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3. Kuczmarski RJ, Ogden CL, Grammer-Strawn LM, Flegal KM, Guo SS, Wei R, et al. CDC growth charts: United States. Advance data from vital and health statistics. No. 314. Hyattsville, Md: National Center for Health Statistics, 2000. (DHHS publication no. (PHS) 2000-1250 0-0431)
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Original Article(s)

Journal of Advances in Environmental Health Research

Noise pollution and its spatial distribution in urban environments (case study: Yazd, Iran)

Mostafa Gholami-Zarchi[1], Leila Khosravanian[1], Behzad Shahmoradi[2]

1 Department of Urban Planning, School of Technology, University of Kurdistan, Sanandaj, Iran
2 Kurdisan Environmental Health Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran

Original Article

Abstract

This study focuses on identifying source and frequency of noise pollution in Yazd, Iran. For data collection, we used two approaches: First we selected 55 monitoring stations for 122 km grid network; the distance between two stations was 2 km. Then, at the same time, we measured the sound pressure level (SPL) at main roads; the distance between two monitoring stations in this case was 1 km. After collecting data required, noise map of each collection and composite map were provided using ArcGIS software in geographic information systems environment. It was founded that the maximum noise pollution was created by vehicles in Yazd. The map based on 2 km network showed that 99% of residential areas were exposed to noise pollution. Composite map showed that 56% of Yazd lands were exposed to noise pollution. In addition, other activity zones such as university, administrative and commercial were in critical pollution conditions. Moreover, due to the surrounding sources such as airport and industries, the SPL in the western and eastern parts of the city was too much, so that it is a limiting factor resulted in the city development most properly toward those parts. We propose a project to define the impact of industrial noise pollution on residential land in the eastern and western parts of the city. Nevertheless, due to the high pollution levels in the city center and because of existence of uses such as bazaar in historic area of the town, residential areas, some small shops and major hospitals of the city, the pollution and exposure must be reduced.

KEYWORDS: Noise Pollution, Cities, Environmental Pollution, Geographical Information Systems, Noise, Urban Development, Iran

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Introduction

No one would be able to escape from the effects of manmade life. Urbanization and industrialization along with growth of population have brought about environmental issues in many metropolitans and cities throughout the world. Noise pollution is one of the most important issues in big cities causing disturbance in urban areas and nowadays is considered as a serious threat to the quality of modern life.[1,2] Noise pollution sources in urban areas mainly include construction activities, workshops, industrial and commercial units, and traffic. In this regard, impact of vehicles and cars on human health is obvious so that it influences the everyday life of inhabitants, work, and sleep; hence, study and finding a suitable approach to reduce noise pollution is demanded in urban areas.[3-6] Noise pollution in urban areas has been globally recognized as a major detriment to the quality of life. The adverse effects of noise include various impacts on people's physical well-being[7] and the disturbance of daily activities,[8] which includes: (a) Physical effects

Corresponding Author:
Behzad Shahmoradi
Email: bshahmorady@gmail.com

such as hearing defects; (b) physiological effects such as increased blood pressure, irregularity of heart rhythms and ulcers; (c) psychological effects such as sleep disorders, irritability, and stress; and (d) effects on work performance such as reduction of productivity, communication gap, and hearing impairment.[9]

In 2002, the European Union (EU) passed directive for the assessment and management of environmental noise stipulated by July, 2008. According to this directive, all EU members must draw up noise map for the main areas of population aggregation and primary road systems for public reference. England then published the London noise map in 2004, which is the first noise map produced by a national government, as a reference for London citizens to avoid noise nuisances.[10] Thus, with regard to the sensitivity of the noise and its adverse effects, sound mapping is essential to the public as a tool to show the areas with intensive pollution.[11]

Therefore, the main aim of the present research is to draw Yazd, Iran, sound map using data obtained from 177 points along the main streets and the motor vehicles, and network points between different parts of the city including residential, industrial, and commercial activities. The division of land was according to the specified zones of Iran Environmental Protection Agency (EPA) approved in 2009, which determines the amount of allowed pollution for a five given zones and critical areas.

Materials and Methods

This study was conducted in summer 2012 by measuring sound pressure level (SPL) at 177 points. The methodology of this research, which was a descriptive case study, is divided into two parts.

According to the type of streets and the density of city, the sound data were collected in two ways in order to determine the SPL in Yazd city. First, we measured the SPL using a sound pressure meter (1353H, TES, Taiwan) at the main streets over a distance of 1 km with respect to the street distances from each other. The number of monitoring stations (points) determined was 122. The distance between these points was determined mainly based on the criteria such as typology and type of road network in the Yazd city, which was linear. Moreover, we considered the activity nodes and their density along the movement axes of Yazd, which were a variety of commercial and workshop uses. Then, the sound level was recorded as a network with a distance of 2 km; the aim of this monitoring was to gain not only the sound level on the roads but also to obtain the amount of sound resulted from different activities within the city. The distance between these monitoring stations (55 points) was determined based on the Yazd city dimension, urban land use zones in different areas of the city, and accessibility hierarchy. After determining monitoring stations, SPL was recorded at crowded hours (8:00-9:00 AM). The reading was repeated twice. Simultaneously, data related to noise were collected during the same time period.

After data collection, for determining the sound intensity in different parts of the city, data processing was carried out using the ArcGIS software (version 10, Esri, Redlands, CA, USA). For creating the sound maps, first position map of the red and blue dots were prepared using the "editor" tool. Then, data interpolation and sound intensity at each point on the surrounding points were investigated using "spatial analyst" tool, such as interpolation and existing extension of these tools, which include under kiriging and natural neighbor. In the next stage, map of different land use zones was prepared, areas exposed to noise pollution were determined, and areas at the risk of noise pollution were clearly defined.

With increasing vehicles traffic, urban activities in the cities, and disturbing tranquility of living, it has been recognized necessary to create sound maps in order to determine the sound intensity level of noise pollution in different urban areas. In recent years, mass vehicle production, increasing car ownership, and travel density have made metropolitan and other cities be affected by sound in Iran. Yazd city, located in the center of Iran (desert region) has experienced expansion and population growth in recent years due to various mineral resources and locating on the motor highway including large industries (Figure 1). Today, the legal area of Yazd is about 9,976 ha, out of which 21.4% equivalent to 2,138 ha is residential lands, 18.8% (1,873 ha) road network, 11.9% (1,191 ha) agriculture lands and gardens, 25.7% (2,564 ha) barren and desolate lands, and the remaining land is allocated to other land uses. Table 1 represents the land use categorization and definition of each zone. Building density in Yazd is averagely 73%.[12] The city has a population of about 550,904 people.

Results and Discussion

According to the Iran Environmental Protection Department classification, Yazd land uses were zoned and the noisy parts of the city were determined, Then Yazd city noise maps were prepared by measuring the SPL. For this purpose, first we measured the noise frequency production in different parts of the city. By preparing the noise maps based on the data points on the road and raster grid and

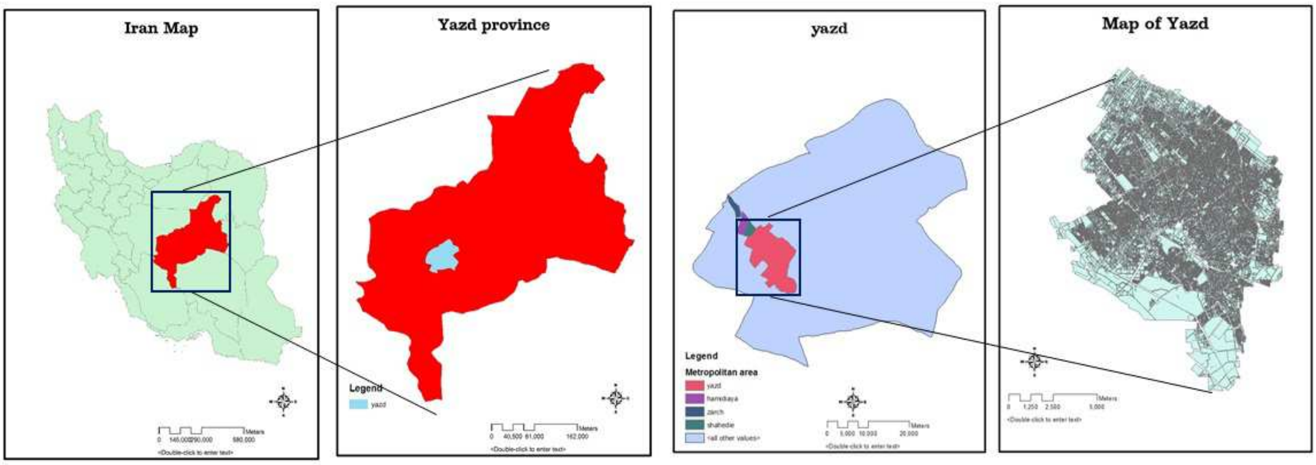

Figure 1. Location of Yazd city

Table 1. Categorizing city land use zones and their definition

Zone	Definition
Residential	A zone, where more than 50% of the land use is pure residential and the rest (in addition to road network) is consisted of unobtrusive residential-related services
Commercial	A zone, where more than 50% of land uses are predominantly related to offices, recreational, cultural activities, etc.,
Commercial-residential	A zone, where ground floors are commercial and upper stories are usually predicted to be residential; however, residential uses are usually more than commercial ones
Residential-industrial	A zone, where some non-intrusive and non-polluting industries (for example, workshops) are located besides residential areas. Here, the major land use is residential
Industrial	A zone with industrial land use located far away from the residential areas and out of city based on the environmental considerations
Administrative	A zone, where more than of 50% of land uses are administrative
University	A zone, where university has the highest performance
Airport	A zone, where includes airport and its surroundings
Agricultural	A zone, where has a predominantly agricultural uses and is within the residential uses limits
Commercial-industrial	A zone, where more than 50% of the land uses include small industries and commercial uses

comparison with Iran National Sound Standard at Ambient Air (INSSAA),[13] it can be said that the most of the noise pollution is due to the motor vehicles in the city of Yazd. According to the map shown in figure 2, the highest SPL is around the streets while they would influence their adjacent lands. In this map with red dots, the lowest SPL is 57 and its highest is 84 dB, which demonstrates that all the noise pollution is around the streets in residential areas. Moreover, it was found that the most of the noise pollution generated by various activities is in the eastern and western parts of the city causing inconvenience to the industrial-residential and commercial-industrial areas.

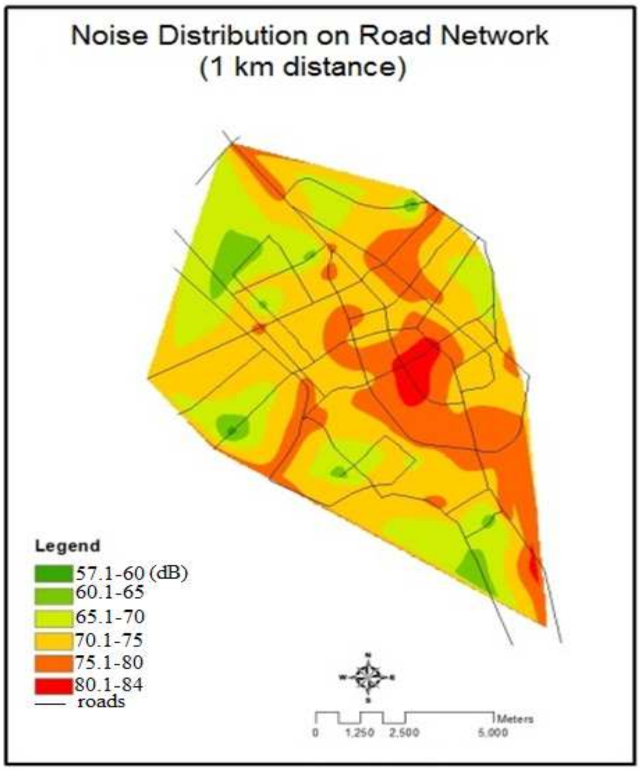

Figure 2. Yazd city noise map of road network

Finally, to obtain a general sound map of Yazd, we combined both types of data (grid and roads network) in order to obtain the effects of pollution. Comparison of this map (Figure 3) with the EPA standards for five urban zones revealed considerable facts.

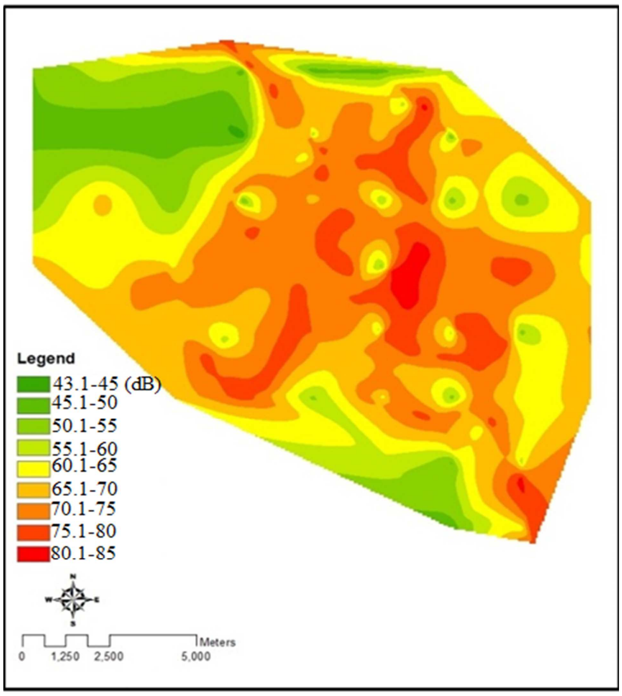

Figure 3. Noise map of Yazd city

Considering the integrated map and noise pollution permitted limit standards (INSSAA, Table 2) in these zones, we found that Yazd is a city, where:

- 99% of the fully residential zones are in the upper permissible level of noise pollution
- 97% of the residential-commercial areas are in the high level of noise pollution
- 100% of the commercial zones are suffering from noise pollution, according to the red and blue sound map and the location of this zone, all the noise pollution could be attributed to the motor vehicles
- 4% of the residential-industrial zones are exposed to noise pollution sprawled around the suburban
- 75% of the commercial-industrial areas are exposed to noise pollution
- According to standards, the industrial area has no noise pollution
- Due to the nearness of the airport to the Azadshahr town, Iran, the sound measurement importance in airport zone has become crucial. 25% of this area is affected by noise pollution

- Administrative zone is also an important zone, which must be monitored because of its vast area. 2% of the area is at risk of noise pollution
- The entire university zone, which is a residential area, is exposed to noise pollution
- 58% of the agricultural zone is also affected by noise pollution, and
- Finally, 56% of the total areas of the city are exposed to noise pollution.

Table 2. Iran National Sound Standard at Ambient Air (INSSAA)

Zone	Leq (30), dB (A) from 7 am to 10 pm	Leq (30), dB (A) from 10 pm to 7 am
Residential	55	45
Residential-commercial	60	50
Commercial	65	55
Residential-industrial	70	60
Industrial	75	65

Leq: Sound equivalent level

Many research works have been carried out for identification and analysis of sound sources in order to reduce its impact. Tsai et al. analyzed the spatial characteristics of urban environmental noise using noise maps produced at 345 noise monitoring stations in Tainan, Taiwan. Noise data were collected at varying intervals: Morning, afternoon, and evening in both summer and winter in various areas of commercial, residential, and cultural zones and they compared it with standards of sound control. Their findings showed that about 90% of Taiwan's population was exposed to unacceptable noise generated by urban development.[10] In another study, it was referred to negative impact of noise on the quality of life that is one of the serious concerns in Vietnam. Cameras were installed to measure quantitative volumes of traffic and compared it with Japan data. It was concluded that the noise of means of transportation is very relevant to car horns, where noise levels decreased with the absence of horn sounds.[14] In another research work conducted in Delhi and India, some suggestions such as land use and transportation planning were given for mitigating and managing the noise pollution problem in the sustainable urban development perspectives.[7] All of the above restudies had a view of improving the environment, while our study aimed at determining the amount of noise pollution generated by different activities. This is very useful in the preparation of urban development plans and prevents unnecessary costs in future development. Moreover, through field observation, this survey indicated that the maximum amount of noise pollution in Yazd city is due to the private vehicles, especially in the downtown, where the existence of bazaar and markets has brought maximum visits in this area. In addition, it was found that the historic fabric of downtown is exposed to noise pollution indicating the importance of conducting this study in future plans in order to attract tourists and preserve monuments.

Figure 4 shows the area of each zone classified. Moreover, table 3 presents the SPL range and related area of each zone. After investigating and preparing Yazd sound map and overlaying this map on the land use map, it was revealed that noise pollution is at a critical level in residential areas in Yazd city (Figure 5). Other lands except industrial and residential-industrial zones had high level of noise pollution. Therefore, we conclude that most of the noise pollution is caused by motor vehicles in the Yazd city, which includes a large area (about 56%). In addition, administrative zone, university and airport were evaluated because these areas are sensitive to noise pollution like other areas. The noise level at airport was remarkable due to exposure in metropolitan and residential settlements of Azadshahr (a district name). Academic and administrative areas are not an exception and were influenced by noise pollution.

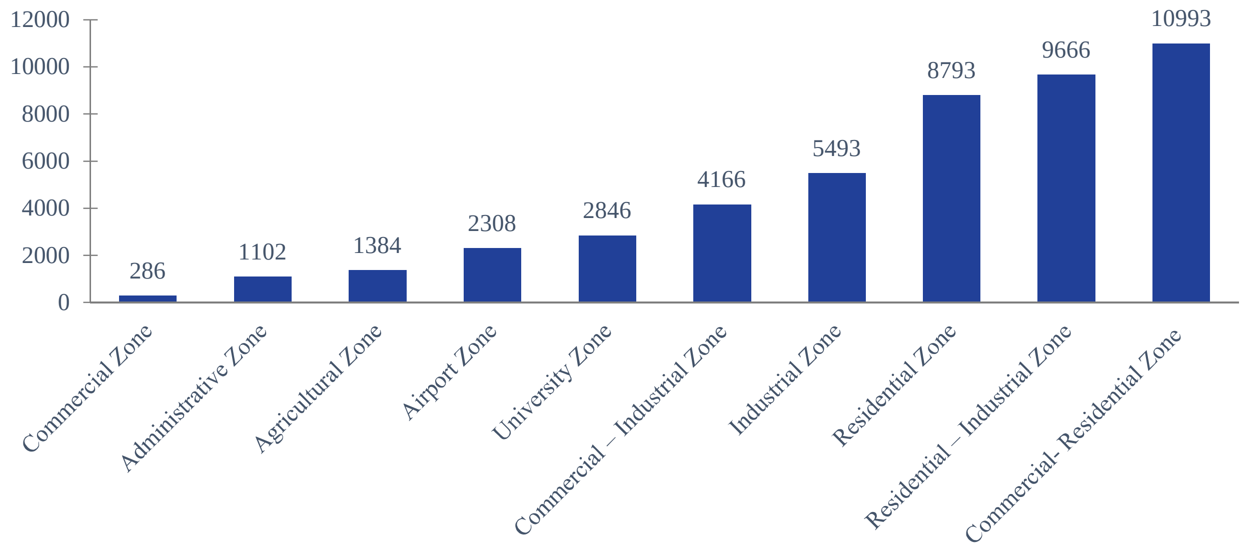

Figure 4. Zone area based on different land uses

Table 3. Sound pressure level (SPL) (dB) range of each zone area (ha)

SPL (dB) range	Agriculture	Commercial	Commercial residential	Residential	Industrial	Industrial commercial	Industrial residential	University	Official	Airport
40.0-45	0	0	0	0	0	33	0	0	0	0
45.1-50	0	0	1	2	80	367	1068	0	0	937
50.1-55	0	0	30	54	886	509	1780	6	0	923
55.1-60	4	0	279	443	613	149	1507	667	1	276
60.1-65	244	0	1499	920	788	223	3014	1103	23	121
65.1-70	340	8	3669	1845	2311	1099	1912	667	291	51
70.1-75	456	154	4489	3600	806	1334	327	381	575	1
75.1-80	323	114	1014	1590	9	675	58	22	212	0
80.1-85	17	10	12	339	0	0	0	0	0	0
> INSSAA*	796	286	10683	8737	9	3108	385	2840	1078	449
≤ INSSAA**	588	0	310	56	5484	1058	9281	6	24	1860

* Total zone area exceeding INSSAA; ** Total zone area not exceeding INSSAA; SPL: Sound pressure level; INSSAA: Iran National Sound Standard at Ambient Air;

Conclusion

We found that 56% of the land in Yazd city is suffering from noise pollution and 100% of residential area is polluted. Moreover, noise pollution has pervaded the entire region in the central area (i.e., downtown) including commercial areas. Administrative zone, the university and the airport had noise pollution, which of course these zones are not in EPA standards. Pollution is, now, very sensitive and crucial in Yazd according to the results achieved. Noise pollution studies must be taken into account in the urban plan processes such as Yazd master plan and strategic development plan. With regard to the results of the industries and factories noise impact in the eastern and western parts of the city, it is essential to make serious steps toward revising the residential construction (which are now growing in all four directions) and preventing the annoying sound. Therefore, we propose a project to define the impact of industrial noise pollution on residential land in the east and west part of the city. Nevertheless, due to the high pollution levels in the city center and because of existence of uses such as

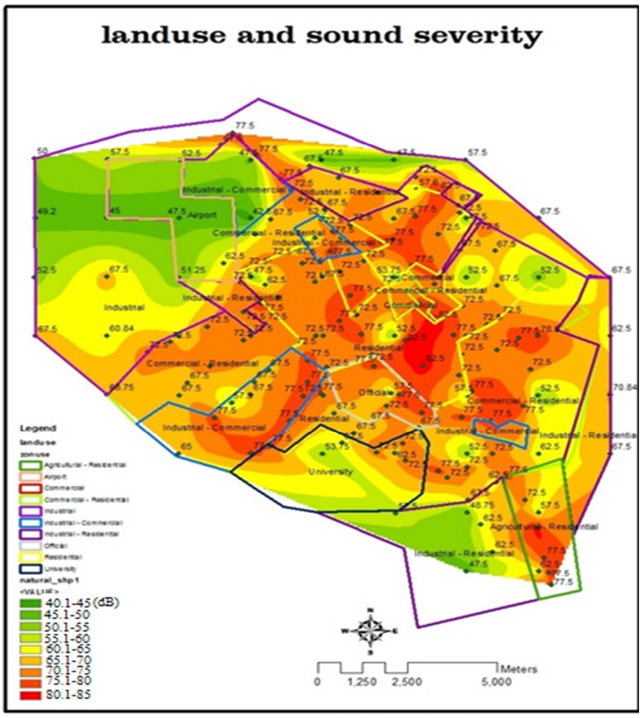

Figure 5. Comparison of the landuse and the sound map of Yazd city

bazaar in historic area of the town, residential areas, some small shops and major hospitals of the city, the pollution and exposure must be reduced. There are some important uses in this area for the local region, which makes it to be considered as the most important part of economy in the city. This research proposed sound barrier to prevent the noise in short-term, and also refers to planning in order to permanent adjustment of the contamination causes in long-term. Thus, factors that increase vehicle traffic should be identified and then modified, changed and improved in this area. About academic, administrative and airport areas covering a considerable area of city due to similarities with other cities, it is recommended that EPA reconsider its standards.

