



Studying Spatial Changes of Groundwater's Nitrate Content in Central District of Khodabandeh, Iran



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ABSTRACT

Background: This research aims to measure and study spatial changes, and the reason behind the increasing nitrate content in water wells in the Central District of Khodabandeh County in the Zanjan Province.

Methods: The nitrate and nitrite content, electrical conductivity, dissolved oxygen, temperature, total hardness and pH were measured at 40 sampling stations in the study area. The obtained features were categorized into four classes by principal component analysis. The kriging and cokriging methods along with electrical conductivity and total hardness covariates were used to study the spatial changes. By using the cross-validation method, the linear model for cokriging method with electrical conductivity covariate was selected as the best model for nitrate zoning in ArcGIS 10.2 software.

Results: The principal component analysis indicated that nitrate feature was in the same classification as that of electrical conductivity and total hardness. The comparison of surface digital elevation model, geology and land use maps with nitrate zoning indicated that nitrate pollution in the south and south-west regions was due to geological content, agricultural activities, and the discharge of human sewage into absorbing wells.

Conclusion: The findings of the present research revealed that lowlands with agricultural use had more nitrate content than other uses, and the most important reason could be attributed to the excessive use of nitrate containing chemical fertilizers.

1. Introduction

Nitrate is one of the important indices for determining the quality of water for drinking and agriculture uses and its presence in water supplies limits the use of these resources. Measuring this element is very important and practical for constructing and designing treatment plants as well as for other managerial plans such as contamination removal and surveying changes in water pollution concentration [1].

Nitrate enters surface and ground waters from the decomposition and corruption of human and animal wastes, industrial products, and runoff from agriculture [2]. It harms the digestive system, causes mouth cancer, creates congenital anomalies on the fetus, increases the size of thyroid gland, creates goiter, increases blood pressure, leads to the emergence of insulin-dependent diabetes, and reduces physiological- and neurological performances [3].

The maximum allowed nitrate content in irrigation water and discharged waste water to surface waters is determined as 50 mg L⁻¹ by the World Health Organization and the Iranian Department of Environment [4, 5].

Due to the ever increasing use of dissolved chemicals and permeation of home and industrial sewage into aquifers, the quality of groundwater has significantly decreased [1, 6-8]. The removal of nitrate from water is very expensive and knowing the spatial distribution of nitrate is very important [1, 6, 8].

Geographic information system (GIS) and geostatistics are the basic instruments to determine dispersion and the way of pollution space distribution.

Contrary to classical statistics in geostatistics that is a subset of spatial statistic science, observations are not independent of each other and depend on each other based on their location in the study area and definitely a relation can be established between the values of a quantity in samples community and the distance and the direction of the samples relative to each other [9].

Considering no stability of nitrate spatial (non-static condition) that results from being affected by the spatial changes of other parameters, surveying spatial changes and the way of influence in a place is gaining importance for future planning. There have been several studies regarding the spatial analysis of water quality indices especially nitrate. The study of the temporal and spatial changes of nitrate by the kriging and cokriging geostatistical methods in ground waters has shown that the cokriging method has higher accuracy in evaluating nitrate concentration [10].

The use of the two methods of ordinary kriging and assimilation to prepare nitrate map in two thresholds of 10 and 50 mg L⁻¹ in Monadi plain of Italy showed that the ordinary kriging method was suitable to evaluate groundwater nitrate in this plain [11]. The evaluation of nitrate monitoring networks in Heretaunga plains, New Zealand, by the kriging method (geostatistics) and vulnerability maps indicated that the simultaneous application of these two methods was suitable for highly vulnerable areas and regions with low number of stations [12]. In addition, the results of nitrate spatial changes using geostatistics indicate higher variation of nitrate compared to electrical conductivity [13]. Zezoli et al. (2013) studied the temporal and spatial variations in nitrate and nitrite concentrations of drinking water supplies in Kohgiluyeh by using GIS and reported that the most important reasons for increasing nitrate were geology and agriculture in this area [14]. Setare et al.

(2014) showed that changes in nitrate concentration has a reverse relation with changes in water table depth and referred to low alluvium thickness, the location of the well in lower parts of agricultural lands, the agricultural nature of the area, washing nitrate from agricultural soils, and the extensive use of nitrogen fertilizers as reasons for water supply pollution [15].

