Damavand Street with an average of 0.029±0.03 mg L^{-1} was the highest in summer and Damavand and eastern Tehran in autumn with an average of 0.003±0.01 mg L^{-1} had the lowest rate. Nitrate concentration in Shariati with a calculated average of 2.3±0.01 mg L^{-1} had the highest rate in summer and Tehranpars was found to have the lowest rate in autumn with an average of 0.8±0.01 mg L^{-1}. In the center of Tehran, the concentration of nitrite in Zartosht with an average of 1±0.02 mg L^{-1} in autumn was the highest rate and Tohid in both summer and autumn with an average of 0.001±0.01 mg L^{-1} had the lowest rate. Nitrate concentration in Lalehzar with an average of 2.9±0.02 mg L^{-1} in summer had the highest and Haft-E-Tir in summer with an average of 0.7±0.01 mg L^{-1} had the lowest rate (Figure 1).

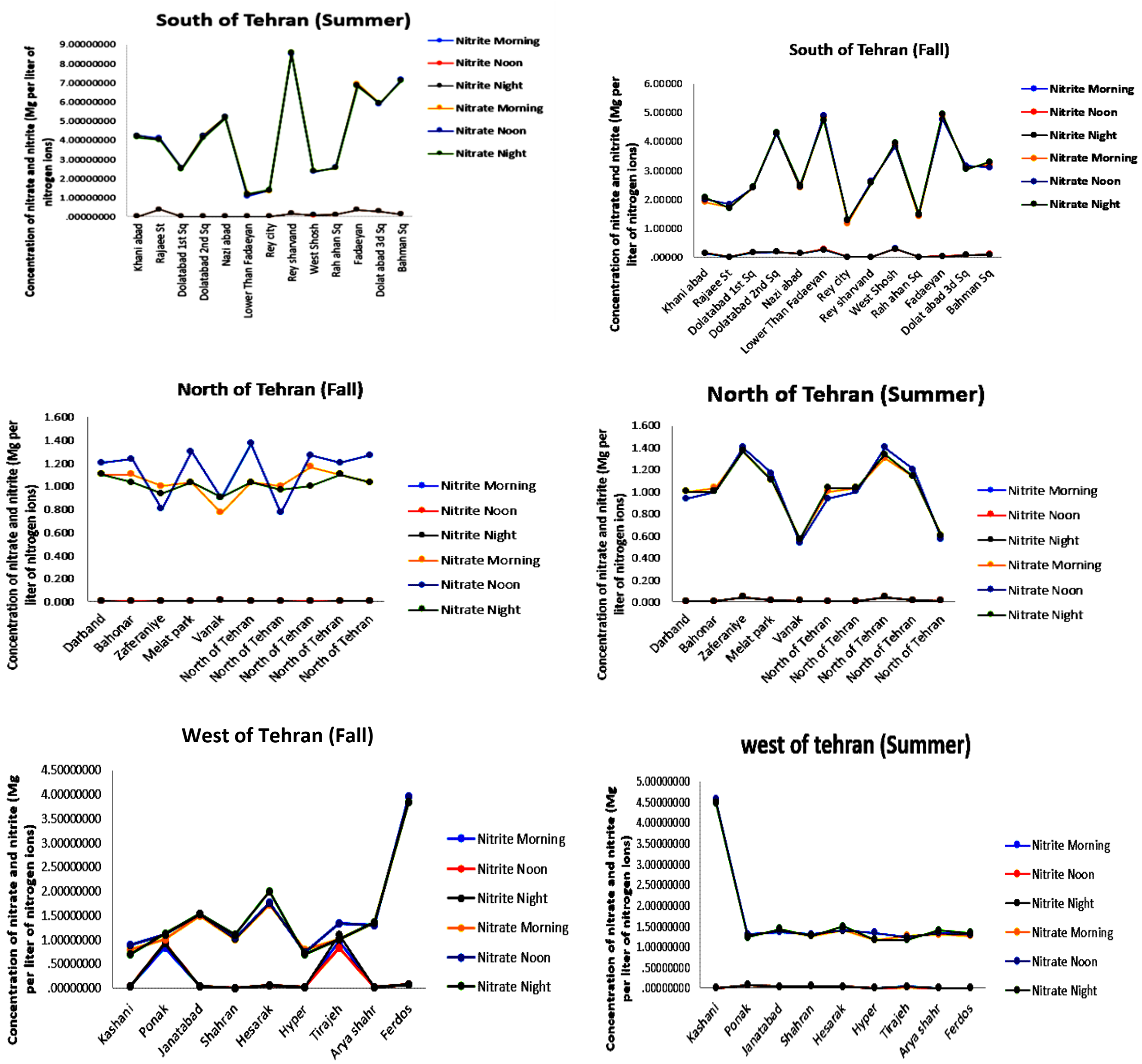

Figure 1. The results of one-way analysis of variance of nitrite and nitrate averages in summer and autumn in the five regions studied.