Conflict of Interests

Authors have no conflict of interests.

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An empirical investigation into the relationship between workshop operations and accidents in local automobile garages in Ghana

Maxwell S. Akple[1], Richard F. Turkson[2], Chizoba I. Ezugwu[3], Robert K. Biscoff[4], Cephas K. Bosrotsi[5]

1 Department of Mechanical Engineering, School of Engineering, Ho Polytechnic, Ho, Ghana AND School of Material Science and Engineering, Wuhan University of Technology, Wuhan, China
2 Department of Mechanical Engineering, School of Engineering, Ho Polytechnic, Ho, Ghana AND School of Automotive Engineering, Wuhan University of Technology, Wuhan, China
3 School of Material Science and Engineering, Wuhan University of Technology, Wuhan, China
4 Department of Mechanical Engineering, School of Engineering, Ho Polytechnic, Ho, Ghana
5 Department of Agricultural Engineering, School of Engineering, Ho Polytechnic, Ho, Ghana

Original Article

Abstract
Local automobile garage workers carry out daily workshop operations, which sometimes lead to accidents and injuries. Therefore, this study was carried out to establish a relationship between automobile workshop operations causing accidents and safety practices among local garage workers in Ghana. Three main data collection approaches were used in the study namely focus group discussions (10 FGDs), observation and survey (250 respondents). Data were analyzed with SPSS. From the FGDs, participants identified workshop operations that had the potential of causing accidents, safety factors and safety practices. Factor reduction analysis was carried out where identified workshop operations were clustered into three factors relating to worker's attitude toward workshop operations, working environment and management practices, and work monotony. Safety practices were clustered into two main components regarding worker's approach to safety measures and provision and storage of chemicals appropriately. Five safety measures were mentioned to be practiced in garages that had a positive moderate correlation with the potential workshop operations causing accidents. Finally, it could be said that local garage workers had some level of knowledge concerning safety measures but the practice does not measure up to standard and best safety practices. Therefore, safety seminars and training sessions should be organized for local automobile garage workers.
KEYWORDS: Accidents, Safety, Automobile, Local Garages, Ghana

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Introduction

Local automobile repair workshops (or garages) are one of the small scale industries which serve as engine for economic growth by providing employment for people.[1] This industry plays a vital role in the economic development of most developing countries. For instance, in Eritrea, garage work is an important source of job creation, provision of potential for improving skills and devising new technology.[2] There are various new technological trials and innovations in progress undertaken by different groups of garage workers. However, previous studies

Corresponding Author:
Richard F. Turkson
Email: riturkus@yahoo.com

indicated that automobile mechanics have higher rates of occupational health hazards compared to workers in other occupations[3,4] leading to injuries, some of which could be fatal.

Accidents in local automobile garages are largely preventable with the use of appropriate occupational safety measures and health services. This is because there are safety standards and practices, which garage workers must adhere to during the execution of garage operations. Adhering to the stipulated safety measures and practices could significantly reduce accidents in garages.

In developing countries such as Ghana, occupational health and safety (OHS) practices have generally been given little or no attention especially in the informal job sector. However, this sector employs a significant proportion of people and contributes to the gross domestic product of the nation. Local automobile garages in Ghana are formed by a group of automotive mechanics who come together to offer automotive maintenance and repair services under the informal job sector. The garages are operated under one or more masters owning the workshop with a number of apprentices. The master mechanics may have different specializations and could be automotive electricians, automotive mechanics, welders, brake binders, interior vehicle liners or vehicle body sprayers.[5] Most of these garages are not officially recognized by governmental authorities and therefore are not provided with the necessary support such as OHS training. However, automobile repair work involves multiple activities that expose workers to many potential accident-causing factors and require multiple approaches for enhanced safety. This study was, therefore, carried out to analyze the relationship between potential workshop operations that could lead to accidents and safety measures practiced among local automobile garage workers in Ghana.

Materials and Methods

The study involved all local automobile garages in three municipalities and two districts in Ghana. The willingness of participants to be part of the study was sought first because the researchers believed unwillingness on the part of participants would not provide reliable information. In all, 250 participants were involved in the study. The participants were local garage mechanics who were engaged in vehicle maintenance and repair work, including automotive electricians and welding technicians operating in the informal small-scale enterprises.

Three main data collection techniques were employed in this study namely focus group discussion (FGD), survey and direct observation. The researchers believed that using more than one data collection approach would enhance the quality and validity of the study by combining and integrating the strengths associated with each method.

Ten focus group discussions were first conducted, two each in the five municipalities and districts. This was done for group members between 8 and 10 people. The composition of the groups was heterogenous involving different ages of participants, experience and positions in workshop (i.e., Masters, Senior and Junior Apprentices) for adequate and in-depth data gathering. It was believed that when people are involved in projects, appreciation of findings is easy because they feel they were part of the work. The main focus for carrying out the FGDs was to ask the participants to identify various workshop operations that had the potential of causing accidents and safety factors in their occupation. This was done to involve participants in the study from the onset, so that any recommendations could easily be adopted.

An observational study was carried out by the researchers to ascertain workshop operations, the availability or otherwise of safety equipment and facilities and safety measures practiced at the various workshops to support responses from the participants.

Based on the factors listed in table 1 a questionnaire was developed using a five-point Likert scale (1 = strongly disagree, 2 = disagree, 3 = indifferent, 4 = agree and 5 = strongly agree) for local garage mechanics to rate their degree of agreement against each of the identified workshop operations and safety variables. In addition, a scale rating using (1-not at all, 2 - sometimes, 3- neutral, 4- often, 5- very often) was used to assess respondents' agreement with their safety practices at their workshops. The questionnaire was pre-tested outside the study area to identify mistakes and avoid ambiguous questions. The questionnaire comprised both open and close-ended questions. The questionnaires were randomly administered to 250 local automobile garage workers from three municipalities and two district capital cities in Ghana.

The questionnaires were coded and entered into using SPSS for Windows (version 16.0, SPSS Inc., Chicago, IL, USA). Descriptive and reliability tests [i.e., Cronbach alpha test and Kaiser-Meyer-Olkin (KMO) and Bartlett's sphericity] were carried out to analyze the demographic characteristics and determine the internal consistency of the data respectively. Furthermore, factor reduction analysis (or principal component analysis) was performed to cluster the workshop operations and safety measures into factors. Correlation analysis was performed to determine the relationship between the workshop operations and safety measures practiced.

Results and Discussion

Analysis of FGDs

The results from FGDs indicated that local garage workers had some level of knowledge concerning OHS. Table 1 shows potential accident causing workshop operations, proposed safety measures and safety measures practiced by the respondents in the various local garages. It is noteworthy that workers mentioned that the accidents in local garages could result from any workshop operation, including the environment of the workshop and attitude of workshop managers.

Demographic characteristics of respondents

The demographic characteristics of respondents are illustrated in table 2. It showed that all the local garage mechanics interviewed were males (100%). This is because the automobile industry is mostly a masculine occupation in Ghana. It is, therefore, not common to see females involved in the vehicle repair business. In addition, most of the mechanics involved in the study were adults (i.e., > 33 years) and had a lot of working experience between 5 and 10 years as mechanics (62.4%). This implied that the majority of the respondents had enough work experience in the local garages and could provide more in-depth information concerning the study.

Table 1. Potential accident causing workshop operations, proposed safety measures and measures practiced by respondents

No.	Accident causing factors and safety measures
	Potential accident causing workshop operations
A1	Use of tools and equipment that are out of order
A2	Violation of standard safety rules and regulation
A3	Inexperience
A4	Inappropriate handling and storage of chemicals
A5	Poor handling of tools and equipment
A6	fatigue and boredom of workers
A7	Reluctance of management towards safety
A8	Working environment
A9	Natural causes
A10	Inadequate operation space for workers
A11	Physical condition of workers
A12	Workers poor job satisfaction
A13	Constant exposure to a particular job
A14	Physical build of workers
	Safety measures
S1	Appropriate storage of chemicals
S2	Enforcement of safety rules by experienced apprentices
S3	Strict compliance to workshop safety rules
S4	Regular orientation on safety practices
S5	Use of safety information materials
S6	Specific safety instructions for particular jobs
S7	Safety awards for motivation workers
S8	Investigation of accident causes
S9	Use of PPEs
	Safety measures practiced
M1	Use of PPEs
M2	Using appropriate tools for operations
M3	Adherence to some specific safety instructions
M4	Proper storage of chemicals
M5	Having first aid box

PPE: Personal protective equipment; A1-A14: Potential accident causing factors; S1-S9: Safety measures; M1-M5: Safety measures practiced

Descriptive and exploratory analysis

The results show that the means for each of the variables appeared to be reasonable as each of the variables was measured on a five-point Likert scale as shown in table 3. No mean values were above 5 or below 1. The standard deviations were all similar suggesting that there were no outliers for any of the variables.

Table 2. Demographic characteristics of respondents

Characteristics	Frequency	Percentage
Gender		
Male	250	100
Female	0.0	0.0
Age (years)		
18-25	66	26.4
26-33	78	31.2
> 33	106	42.4
Workshop experience (years)		
< 5	43	17.2
5-10	156	62.4
> 10	51	20.4

Reliability test

To determine the degree of consistency for the set of variables or scale of measurement, a reliability test was conducted. The reliability test checked the consistency or whether the variables composing the scale were correlated with each other.[6] In this study, the internal consistency reliability was employed to measure the reliability of the research instrument. Internal consistency reliability is used to assess the consistency of the results from the variables within the test.[7] For the purpose of this study, two of the tools namely cronbach alpha test, KMO and Bartlett's sphericity tests were employed.

Cronbach's alpha test (α)

Cronbach's alpha test (α) measures the correlation among the variables of the scale. It is the most common measure of reliability (consistency) of a scale. The higher the correlation among the variables of the scale, the more consistent the research instrument.[8] In general, the accepted Cronbach alpha value is 0.7 and above, whiles a reliability coefficient of 0.6 is acceptable for exploratory research.[6] In this study, the Cronbach alpha reliability test (α) of the scales was 0.778, 0.701 and 0.729 for potential workshop operations causing accidents, safety factors proposed and safety measures practiced respectively (Table 3). This implied that all the variables under the factors were worthy and adequate to be retained on the scale and that there was no need for deletion.

KMO and Bartlett tests of sphericity

KMO is a measure of sampling adequacy and it is used to compare the magnitudes of the observed correlation coefficients in relation to the magnitudes of the partial correlation coefficients. Furthermore, the KMO test is used to assess whether the factor analysis is appropriate for a set of data or not.[8] The value for KMO varies from 0 to 1. Large KMO values are good because correlation between pairs of variables (i.e., potential factors) could be explained by the other variables. According to Field,[7] a value of 0 implies there is diffusion in the pattern or trend exhibited by the correlation and the factor analysis is not appropriate. However, the value of 1 is an indication of correlation and compact pattern or trend and hence factor analysis is suitable for a particular case. Based on this a commonly acceptable KMO value is 0.5 and above whiles a dataset with a KMO below 0.5 is not appropriate for factor analysis. From table 4, it was observed that the KMOs determined for all the factors was above 0.5 indicating or confirming the appropriateness of factor analysis for the variables under each factor.

Furthermore, the Bartlett test of sphericity was used to determine the overall significance of all the correlation within the correlation matrix.[5] According to Field,[6] the Bartlett test of sphericity shows whether a correlation exists between the variables on the scale. The population correlation matrix is an identity matrix; each variable correlates perfectly with itself (r = 1), but has no correlation with the other variables (r = 0). The Bartlett test must be significant for factor analysis to be performed on a dataset. The results indicated that all the factors were highly significant confirming the appropriateness of carrying out a factor analysis (Table 4).

Table 3. Descriptive analysis of potential accident causing workshop operations, safety factors proposed and practiced by respondents

Factors	No.	Workshop operations and safety factors	Mean ± SD	α
Workshop operations	A1	Use of tools and equipment that are out of order	3.25 ± 0.79	0.778
	A2	Violation of standard safety rules and regulation	3.19 ± 0.80	
	A3	Inexperience	3.57 ± 0.87	
	A4	Inappropriate handling and storage of chemicals	3.02 ± 0.75	
	A5	Poor handling of tools and equipment	3.04 ± 0.65	
	A6	Fatigue and boredom of workers	3.09 ± 0.52	
	A7	Reluctance of management towards safety	2.77 ± 0.57	
	A8	Working environment	2.89 ± 0.64	
	A9	Natural causes	2.49 ± 1.00	
	A10	Inadequate operation space for workers	2.67 ± 0.64	
	A11	Physical condition of workers	3.67 ± 0.51	
	A12	Workers poor job satisfaction	2.46 ± 0.67	
	A13	Constant exposure to a particular job	1.88 ± 1.04	
	A14	Physical build of workers	2.03 ± 0.45	
Safety factors proposed	S1	Appropriate storage of chemicals	3.33 ± 0.57	0.701
	S2	Enforcement of safety rules by experienced apprentices	3.19 ± 0.66	
	S3	Strict compliance to workshop safety rules	3.21 ± 0.41	
	S4	Regular orientation on safety practices	3.47 ± 0.61	
	S5	Use of safety information materials	2.83 ± 0.70	
	S6	Specific safety instructions for particular jobs	3.09 ± 0.67	
	S7	Safety awards for motivation workers	3.68 ± 0.88	
	S8	Investigation of accident causes	3.01 ± 0.67	
	S9	Use of PPEs	3.13 ± 0.77	
Safety factors practiced	M1	Use of PPEs	3.03 ± 1.03	0.729
	M2	Using appropriate tools for its operations	1.76 ± 0.43	
	M3	Adherence to some specific safety instructions	2.41 ± 0.99	
	M4	Proper storage of chemicals	2.12 ± 1.02	
	M5	Having first aid box	2.58 ± 1.11	

Figures in bracket are means and standard deviations (SD) respectively, PPEs: Personal protective equipment, SD: Standard deviation; A1-A14: Potential accident causing factors; S1-S9: Safety measures; M1-M5: Safety measures practiced; α: Cronbach's alpha

Table 4. Kaiser-Meyer-Olkin (KMO) and Bartlett tests of sphericity

Factors	KMO	Bartlett tests
Workshop operations	0.79	P < 0.001
Safety factors	0.63	P < 0.001
Safety measures practiced	0.60	P < 0.001

KMO: Kaiser-Meyer-Olkin

Factor analysis and principal component analysis

Factor analysis and principal components analysis were both used to reduce the large set of items or variables under each factor to a smaller number of dimensions and components. These techniques summarized a number of original variables into a smaller set of composite dimensions, or factors. This was done by removing redundant data or variables and reduced them into components that best suits or measures the construction.[9] Factor analysis is performed to assess whether items or variables on a questionnaire can be clustered clearly and meaningfully into small groups of factors or components.[10] Hence factor analysis results in the grouping of individuals, objects or variables into clusters so that objects in the same cluster are homogeneous in a manner that ensures that there is heterogeneity across clusters. This technique is often used to segment the data into similar and natural groupings.[10] The result of factor analysis basically represents measures and factor loading of each variable. According to Field,[7] in performing factor analysis, factor loading values of less than 0.3 must be suppressed and the variable associated with that factor loading excluded from the rest of the variables. The rotated matrix analysis (Table 5) shows that no variable could be excluded from the rest of the group since no factor loading was less than 0.3.

Workshop operations causing accidents

The results showed that the workshop operations that cause accidents in local garages were rotated into three main components (Table 5). Nine workshop operations were classified or clustered as factor one, the main causes of accidents in local garages. Generally, it was observed that the first six workshop operations were closely related to workers' attitudes towards the operations. This showed that most accidents were caused by the attitude of workers in the garages. Among them, the three key ones were the use of tools and equipment that were out of order, inappropriate handling and storage of chemicals and poor handling of tools and equipment. Tools and equipment play an important role in the execution of maintenance and repair works in automobile repair workshops. This implies that out of order tools and equipment have the potential of causing accidents. This was affirmed by one respondent who said "out of order tools cause minor accidents which is often not serious for the victim to attend hospital." One key cause of occupational accidents and injuries in developing countries is the use of obsolete machinery.[11] According to Rantanen[12] various factors are involved in the cause of accidents leading to injuries. The most common cause of accidents in their study was hand tools and equipment which is in line with findings of this study. Reports of other studies also indicated that 55% of workshop accidents leading to injuries per year were from machinery, hand tools, splinters, struck by/against an object.[13-15] Chemicals are very important in garages and their storage should be done according to standard procedure. However, most vehicle repair workers operating in the local garages do not pay attention to this.

Workshop operations clustered as factor two were more focused on the working environment and workshop management practices. This clearly showed that the working environment could be the cause of accidents. However, according to this study, the working environment of local garages has not contributed to any form of accidents, and if any, it was in a non-significant manner. Empirical research carried by Lundstrom[16] on industrial accidents indicated that accident causality is attributed to two major factors: Internal (characteristics of the worker in terms of mood and behavior) and external causal factors (characteristics of the work environment).

Table 5. Rotated component matrix for workshop operations

Workshop operations	Factor ratings	Component 1	Component 2	Component 3
Use of tools and equipment that are out of order		0.887		
Inappropriate handling and storage of chemicals		0.873		
Poor handling of tools and equipment		0.848		
Inexperience		0.761		
Violation of standard safety rules and regulation	Factor 1	0.711		
Fatigue and boredom of workers		0.699		
Inadequate operation space for workers		0.644		
Physical condition of workers		0.518		
Physical build of workers		0.509		
Reluctance of management towards safety			0.815	
Workers poor job satisfaction	Factor 2		0.649	
Working environment			0.624	
Natural causes	Factor 3			0.831

From the FGDs, it was realized that poor job satisfaction on the part of workers could also be another cause of accidents in the local garages. This is because workers who do not derive optimum satisfaction in the work could carry out workshop operations anyhow which could lead to an accident. When people are dissatisfied with their jobs, company or supervisors, they are more likely to experience accidents. The underlying reason for this is that the dissatisfaction distracts one's attention away from the task at hand and leads directly to accidents.[17] A satisfied worker, on the other hand, would always be careful and attentive while performing tasks, leading to reduced chances for accidents.[18] Job dissatisfaction seemed to be linked to the external causal factors responsible for the occurrence of accidents. It was noted that workers (particularly, accident victims) who expressed higher levels of job dissatisfaction significantly attributed accident causality more to work environment factors than to their personal characteristics.[18]

The operations clustered as factor three rarely caused accidents in local garages. Constant exposure to a particular job would increase the experience of workers. However, in some cases, experienced workers prefer to carry out workshop operations haphazardly, thinking they have done that work several times, and this could easily result in an accident.

Safety measures practiced to prevent accidents

The five safety measures practiced by the mechanics were rotated into two main components (Table 6). The first three measures that were clustered as factor one were closely related to worker attitudes. This implied that the attitude of mechanics was very important for workshop safety and accident prevention. According to this study, the use of personal protective equipment (PPE) is a key measure that could help prevent accidents in the local garage. However, it was observed that most of the mechanics did not use appropriate and standard PPEs while working. Anything to protect them is considered as PPE. A similar study conducted in Ethiopia indicated that most of the respondents (97.0%) did not use PPEs at work places.[12] The main reason for not using PPE was absence of it. Another study by Ghebreyohannes also indicated that local mechanics lacked knowledge concerning the selection of appropriate PPEs.[2]

One main requirement for workshops is the provision of first aid boxes. This is because in the event of an accident, the victim could receive some treatment in the workshop before appropriate medical care is sought at a health facility. In this study, result from the survey showed that local garages had first aid boxes, which contained drugs that were used when the need arose. However, a contrary situation was observed at all the local garages sampled with none having a first aid box. Proper storage of chemicals was also identified as a safety measure. An explosion of chemicals could result if not stored appropriately and properly.

A correlation analysis between the accident causing operations (As) and safety practices (Ms) was carried out. This resulted in 65 correlation coefficients, most of which were statistically significant (Table 7).

Table 6. Rotated component matrix for safety measures

Safety measures	Factor ratings	Component	
		1	2
Use of PPEs	Factor 1	0.818	
Adherence to some specific safety instructions		0.774	
Using appropriate tools for its operations		0.685	
Having first aid box	Factor 2		0.761
Proper storage of chemicals			0.725

PPEs: Personal protective equipment

Table 7. Correlation between accident causing operations (As) and safety measures practiced (MS)

	M1	M2	M3	M4	M5
A1	0.520**	0.371**	-0.224**	-0.060	-0.252**
A2	0.252**	0.390**	-0.495**	-0.087	-0.196**
A3	0.313**	0.379**	-0.357**	-0.199**	-0.279**
A4	0.300**	0.401**	-0.285**	-0.193**	-0.381**
A5	0.324**	0.329**	-0.109	-0.040	-0.217**
A6	0.292**	0.503**	0.132*	-0.041	0.006
A7	0.152**	-0.087	0.288**	0.243**	0.394**
A8	0.140*	0.000	0.394**	0.127*	0.231**
A9	0.072	-0.035	0.314**	0.097	0.192**
A10	0.124*	0.180**	0.019	0.187**	-0.133*
A11	0.090	0.109	-0.578**	-0.297**	-0.296**
A12	0.125*	0.296**	0.433**	0.148*	0.284**
A13	-0.009	-0.139*	0.370**	0.105	0.214**

* Pearson correlation is significant at the 0.05 level (two-tailed); ** Pearson correlation is significant at the 0.01 level (two-tailed); A1-A14: Potential accident causing factors; M1-M5: Safety measures practiced

Generally, there were moderate correlations between workshop operations and safety measures practiced. This implied that some of the workshop operations carried out by the local garage mechanics really resulted in accidents and their safety practices did not really prevent accidents. However, the negative correlations indicate that some safety measures reduced the risk of workshop operations causing accidents. This was not surprising because it was observed that most of the safety measures practiced did not conform to the standard safety measures.

Conclusion

The results from this study showed that there were workshop operations carried out by local garage workers that caused accidents. These operations were clustered into three main factors according factor analysis. A critical look at the clusters revealed that factor 1 was mainly concerned with the workers' attitude and conditions regarding workshop tools and equipment. However, factor 2 concerned workshop management and environment while factor 3 was concerned with work monotony and natural causes of accidents. In conclusion, the study also revealed that local garage mechanics had some level of knowledge concerning safety measures but the practice did not measure up to standard and best safety practices. This, therefore, resulted in accidents as shown in the correlation analysis. Safety seminars and training sessions could be organized for the local garage workers in order to improve on their safety behavior and to reduce occupational accidents and injuries.

Conflict of Interests

Authors have no conflict of interests.

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Journal of Advances in Environmental Health Research

Bioaccumulation of mercury in some organs of two fish species from the Sanandaj Gheshlagh Reservoir, Iran

Borhan Mansouri[1], Farshid Majnoni[2], Zahed Rezaei[3], Fardin Gharibi[4]

1 Kurdistan Environmental Health Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran
2 Department of Environmental Sciences, School of Natural Resources, Birjand University, Birjand, Iran
3 Department of Public Health, School of Health, Kurdistan University of Medical Sciences, Sanandaj, Iran
4 Deputy of Research and Technology, Kurdistan University of Medical Sciences, Sanandaj, Iran

Original Article

Abstract

The purpose of this study was to monitor the concentrations of mercury in the edible muscle, gill, liver, and skin of common carp (Cyprinus carpio) and silver carp (Hypophthalmichthys molitrix), in the Sanandaj Gheshlagh Reservoir, Iran. Mercury concentrations were assayed using Shimadzu AA 6600 atomic absorption spectrophotometer, and the results were given as µg/g wet weight. The level of mercury in organs of silver carp was higher than in common carp. Moreover, the highest and lowest level of mercury has been accumulated in the gill and skin organs respectively. The results showed that the maximum allowable fish consumption rate for an adult person with mean 71.5 kg body weight were 21 g/day base on g/day based on mercury levels. In conclusion, results showed that the mercury concentrations in the edible muscle of both fish species are below levels of concern for human consumption.