Considering the importance of nitrate feature in ground water, this research was undertaken due to the location of water supplies in Khodabandeh County in lands with agricultural use and the possibility of nitrate increase. This study was performed in autumn 2015 in Environmental Science Research Group of Zanjan University. Regarding innovations in the present research, we can say that it is the first research regarding nitrate pollution in this study area, using bivariate statistical methods in results interpretation, using bivariate geostatistical methods in nitrate content zoning, and determining the dependence of amount of pollution to land structure characteristics.

2. Materials and Methods

2.1. Study Area

Khodabandeh County, one of the seven counties of Zanjan province, is located in southwest of the province, between 35°, 35' to 36°, 24' northern latitude and 47°, 51' to 48°, 57' western longitude. Qizil Üzan River with an annual runoff volume of 1117420 m³ and Khararud River with annual runoff of 115370 m³ flow in this county. In addition, this county includes 950 springs, 153 qanats, 1450 half-deep wells, 742 deep wells, 27 diversion dams, and five water reservoir pools and the annual water consumption for agriculture is 5013860 m³. Figure 1 shows the location of the sampling stations in the study area.

2.2. Materials and Methods

For analysis of the obtained data and to prepare zoning maps, Excel, STATISTICA 20, SPSS 20 and ArcGIS 10.2 software were used. In statistical tests, a value of $P < 0.05$ was used as the criterion for significance of the tests [9, 16, 17]. Since it is not always possible to sample every location, therefore, unknown values must be estimated from data taken at specific locations that can be sampled. One of the most important methods for estimating unknown values is geostatistics.

Geostatistics is a subset of statistics specializing in the analysis and interpretation of geographically referenced data. Kriging and cokriging are the most

important methods in this field [18]. Based on the kriging method, the value of a variable in unsampled stations is considered as a linear composition of the values of the same variable and to evaluate unknown spots, attributes a weight to each sample (Equation 1).

$$Z_0 = + \sum_{i=1}^n \lambda_i Z(X_i) \tag{1}$$

In this equation, $Z(x_i)$ is the measured value of the variable in (x_i) location, Z_0 is the estimated value of the variable in (x_i) location, λ_i is the weight or significance of a quality dependent to it sample that is attributed to the considered parameter, based on the quantity dependent to the sample in the specified points, and n is the number of points in which the variable has been measured (number of wells, springs, and qanats). The condition for using this estimator is that the variable has a normal distribution [19-21].

In the cokriging method, after determining the dependent variables by bivariate statistics, the value of a variable is estimated by the help of a second auxiliary variable. The evaluation of interpolation methods was performed by Cross Validation. In this method, the Mean Absolute Error, Mean Bias Error indices

(Equations 2 and 3) and R^2 Correlation Coefficient were used [9].

$$MAE = \frac{1}{n} + \sum_{i=1}^n |Z^*(X_i) - Z(X_i)| \tag{2}$$

$$MBE = \frac{1}{n} + \sum_{i=1}^n (Z^*(X_i) - Z(X_i)) \tag{3}$$

In which $Z^*(x_i)$ is the estimated value of the variable in $Z(x_i)$, (x_i) is the observed value of the variable in x_i , and n is the number of data. Based on the above indices, the best method is the one in which the values of MBE and MAE are close to zero and R^2 is close to 1 [22].

One of the important principles in increasing the accuracy of estimation in statistical techniques and the use of statistical methods is normality of data [23, 24].

An abnormal distribution of data causes high fluctuation in variogram and decreases the reliability of analytical results. So in this research, Shapiro–Wilk test and Kolmogorov–Smirnov test were used to survey the status of distribution of the obtained data.

