

The role of domestic wells on Hamadan water supply contamination

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ABSTRACT

The objective of this study was to identify and assess the possible presence of coliform bacteria in Hamadan drinking water during drought seasons that generally influenced the water resources of Hamadan city. For this purpose, 464 water samples were collected during two periods (P1 and P2) between July 2003 to March 2005 and total and faecal coliforms bacteria (Coli.), dissolved oxygen (DO), temperature (T), pH and residual chlorine concentration (Cl) were measured. The results showed that Cl concentrations varied between 0.27 and 0.42 mg/l. The contaminated samples during periods P1, P2 and the total periods (P_T) were 29.5, 12.5 and 21%, respectively, indicating the high coliform levels in water. The results indicated 61.5% of the samples contained Cl concentration between 0 to 0.5 mg/l. An inverse relationship was found between Cl concentration and coliforms of the samples. Contaminated groundwater of domestic wells, collected biofilms in old pipelines of the network, leakage of sewage water and weak chlorine concentration were the major factors that resulted in contamination of coliform-free water supplied from the reservoirs of the city. Further studies are necessary to better understand the exact reasons for the contamination detected during this research.

Key words | coliform bacteria, drinking water, Hamadan, residual chlorine

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INTRODUCTION

The dramatic decline in the incidence of waterborne disease in the early 1900s after introduction of water treatment and disinfection has been documented in detail by numerous authors (Brock *et al.* 1994; Morris & Levin 1995; Ashbolt *et al.* 2001). However, there is a reason to be concerned for the future microbiological safety of drinking water, in both developing and developed countries (Ford & Colwell 1996; Ford 1999). This may be because of the age of water treatment and distribution systems and their deterioration that causes an increase in diseases, or at least an increased diagnosis of disease, caused by pathogens with varying degrees of resistance to treatment and disinfection (Ford 1993; Ford & Colwell 1996).

The presence of any type of coliform organism in treated water, therefore, suggests inadequate treatment and

disinfection, re-growth or infiltration in a distribution system (LeChevallier *et al.* 1996). After the report of the World Health Organization (WHO 1992) nearly half of the population in developing countries suffers health problems associated with lack of drinking water or the presence of microbiologically contaminated water.

There are many steps in water treatment which are being taken to ensure that the public is provided with safe drinking water. The coliform bacteria test is one of the most important steps that must be regularly applied in public water systems. Coliform bacteria are organisms that are present in the environment and in the feces of all warm-blooded animals and humans, and their presence in drinking water indicates that disease-causing organisms (pathogens) may exist in the water system. The test for the

presence of coliform bacteria is relatively easy and inexpensive. In contrast, the possible pathogen identification is more complex, time-consuming and expensive.

Several studies investigated the possibilities of reducing overall fecal contamination of drinking water and its possible health effects (Mintz *et al.* 1995; Conroy *et al.* 1999; Jensen *et al.* 2002). The fecal coliform count is a more reliable indicator of the sanitary quality of water because some coliform generations and species are of fecal or non-fecal origin (Alonso *et al.* 1999).

According to the reports of Hamadan University of Medical Sciences (Rahmani & Kashani 1994), about 150 diseases (based on the international classification) could be transmitted through the drinking water supply network of Hamadan, especially during summer. The objective of this research was to assess and link microbial contaminations in local zones of the city under two different conditions of water distribution, which are: (i) a period of limited flow (about 16 h per day) in summer and (ii) a period that is with normal water circulation (permanent), at different sites of the city.

MATERIALS AND METHODS

Site description

The study was carried out in Hamadan, a major city of Hamadan province situated in west Iran, about 336 km south west of Tehran, at 34°48'N and 48°31'E. The population of the city is about 520,000. The area covers approximately 60 km² with an altitude variation of 1750–1975 m (on average 1890 m). Based on the Emberger climate classification, the climate is cold semi-arid and average annual rainfall is 330 mm. The initial part of the drinking water network of the city was constructed in 1965 and completed in 1985. At present, it consists of 8 zones (numbered from 1 to 8, Figure 1), which are supplied by 11 underground reservoirs. The zones' areas ranged from 1.68 to 20.88 (on average 6.9) km². The reservoirs are connected by the main pipelines that convey water from the resources to the distributor laterals of the network. The total length of pipes in the Hamadan network is about 800 km, comprising 108 km of main pipes of more than 600 mm diameter.