KEYWORDS: Carps, Gills, Liver, Mercury, Iran

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Introduction

The metal contamination of aquatic ecosystems has emerged as a worldwide concern over the past years. Due to their toxicity and long persistence, the addition of metals into the human food chain can introduce the potentially severe health hazards.[1] Metals are categorized into essential and non-essential types. The copper and zinc are essential for maintaining cellular function, enzymatic activities and other biological processes, and hence, the so-called essential metals. Other metals such as mercury and cadmium have no a well-known biological function and exert their toxicity by competing with essential metals to active enzyme or membrane protein sites.[2] Mercury is known as toxic metals for their negative effects on the function of kidney, nervous, and immune systems. Furthermore, long exposure to mercury can permanently damage the brain, kidney, and decline the natural progress of the fetuses and young children.[3]

Fish serve as one of the main sources of protein for humans. It is rich of omega-3 polyunsaturated fatty acids, which can reduce cholesterol level.[4] Fish can easily uptake pollutants from the environment, either from the water or the food. Studies on metals pollution using fish are enormous.[1,5,6] The accumulation of metals in the organs of a fish depends on various factors. Amongst others, the fish age, gender, size, environmental conditions (e.g., water

Corresponding Author:
Borhan Mansouri
Email: borhanmansouri@yahoo.com

hardness, temperature, and pH), metabolic rate, and exposure duration are some examples.[7-9]

The Sanandaj Gheshlagh Reservoir (SGR), Iran, is one of a few fishing sites in the area that supplies a major part of the Sanandaj's demand for fish. However, its safety in providing a healthier food supply is subject to pollution from two external sources; (i) the chemical fertilizers and pesticides from agricultural use on the farms surrounded the reservoir, and (ii) the crude oil and other petroleum products transported by truck from Iraq to Iran and vice versa. Both sources can potentially release a substantial amount of contaminant such as mercury into the SGR. Therefore, the objective of this study was to determine the levels of mercury in edible muscle, gill, liver, and skin organs of two fish species captured daily in the SGR. This can be used to assess whether the mercury levels meet the local and international requirements. We also aim to study whether the species of the fish, their length, weight, and sex are related to mercury accumulation.

Materials and Methods

The Gheshlagh reservoir (35°25′-35°30′ N; 46°57′-47°30′ E) is located 12 km away from Sanandaj city in the west of Iran. The Gheshlagh reservoir was in principal built to supply drinking water for Sanandaj city (main water resources for household use); and the irrigation water for the downstream lands. The average annual temperature of the water ranges from 5.2 °C (January) to 25 °C (August).

Fish samples were caught from random catches in the SGR during October-December, 2013 and carried to the laboratory by a thermos flask with ice. A total of 23 fish was assessed for mercury in the edible muscle, gill, liver, and skin organs. The collection included common carp (males = 5 and females = 8) and silver carp (males = 6 and females = 4). In the laboratory, they were immediately dissected using a stainless steel dissection instrument. Muscle samples were separated from below the dorsal fin without skin.[1] Average of total length and total weight of sampled fish was measured 30.4 (± 4.8) cm and 533.6 (± 175.7) g for common carp; and 39.5 (± 6.8) cm, 34.6 (± 6.8) cm and 664.2 (± 232.1) g for silver carp, respectively. Approximately 1 g wet weight (WW) of gills, skin, and edible muscle, and liver from each sample were dissected, washed with distilled water, and accurately weighed into 150-ml erlenmeyer flasks. To each sample, 10 ml nitric acid (65%) was added. Samples were left overnight in order to digest slowly. Afterward, 5 ml perchloric acid (70%) added to each sample.[10,11] Digestion was performed on a hot plate (sand bath) at 150 °C before diluting the samples with 25 ml deionized water. The concentration of mercury was measured using a Shimadzu AA 6600 atomic absorption spectrophotometer by cold vapor. For mercury metal, we obtained the detection limits as 0.04. Moreover, the mean recovery for mercury was 97.3%.

Statistical analysis was performed using SPSS for Windows (version 16.0, SPSS Inc., Chicago, IL, USA). Data were tested for normality using a Kolmogorov-Smirnov test. Data were normally distributed; therefore, a parametric test was used for analysis. The one-way analysis of variance (ANOVA) was performed to establish the statistically significant differences in the concentration of mercury metal between the organs. Student's t-test was used for group comparison between two species. Pearson correlation (r) was used to determine the correlation between the levels of accumulated mercury metal in the edible muscle, gills, liver, and skin organs of common carp and silver carp and their biometric features (total length and total weight). The mercury concentrations in organs were expressed as microgram per gram WW. Values are given in means ± standard deviation.

Daily consumption limits were obtained using the following equation. It shows allowable daily consumption of mercury contaminated fish based on a contaminant's carcinogenicity, expressed in kilograms of fish consumed per day:[12]

$$CR_{lim} = \frac{RfD \times BW}{C_m}$$

Where CR_{lim} is maximum allowable fish consumption rate (kilograms/day); RfD is reference dose (0.1 µg/kg/day for mercury); BW is consumer body weight (kilograms); and C_m is measured concentration of chemical contaminant m in a given species of fish (milligrams per kilogram).

The consumption limit is determined in part by the size of the meal consumed. We assumed the meal size as 0.227. The following equation can be used to convert daily consumption limits to the number of allowable meals per month:

$$CR_{mm} = \frac{CR_{lim} \times T_{ap}}{MS}$$

CR_{mm} is maximum allowable fish consumption rate (meals/month); CR_{lim} is maximum allowable fish consumption rate (kilograms/day); MS is meal size (0.227 kg fish/meal); and Tap is time averaging period (365.25 days/12 months = 30.44 days/month).

Results and Discussion

The mean concentration of mercury in the organs of liver, gill, edible muscle, and skin of two fish species (common carp and silver carp) is presented in table 1. From table 1, it can be seen that for both fish species, the highest and lowest level of mercury has been accumulated in the gill and skin organs respectively. One reason for high level of metal concentrations in the gill organs of fish samples can be due to absorption and adsorption as the main sites of metallothionein (MT) production and metal retention, after making direct contact with the surrounding waters.[13] MT is low-molecular-weight proteins with many sulfhydryl groups binding a variety of metals such as copper, zinc, cadmium, and mercury, showing a strong affinity toward certain essential and non-essential metals.[2] In contrast, one reason may be due to this fact that the skin involve in lower metabolic activities in accumulating metals. Fish skin typically provides less surface area, a thicker and less permeable diffusion barrier, slower transport of water to the exchange surface, less blood flow, and no countercurrent flow of water and blood.[14]

The mercury is a persistent toxic for humans and wildlife with well-known negative neurological and reproductive effects. Hence, the level of mercury must be monitored in food chains to avoid its negative consequences.[15] The concentrations of mercury in the organs ranged from 0.1 to 0.3 µg/g WW for common carp and from 0.2 to 0.5 µg/g WW for silver carp. These levels were lower than those reported by 6. Majnouni et al.[6] in same fish species in Zarivar

Table 1. Mercury concentrations (mean ± standard deviation) in the organs of common carp and silver carp

Species/sex	Organs			
	Gill	Liver	Muscle	Skin
Common carp				
Male	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
Female	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.1 ± 0.1
Mean	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.1 ± 0.1
P sex	0.05	NS	NS	NS
Silver carp				
Male	0.3 ± 0.1	0.3 ± 0.2	0.3 ± 0.1	0.1 ± 0.1
Female	0.6 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.2 ± 0.1
Mean	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.2 ± 0.1
P sex	0.050	NS	NS	NS
P species*	0.020	NS	0.020	NS

* P value for Student's t-test to compare between species; NS: not significant at P > 0.050

Lake known as an area with high source of pollutions such as wastewater discharge, chemical fertilizers, and pesticides from farmlands. Mercury concentrations in the common carp (0.3-0.1 µg/g) and silver carp (0.4-0.2 µg/g) edible muscles and skin were lower than the maximum acceptable concentrations established by Food and Agriculture Organization and World Health Organization (i.e., 0.5 µg/g on WW).[16] However, it should be noted that the Environmental Protection Agency has defined the published maximum acceptable concentration of mercury by 0.3 µg/g on WW basis.[17] The results presented in table 1 show that apart from the edible muscle organ of silver carp, the mean of accumulated mercury level was in general lower than this threshold in all organs of both fish species. In similar study by Khoshnamvand et al.[18] from July to December 2009 in SGR reported that the T-mercury in the muscle organs (0.31-0.36 µg/g on WW) was higher than this standard.

Assessments of the human health risks associated with the consumption of mercury content contaminated fish are given according to daily (kg/day) and monthly (meals/month) limits for the 3-75 years old population demographic in table 2. The results of this study showed that the maximum allowable fish consumption rate for an adult person with mean body weight of 71.5 kg was 21 g/day based on mercury levels. The maximum allowable consumption rate has been reported equal to 8-56 g/day for cultured fish from Persian Gulf in Iran base on the Hg content.[12] Kannan et al.[19] found that consuming fish from South Florida Estuaries at rates > 70 g/day was estimated to be hazardous to human health. We found that the level of mercury in the muscle of common carp and silver carp (0.3-0.4 µg/g WW) was lower than the reported mercury in the same fish species (1.1-0.8 µg/g WW) from the Zarivar lake.[6]

The analysis of Pearson correlation coefficients of length, weight and metal concentrations in two fish species showed that the significant association between total length, weight and mercury concentrations ($P < 0.050$; Table 3). The results of our study also showed that the mercury concentrations in silver carp were in general higher than what we observed in common carp. We studied two fish species of Cyprinid family. Based on our findings, we may conclude that different species of this family may follow different foraging strategy. However, with a few exceptions (mercury in the gill of both species and mercury in the muscle of silver carp), we found the differences between male and female fish was statistically non-significant (t-test, $P > 0.050$).

Table 2. Maximum allowable fish consumption rate according to the metals content

Age (year)	Average body weight for male and female (kg)	Maximum allowable fish consumption rate (kg/day) mercury	Maximum allowable fish consumption rate (meals/month) mercury
3-6	11.6	0.0033	0.4425
6-9	25.0	0.0071	0.9520
9-12	36.0	0.0102	1.3677
12-15	50.6	0.0144	1.9309
15-18	61.2	0.0174	2.3332
18-25	67.2	0.0192	2.5746
25-35	71.5	0.0204	2.7355
35-45	74.0	0.0211	2.8294
45-55	74.5	0.0212	2.8428
55-65	73.4	0.0209	2.8026
65-75	70.7	0.0202	2.7087

It is believed that the sex-related differences in metal concentration may cause by a combination of some factors, such as dietary preferences, physiological metabolism in relation to stage in the reproductive cycle or foraging behavior. In our study, sex did not exert a significant effect on metal concentrations in most organs of both fish species. However, Al-Yousuf et al.[7] found higher average zinc and cadmium concentrations in the liver, skin, and muscle of female fish compared to male fish. In contrast, metals accumulated in both fish species were significant differences among the organs of liver, gill, edible muscle, and skin (one-way ANOVA, P < 0.001; Table 4). Al-Yousuf et al.[7] and Usero et al.[20] reported that the differences in zinc and cadmium concentrations of the organs might be a result of their capacity to induce production of metal-binding proteins such as MT.

Table 3. Correlation of size, weight, and metals levels in the muscle of common carp and silver carp

Species	Mercury	Size	Weight
Common carp			
Mercury	1	0.08	0.16
Size			0.50
Weight			1
Silver carp			
Mercury	1	0.64*	0.65*
Size		1	0.97**
Weight			1

* Correlation is significant at the 0.050 level; ** Correlation is significant at the 0.050 level

Table 4. Statistical analysis of mercury levels in the edible muscle, gill, liver, and skin of both species

Fish species	One-way ANOVA	
	F	P
Common carp	7.3	< 0.001
Silver carp	7.9	< 0.001

P significance level; NS: Not significant; ANOVA: Analysis of variance

Conclusion

This study reveals that the highest and lowest level of mercury has been accumulated in the gill and skin organs respectively. The statistical analysis indicated that the mercury concentrations differed significantly among liver, gill, edible muscle, and skin in common carp and silver carp. Metal concentrations in both fish species were higher in silver carp than in common carp. In general, results shows metal concentrations in the edible muscle of both fish species are below levels of concern for human consumption. In conclusion, the accumulation and uptake of mercury in the organs of fish depends on the organs, genders, and species.

Conflict of Interests

Authors have no conflict of interests.

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Journal of Advances in Environmental Health Research

Evaluating efficiency of radio waves for microbial removal in water samples

Sommayeh Saadi[1], Mahmood Alimohammadi[1], Ramin Nabizadeh[1], Alireza Mesdaghinia[1], Hassan Aslani[1], Shahrokh Nazmara[1], Maryam Ghani[1], Babak Mahmoodi[1], Masoomeh Asgari[1], Nejat Mousavipour[1]

1 Department of Environmental Health Engineering, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

Original Article

Abstract

The most common used methods for water disinfection were chemicals like chlorine, ozonation, ultraviolet radiation, membrane processes, etc. Water disinfection using irradiation techniques is new in water treatment industry which has been developed recently. The aim of the present study was to investigate radio frequency (RF) efficiency for the inactivation of total coliform (TC), fecal coliform, and heterotrophic bacterial count of water pellets. Tap water samples were taken from School of Public Health, Tehran University of Medical Sciences and irradiated using hydropad device, steam KLEAR model S-38 (1.2 W and frequency of 120-200 kHz). Microbial concentration was measured in cycles 1, 5, 10, 15, 20, 25, 30, 35, and 40 in 1 and 2 h contact time. Indicator bacteria were counted using plate count method and multiple fermentation tube technique. According to the microbial results, after 40 cycles and without chlorine residual, TC, fecal coliform, and heterotrophic bacteria were reduced by 86, 90, and 85%, while after 15 cycles and 0.8 mg/l chlorine residual, removal rate was 89, 91, and 89%, respectively. Furthermore, it was observed that after 2 h of contact time, TCs, fecal coliforms, and heterotrophic plate count were reduced by 78.2, 80, and 60%, respectively. Although RF efficiency in water disinfection has not been studied, our findings suggested its possible use due to more than 75% efficiency. From the standpoint of practical use, more studies should be done, especially to find a fine synergist agent, determining power, frequency, and suitable contact time and also the method should be modified.

KEYWORDS: Drinking Water, Radio Waves, Disinfection, Coliform

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Introduction

Disinfection is the most important and common step of water treatment process for ensuring the health of the community. Numerous chemicals have been used for water disinfection, the most famous and common of which is chlorine due to its high efficiency in bacterial removal. In addition, simple control of the process and the residual chlorine remaining in distribution systems is other factors that make its use popular.[1] After commencing drinking water disinfection with chlorine compounds in 1904, the occurrence of outbreaks associated with the consumption of contaminated water decreased.[2,3] On the other side, by using chlorine and formation of more toxic and harmful byproducts (carcinogens, mutagens) such as trihalomethanes and haloacetic acids and their adverse effects on aquatic systems and human health, some studies have addressed the

Corresponding Author:
Mahmood Alimohammadi
Email: m_Alimohammadi@tums.ac.ir

relationship between long-term exposure to chlorine by products and risk of carcinogenic side effects.[4,5] Furthermore, considering the scientific experiences about microbial immunity against chemical disinfectants and human sensitivity about the health and environment, it is necessary to find some alternative disinfectants using nonchemical materials with complete pathogen removal.[6,7]

There is an old and well-known method for water purification using electromagnetic. Radio frequency (RF) is a kind of 300 kHz-300 GHz electromagnetic wave and unlike ionizing radiation; it does not have any ionization ability and molecular change in the cell structure. The amount of photon energy in RF is not strong enough to make the ionizing operations. This energy may provoke vibration of large molecules or may cause polarization of molecules and atoms. RF field affects the ions and molecules and may change the electrical flux distribution which finally can change their direction in space. RF waves can potentially disinfect and sterilize pest and various substances in food, agricultural, and environmental components due to its high penetrating power and increasing temperature.[8,9]

RF effects are divided into two categories of thermal and non-thermal. In the case of thermal effects, entry the radio waves into the water sample can destroy the bacteria by changing the energy of waves to heat. Nonthermal effects induce electromagnetic currents into the living tissue which can provoke vibration of large molecules and result in the polarization of molecules and atoms. Fields with lower frequency cause little energy absorption and less heat increase and the results are related to the induced current, while the fields with higher frequency are mostly in the heat form.[7,8] Studies have shown that weak RF fields, especially those with low frequencies, change the functional properties of the membrane structure and responses to cellular stimulation. These fields do not make any changes in membrane potential; however, they penetrate into the cell membrane and affect the structure and function of the cytoplasm.[10-12] In an electric field, a potential difference is formed across the membrane by opposite charges attracting each other on either side of the membrane; thus, the membrane becomes thinner. When intensity of the electric filed is sufficiently high, formation of pores in the membrane will eventually rupture the cell.[13,14]

RF efficiency in removal and inactivation of bacterial species, agricultural pests, etc., has been investigated in several studies. Kim et al. examined RF for the inactivation of Salmonella and Escherichia coli on red and black pepper. Results showed 29.4-80.2% decrease in Salmonella and E. coli in black pepper and 38.3% to more than five log decreases of pathogens in red pepper, respectively.[15] Previous studies have investigated the RF and microwave radiation efficiency for the inactivation of Listeria, E. coli and other microbial community in contaminated milk and biofilms. The results showed that, due to the retention time and power of RF, heating was powerful enough to inactivate bacteria.[16-18] The aim of this study was to determine RF efficiencies in the removal and inactivation of total coliform (TC), fecal coliform, and heterotrophic plate count (HPC) as microbial indicators of water samples. Different contact times and microbial load were also examined.

Materials and Methods

This experimental study investigated the efficiency of hydropad device in reducing the microbial load of water samples containing TCs, thermotolerant coliforms (TTC), and HPC. Providing RF, the hydropad device, steam KLEAR model S-38 made in England, was used with the dimensions of 3 × 7 × 11 cm, power of 1.2 W, and frequency of 120-200 kHz. Hydropad is an electronic device that releases an electric field induced in the piping system without the use of electrodes. System performance made the RF of 120-200 kHz by a four wing frequency transformer. This frequency was induced to the water by strontium ferrites around the tubes and the coil winding which made the first orbit and generated the sinuses voltage of 5-35 V with the wavelength of 1200-1600 m in the tube. In these experiments, two 70 L plastic tanks provided with taps on them were used. The tanks were joined together in series with iron tubes of 2 inch in diameter and 6 m in length. Hydropad device was placed between the two tanks and a pump named LONKLY SIRO (model no LSR 40-6s/180, F PN10 class), model F PN10, was used for water circulation. Figure 1 shows the used pilot with details.

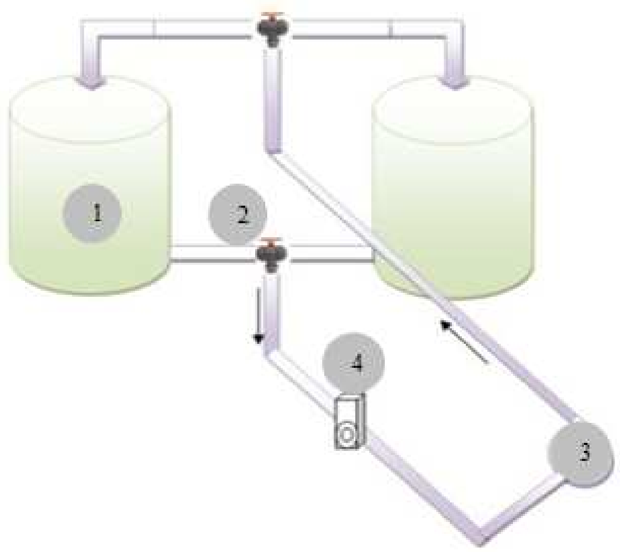

Figure 1. Schematic representation of disinfection pilot: (1) Tank, (2) three-way tap, (3) pump, (4) hydropad device

Water samples were collected from the tap water from School of Public Health. A particular amount of secondary treated, but not disinfected, wastewater from Zargandeh treatment plant, located in north of Tehran, Iran, was added to introduce microbial contamination to water samples and sodium thiosulfate was added to the tap water to neutralize residual chlorine before mixing the sample with wastewater. A proper sample was prepared after completely mixing water and wastewater using a pump. At the beginning of each set of the experiment, sampling was done to determine the initial bacterial count and the efficiency of bacterial removal was measured at the end of each experiment.

Bacterial enumeration was done according to the standard methods for water and wastewater examination (APHA 21ed). Briefly, multiple fermentation tube procedure was used for the TC count (section E9921), bacteria were incubated on lactose broth for 24 h. Fecal coliforms were analyzed by the direct (without enrichment) multiple fermentation tube technique (standard methods, 9221E-2). As mentioned by Nabizadeh et al., the samples were inoculated and incubated on A1-medium and their ability to produce gas was determined. For the enumeration of thermotolerant coliform bacteria, the samples were incubated at 37 °C for nearly 3 h and the tubes were then transferred to a 44.5 °C water bath for 19-21 h.[19] Production of turbidity and/or gas accumulation in the tubes constitutes a positive result. HPC with power plate method (section 9215) was used for the enumeration of heterotrophic bacteria on the R2A agar medium. All the materials were purchased from Merck Company, Germany. Total and fecal coliforms were reported in terms of most probable number at 100 ml and heterotrophic bacteria were reported in terms of CFU/ml. HPC was used for evaluating the microbiological quality of drinking water and controlling water treatment processes, in which different types of colonies of more than 500 were important. All the glassware used in the experiments were sterilized at 180 °C for 2 h.[20]

In these experiments, the water sample which was contaminated with municipal sewage effluent filled the tank number 1. In order to complete mixing of sewage effluent with water, the pump was started and the sample was circulated between the two tanks. Hydropad system was started and the sample circulated from tanks 1 to 2 in contact with the produced waves. The sample cycle from tanks 1 and 2 was named the first cycle and its return from tanks 2 to 1 was called the second cycle. In order to determine the efficiency of RF to inactivate different indicator organisms with different initial concentrations of TCs, TTCs, and HPC, 40 cycles and different sampling times were examined. There was no chlorine residual in all the samples, except for the last cycle, in which RF effect was evaluated beside chlorine residual between 0.5 mg/l and 0.8 mg/l as chlorine. In order to evaluate effect of contact time, only one of the tanks was operated and the effect of 1 and 2 h of contact time was studied.

To determine the initial bacterial load before starting the hydropad device, water sampling was done and TCs, TTCs, and HPC were measured as the base point or cycle zero. Then, hydropad was turned on to determine the efficiency of radio wave on disinfection of target microorganisms. The sample was circulated between two tanks by the pump in cycles 1, 5, 10, 15,…, 40 and the microbial tests were done on the samples once more.