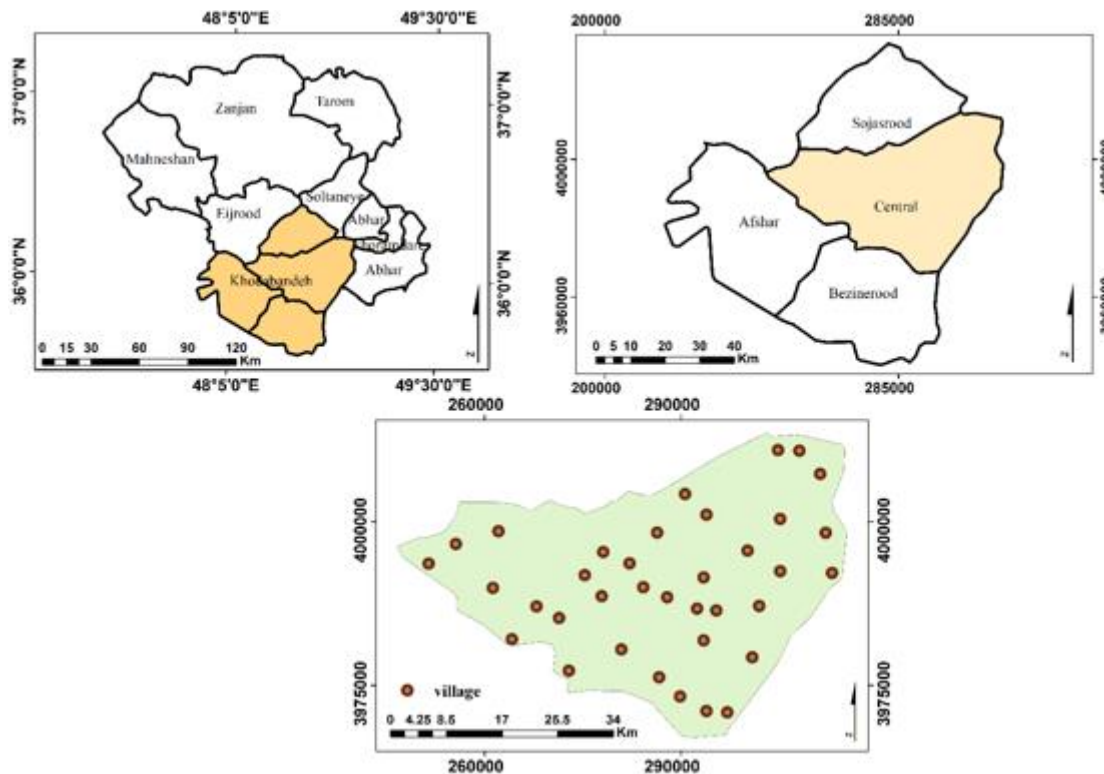


Figure. 1: Location of study area in zanjan province and khodabandeh county, besides the location of sampling spots

2.3. Sampling

Considering the map of wells, villages and access roads to the central district of Khodabandeh County, 40 sampling stations were selected by considering their suitable dispersion and covering all the areas of the central district of the county. The samples were collected through three replications and transmitted to the laboratory of Faculty of Environment Sciences of Zanjan University in less than 24 hours. In the sampling location, the amount of dissolved oxygen, temperature, ph, and electrical conductivity of the sample were measured by portable multimeter (Hach HQ 40d). Nitrate and nitrite in the samples (DR 5000 HACH, nitrite 3 reagent, LR; DR890 HACH, nitrate high range, Chromotropic acid method) were measured in a laboratory.

3. Results and Discussion

Table 1 lists the statistical characteristics related to the measured samples. The research results revealed that the measured temperature, ph, dissolved oxygen, electrical conductivity, total harness, nitrate and nitrite in the water samples were unrecognizable in the ranges

of 4°-16°C, 6.99-8.70, 6.00-8.96 mg l⁻¹, 188-3870 μs cm⁻¹, 164 -1460 mg L⁻¹ caco₃, -0.022 and 3.18-150.150 mg l⁻¹, respectively. Regarding drinking water standards, the temperature, ph, and dissolved oxygen in these samples were found suitable and acceptable, but water hardness in 40% of the water samples was more than the maximum acceptable level (300 mg L⁻¹). Electrical conductivity in 65% of the water samples was more than the acceptable level prescribed by WHO (300 μs cm⁻¹). In addition, all the water samples included less than 1 mg L⁻¹ of nitrite (maximum limitation of United States Environmental Protection Agency).