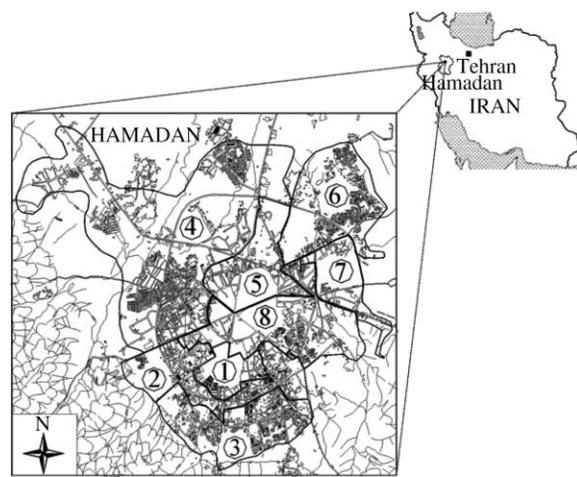


Figure 1 | Map of Hamadan city showing the sampling zones.

The city water resources consist of the water of the Ekbatan Dam (about 40% of the total) constructed about 21 km south east of Hamadan and the groundwater of the Bahar plain (about 60%), located 23 km north of the city. The Bahar plain is generally an agricultural area and substantial amounts of inorganic pollutants are used by farmers each year.

A network contributed by 65 wells has been developed in Bahar groundwater. Three pipelines convey the pumped water of the Bahar wells as well as the water of the Ekbatan Dam toward Lonapark, the major reservoir of the Hamadan water supply network.

The disinfecting process of Hamadan water involves chlorine (solid and gas) which is a very reactive chemical and the most important disinfectant as well as ozone (gas), which is now increasingly used in drinking water treatment. The treatment and disinfecting processes of the Ekbatan Dam water is normally carried out before distribution into the city, whereas Bahar wells water is disinfected after pumping into the Lonapark Reservoir (Marofi 2006).

Water samples

The sample sites were selected from the 8 zones of the network. Sampling was according to the procedure recommended by the American Public Health Association (*Standard Methods* 1995). The samples were stored in three sterile Shcott glass bottles of 300 ml (one sample for the presence/absence examination of coliforms) and 500 ml

(two samples for identification of pathogen populations). Once collected, the samples were immediately stored at 4°C in a dark cooler box (ice bag) and transported to the laboratory. They were analysed within 2–4 h of collection. Total coliforms and fecal coliform bacteria, dissolved oxygen, temperature, pH and chlorine concentration of the water samples were examined and analyzed.

The laboratory analyses were carried out according to the generally accepted basic laboratory procedures of the American Public Health Association *Standard Methods for the Examination of Water and Wastewater (Standard Methods 1995)*.

The multiple tube fermentation method (which is a quantitative method and uses cultivation to detect/confirm the presence of coliform organisms) was applied for the presence/absence examination of coliforms. It consisted of five tubes for the presumption and confirmation tests. A detailed description of this method is given in *Standard Methods (1995)*.

The existence of total or fecal coliforms in a water sample (contamination value) was showed by the Most Probable Number (MPN). The MPN index per 100 ml at 95% confidence limit, for the series used of decimal dilution, was determined based on the *Standard Methods* recording tables.

In order to identify the pathogenic microorganisms isolated from those water samples when their preliminary tests were positive (MPN > 0), two different tests were applied: (i) membrane filter method and (ii) sediment method (Baron *et al.* 1994). In both tests, a sample of sterile water was utilized as control.

- (i) Membrane filter: a volume of 500 ml of water was filtered through a 0.45 mm pore size, 47 mm diameter membrane Millipore sterile filter and placed over the surface of a blood agar plate and incubated at 37°C for 24 h. After this period, the growth of colonies could be detectable. For isolation of fastidious pathogens, the samples were again cultured on a selective media such as Brain Heart Infusion agar, Eosin Methylene Blue and MacConkey agar and incubated at 37°C for 48–72 h. Finally, biochemical tests such as carbohydrate fermentation tests, oxidation, carbon consumption, hydrolysis of Esculine and Indole production were used for detection of isolated pathogens.

- (ii) Sediment method: to provide the sediment of the sample, 50 ml of the sample water was centrifuged at 2,500 rpm for 15 min. The insoluble solid portion (pellet) of the suspension was separated from the remaining water. The sediment was cultured onto a blood agar plate and incubated at 37°C for 24–48 h. After growing the pathogens, colonies were counted in 10, 50, 500 and 1,000 per ml.