In the second phase of the experiments, the amounts of TCs, TTCs, and HPC were reduced in comparison to the earlier tests and water was circulated in one of the tanks for 2 h, instead of circulating between two tanks. Finally, bacterial removal was investigated in the range of 0-2 h. The experiments were conducted in six phases with different initial microbial loads.

Results and Discussion

Results related to the effect of radio waves on water disinfection using the hydropad device at different microbial concentrations and cycles are presented in table 1. As can be observed, when the initial bacterial concentration was high (HPC = 41000-95000, TTCs = 15000-33000 and TCs = 22000-50000), removal efficiency was very low between 5.2 and 9%, while in lower microbial load (HPC = 6200, TTCs = 2300 and TCs = 3500), the removal efficiency was 19.3, 41, and 28.6% in the 20th cycle and 86, 87, 90% in the 40th cycle. Thus, it is seen that increasing the number of cycles from 20 to 40 and reducing the initial bacterial concentration increased the efficiency of the disinfection process. The removal efficiency of pathogens at the initial concentration of HPC = 500, TTCs = 200, and TCs=300 and in the presence of residual chlorine after 10 cycles was 89, 91, and 89, respectively. Therefore, it is obvious that in the presence of both disinfecting agents, the microbial load of the samples was effectively reduced.

Table 1. Hydropad steam KLEAR S38 device results after each cycle

Cycle number	Bacteria name	Before disinfection	After disinfection	Log inactivation	Removal percent
20	TC	5.00E + 04	4.55E + 04	0.04	9.0
	TTC	3.30E + 04	2.90E + 04	0.06	12.0
	HPC	9.50E + 04	9.20E + 04	0.02	3.0
20	TC	2.20E + 04	2.00E + 04	0.04	9.0
	TTC	1.50E + 04	1.40E + 04	0.03	6.6
	HPC	4.10E + 04	4.00E + 04	0.01	2.5
20	TC	3.50E + 03	2.50E + 03	0.20	28.6
	TTC	2.30E + 03	1.35E + 03	0.20	41.3
	HPC	6.20E + 03	5.00E + 03	0.10	19.3
40	TC	3.50E + 03	4.90E + 02	0.85	86.0
	TTC	2.30E+ 03	2.20E + 02	1.02	9.0
	HPC	6.25E + 03	8.00E + 02	0.87	87.2
10	TC	3.00E + 02	3.30E+01	1.00	89.0
	TTC	2.00E + 02	1.80E + 01	1.11	91.0
	HPC	5.00E + 02	5.50E + 01	0.96	89.0

TC: Total coilforms, TTC: Thermotolerant coliforms, HPC: Heterotrophic plate count

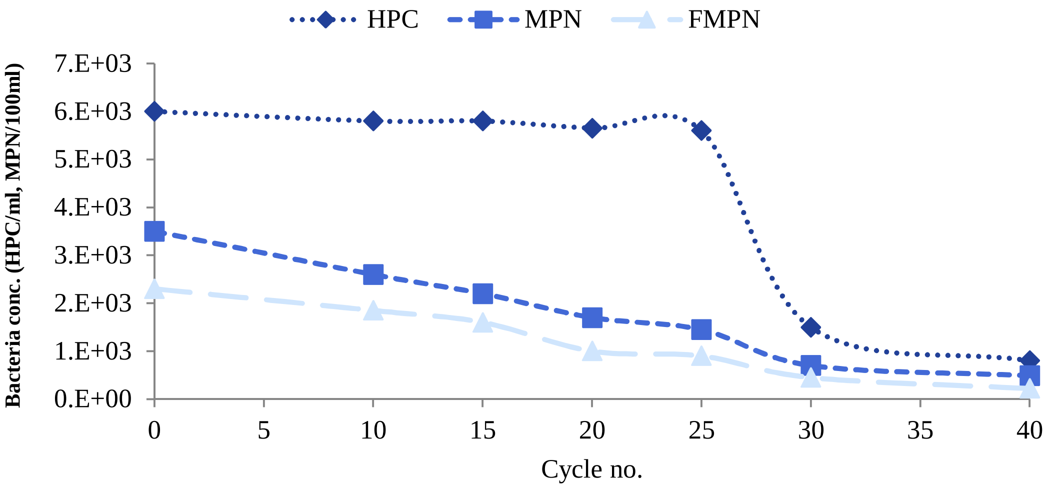

Figure 2. Total ciloforms, thermotolerant coliforms, and heterotrophic plate count removal trend after different cycles in absence of residual chlorine
HPC: Heterotrophic plate count; MPN: Most probable number; FMPN: fecal coliforms most probable number

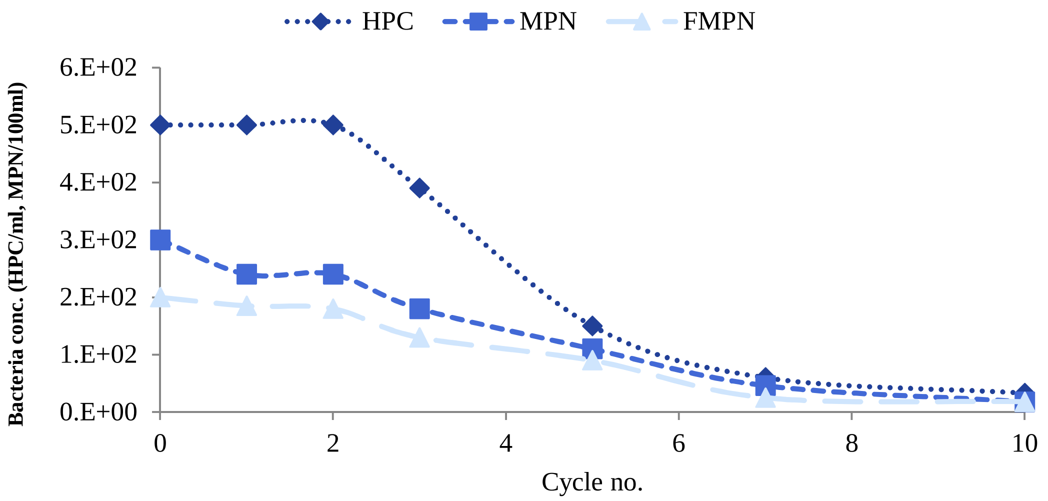

Figure 3. Total ciloforms, thermotolerant coliforms, and heterotrophic plate count removal trend after different cycles in the presence of residual chlorine
HPC: Heterotrophic plate count; MPN: Most probable number; FMPN: fecal coliforms most probable number

Figure 2 shows the efficiency of radio waves at the initial microbial concentrations during 40 cycles and in the absence of residual chlorine. As can be observed in the figure, the removal efficiency was constant until the 25th cycle, while it was highly increased by passing the cycles from 25 to 35.

Figure 3 shows the number of TCs, TTCs, and HPC after disinfection by radio waves and in the presence of residual chlorine over 10 cycles. Efficiency of removing pathogens at the initial concentration of HPC = 500, TTCs = 200, and TCs = 300 was constant until the third cycle and was effectively increased after the 7th cycle. Efficiency of disinfection improved when RF was used along with residual chlorine (Figures 2 and

3). From this point of view, the results were similar to those of Ukuku et al. which the twin method of RF and ultraviolet (UV) was applied for inactivation of E. coli in juice.[21] In the presence of both RF and UV, RF destroyed cell structure first and chlorine inactivated the cells with its expected mechanisms such as affecting nucleic acid, breathing system, and electron exchange system.[22] Thus, observing an increase in bacterial inactivation is expected with the presence of these two disinfecting agents. Simultaneous use of chlorine and RF is important because, in addition to improving RF effects, chlorine can provide a residual which may prevent secondary pollutions (as RF leaves no residual after disinfecting the samples, similar to UV). In addition, the application of these disinfectants can be discussed in pool water samples. As with a proper operation system, RF can be used in pool disinfection and chlorine can be useful with its residual. Indeed, the power needed for bacterial inactivation will reduce in the presence of chlorine; i.e., the power and retention time of RF can be reduced. In this case, the effective factors such as chlorine dose, RF power, thermal effects, pH, and the costs should be carefully studied.

Furthermore, efficiency of hydropad device is shown in table 2 during cycles 1-6 (at different microbial concentrations) and in 1 and 2 h of contact time. As shown by the results, at different microbial concentrations, there was no important change in removal efficiency by increasing the contact time from 1 to 2 h.

Results showed that at the frequency between 120 kHz and 200 kHz and flow intensity of 1.2 W/cm, microbial contamination of the samples was reduced by 50-80%. However, this frequency and the related power were unable to reduce total and fecal coliforms to zero per 100 mL and HPC to 250 CFU/mL in drinking water (standard of Iranian Environmental Protection Organization) (Tables 1 and 2). Therefore, more studies are needed to modify the system and improve the efficiencies for using in disinfection process. On the other side, in these systems, efficiency of disinfection is related to the used power and wave frequency. Some studies have reported that the capacity of bacterial inactivation by RF is related to the field intensity used in the system.[13,14]

Table 2. Hydropad steam KLEAR S38 (Hydropad Co. UK)device results after 1 and 2 h contact time

Cycle number	Bacteria name	Before disinfection	After disinfection		Log inactivation		removal percent	
			1 h	2 h	1 h	2 h	1 h	2 h
1	TC	5.40E + 02	2.20E + 02	1.30E + 02	0.39	0.39	59.3	75.9
	TTC	4.00E + 02	1.75E + 02	1.75E + 02	0.36	0.36	56.0	56.0
	HPC	8.20E + 03	7.50E + 03	5.00E + 03	0.04	0.20	8.5	40.0
2	TC	5.00E + 02	2.30E + 02	1.70E + 02	0.34	0.47	54.0	66.0
	TTC	3.80E + 02	1.85E+ 02	1.20E+ 02	0.31	0.50	51.3	68.4
	HPC	8.10E+03	7.50E + 03	5.00E + 03	0.03	0.20	7.5	38.3
3	TC	2.40E + 03	4.90E + 02	4.90E + 02	0.69	0.69	79.6	79.6
	TTC	1.50E + 03	3.50E + 02	3.50E + 02	0.63	0.63	76.0	76.0
	HPC	5.00E + 03	2.00E + 03	2.00E + 03	0.40	0.40	60.0	60.0
4	TC	2.30E + 03	4.50E + 01	4.50E + 01	0.71	0.71	80.4	80.4
	TTC	1.50E + 02	3.00E + 01	3.00E + 01	0.69	0.79	80.0	80.0
	HPC	5.00E + 03	1.90E + 03	1.90E + 03	0.40	0.50	62.0	62.0
5	TC	7.80E + 01	2.00E + 01	1.70E + 01	0.59	0.66	47.4	78.2
	TTC	5.00E + 01	1.40E + 01	1.00E + 01	0.55	0.69	72.0	80.0
	HPC	1.50E + 03	6.50E + 02	4.80E + 02	0.36	0.50	56.6	68.0
6	TC	2.30E + 01	1.30E + 01	5.00E + 00	0.25	0.66	43.5	78.3
	TTC	1.50E + 01	8.00E + 00	3.00E + 00	0.27	0.69	46.6	80.0
	HPC	2.50E + 03	1.00E + 03	1.00E + 03	0.40	0.40	60.0	60.0

TC: Total ciloforms, TTC: Thermotolerant coliforms, HPC: Heterotrophic plate count

In fact, these two factors are the main reasons for increasing temperature in the environment where exposure to RF is occurred,[16] and the high temperature is the main reason of inactivation by RF, which is called thermal effect. Ukuku et al. showed that by increasing temperature from 25 to 40°C, E. coli survival was reduced by more than 1.5 log.[21] Awuah et al. studied RF efficiency for the E. coli removal in milk, and declared that the main reason of bacterial inactivation by RF was temperature increase by radio waves. It was also mentioned that using RF depended on its contact time and radiation power and the results showed that using RF was a suitable method for inactivating L. innocua and E. coli in milk for a short retention time i.e., 1 min, and 1200 W energy used 5-7 log inactivation was observed. Therefore, they concluded that it can be an effective method for milk pasteurization.[16] Besides thermal effects, it is believed that other mechanisms like nonthermal effects are effective in the efficiency of RF in biological systems. In fact, temperature is the only factor that might lead to cell proliferation damage. Changes which occur in cell proliferation from exposure to RF are not solely the result of heat production although high temperature can lead to such a kind of changes. In sum, it has been proven that temperature changes cannot lead to biological changes in the normal range of RF.[21] In the present study, RF in the used range made no changes in the solution temperature; hence, it seems that the microbial changes were related to nonthermal effects of RF. Velizarov et al.[23] reported that stress induction was one of the most important non-thermal effects of RF and such an effect can increase the release of stress proteins like heat-shock proteins (HSP), which are not produced only by temperature increase. It has been proven that biological cells in exposure to stresses like hyperthermia, chemical substances, etc., would excrete the HSPs. One of the factors which cause increasing such proteins is electromagnetic flow with low frequency (such as RF). In the case of inactivation increase over time (Figures 2 and 3), the present findings were in good agreement with Velizarov et al., since they stated that longer contact times did not improve the removal efficiency owing to the improved cell adaptation to the new condition.[23]

As shown in table 2, the removal efficiency of TCs, TTCs, and HPC with contact time did not increase significantly. Although in the Velizarov et al. study, longer contact time was ranged from several hours to some days; it seems that increasing contact time from 1 to 2 h was enough for cell adaptation.[23]

For RF application in water and wastewater disinfection, it should be considered that no similar mechanism such as decreasing microbial load in milk and other food product is expected. For example, in studies related to E. coli and Listeria inactivation in milk, there are two factors which distinct the findings from water and wastewater samples; first, by irradiation with RF, as mentioned above, the main reason of bacterial inactivation in the milk samples is related to heating mechanisms and the second is that even the RF energy and frequency used for water samples would be the same with the amounts used for milk samples due to the fact that milk samples on average are heated 16% faster than water samples; this issue can be attributed to milk proteins, minerals, and vitamins. It is not possible to claim the same effects in both samples. It has been claimed that milk warming rate has a linear relationship with RF energy.[16] In water samples, there is also no reason for extra heating and RF power to inactivate microorganisms with thermal mechanisms, because there are far better methods for heating up to pasteurization point both practically and operationally.

Conclusion

Although RF role for water and wastewater disinfection was not investigated completely, the results showed some possibilities for its use in disinfection process. RF was able to eliminate some percentage of the microorganisms in this study; but, it was not sufficient for reaching the environmental standards. Hence, more studies should be done for determining the power, frequency, and suitable contact time and also the method should be modified.

Conflict of Interests

Authors have no conflict of interests.

Acknowledgements

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Journal of Advances in Environmental Health Research

Assessment of household reverse-osmosis systems in heavy metal and solute ion removal in real and synthetic samples

Afshin Maleki[1], Hiua Daraei[1], Ebrahim Akhgar[1], Shirzad Amini[1], Yahya Zandsalimi[1], Siroos Shahsavari[1]

1 Kurdistan Environmental Health Research Centre, Kurdistan University of Medical Sciences, Sanandaj, Iran

Original Article

Abstract

In this study, the efficiency of household reverse-osmosis system (HROS) with and without neutralizer accessory was investigated in both real and synthetic samples. The real samples were collected from rural and urban public drinking-water systems with and without primary refinery treatment. The selected areas were situated in the Kurdistan province, Iran. The HROS model RO100GPD with and without neutralizer accessory was used in all experiments to prevent effects of the membrane used, age of devices, and length of time in service. In order to assess sample quality, some more common physico-chemical analyses consisting of hardness, Ca^{2+}, Mg^{2+}, total dissolved solids (TDS), electrical conductivity (EC), alkalinity, Cl^-, Br^-, SO_4^{2-}, PO_4^{3-}, NO_3^-, NO_2^-, and heavy metals were performed based on standard methods. The results indicate that HROS and neutralizer accessory have significant effects on the physico-chemical properties of feed water. However, the results indicate the instability of HROS output water quality, but they verify that this instability cannot reduce the output quality. Finally, results emphasize that HROS output water meets standard levels regardless of the input water quality and application of neutralizer accessory.

KEYWORDS: Membranes, Drinking Water, Heavy Metals, Solute Ion, Hardness, Water Quality, Iran

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Introduction

The Iranian National Drinking Water Standards and other international standard systems specify a maximum contaminant level (MCL) and a maximum desirable level (MDL) for a number of chemical species, including anions, cations especially heavy metals, and some organic compounds [1]. The MCLs are specified to minimize potential health effects arising from the ingestion of these species in drinking water.[2]. For instance, high levels of nitrite and nitrate can cause Methemoglobinemia, which can be fatal to infants. Moreover, the MDLs are specified to maximize quality and desirability arising from the ingestion of these species in drinking water.[3]

Public drinking-water systems and local aquifers in Iran produce different drinking water regarding their quality. Sometimes concentrations of arsenic, nitrate, and hardness are not at the standard levels for drinking water, or the supplied water has an unpleasant smell or taste. Household reverse-osmosis systems (HROS) are efficient, economic, and simple to install or maintain. The application of HROS in Iran has promptly increased especially in areas that water is not supplied by public drinking-water systems or the supplied water does not meet standard level or satisfy the customers. [4,5]

In HROS, water is forced to pass through membranes with angstrom size pores. The solutes

Corresponding Author:
Hiua Daraei
Email: hiua.daraei@gmail.com

in the feed water are rejected by the membrane, and thus, the treated water contains lower concentrations of solute than feed water. It has been reported that the efficiency of HROS in removing contaminants and other chemical species varies with the specific specie, membrane used, age of devices, and length of time in service. However, the effects of physico-chemical properties of feed water on the HROS efficiency have not been fully investigated. The size and selectivity of RO membranes and other membranes that operate based on size are illustrated in figure 1.[6-9]

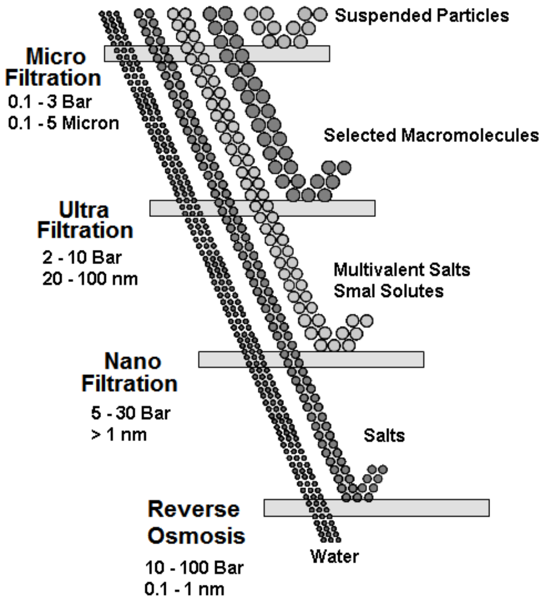

Figure 1. Reverse-osmosis membrane size and its selectivity rather than other membrane work with size

Improvements in taste of drinking water and promising results of simple tests, like conductivity, are misleading in the evaluation of HROS. Therefore, because water treated by HROS has recently become a significant part of the water consumed by the public, it has been urged to precisely investigate these systems.[6,10-12]

Extremely low concentrations of total dissolved solids (TDS) result in undesirable drinking water with flat and insipid taste. Therefore, an optional neutralizer accessory has been presented as a solution by the companies to replace minerals and to give it a taste of spring water. Albeit it is essential to evaluate this new accessory, no study has been reported yet.

Finally, the purposes of this study were first, evaluation of HROS output water quality, second, investigation of HROS influences on feed water, and third, evaluation of the neutralizer accessory and its influences and urgency.

Materials and Methods

The Kurdistan province of Iran was selected as the study area (Figure 2). In addition, 2 urban drinking water samples were collected from Sanandaj and Sarvabad, Iran, and 3 rural drinking water samples were collected from Daraki (Sarvabad, Kurdistan, Iran), Goor-Baba-Ali (Divandare, Kurdistan Iran), and Gharakhlar (Bijar, Kurdistan, Iran). This area was selected due to availability and low quality of supplied water in this area. High levels of nitrate, arsenic, and hardness were reported for drinking water in this area. All samples were ground water without any further treatment, except the Sanandaj sample that was treated in the refinery unit of Sanandaj. In addition to the real samples, 1 synthetic sample containing common heavy metals of drinking water in Iran was prepared to investigate the heavy metal removal efficiency of HROS.

Samples were collected based on standard methods of water and wastewater examination (No. 1060) using 3 heavy polyethylene containers. The 3 samples consisted of 20 liters sample as feed water of HROS, 2 liters sample for heavy metal analysis with 1% acid nitric addition, and 2 liters sample for other physico-chemical analysis. All containers were kept sealed and refrigerated at 5 °C until the time of analysis.

The HROS model RO100GPD (Luna Water Co., Canada) with and without neutralizer accessory was used in this study. In addition to neutralizer accessory, RO100GPD contains five filters containing 25 microns sediment pre-filter, 10 microns active carbon, and 1 micron sediment pre-filter and osmosis membrane. In the filtration

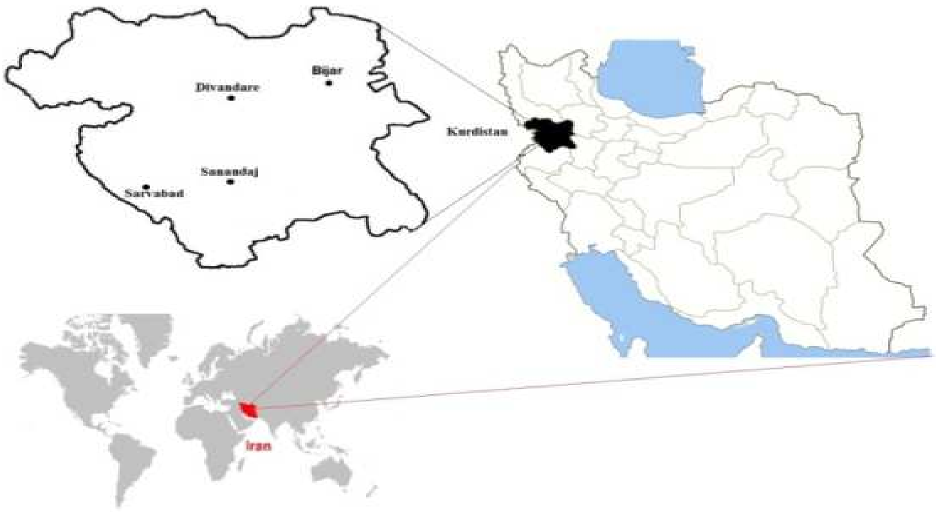

Figure 2. Study area (Kurdistan province, Iran)

stage, feed water first passes through sediment filters using a primary pomp where silt, sediment, sand, and clay particles are removed. Water is then forced through a high efficiency carbon block filter where micro-pulverized carbon efficiently adsorbs chlorine, chloramines, pesticides, and other harmful organic chemicals and pollutants. Then, pre-filtered feed water stripped of membrane-damaging particles and chemicals flows into the RO membrane module using a 6 bar (85 psi) secondary pump where pure water molecules are forced through the RO membrane leaving salts, metals, and other impurities to be flushed from the system. Reverse-osmosis membranes, although very efficient in removing contaminants, still allow trace amounts to pass through. Nitrates, phosphates, and silica are among the substances not fully removed.