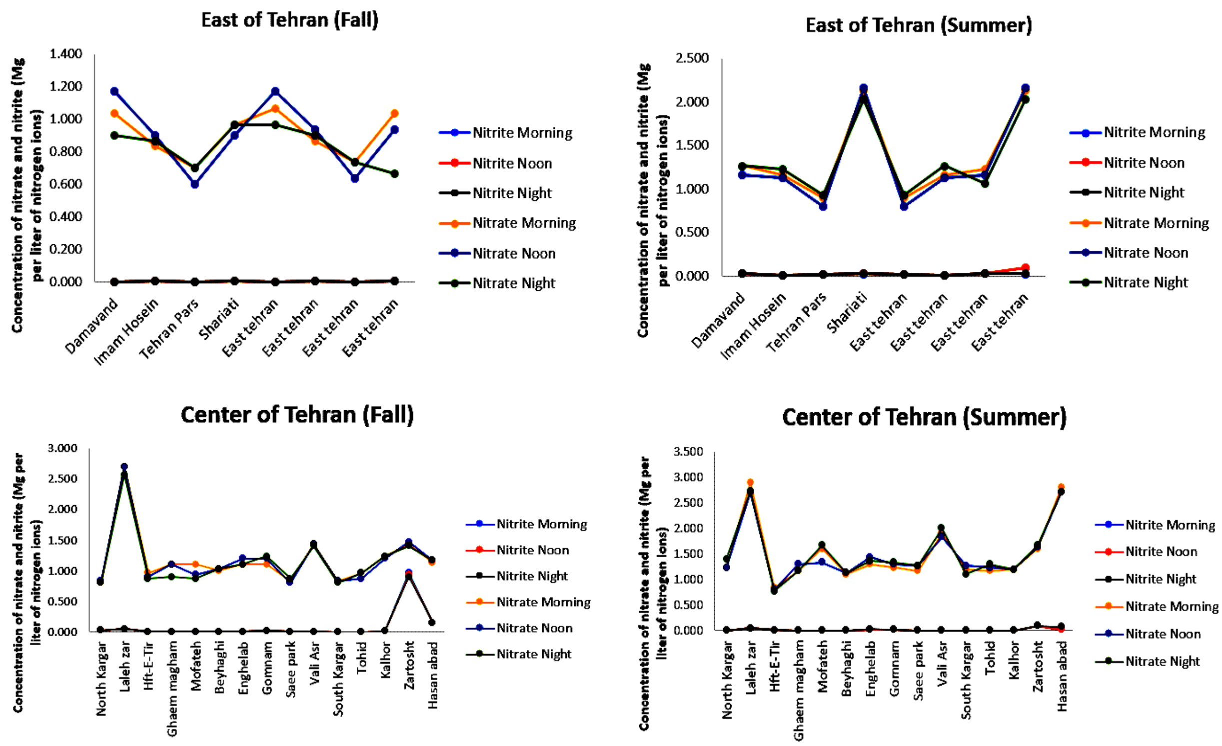

Figure 1. Continued.

The results of statistical analysis of water samples collected in the morning, at noon, and night showed that nitrite concentration only is significantly different among these times of the day in autumn in the south of Tehran in areas below Fadaiyan-e-Islam, West Shoush, Rah Ahan Square, Dolatabad 3rd Square, and Bahman Square. Meanwhile, nitrate concentrations in the south of Tehran, in autumn and summer, showed no significant differences among morning, noon, and night. Nitrite concentration in the north of Tehran, in autumn and summer, was not significantly different among these times. However, nitrate concentration in Mellat Park and four areas in the north of Tehran in autumn and one area in the north of Tehran in summer were significantly different. Nitrite concentration in the west of Tehran, in autumn and summer, had no significant differences among morning, noon, and night. Meanwhile, nitrate concentration in the west of Tehran, in Tirajeh, in autumn, was significantly different. Nitrite concentration in eastern Tehran was significantly different in only one area of it and just in autumn whereas nitrate concentrations in autumn and summer were not significantly different among morning, noon, and night. Nitrite concentration in the center of Tehran, in Ghaem Magham and Mofteh, was significantly different in autumn while nitrate concentrations in autumn and summer were not significantly different among morning, noon, and night (Table 1).