The samples were taken during two different periods, which were: a special case, in which the network operation was periodic (P1) as well as the normal case of water distribution (P2). During period P1 (which was in summer), because of a severe drought that occurred in some western regions of Iran, the water drinking resources of Hamadan were very limited. Therefore, a drought season calendar that consisted of a reduced water distribution time of 16 h was applied (in the network). During this period, water was available just between 6:00 am and 10:00 pm each day.

The samples were taken on a regular basis from July 2003 to March 2005. Total samples for the microbiological pollution tests that verified the presence/absence of total and fecal coliforms were 400 (200 samples during each period). In addition to the samples taken for the presence/absence tests of coliforms, more than 64 samples were randomly taken of the positive samples, to identify their pathogenic microorganisms (Table 1). The sample distribution was not only based on the surface of each zone but also on their populations. It ranged from 4.2 to 30.5 (on average 10.85) samples per km². However, though bilofilm investigation was not a part of this study, four old pipelines of the network were trenched for bilofilm detection.

Statistical analysis

Statistical parameters of total and fecal coliforms, DO, T and pH of the samples were calculated to present the variation of these water quality characteristics during periods P1, P2 and P_T. Pearson's correlation coefficient (*r*) was used to show a correlation between the microbiological collected data using SPSS software. The Student's *t*-test was used to determine the statistical significance at levels of $p < 0.01$, $p < 0.05$ and $p < 0.001$.

Table 1 | The statistics of pH, T, Cl and DO collected in the Hamadan drinking water network

No. of samples for pathogens identification	No. of samples for coliforms detection	No. of samples for pH, Cl, DO and T investigation	T (°C)	DO (mg/l)	Cl (mg/l)	pH	Statistics	Period
32	200	200	20.41	4.92	0.27	7.31	Average	P1
			25	7.2	1	8.4	Max.	
			9	2.30	0	6.33	Min.	
			2.63	1.12	0.22	0.25	SD	
32	200	200	12.83	6.06	0.42	7.31	Average	P2
			28	7.2	0.8	7.8	Max.	
			5	3.1	0	6.5	Min.	
			5.84	0.81	0.24	0.22	SD	
64	400	400	16.75	5.44	0.34	7.31	Average	P _T
			28	7.2	1	8.4	Max.	
			5	2.3	0	6.33	Min.	
			5.86	1.14	0.24	0.24	SD	

RESULTS

This research was introduced for two different water distributions, including: period P1 (which was a periodic water circulation) and period P2 (which was a permanent water circulation). The statistical examination (Fisher and Student *t*-tests) showed that differences between the parameters, observed during these periods, were significant ($p < 0.001$), except for pH ($p > 0.05$). Therefore, the statistical analyses were separately calculated for each period (P1, P2), as well as the total period of observation (P_T).

Temporal variations of pH, Cl and DO

The average values of pH, Cl and DO of the collected data were calculated during periods P1, P2 and P_T of observation and presented in Table 1. During periods P1, P2 and P_T, the chlorine concentration were 0.27, 0.42 and 0.34 mg/l, respectively, lower than the minimum value indicated by the usual international standards.

To distinguish the characteristics of the data, the measured pH, DO and Cl were divided into three classes (Table 2). The results showed that 75.5% of the samples maintained a pH of 7–7.5 and about 61.5% of the samples contained residual chlorine concentrations between 0 to 0.5 mg/l. Furthermore, 19% of the samples were seen to have no chlorine concentration (0 mg/l). DO levels in 57%

of the samples were sufficient but in the others (43%), which drop below 5 mg/l, were low.

Temporal variations of total and fecal coliforms

The contaminated samples (based on the detected total coliforms) during periods P1, P2 and P_T were 29.5, 12.5 and 21%, respectively (Table 3). In the case of the fecal coliforms, which are more important contamination indicators, they were about 19.5, 9 and 14.25%, respectively. The mean total coliform counts during periods P1, P2 and P_T were 6.54, 5.31 and 6.2 MPN/100 ml, respectively. The mean fecal coliform counts during periods P1, P2 and P_T were 3.97, 1.76 and 3.33 MPN/100 ml, respectively. To assess the contamination density, total and fecal coliform counts detected in the contaminated samples were classified into four groups (Table 4). This classification illustrates that, during periods P1, P2 and P_T, about 72.9, 68 and 71.4% of the contaminated samples had total coliform counts less than 5.1 MPN/100 ml, respectively. The same results were