The common physico-chemical analyses consisting of anions, heavy metals, hardness, calcium, magnesium, alkalinity, TDS, and electrical conductivity (EC) were performed based on standard methods. [13] Phosphate, nitrate, nitrite, chloride, bromide, and sulfate levels were determined by standard ion chromatography method. Heavy metals analysis was determined by standard inductively coupled plasma (ICP) method.

Ion chromatography was carried out based on the United States Environmental Protection Agency (EPA) method 300.0 using 882 compact IC plus (Metrohm, Switzerland) equipped with a conductivity detector and a 20 µl injection loop. Separation of anions was carried out on a Metrosep A Supp 4-250 analytical column at 25 °C with a 1 ml/min flow rate of eluent. Metrosep A Supp 4.5 Guard column and suppressor systems were also connected to the analytical columns. A mixture of sodium hydrogen carbonate (1.7 mm) and sodium carbonate (1.8 mm) was used as the mobile phase for eluting anions. Data acquisition and instrument settings were performed by Magic Net software (version 2.1; Metrohm, Switzerland). The ultra-pure water type 1 was used as blank. Mixed standard solutions were used to plot the calibration curve with appropriate concentrations of each desired anions. The linear relationship between peak area and concentration were confirmed experimentally.

Determination of the heavy metals was achieved by ICP-OES (model Spectro arcos., SPECTRO Inc., Germany). The main operation parameters were torch type (flared end EOS Torch 2.5 mm), detector type (CCD), nebulizer type (cross flow), nebulizer flow (0.85 l/min), plasma power (1400 W), coolant flow (14.5 l/min) and pump rate (30 RPM). The ultra-pure water type 1 was used as blank. Mixed standard solutions were used to plot the calibration curve with appropriate concentrations of each desired heavy metal. The linear relationship between peak area and concentration were confirmed experimentally.

Results and Discussion

In each sample, the mentioned physico-chemical tests were performed for both feed and output sample water. The experimentally obtained data are summarized in table 1 and figure 3. The Iranian National Drinking Water Standards' levels [maximum acceptable concentration (MAC), and maximum desirable concentrations (MDC)] were presented for each parameter in the same table along with the experimental data.

The HROS influences

The results clearly show the influences of HROS on the feed water. As presented in table 1 and figure 3, the concentrations of species were affected by HROS for all investigated parameters. The paired t-test analysis also confirmed the significant influences of HROS (Table 1).

Table 1. Experimental and standard values of investigated physico-chemical parameters

Sample	TDS (mg/l)	Hardness (mg/l)	Ca^{2+} Hardness (mg/l)	Mg^{2+} Hardness (mg/l)	HCO_3^- Alkalinity (mg/l)	SO_4^- (ppm)	NO_3^- (ppm)	Cl^- (ppm)	As (ppb)	Cu (ppb)	Pb (ppb)
MDC	1000	200	-	-	-	250.0	-	250.0	-	1000	-
MAC	1500	500	300	30	-	400.0	50.0	400.0	10	2000	10
Daraki*	222	220	200	20	196	10.6	5.6	8.2	< 1.2	22	3
Daraki**	171	20	12	8	26	2.1	0.4	1.3	< 1.2	5	2
Exclusion (%)	23	91	94	60	87	80.0	93.0	84.0	-	77	33
GoorBabaAli*	454	400	280	120	336	52.0	5.5	28.7	68.0	3	2
GoorBabaAli**	26.6	68	36	32	30	2.8	0.4	1.4	< 1.2	< 0.3	1
Exclusion (%)	94	83	87	73	91	95.0	92.0	95.0	> 98	> 90	50
Gharakhlar*	305	232	180	52	222	55.0	14.9	11.6	< 1.2	3	2
Gharakhlar**	61	44	28	16	48	9.8	1.1	3.0	< 1.2	< 0.3	1
Exclusion (%)	80	81	84	69	78	82.0	93.0	74.0	0	> 90	50
Sarvabad*	371	348	260	88	286	29.0	16.2	13.8	< 1.2	< 0.3	2
Sarvabad$_o$	328	320	232	88	272	27.7	10.9	12.7	< 1.2	< 0.3	1
Exclusion (%)	12	8	11	0	5	4.0	33.0	8	-	-	50
Sarvabad*	332	322	242	80	286	-	-	-	-	-	-
Sarvabad**	244	208	180	28	210	-	-	-	-	-	-
Exclusion (%)	27	35	26	65	27	-	-	-	-	-	-
Sanandaj*	196	180	140	40	168	22.5	3.4	10.8	< 1.2	1	4
Sanandaj**	155	160	104	18	116	16.1	1.5	8.4	< 1.2	< 0.3	2
Exclusion (%)	21	11	26	55	31	28.0	56.0	22.0	-	> 70	50
Sanandaj*	177	175	140	35	164	-	-	-	-	-	-
Sanandaj**	99	64	48	16	80	-	-	-	-	-	-
Exclusion (%)	44	63	66	54	51	-	-	-	-	-	-
Sanandaj*	177	175	140	35	164	-	-	-	-	-	-
Sanadaj**,***	159	104	72	32	130	-	-	-	-	-	-
Exclusion (%)	10	41	49	9	21	-	-	-	-	-	-
Synthetic*	-	-	-	-	-	-	-	-	24	52	34
Synthetic$_o$	-	-	-	-	-	-	-	-	< 1.2	< 0.3	2
Exclusion (%)	-	-	-	-	-	-	-	-	95	99	94
T-test objects					P of T-test results						
T-test (t_1)	< 0.001	0.09	-	-	-	< 0.001	-	< 0.001	-	-	-
T-test (t_2)	< 0.001	< 0.001	0.01	0.05	-	< 0.001	< 0.001	< 0.001	-	-	-
T-test (t_3)	< 0.001	0.12	-	-	-	< 0.001	-	< 0.001	-	-	-
T-test (t_4)	< 0.001	< 0.001	< 0.001	0.96	-	< 0.001	< 0.001	< 0.001	-	-	-
T-test (t_5)	0.04	0.01	0.01	0.03	0.02	0.10	0.03	0.12	-	-	-

MAC: maximum acceptable concentration; MDC: maximum desirable concentrations; *Feed water; **Output water; ***Optional neutralizer accessory was installed; t_1: comparison of feed water and MDC; t_2: comparison of output water and MAC; t_3: comparison of output water and MDC values; t_1: comparison of output water and MAC; t_5: comparison of feed water and output water

Quality evaluation of output water

The comparison between the Iranian National Drinking Water Standards (MAC and MDC) and experimental data for feed and output water clearly illustrate that the HROS output water meets both MAC and MDC standards regardless of feed water quality. The one-sample t-test was applied to evaluate the difference between experimental data and MAC/MDC (Table 1).

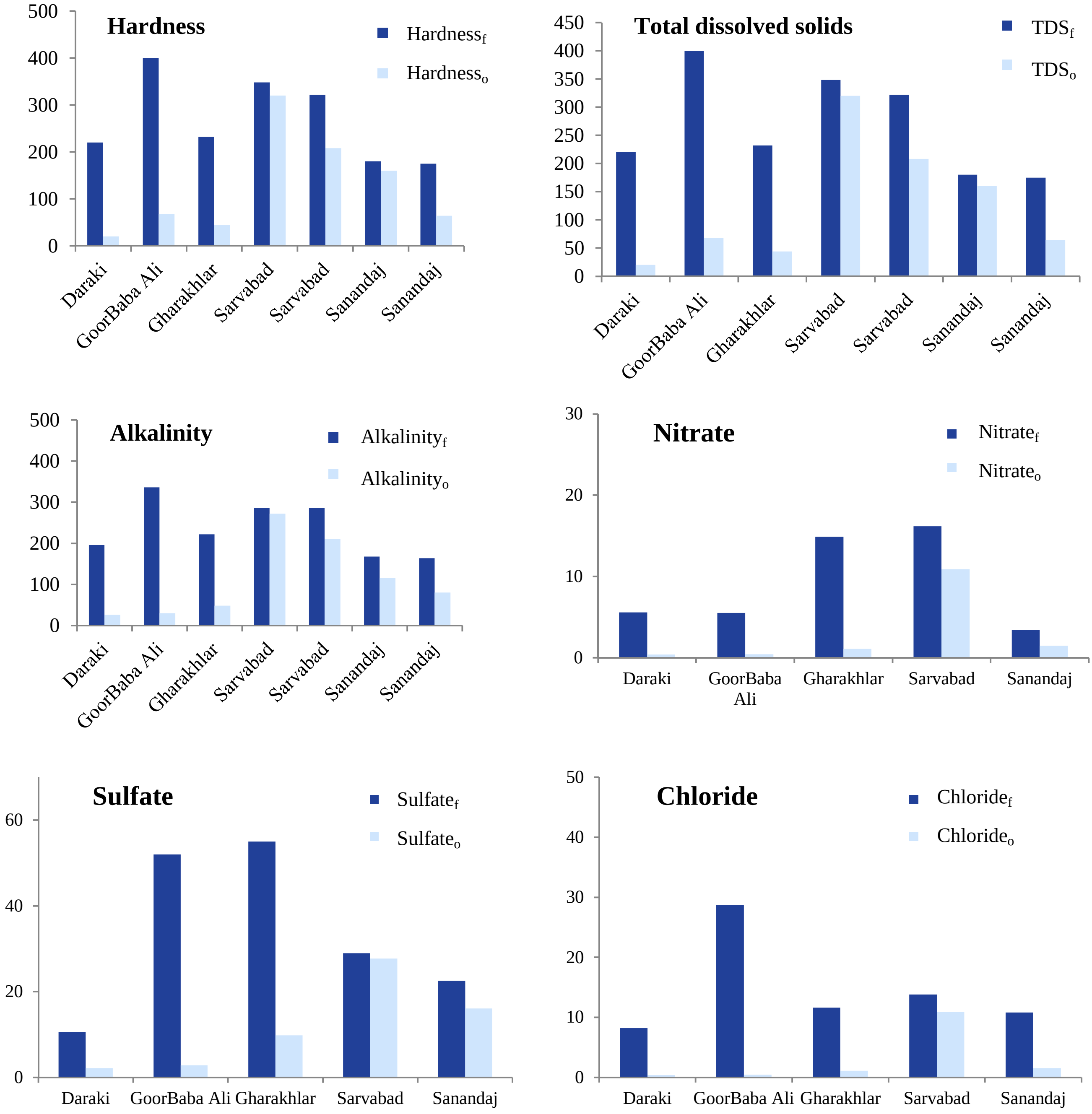

Figure 3. Physico-chemical compression for whole investigated species in feed and output water quality

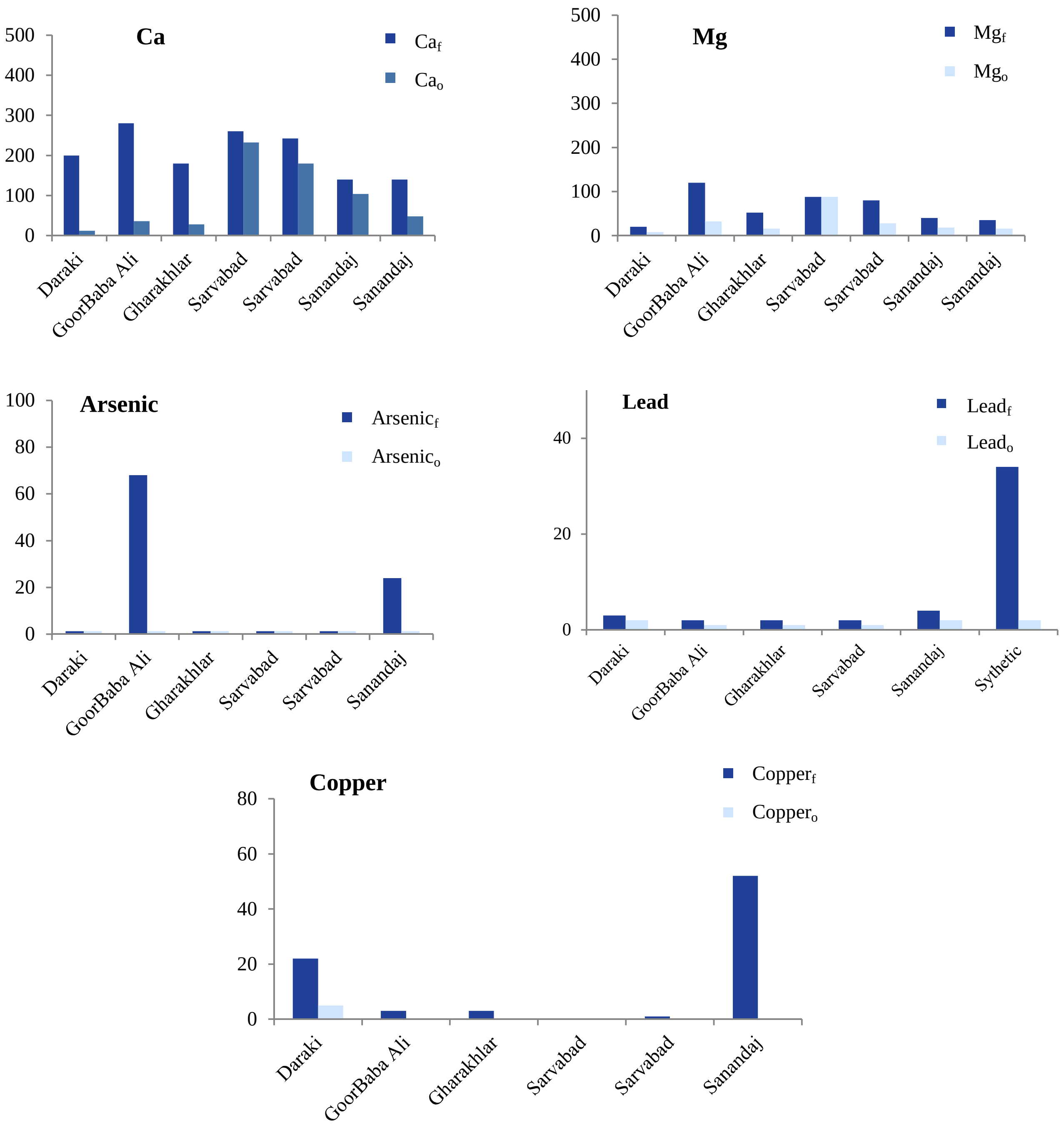

Figure 3. Physico-chemical compression for whole investigated species in feed and output water quality (Continue)

The stability evaluation of HROS output

The results of two repeated experiments for Sanandaj and Sarvabad were presented in table 1 and figure 4. The results clearly demonstrate that although the output water in both cases was lower than standard levels, there are significant differences between outputs. Similar differences cannot be seen for feed water. Then, it can be said that HROS cannot produce stable and repeatable output, but it can produce standard output.

Investigation of neutralizer accessory influences

Evaluation of neutralizer accessory results was presented at figure 5 and table 1. However neutralizer accessory affects the output, but this influences is not urgent. On the other hand, the compensation of TDS as a most important duty of this accessory was occurred when increasing of TDS more than 100 mg/l prevents the flat, insipid taste of output water.

Finally, paired t-test was applied for statistical evaluation of differences between the HROS output with and without installing neutralizer accessory, and differences between the repeated input/output samples (Table 2). The statistical evaluation confirmed the presented discussion.

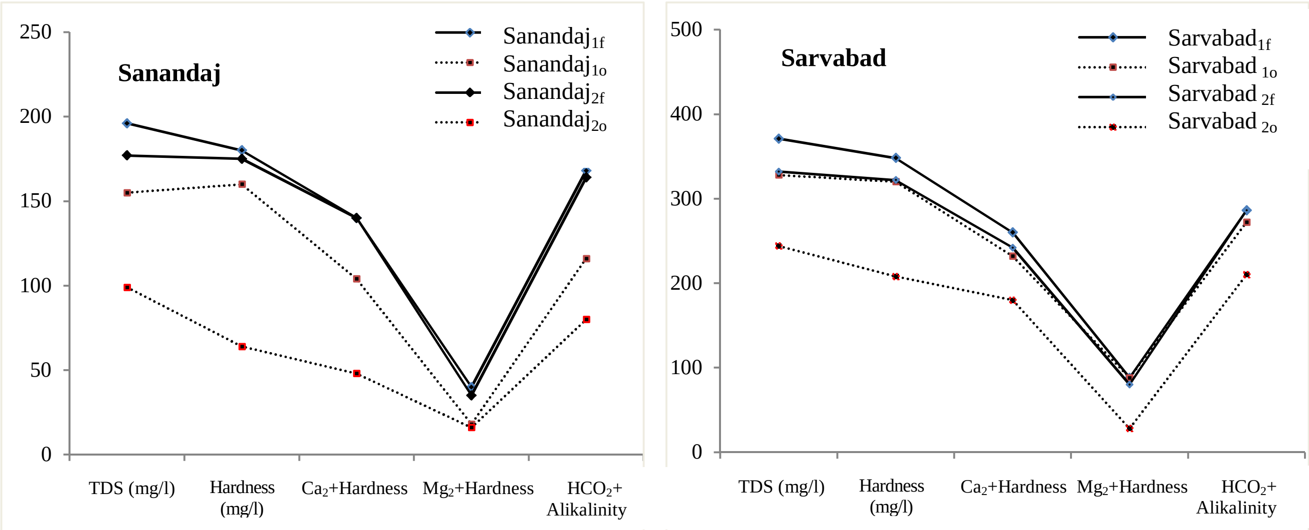

Figure 4. Repeated samples of Sanandaj and Sarvabad
TDS: Total dissolved solids

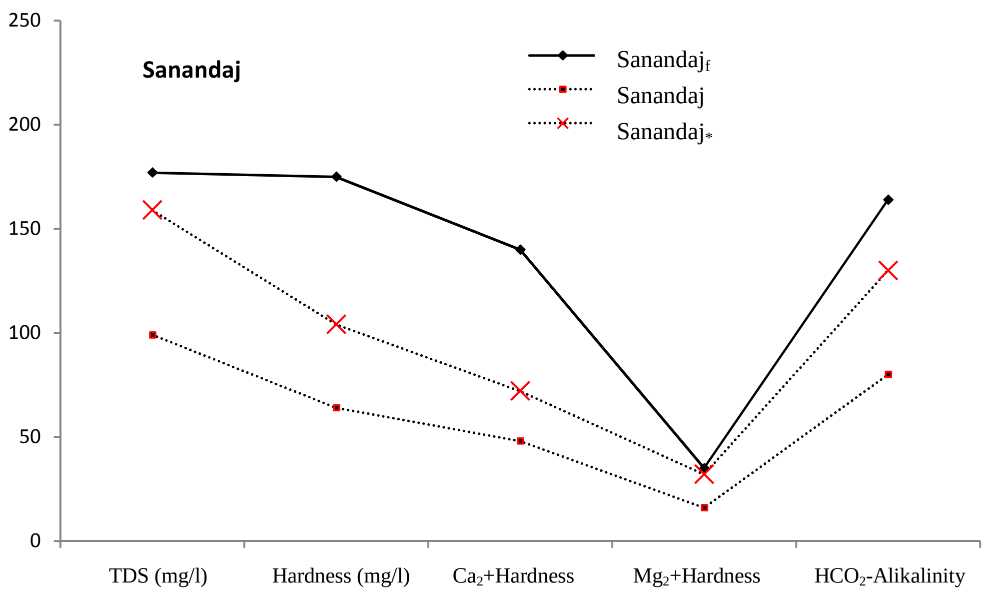

Figure 5. Neutralizer accessory efficiency
TDS: Total dissolved solids

Table 2. T-test results for evaluation of neutralizer accessory and repeated samples

T-test objects	P
T-test for HROS output with and without neutralizer accessory	0.010
T-test for repeated output of Sanandaj	0.030
T-test for repeated feed HROS samples for Sanandaj	0.110
T-test for repeated output of Sarvabad	< 0.001
T-test for repeated feed HROS samples for Sarvabad	0.060

HROS: Household reverse-osmosis system

Conclusion

In this study, the influences of household HROS on feed water, quality of output water of HROS regarding standard MAC and MDC levels, influences of the new neutralizer accessory, and the stability (repeatability) of physico-chemical characteristics of the output water were investigated. Several rural and urban samples with different initial physico-chemical characteristics were used as feed water. It can be concluding from the results that the output water is different from the feed water. This means that these systems strongly influence the feed water. Moreover, the results show that the output water meets the standard levels of MAC and even MDC completely regardless of feed water quality. Based on the results, however, the output water did not show suitable repeatability, but output water was always of standard quality. Thus, it can be concluded that, for both polluted and standard feed water, application of HROS results in high quality output water regarding the investigated physico-chemical properties, but it is more urgent and applicable for polluted feed water specially feed water containing heavy metal pollutants. Although the new neutralizer accessory influences the output water significantly, it is not a necessity for HROS because the output of this system is not pure water and contains a suitable amount of TDS. This means that the membranes used in these systems are not reverse-osmosis membranes and are probably nano-filters. The acceptable TDS and hardness levels of the output water are clear evidences for the use of nanofilters instead of reverse-osmosis systems. This neutralizer accessory may be more applicable to real reverse-osmosis membrane-based systems.

Conflict of Interests

Authors have no conflict of interests.

Acknowledgements

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Temporal and spatial variation of drinking water quality in a number of Divandareh villages, Iran: With emphasis on fluoride distribution

Afshin Maleki[1], Esmail Ghahremani[1], Yahya Zandsalimi[1], Pari Teymouri[1], Hiua Daraei[1], Reza Rezaee[1], Pegah Bahmani[1], Behroz Davari[2], Dariush Naghipour-Khalkhaliani[3], Enayatollah Kalantar[1]

1 Kurdistan Environmental Health Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran
2 Department of Entomology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
3 Department of Environmental Health Engineering, School of Health, Guilan University of Medical Sciences, Rasht, Iran

Original Article

Abstract

Fluoride is found in all water resources at different concentrations and drinking water is the major source of fluoride exposure. Thus, because of the adverse effect of fluoride in low and high concentrations, the evaluation of its content in drinking water is necessary. In the present study, the temporal variations and spatial distribution of fluoride concentrations in the drinking water of villages in Divandareh (Kurdistan, Iran) were determined. Thus, 30 villages were selected and 180 groundwater samples were taken in 2 dry and wet seasons in the year of 2013. The concentrations of fluoride and other anions were measured using the ion chromatography (IC) method. Geospatial analysis of the data was performed using the ArcGIS geographical information system (GIS) software. The results showed that the average fluoride concentration in drinking water ranged from 0.136 to 0.736 mg/l; 90.56% of samples had a concentration less than 0.50 mg F/l, and the rest had concentrations between 0.51 and 1.0 mg F/L. Based on the results of the nonparametric Wilcoxon test, a significant difference was found between the concentrations of fluoride in the two-stage sampling ($P < 0.01$).