Table 1. Results of one-way analysis of variance and Duncan test obtained by collecting water samples in the morning, at noon, and night Sig< 0.05 indicates significant difference in nitrate and nitrite concentrations in the morning, at noon, and night

	Concentration (mg per liter of nitrogen ions)		
	Morning	**Noon**	**Night**
South of Tehran (nitrite concentration) autumn			
Bahman Sq	0.09800	0.098133	0.09800
North of Tehran (nitrate concentration) autumn			
Mellat park	1.033	1.300	1.033
North of Tehran	1.033	1.367	1.033
North of Tehran	1.000	0.767	0.967
North of Tehran	1.167	1.267	1.000
North of Tehran	1.100	1.200	1.100
North of Tehran	1.033	1.267	1.033
West of Tehran (nitrate concentration) autumn			
Tirajeh	1.000	1.333	1.000
East of Tehran (nitrate concentration) autumn			
East of Tehran	1.067	1.167	0.967
Tehran downtown (nitrite concentration) fall			
Ghaem magham	0.004	0.003	0.004
Mofatteh	0.003	0.003	0.004

Results of the evaluation of harmful amounts of nitrate and nitrite in drinking water in the five areas of Tehran based on standard 1053

The results of the evaluation of harmful amounts of nitrite showed that in summer, south of Tehran and in autumn, west of Tehran has the highest risk of harmful amounts of nitrate and nitrite (Table 2).

Table 2. Evaluation of harmful amounts of nitrate and nitrite in drinking water in the five areas of Tehran in summer and autumn 1995

Row	Region	The number obtained according to the standard 1053 in the fall of 2016	The number obtained according to the standard 1053 in the summer of 2016
1	Downtown	0.58	0.33
2	South	0.54	0.88
3	East	0.12	0.59
4	North	0.13	0.14
5	West	0.75	0.21

Results from the presence of reducing bacteria in the five areas of Tehran in summer and autumn 1995

The water samples extracted in summer and autumn of two areas located in the south of Tehran (Fadaiyan-e-Islam and Shahid Rajaei) had a positive nitrate reduction test. Therefore, to identify the type of bacteria, diagnostic and differential tests were performed, through which two bacteria, namely *Pasteurella* sp. and *Acinetobacter* sp., were identified (Table 3).

Table 3. Results of diagnostic and differential tests for nitrate-reducing bacteria

Bacteria	Broth nitrate	Warm colouring	Growth in Mekanki environment	Oxidase	Motion	Indole	Hydrogen sulfide gas
***Pasteurella* sp.**	+	-	+	+	-	+	+
***Acinetobacter* sp.**	+	-	+	-	-	-	-

DISCUSSION

Over the recent decades, the use of nitrogen fertilizers and the rapid growth of industry, regardless of their effects on soil and water properties, has increased environmental pollution. Nitrate, as the main form of nitrogen, is easily washed from the soil by irrigation or rainfall and transferred to groundwater and causes water pollution due to its negative charge. Another harmful consequence of nitrate occurs when it is leached into surface water sources, such as lakes and seas. Due to the pollution of aquatic environments with nitrate, ecological disturbances appear, which in turn result in detrimental impacts on aquatic life due to the lack of available oxygen, intoxication, and so forth. Since high levels of nitrate in drinking or agricultural water directly enter the human lifecycle and cause problems and diseases, such as methemoglobinemia, leukemia, stomach, bladder, brain cancer, and other cancers, measuring these ions could be a warning factor to prevent numerous diseases [26]. Brender et al. [27] examined the relationship between nitrate-containing drinking water consumption and congenital malformations in the fetus. The results showed that drinking high-nitrate water within the first three months causes the fetuses of mothers to have limb defects, cleft palate, and cleft lip. According to the EPA standard, the permissible amount of nitrate in water is about 50 mg per liter and if it exceeds this amount, it causes a disease called cyanosis (especially in children). In this disease, through the reduction in the amount of stomach acid, special bacteria are activated and nitrate ions are converted to nitrite ions. Many papers have been conducted on drinking water in Iran by measuring the concentration of nitrate and nitrite. Several studies have identified the drinking water of Lahijan, Forg section of Darab city, Kashan, Bushehr, Urmia, Jiroft, Babol, Sabzevar, Sari, Bardsir, Divandere, and Asadabad as healthy and their nitrate and nitrite concentration as permissible, including the results of research by 5-10, 12, 16, 19, and 28-30.