Table 2 | Classification of pH, DO and Cl measured during all of periods (P_T)

Class	pH		Cl		DO	
	Range	(%)	Range (mg/l)	(%)	Range (mg/l)	(%)
I	6–7	9.5	0	19	1.5–3.5	11.5
II	7–7.5	75.5	0–0.5	61.5	3.5–5.5	31.5
III	7.5–8.5	15	0.5–1	19.5	5.5–7.5	57

Table 3 | Contaminated samples and the average of total and faecal coliform counts

Coliforms counts (MPN 100 ml ⁻¹)		Contaminated samples (%)		Period
Faecal coliforms	Total coliforms	Faecal coliforms	Total coliforms	
3.97	6.54	19.5	29.5	P1
1.76	5.31	9	12.5	P2
3.33	6.2	14.25	21	P _T

observed for faecal coliforms. They were 40.7, 44 and 41.7 MPN/100 ml, during periods P1, P2 and P_T, respectively.

The results confirmed that the mean faecal and total coliform counts throughout all of the study periods, (specially period 1) were high. They also illustrated a significant inverse relationship between the residual chlorine concentrations and total and faecal coliform counts of the contaminated samples. The Pearson correlation coefficients (r) of total and faecal coliforms counts during P_T were -0.40 and -0.29 ($p < 0.01$), respectively.

Spatial variations of pH, Cl and DO

In order to understand the local variability of Hamadan drinking water, based on the age of the pipes and location of the supplying reservoirs, the network was separated into eight zones (Figure 1). Table 5 shows the mean values of water quality parameters of the sampled water at each zone. Cl concentration varied between 0.2 and 0.4 mg/l. This evaluation confirms that generally the concentration of chlorine in all zones of the network was low.

Based on residual Cl measured in the zones, the following comments could be summarized. (i) Generally in all zones, the class in which the samples had chlorine concentrations equal to 0 mg/l was the least of the classes. In this case, zones 1 and 6 were identified as the sites with minimum (5.9%) and maximum (28.6%) samples without

chlorine concentrations (0 mg/l), respectively. (ii) Zones 3 and 6 were recognised as the sites with minimum (23.7%) and maximum (57.1%) samples of chlorine concentrations between 0 and 0.5 mg/l, respectively. (iii) Zones 6 and 3 were found as the sites with minimum (14.3%) and maximum (57.9%) samples of chlorine concentrations higher than 0.5 mg/l, respectively.

Local variations of total and faecal coliforms

Based on the total coliform counts, the results showed that zones 1 and 6 were identified as the sites with minimum (1.96%) and maximum (42.86%) contaminated samples, respectively. Moreover, in the case of faecal coliforms the results were relatively similar. Zones 1 and 6 were identified as the area with minimum (0%) and maximum (39.29%) contaminated samples, respectively (Table 6). The coliform count analysis showed that the contamination densities of total and faecal coliforms ranged from 3.6–9.74 and 1.1–6.54 MPN/100 mL, respectively. Based on the contamination densities, the more critical zones were 2 and 7.

Identification of isolated pathogens from contaminated samples

Results of the pathogenic microorganisms detections (by the membrane filter and sediment methods) of the contaminated samples showed that the samples which had microorganisms grown through the membrane filter and sediment methods were 93 and 28%, respectively. This result suggests that the amount of identified microorganisms through the membrane filter were three times more than the sediment method value. Based on the identified microorganisms, the greatest population frequency (total number of each pathogen's identification of the 64 contaminated samples) through the membrane filter was as follow: *Bacillus species* (28), *Staphy-*

Table 4 | Classification of both total and faecal coliforms of the contaminated samples

Contaminated samples (%)							
P _T		P2		P1		Coliform counts (MPN 100 ml ⁻¹)	Class
Faecal coli.	Total coli.	Faecal coli.	Total coli.	Faecal coli.	Total coli.		
47.6	0	56	0	44.1	0	<1.1	I
41.7	71.4	44	68	40.7	72.9	1.1–5.1	II
7.1	23.8	0	32	10.2	20.3	5.1–16.1	III
3.6	4.8	0	0	5.1	6.8	>16.1	IV

Table 5 | Average chemical parameters observed at the sampling zones

DO (mg/l)	Cl (mg/l)	pH	Samples	Total length of main pipes (km)	Area (km ²)	Zone
5.24	0.4	7.3	51	4	1.7	1
5.02	0.4	7.3	38	10	5.1	2
5.43	0.4	7.4	38	9	5.2	3
5.75	0.3	7.3	88	43	21	4
5.65	0.3	7.2	52	9	4.4	5
5.65	0.2	7.3	28	13	6.5	6
5.3	0.3	7.3	32	6	2.8	7
5.3	0.4	7.3	73	14	7.8	8

lococci (23), *Micrococci* (7), *Alcaligenes species* (4), *Acinetobacter* (3) and *Fungi* (2 times in the 64 contaminated samples). Other microorganisms with just one occurrence in all of the contaminated samples included: *Escherichia Coli*, *Pseudomonas Aeruginosa*, *Neisseria Weaveri* and *Enterobacter*.