KEYWORDS: Drinking Water, Fluoride, Geographic Information Systems, Groundwater, Water Resources

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Introduction

Groundwater is one of the most important sources for drinking and agricultural uses, and its quality is mainly affected by both natural and human factors.[1] In other words, the chemical composition of groundwater is a complex function of several variables including geological structure and mineralogy of the watersheds and aquifers, hydro-geological conditions, the evaporation of the water table, hydro-chemical processes within the aquifer, lithology, groundwater velocity, interaction of water with soil and rock, precipitation, and human activities.[2,3] The interaction of these factors leads to different types of water. Thus, the chemical composition of water in each area is different and can affect the health aspects of drinking water quality.[4] For this reason, It is necessary to monitor the groundwater quality, especially in areas where water quality is not desirable, the values of cations and anions (such as fluoride) in

Corresponding Author:
Afshin Maleki
Email: Maleki43@yahoo.com

water are above the permitted standards, and water-borne diseases have been reported.[5,6]

According to the World Health Organization (WHO) guidelines for drinking water quality, fluoride, arsenic, and nitrate are key chemicals that cause large scale health effects through drinking water exposure.[5,7] Fluoride is necessary for human life and drinking water is the main source of its intake. However, excessive intake of fluoride can cause a wide range of adverse health effects such as dental mottling and fluorosis.[8-10] The concentration of fluoride in water resources is a function of the factors mentioned above; therefore, determination of fluoride concentration in drinking water and its correlation with other drinking water parameters is important. Therefore, the aim of this study was to determine the concentration of major anions and cations, their correlation with F content of groundwater, and the temporal variations and spatial distribution of fluoride concentration in Divandareh villages, Kurdistan, Iran.

Materials and Methods

This research was a cross-sectional study performed to determine the quality of drinking water of 30 villages in Divandareh, Kurdistan, Iran. A total of 180 samples were taken in 2 dry and wet seasons and were analyzed according to standard methods.[11] Fluoride concentration and other anions were measured with ion chromatography (IC) method using a Metrohm 882 compact IC plus (Metrohm AG, Switzerland). Descriptive statistics and the Piper, Schuler, and Wilcox diagrams were used to interpret the results. In order to compare the results of the 2 phases of the study, SPSS software (version 16, SPSS Inc., Chicago, IL, USA) and Wilcoxon test were used. To determine the correlation between physical and chemical characteristics of water quality, Pearson correlation coefficient was used. RockWorks software (RockWare Inc., Golden, CO, USA) was used to analyze the results of chemical analysis in the studied samples. Using the results obtained from previous phases, groundwater type was determined and its application for drinking, agricultural, and industrial purposes was assessed. Moreover, Esri's ArcGIS, a geographical information system (GIS) (version 10, Esri, Redlands, CA, USA) was used to study the spatial variation of F.

Results and Discussion

Table 1 shows the average concentrations of major anions, cations, and other physicochemical parameters in the studied groundwater samples. The variation of fluoride concentration in the studied water supplies (in dry and wet seasons) is provided in Figure 1 As can be seen in Figure 1, the average fluoride concentration varied from 0.136 mg/l (sample no. 17) to 0.736 mg/l (sample no. 1). About 90.56% of samples showed a fluoride concentration below 0.5 mg/l and 9.44% of them had a fluoride concentration between 0.5 and 1 mg/l. These results are in accordance with another study in Iran which showed a low concentration of fluoride in drinking water.[12] Compared to drinking water quality standards for fluoride concentration, set by the Institute of Standards and Industrial Research of Iran (ISIRI)[13] and the WHO[5], only 9.44% of water samples have a fluoride concentration in the permissible range and 90.56% of them have lower concentration than the permissible limit (0.5 mg/l), indicating a high probability of dental caries in the study area. Therefore, supplying fluoride through other sources, such as foodstuff, tea, and toothpastes, is recommended. Indeed water fluoridation is not recommended because of its cost especially in rural areas. Cartona's study comes to the same conclusion.[14] On the other hand, the disadvantages of high concentrations of fluoride in water are much greater than that of its scarcity. According to the Iranian Fluoride Scientific Association, fluoride concentration exceeding 0.7 mg/l in drinking water may increase disadvantages rather than desirable effects of caries prevention.[10] Thus, although the lack of fluoride is a risk for consumers, it is essential to monitor its concentration in water, and tooth decay especially in children.

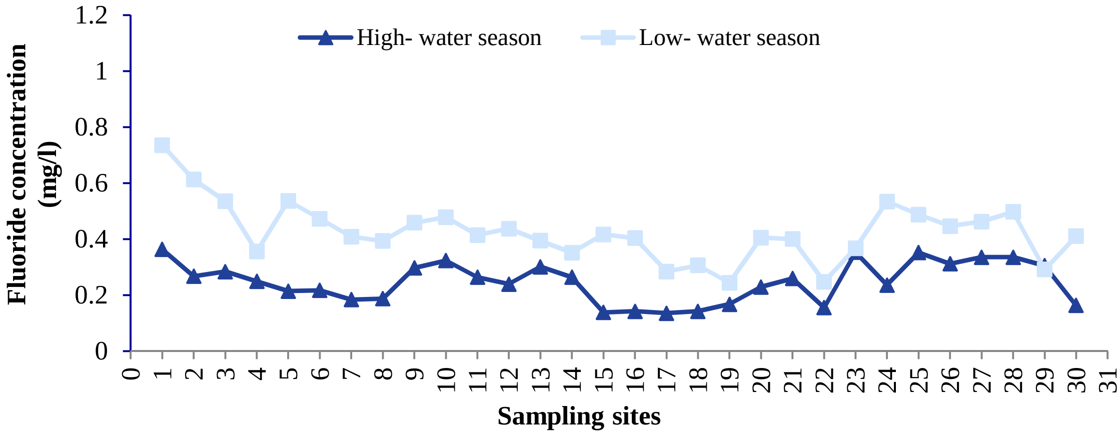

Figure 1. Fluoride concentration of different sampling sites in low- and high-water seasons

Table 1. Average values of physicochemical parameters of groundwater in rural areas

Village	Site Code	Ca	Mg	K	Na	NO_3	SO_4	F	Cl	pH	TDS*	EC†	Alkalinity	TH‡
Haydardideban	1	106.0	20.8	7.5	34.0	53.3	20.6	0.55	22.5	7.7	625	943	314	346
Kanichay	2	61.6	4.8	0.3	12.0	67.5	6.4	0.44	2.9	7.7	312	455	145	172
Kasnazan	3	76.7	14.6	0.2	16.0	42.5	8.5	0.51	13.3	7.7	428	624	215	248
Zarineh	4	94.0	23.0	0.9	48.5	1.6	26.1	0.30	25.9	7.1	626	951	330	325
Kalkan	5	121.6	15.8	18.1	41.0	56.3	74.8	0.35	35.5	7.5	707	1060	281	334
Kanisefid	6	69.2	14.3	0.2	18.5	72.1	9.2	0.36	8.6	7.5	398	586	171	226
Ghalerotaleh	7	115.5	20.3	4.8	23.0	121	30.6	0.40	36.5	7.5	648	938	231	368
Gavsheleh	8	97.8	19.2	3.5	44.5	5.2	20.7	0.29	25.5	7.6	613	928	328	318
Darehasb	9	103	21.0	4.4	71.0	9.2	41.6	0.38	66.0	7.8	726	1096	336	339
Jafarabad	10	103	20.7	1.9	38.5	6.8	16.2	0.40	19.8	7.6	625	932	344	340
Rashidabad	11	93.4	9.2	2.9	14.5	63.3	21.1	0.34	5.1	7.4	448	665	199	267
Koleh	12	87.9	18.0	3.0	34.0	5.3	33.2	0.34	16.8	7.8	537	808	282	290
Ghajar	13	73.0	13.4	0.7	24.0	25	9.1	0.35	5.0	8.0	425	618	228	234
Kapak	14	87.7	11.9	0.4	21.0	5.0	12.1	0.30	4.3	7.8	451	674	259	264
Kool	15	66.0	12.5	0.85	10.5	5.9	7.5	0.26	2.3	7.9	354	526	207	213
Dozakhdareh	16	55.0	7.5	0.4	8.5	2.5	5.2	0.27	1.3	7.9	269	402	160	165
Ghezelbelagh	17	64.5	9.5	0.6	8.5	4.3	7.1	0.20	2.0	7.8	322	478	187	198
Hezarkanian	18	80.0	10.4	3.1	17.0	4.8	16.8	0.22	14.2	7.8	413	618	219	240
Zagheolya	19	100.0	17.0	2.5	16.0	8.9	14.7	0.20	7.7	7.8	525	780	293	315
Zaghesufla	20	100.0	20.6	1.1	21.5	9.5	13.0	0.39	10.5	7.9	554	836	315	332
Nesareolya	21	78.5	17.8	3.7	45.0	22.5	28.1	0.33	23.3	7.7	534	757	254	266
Ghalerehaneh	22	74.3	21.6	0.5	9.5	50.0	18.9	0.20	12.0	7.8	427	648	198	270
Kahrizeh	23	87.0	10.6	0.1	33.5	28.2	7.5	0.36	5.6	7.7	493	720	267	254
Sarghaleh	24	47.0	12.3	0.9	43.5	23.5	11.0	0.40	4.3	8.0	387	571	205	166
Berkeh	25	65.4	12.1	0.4	42.0	27.5	9.6	0.42	6.2	7.9	448	657	239	210
Aghajari	26	47.5	13.3	1.0	50.0	21.2	5.9	0.43	4.9	7.9	443	650	254	222
Baghcheleh	27	87.7	20.3	1.5	77.5	18.9	41.5	0.41	16.8	7.8	699	1030	361	324
Dalan	28	101.5	19.0	4.5	81.5	15.3	76.2	0.44	27.2	7.8	761	1123	339	326
Ahmadabad	29	72.3	12.9	0.4	19.5	22.3	8.5	0.32	4.3	8.0	402	595	221	226
Aldareholya	30	50.5	15	0.2	11.5	18.5	13.2	0.35	3.1	7.9	300	461	159	185
National Standard of Iran (Max. Permissible) [13]		-	-	-	200	50	400	1.5	400	-	1500	-	-	500

* Total Dissolved Solids; † Electrical Conductivity; ‡ Total Hardness

All parameters are expressed in mg/l, except for pH, EC (μS/cm), Alkalinity (mg/l $CaCO_3$), and TH (mg/l $CaCO_3$)

Other physicochemical parameters such as pH, Ca^{2+}, Mg^{2+}, NO_3, total dissolved solids (TDS), and total hardness (TH) were also studied. An important parameter affecting the solubility of fluoride is pH. According to Saxena and Ahmed, in acidic pH, fluoride remains in the soil; however, in alkaline pH, it is released into the water.[15] According to the results, the pH value varies from 7.1 to 8 and represents an alkaline condition that is appropriate for the solubility of fluorine-bearing minerals. However, the low fluoride concentrations in the studied groundwater indicate that there are small quantities of fluorine-bearing minerals in the soil. On the other hand, fluoride has a unique chemical ability to replace other anions. Ca^{2+}, Na^+, OH^-, and some complexing ions may change fluoride concentration in groundwater.[16,17] Therefore, fluorite dissolution is limited when the concentration of Ca^{2+} exceeds the limit of fluorite solubility.[18] A strong reverse correlation between F and Ca^{2+} in the Ca^{2+}-containing groundwater, with concentrations higher than that required, has been observed by Raju et al. for the solubility of fluoride minerals.[18] The Ca^{2+} and Na^+ concentrations in groundwater vary from 47 to 121.6 mg/l and 8.5 to 81.5 mg/l, respectively. According to table 2, the average concentration of Ca^{2+} (82.35 mg/l) was higher than Na^+ (31.3 mg/l), which may be the reason for the low fluoride concentrations in the groundwater.

Electrical conductivity (EC) and TDS showed good correlation with fluoride concentration compared to other studied physicochemical parameters. Minimum EC of groundwater samples was around 700 µS/cm (Table 2), which placed them in the C2-S1 and C3-S1 classes; classified as slightly salty and usable brackish waters for agriculture, respectively.[19] The amount of TDS in water samples ranged between 260 and 790 mg/l. The major ions contributing to TDS are calcium, magnesium, sodium, potassium, carbonate, bicarbonate, chloride, fluoride, sulfate, and nitrate.[20] According to Gaillardet et al., variation in TDS may be related to land use and pollution.[21] Hence, animal waste, agricultural fertilizers, and industrial and municipal wastewater are the main source of nitrate, sulfate, sodium, and chloride ions in the water environment and can be related to the TDS variation.[3,22]

Based on the results, TH in the groundwater samples is carbonate hardness with a mean concentration higher than 250 mg/l $CaCO_3$. Dutta et al. have stated that water with a fluoride concentration higher than 1.5 mg/l has a hardness less than 200 mg/l.[23] Considering the amount of fluoride and hardness in this study, the results seem reasonable. A positive correlation was observed between fluoride and Ca^{2+}, Mg^{2+}, and Na^+. Similarly, a direct correlation was also observed between fluoride and bicarbonate (R^2 = 0.28) followed by chloride (R^2 = 0.16), sulfate (R^2 = 0.11), and nitrate (R^2 = 0.08). The results are in agreement with the findings of other researchers.[4,8]

Table 2. Descriptive statistics of elemental concentration for the studied parameters

Parameter	N (in each season)	Low-water season				High-water season			
		Mean	SD	Min	Max	Mean	SD	Min	Max
EC	90	782.00	217	396.00	1180.0	700.00	194.0	399.0	1100.0
TDS	90	525.00	146	261.00	784.0	468.00	130.0	275.0	737.0
pH	90	7.70	0.29	7.00	8.1	7.70	0.3	7.0	8.1
Ca	90	88.90	22.00	49.00	131.0	75.80	20.5	45.0	126.0
Mg	90	15.70	6.40	4.90	25.0	14.90	4.5	4.8	21.7
Na	90	31.80	21.20	9.00	83.0	30.60	19.7	8.0	80.0
K	90	2.60	4.30	0.20	22.8	2.10	2.8	0.1	13.4
HCO_3	90	328.00	81.00	189.00	512.0	285.00	75.2	140.0	410.0
Cl	90	15.40	16.10	1.00	75.3	13.40	12.1	1.5	56.0
SO_4	90	20.80	18.40	3.50	79.0	19.90	17.9	4.8	82.2
F	90	0.25	0.08	0.14	0.4	0.43	0.1	0.2	0.7

N: number of samples; SD: Standard deviation; EC: Electrical conductivity; TDS: Total dissolved solids

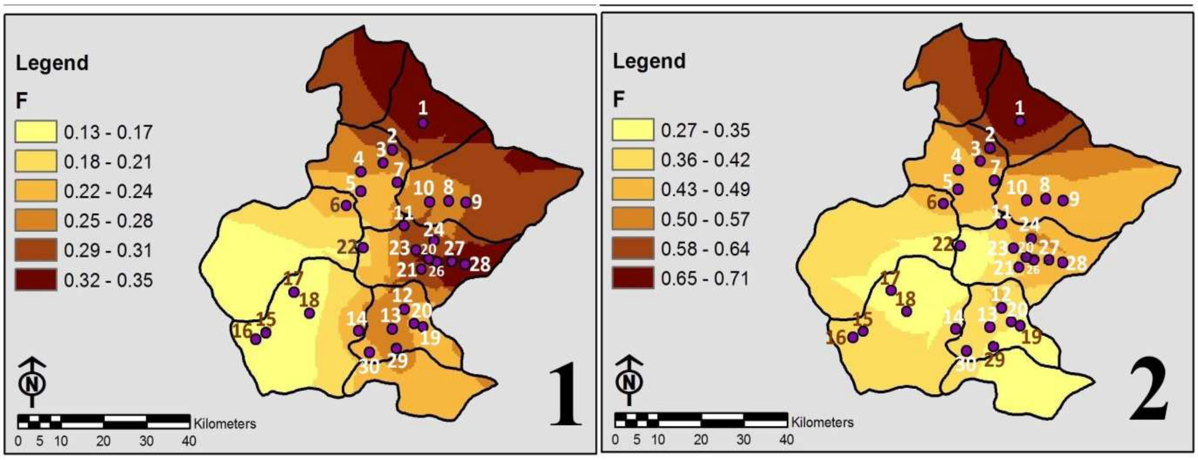

Figure 2. Spatial distribution of fluoride in groundwater in 1: low-water seasons and 2: high-water seasons

According to Figure 2, the concentration of fluoride is related to the North and Northeast part of the aquifer, which is an agricultural area with a high concentration of Ca^{2+}, TDS, and TH. According to the Wilcoxon test and its correlation coefficient, there is a significant difference between the fluoride ions concentration in samples taken in the dry and wet seasons ($P < 0.01$). However, due to the low concentration of fluoride in both periods, this difference is not important.

The average concentrations of Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+} were 62.8%, 11.7%, 23.8%, and 1.8% of all cations, respectively (Table 1). Average concentration of HCO_3^-, NO_3^-, SO_4^{2-}, and Cl^- were 83%, 7.5%, 5.6%, and 3.9% of all anions, respectively (Table 1). NO_3^- concentration in around 20% of water samples was higher than the recommended standard set by ISIRI.[13] This is mostly due to agricultural activities in the area; application of different fertilizers and pesticides.
Geochemical facies showed that HCO_3^- and Ca^{+} are the dominant anion and cation in the studied samples, respectively, introducing calcic-bicarbonate as the water type. Results obtained from RockWorks software showed that the rock bed in the studied villages is mainly made up of limestone, dolomite, and feldspar. Distribution of carbonate rocks in the study area and dissolution of carbonate minerals are the main source of Ca^{2+} and Mg^{2+} ions in the water. The high concentration of HCO_3^- ions in the water is due to erosion and weathering of carbonate and silicate minerals. Correlation coefficients between the different chemical parameters measured in the studied villages showed that the highest correlation exists between HCO_3^- and Ca^{2+} (Table 3). The Piper diagram also confirmed that the facies of water was calcic bicarbonate, showing that the main chemical composition of water is $Ca(HCO_3)_2$. Considering the presence of other cations and anions in the water and existence of the correlation coefficient between them, other chemical compounds present are $CaSO_4$, $CaCl_2$, $Mg(HCO_3)_2$, $MgSO_4$, $MgCl_2$, $NaHCO_3$, Na_2SO_4, NaCl, and KCl (depending on the specific terms of the ratio of Ca^{2+} to Mg^{2+} and Na^{+} to Ca^{2+} in each of the studied sources).

Conclusion

This study provides an overview of groundwater quality with emphasis on fluoride concentration in rural areas of Divandareh County. It was found that groundwater is slightly alkaline and hard in nature. Fluoride concentration in 90% of groundwater samples was less than the permissible limit set by ISIRI. It is evident from the results that the consumers in study area are at risk of dental caries. Therefore, it is essential to monitor the fluoride concentration of water and tooth decay especially in children.

Table 3. Correlation matrix of studied water quality parameters

	Ca	Mg	Na	K	F	HCO_3	Cl	SO_4	NO_3	TH	TDS	EC	pH
Ca	1												
Mg	$0.332^{£}$	1											
Na	$0.307^{£}$	0.480^{**}	1										
K	0.476^{**}	$0.244^{£}$	$0.293^{£}$	1									
F	$0.266^{£}$	$0.072^{£}$	0.554^{**}	$0.005^{£}$	1								
HCO_3	0.685^{**}	0.644^{**}	0.747^{**}	$0.253^{£}$	0.276	1							
Cl	0.593^{**}	0.595^{**}	0.637^{**}	0.542^{**}	$0.162^{£}$	0.571^{**}	1						
SO_4	0.509^{**}	0.467^{**}	0.646^{**}	0.757^{**}	0.112^{3}	0.489^{**}	0.715^{**}	1					
NO_3	$0.258^{£}$	-0.059^{3}	$0.184^{£}$	$0.219^{£}$	$0.079^{£}$	$0.206^{£}$	$0.141^{£}$	$0.037^{£}$	1				
TH	0.873^{**}	0.678^{**}	0.489^{**}	0.477^{**}	$0.304^{£}$	0.846^{**}	0.699^{**}	0.571^{**}	$0.174^{£}$	1			
TDS	0.794^{**}	0.654^{**}	0.782^{**}	0.499^{**}	0.450^{*}	0.904^{**}	0.787^{**}	0.730^{**}	$0.100^{£}$	0.907^{**}	1		
EC	0.786^{**}	0.687^{**}	0.781^{**}	0.514^{**}	0.427^{*}	0.906^{**}	0.805^{**}	0.739^{**}	$0.059^{£}$	0.911^{**}	0.996^{**}	1	
pH	-0.628^{**}	$-0.267^{£}$	$-0.069^{£}$	$-0.342^{£}$	$-0.116^{£}$	$-0.320^{£}$	-0.374^{*}	$-0.232^{£}$	-0.401^{*}	-0.562^{*}	-0.450^{*}	-0.454^{*}	1

* Correlation is significant at the 0.05 level; ** Correlation is significant at the 0.01 level; $^{£}$ Non-significant; EC: Electrical conductivity; TDS: Total dissolved solids; TH: Total hardness

Conflict of Interests

Authors have no conflict of interests.

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Journal of Advances in Environmental Health Research

Adsorption of nitrate from aqueous solution using activated carbon-supported Fe^0, $Fe_2(SO_4)_3$, and $FeSO_4$

Saeed Dehestaniathar[1], Abbas Rezaee[2]

1 Kurdistan Environmental Health Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran
2 Department of Environmental Health Engineering, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran

Original Article

Abstract

In this laboratory scale study, impregnated almond shell activated carbon was used as adsorbent to investigate its feasibility for nitrate adsorption from aqueous medium. The effects of activated carbon dosage and contact time have been examined in batch experiments. Experimental data show that Fe^0, $Fe_2(SO_4)_3$, and $FeSO_4$ impregnated activated carbons were more effective than virgin almond activated carbon in nitrate removal. The maximum nitrate removal was 70% and 10-15% for modified activated carbons and virgin activated carbon, respectively. These experiments were conducted at pH 6.2, 20 °C, and initial concentrations of 20 mg/l nitrate-N. The increase in modified activated carbon dosages increased the removal of nitrate. The equilibrium time was found to be 30 min for modified activated carbons.

KEYWORDS: Activated Charcoal, Adsorption, Nitrate Removal, Wastewater

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Introduction

Due to increased anthropogenic activities nitrate contamination of soil has become an environmental and health problem in many countries. The intensive application of nitrogen fertilizers, irrigation with wastewater, and manure application are the most significant causes of nitrate pollution.[1] Increased levels of nitrate can create the potential for eutrophication and toxic algal blooms in the receiving water.[2] High concentrations of nitrate result in diseases in newborns such as infant cyanosis or blue baby syndrome and cancer.[3,4] The European Union and the US Environmental Protection Agency determined 5.6 mg (NO3-N)/l and 10 mg (NO3-N)/l as the standard values of drinking water, respectively.[5] Several physicochemical and biological processes have been investigated for the removal of nitrate from water and wastewater. Adsorption method is promising, since it is simple and economically efficient. Several adsorbents such as fly ash[6], agricultural waste[7], natural clays[8], and bamboo powder charcoal[9] have been investigated as adsorbents for the removal of nitrate.