The present study was the first work in Iran to simultaneously investigate the correlation between nitrate and nitrite concentrations and the microbial flora of drinking water, in which there was a significant difference ($p < 0.05$) between the nitrite and nitrate concentrations in the study areas of Tehran city center in summer and autumn. It was shown that in all the regions, the amount of nitrate was significantly higher than that of nitrite in autumn and summer. Nitrate ion is the product of the final oxidation of ammonia, which due to the vital activity of bacteria, is first converted to nitrite and then to nitrate. That is why the amount of nitrate is always higher than that of ammonia and nitrite. In addition to organic matter, fertilizers are a major source of nitrate in water sources. This anion (nitrate) is present in surface water and mostly enters running water by oxidation of ammonia, factory effluents, production of materials, such as ammonia, nitric acid, ammonia compounds, sodium carbonate, explosives, or leaching of agricultural land by irrigation or rain. The results of one-way analysis of variance indicated that the highest mean concentrations of nitrite and nitrate were found in all the five areas, except for the west of Tehran in summer. However, the results of statistical analysis and Duncan's test implied that the highest nitrite concentration in the west of Tehran in Tirajeh with an average of 1.2 ± 0.01 mg L^{-1} was found in autumn. This indicated that the factor of season has a significant effect on the amount of nitrite in the waters of the circulation area. This is while Kashani with an average of 4.6 ± 0.02 mg L^{-1} in summer had the highest amount of nitrate compared to other regions; this result showed that summer has a significant effect on the presence of reducing bacteria. This result is consistent with the results of Heydari Kochi in 2008. Studying the trend of nitrate changes with rainfall in the drinking water of villages in Fasa city, these researchers found that with the decrease in rainfall, the amount of nitrate in groundwater increases [31]. In addition, the results of the present study showed that the concentration of nitrite and nitrate in the south of Tehran in the first square of Dolatabad with an average of 0.001±0.07 mg. L^{-1}, in the center of Tehran, Tohid region, with an average of 0.001±0.01 mg L^{-1}, and lower than Fadaiyan-e-Islam in summer with an average of 0.06±0.02 mg L^{-1} had the lowest rates. Meanwhile, in the southern regions of Tehran (Fadaiyan-e-Islam and Shahid Rajaei), the samples of pathogenic bacteria, such as *Pasteurella* sp. and *Acinetobacter* sq. were observed.

In evaluating the harmful amounts of nitrate and nitrite in the studied samples, the concentration of nitrate and nitrite in the studied drinking water in Tehran was in accordance with the 1053 standard and the WHO standard. The samples of the northern, eastern, and central regions had the lowest risk of nitrite and nitrate. The samples of the west and south of Tehran showed the highest risk and adverse effects of nitrite and nitrate, which meets the results of the highest amounts of nitrate in the west of Tehran and the presence of *Pasteurella* sp. and *Acinetobacter* sp. in the south of this city.

In several studies conducted on drinking water of Semnan, Hamedan, Ilam, Amol, Zanjan, west of Tehran industrial units, Ardabil, Saveh, Dehlagan, and Bojnourd, the amount of nitrite and nitrate has been detected to be higher than the permissible and standard limit [11-14, 21-24, 32-35]. These results suggest that drinking water, even after treatment, may not be within the permitted standards. This condition is so important that researchers have even examined the concentration of nitrate and nitrite in bottled mineral water. Forouzan et al. [35] investigated the mineral waters available in the market of West Azerbaijan province and showed that the average amount of nitrite and the total concentration of nitrate and nitrite are higher than the standard. In addition, Aghalari and Jafarian [36], in a study conducted on bottled mineral water supplied in the city of Babol, reported that the measured nitrate values are very different from the values recorded on the label. On the other hand, Ashrafi [37] conducted a study on mineral waters in the market of West Azerbaijan province and showed that the concentration of nitrite and nitrate in the samples is less than the maximum national standard of Iran; thus, there is no threat to the use of bottled mineral water for the health of consumers. The results of this experiment indicated that although the average concentration of nitrate and nitrite in all the samples was less than the standard rate of the World Health Organization and the latest national standard, the protection of groundwater resources against the entry of pollutants and also providing practical plans for acceleration of the implementation of wastewater collection and treatment networks in order to prevent the increase of these ions in water seems necessary. Hence, if the necessary measures are not taken in the future, we will see the concentration of nitrate ions above the standard.

CONCLUSIONS

The obtained results herein revealed the poor quality of drinking water in areas of the west and south of Tehran in terms of nitrite and nitrate concentration and the presence of nitrate-reducing bacteria compared to other studied areas. Therefore, even though the values of nitrate and nitrite concentrations were obtained within the allowable limit, if the necessary precautions are not taken in the future, we will see the concentration of nitrate ions above the standard. In addition, due to the fact that nitrate and nitrite, in addition to drinking water, enter the human body through some foods as well as fruits and vegetables, the effective factors in the entry of nitrate and nitrite into the human body should always be considered. Therefore, all those in charge of the food industry and all drinking water treatment plants should be obliged to maintain standards by considering other sources of nitrate and nitrite.

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Conflict of interests

The authors declare that they have no conflict of interest.

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