DISCUSSION

The presence of fecal coliform bacteria in water is indicative of contamination by fecal material and is therefore considered indicative of a health risk because many enteric pathogens (bacterial, viral and parasitic) are present in feces (Boualama *et al.* 2002). Furthermore, the significance of the coliform group's density has been established as an indication of the degree of pollution and thus the sanitary quality of water (Feachem *et al.* 1983).

The significant incidence of microbial contamination (about 21% of the samples) was observed by total coliform identification between July 2003 and March 2005 in the

Table 6 | Contaminated samples, total and fecal coliform values detected at the sampling zones

Coliform counts (MPN 100 ml ⁻¹)		Contaminated samples (%)		Samples	Zone
Fecal coli.	Total coli.	Fecal coli.	Total coli.		
<1.1	3.6	0	1.96	1	1
6.54	7.68	7.89	13.16	5	2
1.7	5.38	15.79	21.05	8	3
3.55	7.18	11.36	19.32	17	4
1.8	4	15.38	21.15	11	5
3.82	8.23	39.29	42.86	12	6
6.54	9.74	18.75	28.13	9	7
2.45	4.29	17.81	28.77	21	8

Hamadan drinking water network. The period of observation consisted of two different steps: period P1 (in which water circulation was periodic and limited to 16h per day) and period P2 (in which water circulation was normal and permanent). The results illustrated that the microbial quality of Hamadan drinking water was very different during P1 and P2. During period P1, the average residual chlorine concentration was lower than the minimum standard level. Therefore, that situation provoked microbial contamination of the network. During period P2, when water distribution was permanent, the contamination was significantly reduced (from 29.5 to 12.5%). An inverse relationship between residual chlorine concentration and total and fecal coliform counts of contaminated water was suggested, which was similar to that from earlier research studies (White 1986; Roberts *et al.* 2001).

In spite of the production of coliform-free water (from the Ekbatan Dam and Bahar groundwater), the contamination originally occurred in the water supplying the Hamadan network. Traditionally, in old houses of the city many domestic wells were in use. Therefore, when water distribution of the network was stopped (because of water resource limitations), the household wells' water was used and contaminated groundwater was pumped into the household piping network.

The intermittent water supply program (systematic off-on flow) and the considerable difference between the zone elevations (which in some areas is more than 210 m) produced air blockages in the pipes, and therefore the air pressure in the pipes was locally increased. The high air pressure was removed and distributed coliform bacteria from the contaminated domestic pipes towards other places in the network, especially in lower elevation zones of the city. They then grew where chlorine concentration was inadequate (based on the standard values), such as zones 6, 4 and 8 which were more critical sites regarding microbiological contamination (LeChevallier *et al.* 1996). The coliform growth also occurred at the end of pipelines where chlorine concentrations were generally inadequate (especially during P1).

These results suggest an important spatial variation of coliform contamination between zones of the city. The two different water resources (Ekbatan Dam and Bahar groundwater) of the network, the difference in ages of the pipelines as well as the zones' elevations and domestic well usage (in some place of the city during the periods that

water distribution was stopped) were all factors that caused the local variability in the supplied water contamination (Kempster *et al.* 1997).

CONCLUSIONS

The results of this study present some important information about Hamadan drinking water quality as well as its temporal and spatial contamination. The existence of contamination depended on a complex interaction between several factors, especially during the period of intermittent water supply (P1) that resulted in contamination of approximately 29.5% of the water samples. This contamination could occur because of several factors such as (i) the age of the pipelines, (ii) water leakage in some places of the network that allows coliform bacteria to enter the safe water and (iii) collected biofilms in old pipelines of the network (Tall *et al.* 1995; Van der Kooij *et al.* 1995). Further studies are necessary for a better understanding of the exact reasons for these contaminations detected during the period of study.

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