The activated carbon (AC) mainly contains micropore, which is efficient in the removal of some pollutants. In some cases, mesopores are needed to improve adsorption velocity and mass transfer. Therefore, it is necessary to increase the mesopore content of AC. One of the most effective approaches to increasing the mesopore and micropore volume of AC is to catalyze the steam activation reaction of carbon by using

Corresponding Author:
Saeed Dehestaniathar
Email: saeed_dehestani@yahoo.com

transition metals or rare earth metal compounds, which can promisingly promote the mesopore formation.[10]

The generation mechanism of mesopore and micropore is the activation reaction which takes place in the immediate vicinity of metal particles, leading to the formation of mesopores and micropores by pitting holes into the carbon matrix. The impregnation optimizes the existing properties of the AC, giving a synergism between the chemicals and the carbon.[11,12] This facilitates the cost-effective removal of certain pollutants which would be impossible otherwise. The unique structure of AC provides a very large surface area. AC has an extraordinarily large surface area and pore volume that gives it a unique adsorption capacity.[13] Organic molecules and AC are similar materials; therefore, there is a stronger tendency for most organic chemicals to associate with the AC rather than stay dissolved in a dissimilar material like water. Generally, the least soluble organic molecules are most strongly adsorbed. Often the smaller organic molecules are held the tightest, because they fit into the smaller pores.[14]

Adsorption usually increases as pH and temperature decrease. Chemical reactions and forms of chemicals are closely related to pH and temperature. When pH and temperature are lowered, many organic chemicals assume more adsorbable forms.[14] AC is not widely used in practice for nitrate control due to the low capacities and slow adsorption kinetics of nitrate by commercially available ACs. In the present work, nitrate removal was carried out by a series of modifications and well characterized almond shell ACs with Fe^0, $Fe_2(SO_4)_3$, and $FeSO_4$.

Materials and Methods

Almond activated carbon was stirred in a boiling solution containing Fe^0, $Fe_2(SO4)_3$, and $FeSO_4$ (5 wt. % based on AC) at room temperature, and filtered and dried at 120°C. At the end of preparation, modified carbon was extensively washed with normal perchloric acid and deionized water. After washing, the AC were dried at 90 °C in a drying oven and stored in a dissector until use. The AC particles between 10 and 20 mesh sizes with 1 mm effective size and 1.18 uniformity coefficients were used in all experiments.

Batch adsorption experiments were conducted in different conical glass flasks in a shaking thermostat with a constant speed of 120 rpm. For each batch adsorption, 50 mL of solution containing 20 mg/l NO_3-N concentration of nitrate with desired level of adsorbents (0, 1.5, 3, 4.5, and 6 g AC) and contact time variations (10, 20, 30, 40, 50, 60, 90, and 120 min) were used. All experiments were conducted in triplicate and average values of adsorption are presented in subsequent sections. Potassium nitrate was used as the source of nitrate in all the experiments (Merck, Darmstadt, Germany). The percentage of nitrate removed has been measured calorimetrically using a UV-visible spectrophotometer (Philips PU 8700 Series model). The experiments have been conducted at 20°C for the impregnated AC and AC.

AC was characterized for surface morphology and composition. The surface morphology of the adsorbents was observed using scanning electron microscopy (SEM) technique (XL30-Philips model). The metals on ACs were demonstrated by SEM (Model XL30, Philips, Eindhoven, Netherlands) equipped with energy dispersive x-ray microanalysis (EDX) and analysis system of ZAF software (Model XL30, Philips, Eindhoven, Netherlands).

Nitrate was analyzed using UV-visible spectrophotometer. The concentration of ammonia was measured using the Nesslerization method, as described in the standard methods of water and wastewater examination, and the concentration of nitrite was analyzed by sulphanilamide method at 543 nm. Fe and sulfate were regularly checked for untreated and treated effluents according to the standard methods.[15] AC properties such as apparent and bulk densities, iodine adsorption test, moisture content (%), and particle size were analyzed according to ASTMD 2854, ASTM D 4607, ASTM D 2867, and ASTM D 2862, respectively.

Results and Discussion

After modification of activated carbons, physicochemical characteristics were determined. Physicochemical characteristics of almond shell AC and impregnated AC are shown in table 1. The results were obtained from the mean of triplicate samples for every variable. Surface morphology and chemical characterization of ACs were estimated by SEM and analysis system of ZAF software, respectively. For preparation of SEM, The ACs were dried in a CO_2 atmosphere under critical conditions. The SEM micrographs of AC and impregnated AC are displayed in figure 1.

The x-ray microanalysis of ACs is demonstrated in figure 2. Fe was not indicated in EDX elemental analysis of no modified AC (AC_0), while Fe increased on the surface of the impregnated ACs (carbon exception). The effects of contact time on the removal of nitrate by AC with the initial concentration of 20 mg/l NO^3-N, 20°C temperature, and pH 6.2 are shown in figures 3 and 4.

Batch adsorption experiments have been carried out by taking various doses of adsorbent (1.5, 3, 4.5, and 6 g ACs/50cc nitrate solution) in different conical glass flasks in a shaking thermostat with a constant speed of 120 rpm. Initial solution pH and temperature were adjusted to 6.2 (using HCl and NaOH) and 20 °C, respectively. The effect of carbon dosages on the adsorption process is shown in figure 5.

Table 1. Physicochemical characteristics of almond activated carbons and activated carbon modifications

Parameters	AC-0	AC-Fe	AC-$FeSO_4$	AC-$Fe_2(SO_4)_3$
Apparent (bulk) density (g/ml)	0.42	0.38	0.42	0.44
Iodine adsorption test	375.00	222.00	395.00	350.00
Moisture content (%)	3.30	4.79	8.60	8.75
pH	3.72	3.04	2.76	2.65

AC: Activated carbon

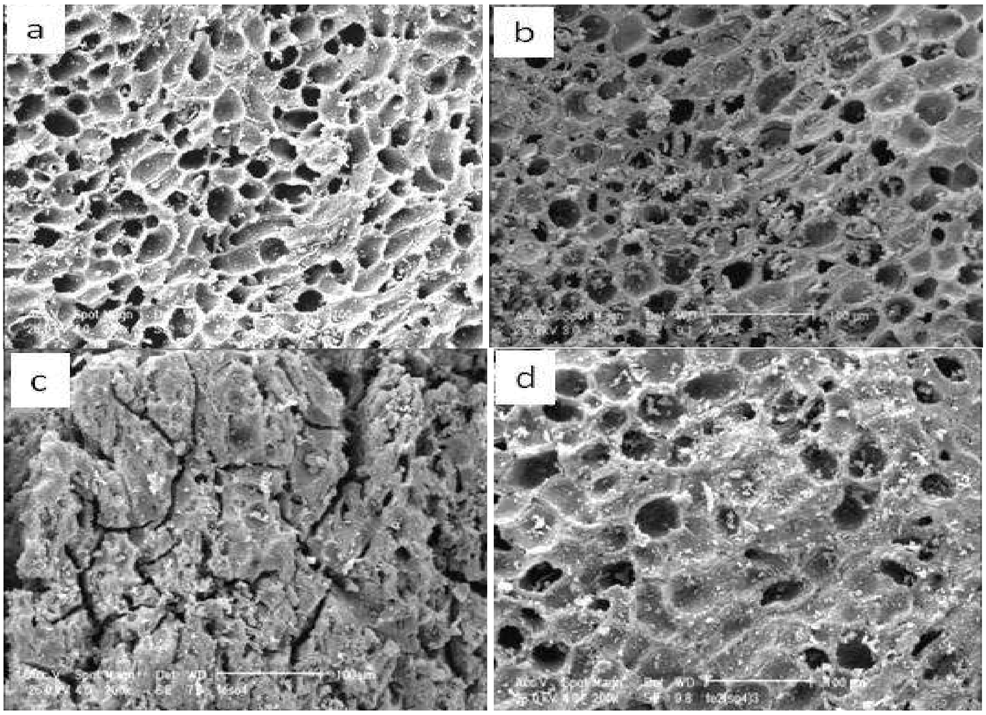
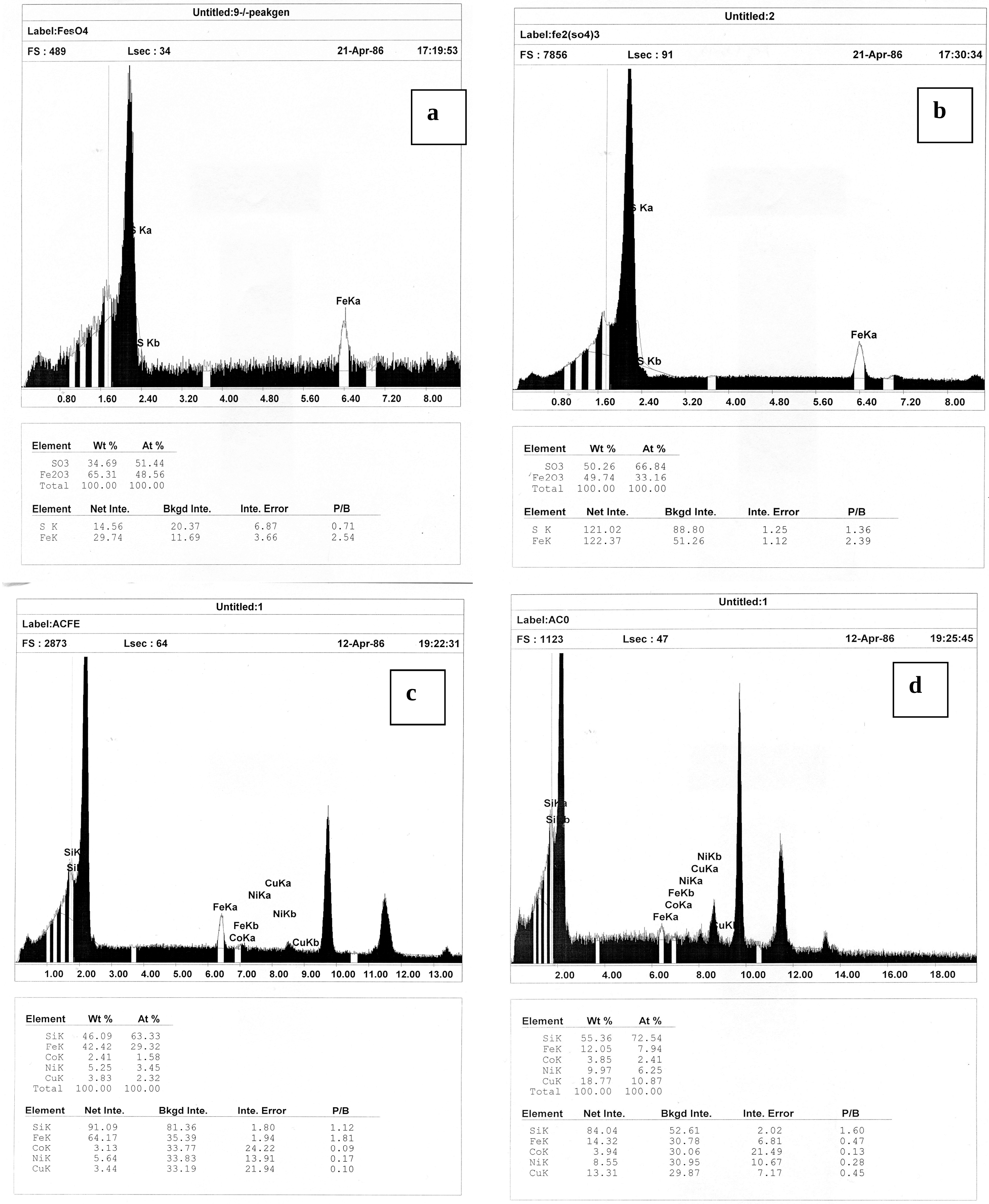


Figure 1. Morphological image of a: AC-0, b: AC-Fe, c: AC-$FeSO_4$, and d: AC-$Fe_2(SO_4)_3$
AC: Activated carbon



a

Element	Wt %	At %
SO3	34.69	51.44
Fe2O3	65.31	48.56
Total	100.00	100.00

Element	Net Inte.	Bkgd Inte.	Inte. Error	P/B
S K	14.56	20.37	6.87	0.71
FeK	29.74	11.69	3.66	2.54

b

Element	Wt %	At %
SO3	50.26	66.84
Fe2O3	49.74	33.16
Total	100.00	100.00

Element	Net Inte.	Bkgd Inte.	Inte. Error	P/B
S K	121.02	88.80	1.25	1.36
FeK	122.37	51.26	1.12	2.39

c

Element	Wt %	At %
SiK	46.09	63.33
FeK	42.42	29.32
CoK	2.41	1.58
NiK	5.25	3.45
CuK	3.83	2.32
Total	100.00	100.00

Element	Net Inte.	Bkgd Inte.	Inte. Error	P/B
SiK	91.09	81.36	1.80	1.12
FeK	64.17	35.39	1.94	1.81
CoK	3.13	33.77	24.22	0.09
NiK	5.64	33.83	13.91	0.17
CuK	3.44	33.19	21.94	0.10

d

Element	Wt %	At %
SiK	55.36	72.54
FeK	12.05	7.94
CoK	3.85	2.41
NiK	9.97	6.25
CuK	18.77	10.87
Total	100.00	100.00

Element	Net Inte.	Bkgd Inte.	Inte. Error	P/B
SiK	84.04	52.61	2.02	1.60
FeK	14.32	30.78	6.81	0.47
CoK	3.94	30.06	21.49	0.13
NiK	8.55	30.95	10.67	0.28
CuK	13.31	29.87	7.17	0.45

Figure 2. The x-ray microanalysis of activated carbons a: AC-$FeSO_4$, b: AC- $Fe_2(SO_4)_3$, c:AC-Fe, and d:AC-0

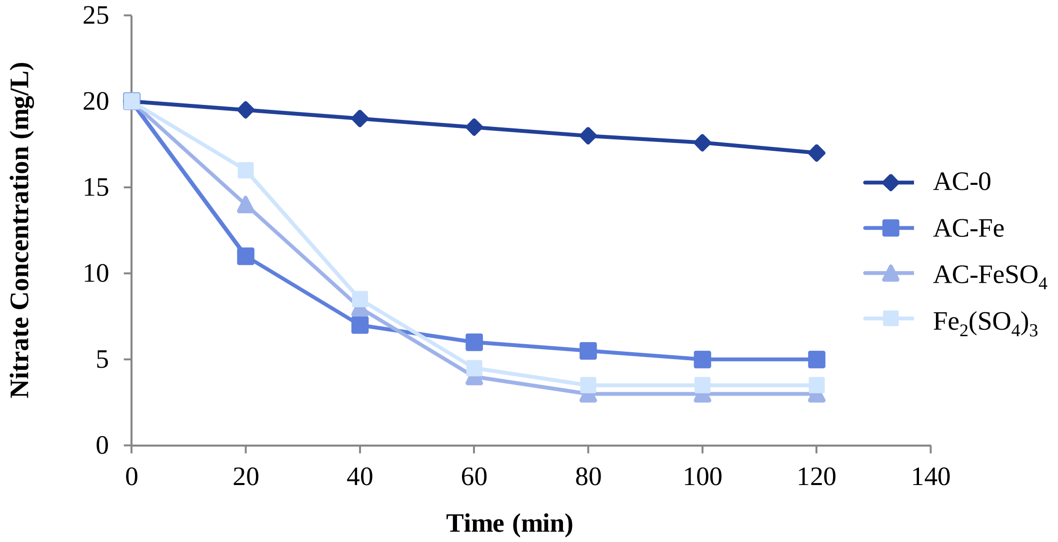


Figure 3. Effect of different activated carbons (AC) on nitrate-N removal (T = 20°C, C_o = 20 mg/l, and pH = 6.2)

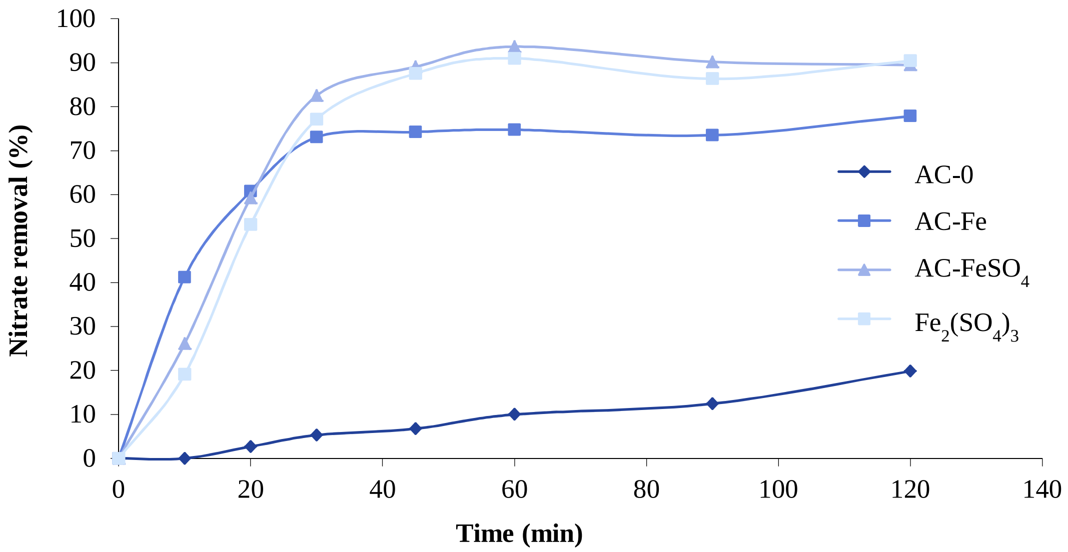


Figure 4. Nitrate removal curve for different activated (AC) carbons (T = 20oC, Co = 20 mg/l, and pH = 6.2)

To identify the regeneration capacity of the adsorbent, the ACs (AC-0, Fe^0, $Fe_2(SO4)_3$, and FeSO4) were agitated with 50 ml of nitrate solution at different concentrations and nitrate isotherms were determined. Nitrate isotherms of all activated carbons in mass basis are shown in figure 6. Water quality after AC treatment is shown in table 2. The water quality was in the range of WHO guideline.

Nitrate could be effectively removed by AC that was impregnated in the presence of Fe^0, $Fe_2(SO4)_3$, and $FeSO_4$. Bulk density and apparent density increased by modification of almond shell AC (Table 1). The SEM micrographs of ACs (Figure 1) show that original AC (a) became dark after modification (b, c, and d); the surface of modified AC lost its metallic glaze and its color turned black. The comparison of figures a, b, c, and d shows that macropore AC is converted to micropore AC by modification of AC using Fe_0,

$Fe_2(SO_4)_3$, and $FeSO_4$. This phenomenon increased ions adsorption such as nitrate. The SEM micrographs and energy dispersive x-ray microanalysis of ACs (Figure 2) support this fact. It was shown that AC-0 have 0% Fe of total elements (carbon exception) on the surface (Figure 2), while with modification by Fe^0, $Fe_2(SO4)_3$, and FeSO4, Fe increased on the surface of AC-0.

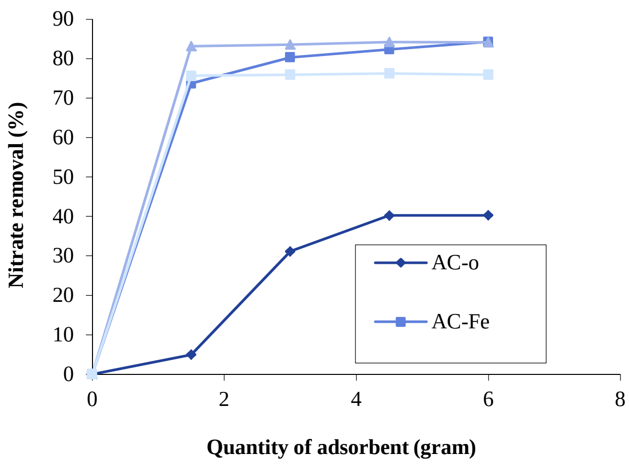


Figure 5. Effect of the quantity of adsorbent activated carbon (AC) and variation in nitrate-N removal percentage with weight of adsorbent on nitrate removal (T = 20 °C, C_o = 20 mg/l, and pH = 6.2)

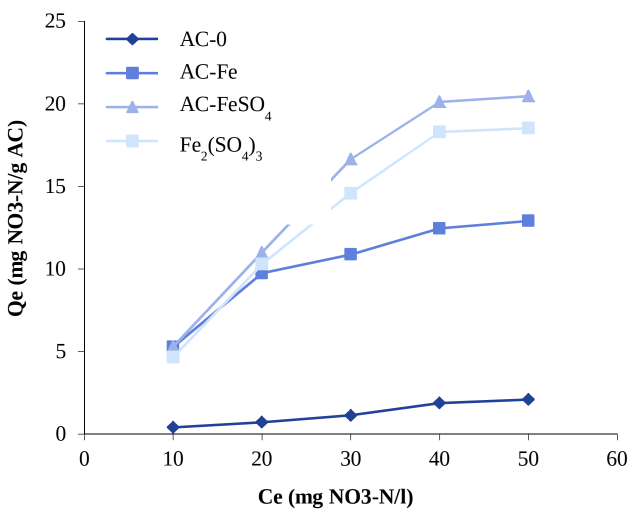


Figure 6. Nitrate-N isotherms of all activated carbon in mass basis

The studies conducted by Shen et al.,[10] and Namasivayam and Sangeetha[12] support this finding. Figures3 and 4 show the effect of different ACs on nitrate removal from solution with 20 mg/l nitrate-N concentration. When AC_0 is used, 15 mg/l nitrate is removed in 30 minutes reaction. The final nitrate concentration gained was in the standard range of the WHO recommendation for water quality when impregnated ACs is used. It was evident that the modification of AC with Fe^0, $Fe_2(SO4)_3$, and $FeSO_4$ greatly enhanced nitrate removal. This phenomenon may be caused by adsorption and chemical reactions. Moreover, AC-$FeSO_4$ and AC-$Fe_2(SO4)_3$ showed the highest removal capacity for nitrate, which was almost 70-80% in a 30 minute reaction. The equilibrium time for impregnated ACs was found to be 45 minutes. Namasivayam and Sangeetha investigated the removal of anions, heavy metals, organics, and dyes from water by adsorption on to $ZnCl_2$ activated coir pith carbon.[12] Their findings showed a 95% removal of nitrate by $ZnCl_2$ coir pith carbon at initial concentration of 20 mg/l, temperature of 35°C, 200 rpm, and agitation time of 3 hour. However, this reduction was insignificant for coir pith carbon without $ZnCl_2$ modification. The mechanisms of nitrate removal included adsorption and chemical reactions. In the modification process, micropores were formed after impregnation of AC with $FeSO_4$ and $Fe_2(SO4)_3$. The SEM micrographs showed that modified-AC has superior micropore content in comparison to its parent sample (Figure 1). Another other advantage of impregnation by $FeSO_4$ and $Fe_2(SO4)_3$ is increasing of AC positive charge. This phenomenon increased adsorption of anions such as nitrate. Previous studies which have conducted the same experiments as those in the present study have confirmed its results.[10,16]

Mizuta et al. studied the removal of nitrate from drinking water using bamboo powder charcoal.[9]

Table 2. Water quality after activated carbon treatment

Adsorbent	pH	nitrite (mg/l)	Ammonia (mg/l)	Sulfate (mg/l)	Fe (µg/l)
AC-0	6.71	0.0175	0.68	0	0
AC-Fe	6.65	0	0.65	0	750
AC-$FeSO_4$	5.40	0.0180	0.57	0	1100
AC-$Fe(SO_4)_3$	5.45	0	0.45	0	337

AC: Activated carbon

Adsorption isotherm of nitrate-nitrogen on to commercial AC at 10°C has shown that maximum amount of nitrate adsorption is 1.17 and 0.93 for bamboo powder charcoal and AC, respectively. Shen et al. studied the effect of AC fiber structure and loaded metals on the adsorption of dichloroethylene.[10] In their work, AC was modified and impregnated with copper, which was converted to metal oxides and reduced to elemental substance, and the adsorption properties of dichloroethylene on AC fiber was investigated. The results showed that both the pore structure of AC fiber and metal/oxide loading affected the adsorption capacity of dichloroethylene.

The amount of AC adsorption increased with the increase in carbon dosage and reached a maximum value after a particular dose. The effect of the quantity of adsorbent AC and variation in nitrate removal percentage with weight of adsorbent on nitrate removal is shown in figure 5. Another study which had conducted the same experiments as those in the present study have showed that an increase in adsorbent dosage increased the percentage of nitrate removal and it reached a maximum value after a particular dose.[17] This was in agreement with the results of the present study. Nitrate isotherms of all AC in mass basis are shown in figure 6. Ozturk and Bektas studied nitrate removal from aqueous solution by adsorption on to various materials.[17] The equilibrium time was found to be 45 minutes for AC. Maximum amount of removal in time was 4 mg NO_3^- per gram of AC. However, in this paper, maximum removal is over 14 and 15 mg nitrate-N per 1 gram AC for AC-$Fe_2(SO4)_3$ and AC-$FeSO_4$, respectively.

Water quality after activated carbon treatment is shown in table 2. As illustrated in table 2, all parameter were within the acceptable range recommended by WHO guideline. However, pH modification is needed.

Conclusion

In the present study, impregnated almond shell activated carbon by Fe, $Fe_2(SO_4)_3$, and $FeSO_4$, were used as adsorbent to remove nitrate from water. Nitrate concentrations, activated carbon dosage, and time of contact between modified activated carbon and nitrate solution were studied. The mechanisms of nitrate removal included adsorption and chemical reactions. Impregnated activated carbons by Fe^0, $Fe_2(SO_4)_3$, and $FeSO_4$ were more effective in nitrate removal than virgin almond activated carbon. The maximum nitrate removal was 70% and 10-15% for modified activated carbons and virgin activated carbon, respectively.

Conflict of Interests

Authors have no conflict of interests.

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Anammox enrichment and constructed wetland inoculation for improvement of wastewater treatment performance

Oleksa Shved[1], Saeed Dehestaniathar[2], Volodymyr Novikov[3]

1 Department of Biologically Active Substances, Pharmacy and Biotechnology, Lviv Polytechnic National University, Lviv, Ukraine AND Department of Environmental Biotechnology, Helmholtz Centre for Environmental Research, Leipzig, Germany
2 Kurdistan Environmental Health Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran AND Department of Environmental Biotechnology, Helmholtz Centre for Environmental Research, Leipzig, Germany
3 Department of Biologically Active Substances, Pharmacy and Biotechnology, Lviv Polytechnic National University, Lviv, Ukraine

Original Article

Abstract

This study contributes to the improvement of low-cost biotechnology for wastewater treatment in constructed wetlands (CWs). Constructed wetlands are energy efficient engineered systems that mimic the treatment processes of natural wetlands, removing polluting organic matter, nutrients, and pathogens from water. The aim of this study was to investigate the advisability of the inoculation of horizontal subsurface flow constructed wetlands with the enriched biomass of anaerobic ammonium oxidation (anammox) bacteria to enhance nitrogen removal. Contaminants removal in constructed wetlands occurs mainly due to the biological transformations caused by indigenous water-borne microorganisms. However, the role of different microbial mechanisms is still unknown. To estimate the role of the anammox process in wetlands the laboratory-scale fixed bed reactor planted with Juncus effusus was inoculated with enriched biomass of anammox bacteria and fed with synthetic wastewater containing ammonium-nitrogen as the main contaminant. In order to obtain the active enriched culture of anammox bacteria, an upflow anaerobic fixed bed reactor inoculated with activated sludge from a municipal wastewater treatment plant was run. The reactor was fed with enrichment medium containing ammonium and nitrite in high concentrations. After 270 days of operation, nitrite was not found in measurable levels, the concentration of ammonium had slightly increased, and the concentration of nitrate in the reactor had significantly dropped compared to its level at the initial phase. The microbial association, which had developed in the enrichment reactor, allowed continuous removal of ammonium and nitrite. The anammox bacteria abundance in the reactor accounted for approximately 95% of total biomass.

KEYWORDS: Wastewater, Wetlands, Bioreactors, Nitrogen, Ammonium, Bacteria

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Introduction

Water is essential to human life, and therefore, water resources must be protected from contamination. Discharge of untreated wastewater into the natural water bodies is one of the main causes of water pollution. However, accurate wastewater treatment is a serious challenge for many developing countries due to the high construction and exploitation costs of

Corresponding Author:
Oleksa Shved
Email: oleksa.shved@gmail.com

the treatment systems. This problem can be solved by the implementation of inexpensive competitive alternatives to conventional treatment technologies.

Constructed wetlands (CWs) are energy-efficient, chemical-free, and easy-to-operate robust wastewater treatment systems. The design of CWs utilizes the principles and properties of natural wetlands, but provides better control over the treatment process. The horizontal subsurface flow (HSSF) constructed wetlands are one of the most widespread and reliable types of near-natural treatment technologies used around the world.[1] Specified CWs comprise macrophytes that are introduced into porous medium (gravel, sand, and etc.) and wastewater that flows under the surface of the bed mainly in a horizontal direction. Horizontal flow constructed wetlands efficiently remove organic matter, suspended solids, and pathogenic microorganisms from sewage. However, nutrient (nitrogen and phosphorous) removal rates in these systems are considerably low.[2] In particular, nitrogen removal efficiencies have been reported as only between 30% and 50% in long term studies.[3-5]

It was clarified that if the loading of ammonia to a wetland exceeds the growth requirements of the plants, bacteria, and algae, microbial transformation of nitrogen to its gaseous forms is generally the dominant N removal process in CWs.[5-7] Moreover, Kadlec[8] has stated that the loading limit for bacterial transformation to predominate in CW is equal to the loading rate of approximately 120 g-N m^{-2} yr^{-1}. However, the function of emergent water plants is to provide favourable conditions and habitat for the microbes, and they can enhance nitrogen removal mainly via indirect effects on the physicochemical and microbial processes.[7] Harvesting the above ground biomass of macrophytes in HSSF CWs can contribute to a less than 10% removal of nitrogen , as compared to the inflow load.[9] In addition, it should be considered that elevated pH values promote ammonia volatilization from the system; however, nitrogen removal through this process is generally insignificant if the pH is below 8.0.[5,7]

As was explained, researchers have stated that the major mechanism of nitrogen removal from wastewater in CWs is biological transformation by indigenous water-borne microorganisms. Nevertheless, there is no "conventional wisdom" that explains the low efficiency of nitrogen removal in horizontal flow CWs, because present understanding of the relative importance of different microbial pathways in wetlands is very poor. Therefore, nitrogen removal is the issue that receives probably the most attention today from researchers who work in the field of constructed wetlands. Still, it should be taken into account that constructed wetlands are very complex bioreactors, where numerous physical, chemical, and biological processes caused by plants, microorganisms, and contaminants take place simultaneously. Thus, they are difficult to study.[6,10]

Estimated oxygen fluxes into HSSF wetlands are generally insufficient to supply conventional nitrification-denitrification; nevertheless, microbial denitrification is still considered to be the primary nitrogen removal mechanism.[7] Moreover, the denitrification process was rarely measured directly in treatment wetlands. Therefore, recently discovered alternative microbial pathways of nitrogen transformation common for low-oxygen aquatic environments are considered to play an important role in the nitrogen cycling process in treatment wetlands.[11] A few studies have been conducted to investigate the newly discovered anammox process in constructed wetlands.[12-15] Thus, the goal of the present study was to inoculate the model horizontal subsurface flow constructed wetland with enrichment culture of anammox bacteria in order to enhance removal efficiency of nitrogen.

Materials and Methods

The enrichment culture of anaerobic ammonium oxidation (anammox) bacteria was cultivated in an anaerobic upflow fixed bed reactor (henceforth referred to as anammox-reactor). Figure 1 shows the principal configuration of the experimental setup. The experimental setup consisted of the following major components:

- Anammox-reactor [(1) in Figure 1];
- A peristaltic pump for continuous recirculation of the reactor medium;
- A water-lock [(2) in Figure 1] for elimination of the formed N_2 gas;
- A water heating device [(3) in Figure 1] with a circulation pump;
- The trace mineral solution (TMS) storage vessel [(4) in Figure 1] connected to a gas bag [(7) in Figure 1];
- Storage vessels [(5) and (6) in Figure 1] for two enrichment half-media, which were also connected to the gas bag;
- Magnetic stirrer [(8) in Figure 1] for vessel 5;
- Two peristaltic pumps for feeding of the enrichment media and TMS.

The reactor itself was a vertical glass column (0.15 m diameter), which was wider in the upper end (0.2 m diameter) in order to promote the gas separation. The reactor was equipped with a peripheral twin-wall enclosure (thermostatic jacket) and operated at the temperature of 30 ˚C. Warm water (34 ˚C) from the heating device circulated inside the enclosure. Total volume of the reactor was equal to 25 l and volume of the liquid medium was 20 l (fill factor = 0.85). The anammox-reactor was completely filled with Kaldnes type K1 polyethylene biofilm carriers (density = 0.95 g/cm³, void fraction = 95%).[16]

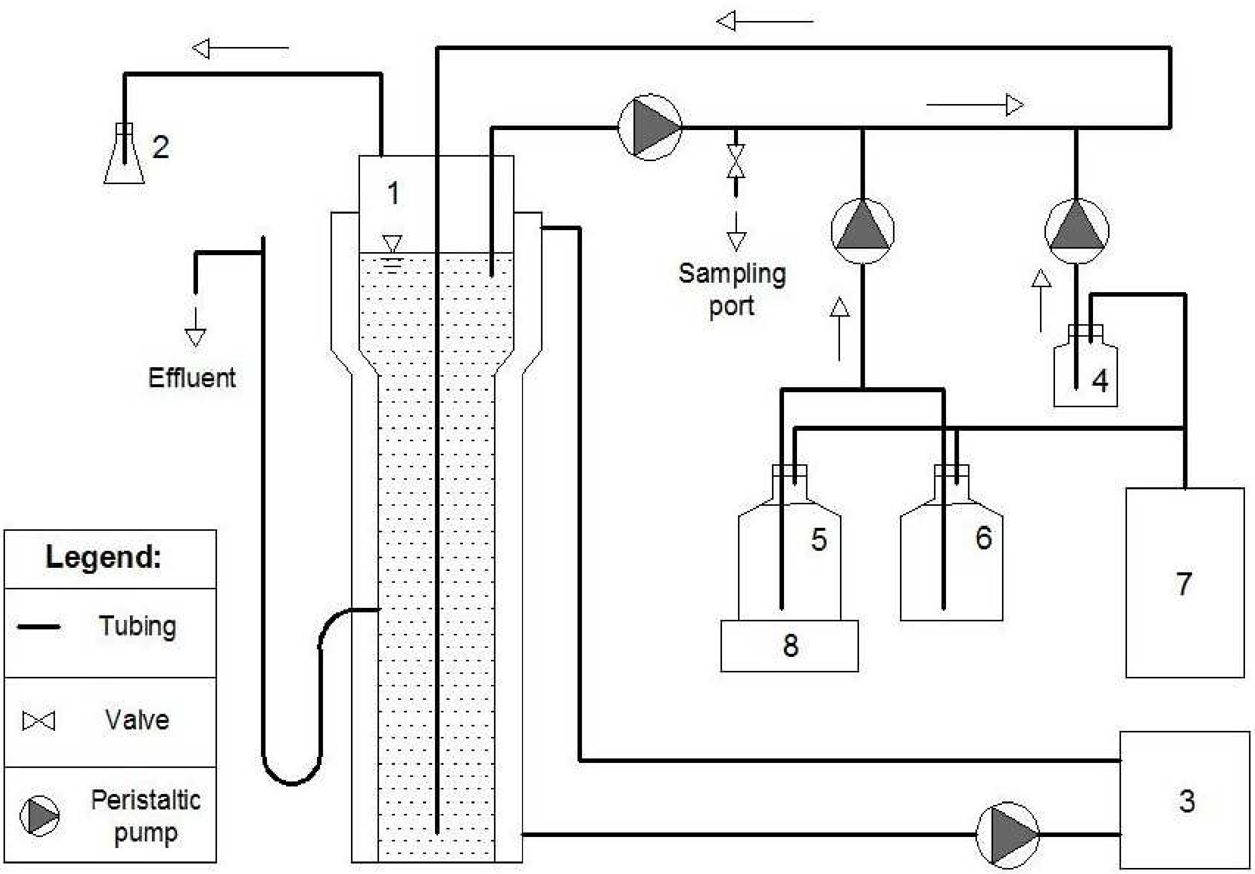

Figure 1. Schematic diagram of the lab-scale setup of anammox-reactor

The reactor was filled with enrichment liquid medium of a given composition which permits preferential emergence of anammox bacteria. The reactor was inoculated with pre-enriched activated sludge from the Leipzig-Rosenthal municipal wastewater treatment plant [440000 population equivalent (PE)]. The highly concentrated medium (henceforth referred to as end-medium) was separated into 2 half media (Table 1), which were stored in separate 5 l Schott bottles, in order to prevent precipitation, and kept in anaerobic conditions to prevent oxygen input into the reactor. The concentrations of $NaNO_2$ and NH_4Cl in the end-medium (which was formed after unification of the streams from 2 storage tanks) were initially set to 10 mm. The media were supplied periodically by one peristaltic inflow pump with 2 channels. During start-up period, 32 ml of the end medium was supplied twice a day (two cycles), resulting in 20 mg-N day^{-1}. The ammonia and nitrite concentration in the end-medium were increased gradually to 18 mm each. Number of feeding cycles was gradually increased to 12 per day and feeding volume was increased to 48 ml per cycle, resulting in 290 mg-N day^{-1} or 14.5 g-N m^{-3} reactor day^{-1}. TMS, containing (mg l^{-1}) NaEDTA, 15.0 g; $ZnSO_4 \cdot 7H_2O$, 0.43 g; $CoCl_2 \cdot 6H_2O$, 0.24 g; $MnCl_2 \cdot 2H_2O$, 0.81 g; $CuSO_4 \cdot 5H_2O$, 0.25 g; $Na_2MoO_4 \cdot 2H_2O$, 0.22 g; $NiCl_2 \cdot 6H_2O$, 0.19 g; $Na_2SeO_3 \cdot 5H_2O$, 0.11 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; and H_3BO_3, 0.07 g, was supplied with a separate peristaltic pump in amount of 1 ml per liter of end-medium.

Table 1. Enrichment medium composition

Component	Concentration in the end-medium (gl^{-1})
1st half-medium (N and C supply)	
$NaNO_2$	1.240
NH_4Cl	0.960
$KHCO_3$	1.500
2nd half-medium (Minerals supply)	
$MgCl_2 \cdot 6H_2O$	0.110
KH_2PO_4	0.014
$FeSO_4 \cdot 7H_2O$	0.012
$CaCl_2 \cdot 2H_2O$	0.300
Na_2SO_4	0.200

To emulate the horizontal subsurface flow constructed wetland, planted fixed bed reactor (PFR) with continuous recirculation (providing free from macro-gradient conditions within the root bed) developed by Kappelmeyer et al.[17] was run. PFR was operated under the defined conditions of an average summer day in a moderate climate in a greenhouse (the Phytotechnicum of the Helmholtz Centre for Environmental Research). The plastic PFR with a diameter of 0.3 m and a height of 0.28 m was filled with 17 kg of gravel (particle size 4-8 mm) and planted with Juncus effusus. The pore water volume was equal to 14 L with hydraulic retention time (HRT) approximately 7 days. The PFR was fed with synthetic wastewater containing ammonium-nitrogen as the main contaminant (50 mg-NH_4^+ l^{-1}). Composition of the wastewater is presented in table 2. TMS (with the same composition as that for anammox-reactor) was supplied in 1.5 ml per liter of the synthetic wastewater. Wastewater inflow rate was equal to 1.85 l d^{-1}.

Table 2. Synthetic wastewater composition

Component	Concentration (gl^{-1})
NH_4Cl	0.150
$KHCO_3$	1.180
$MgCl_2 \cdot 6H_2O$	0.110
KH_2PO_4	0.014
$FeSO_4 \cdot 7H_2O$	0.012
$CaCl_2 \cdot 2H_2O$	0.300

After the enrichment of bacterial culture in the anammox-reactor the biofilms were mechanically transferred from the plastic carriers to the liquid phase, and then, the liquid phase was homogenized by high rate recirculation. Then, 3 l of the liquid enriched culture were anaerobically taken into 1 L glass bottles. Inoculation of the PFR was performed using a metal needle of a large diameter and a peristaltic pump. The enriched culture was slowly introduced into the PFR. After the inoculation, the feeding of the PFR was paused for 48 hours, while the recirculation was run.

To analyze treatment performance of the PFR, different parameters were monitored, including temperature, pH, redox potential (Eh), dissolved oxygen, pore volume, inorganic nitrogen concentrations, and microbial community development. The analytical methods were based on the publication by Wiessner et al.[18] Real-time quantitative polymerase chain reaction (RT-PCR)[19] with non-specific (bacterial) and group-specific PCR oligonucleotide primers were used for microbial community analysis. Total DNA was isolated from enrichment culture samples (40 ml) using DNeasy® Blood & Tissue Kit (Qiagen GmbH, Hilden, Germany) and the FastPrep® Instrument (MP Biomedicals, Irvine, CA, USA). Group-specific primer set targeting 16S ribosomal RNA gene sequences of anammox bacteria (AMX) was used.

Results and Discussion

As can be observed in figure 2, during the first month of the operation, nitrate-nitrogen was strongly prevalent among nitrogen species in the anammox-reactor. This could be a result of nitrifying bacteria (Nitrosomonas and Nitrobacter), dominant in the reactor at the initial phase.

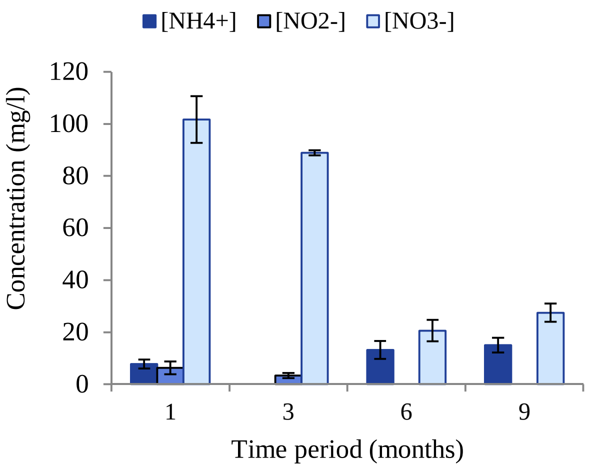

Figure 2. Nitrogen species composition dynamics in anammox-reactor

After 3 months, the concentrations of both nitrate and ammonium decreased (Figure 2), while the inflow load was considerably increased. This could be evidence, to some extent, of nitrogen removal via the anammox pathway by direct oxidization of ammonium by nitrite to dinitrogen gas. After 6 months of operation, considerable elevation in activity was observed (Figure 2). The concentration of nitrite had dramatically dropped, but nitrite was not measurable; however, the concentration of ammonium had increased. This confirmed the possibility of the anammox activity, due to the following facts: nitrate was no longer produced in high quantities, while ammonium was present in higher amounts in the system, meanwhile nitrite was completely removed, while ammonium was still present. The presence of ammonium can be explained by the ammonium to nitrite ratio in the feeding medium, which was equal to 1:1. However, stoichiometric ratio amounted to 1:1.32 as calculated by Strous et al.[20]:

$$NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13\ H^+ \rightarrow 1.02\ N_2 + 0.26\ NO_3^- + 0.066\ CH_2O_{0.5}N_{0.15} + 2.03\ H_2O$$

Experimental ammonium to nitrite ratio of 1:1 was used in order to prevent accumulation of nitrite in the system, which is toxic to living organisms in high concentrations. The stoichiometry of the anammox process can also explain the accumulation of ammonium in the system with increasing of the nitrogen load to the system.

After 9 months of operation under anaerobic conditions, the microbial association, which has developed in the anammox-reactor, allowed continuous removal of ammonium and nitrite in the ratio that was similar to one of the anammox processes. After 270 days of operation, the liquid samples of the enrichment culture from the anammox-reactor were taken for genetic analysis using molecular biological techniques. Since attached-growth biomass has grown on the surfaces of support media, the biofilm was previously mechanically transferred to the liquid phase.

Table 3. Real-time quantitative polymerase chain reaction (RT-PCR) results for the water samples from anammox-reactor

Sample	Genes copy number ± SD (copies µl^{-1})		Relative abundance of anammox bacteria (%)
	Total prokaryotes	Anammox	
AMX1	9.26E + 6 ± 7.70E + 5	8.82E + 6 ± 6.64E + 5	95.3
AMX2	9.12E+ 6 ± 6.33E + 5	8.77E+ 6 ± 8.58E + 5	96.1
AMX3	1.11E+ 7	1.04E + 7 ± 2.65E + 5	93.7

SD: Standard deviation; AMX1: Anammox reactor

Table 3 presents results of the real-time PCR analysis of 3 water samples (AMX1, AMX2, and AMX3) taken from the anammox-reactor. Results have therefore provided evidence of anammox bacteria dominance in the developed microbial association.

At the same time, the model planted fixed bed reactor system was operated. The PFR reached generally stable physicochemical conditions and removal efficiencies. In particular, the total nitrogen (TN) removal efficiencies were equal to about 50%. Furthermore, residual nitrogen was present only in the form of ammonium and nitrate. The redox potential (Eh) during daylight hours amounted to 300 ± 50 mV. However, it should be considered that CWs, are interpreted as redox multi-gradient systems, with both reducing and oxidizing conditions occurring at the same time in different zones (root surfaces, gravel bed, microbial biofilms). These factors have created favourable living conditions for both aerobic and anaerobic organisms.

To estimate the role of the anammox process in nitrogen transformations and its influence on the treatment performance of CWs, the PFR was inoculated with obtained enrichment culture of anammox bacteria. Inoculation is essentially important for the study, because anammox bacteria are slow growers (cells double only once per 11-20 days). The changes in nitrogen removal rates and microbial community composition will be further determined, while the achievement of higher nitrogen removal rates can be expected. When anammox bacteria adapt to the environmental conditions of CWs, the main limitations for the classic process of nitrogen removal (not enough oxygen and organic carbon) could become an advantage for anammox.

Conclusion

The anammox bacteria abundance in the anammox-reactor accounted for approximately 95% of the total microbial biomass. This is a suitable amount for inoculation of the experimental planted-fixed bed reactor, which was used as a model constructed wetland system. The results showed that upflow anaerobic fixed bed reactor filled with plastic carriers can be used for anammox enrichment. The obtained bacterial association is capable of anaerobic ammonium oxidation and can more efficiently remove high concentrations of nitrogen. Therefore, the inoculation of constructed wetlands with enrichment culture of anammox can improve poor performance of the system in terms of nitrogen removal.

Conflict of Interests

Authors have no conflict of interests.

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