Table 2 shows that there is a positive linear correlation between the nitrate amount and the EC. The presence of nitrate in water increases the number of dissolved ions and water EC. Nitrate has a negative linear correlation with pH and the negative correlation of PH with EC can be justified by their relation with nitrate [25]. Nitrate has positive linear correlation only with temperature. In addition, among other significant binary correlations, we can refer to -Nitrate/ph, Nitrate/EC, -ph/EC, ph/DO, -ph/TH, EC/DO, EC/TH, -DO/TH and nitrite/temperature.

Table 1: Statistical summary of measured characteristics of ground water samples in central district of khodabandeh county in zanzan province

	Nitrate (NO ₃)	Nitrite (NO ₂)	Electrical conductivity(Ec)	Total hardness* (Th)	Dissolved oxygen (Do)	(pH)	Temperature (T)
	mg L ⁻¹	mg L ⁻¹	scm ⁻¹	mg L ⁻¹	mg L ⁻¹		C°
Minimum	3.18	0	188	185	6	6.99	4
Maximum	150.15	0.022	3780	1640	8.96	8.7	16
Range	146.96	0.022	3592	1455	2.96	1.71	12
Mean	28.21	0.003	416	287.5	7.76	7.65	8
Average	32.07	0.003	677	393.62	7.76	7.64	8
Standard Deviation	25.44	0.004	188	292.39	0.61	0.32	2.59

* mg L⁻¹ (CaCO₃)

Following a comparison of the univariate and multivariate geostatistical methods, it became clear that the cokriging method with covariate of EC and the least amounts of MAE (8.95) and MBE (1.08) and the maximum value of R² (0.75) indicated nitrate changes in the study area better than the other models.

Figure 2 shows the nitrate zoning in this area. What is considerable in this map is the heterogeneous distribution of nitrate values, so that the maximum amounts are observable in the western, southern, and southwestern areas. The least amount of nitrate was recorded for the northwestern and northeastern areas in the district. Figure 2 shows the digital elevation model (DEM) map of the central district of Khodabandeh County. Studying the geological characteristics in the area, nitrate zoning and DEM (Figure 2) indicated that the places with higher elevation have lower amounts of

nitrate, and places with lower elevation have higher amounts of nitrate, while in the highlands, the possibility of agriculture and other human activities were less and these areas could be used as the control.

Of course, in the southwestern region, which has a cover of gypsum and marl, we observed high amounts of nitrate in elevations as well. In the southern and southwestern regions, the direction of surface waters and consequently ground waters stream is from the highlands toward the lower lands and therefore the direction of waters is from the northeast toward the southwest. Due to the concentration of geological units capable of nitrate concentration (marl, gypsum marl, layers of gypsum, and salt units) in the southern elevations of the study area [26], nitrate is washed from elevations by permeating waters and runs to the lower lands and thereby increases the nitrate concentration in

the ground waters of wells, springs, and qanats. The obtained values for nitrate concentration in ground waters indicated that natural pollution could not be the only reason for such an increased amount of nitrate and

agricultural activities as well as human and animal wastes in water were also important factors for pollution in these areas.

Table 2: Spearman Correlation between Measured Characteristics in Ground Water Samples of Central District of Khodabandeh County in Zanjan Province

		Nitrate	Ph	Ec	Dissolved oxygen	Nitrite	Total hardness
Ph	Correlation Coefficient	-0.35					
	P value*	0.027					
Ec	Correlation Coefficient	0.395	-0.481				
	P value	0.012	0.002				
Dissolved Oxygen	Correlation Coefficient	-0.174	0.458	0.432			
	P value	0.447	0.003	0.005			
Nitrite	Correlation Coefficient	-0.295	0.159	0.104	0.167		
	P value	0.119	0.327	0.524	0.304		
Total Hardness	Correlation Coefficient	0.185	-0.337	0.812	-0.368	0.151	
	P value	0.445	0.033	0.000	0.019	0.353	
Temperature	Correlation Coefficient	0.224	-0.038	0.194	-0.172	0.330	0.150
	P value	0.108	0.816	0.231	0.288	0.037	0.357

*P < 0.05 Indicates Significance of Correlation Coefficient

A similar study by Uyan and Cey (2010) has shown that most of the nitrate pollution in urban ground waters is due to human activities [7]. Additionally, the results of a study by Trivedi and Vediya (2012) on nitrate and nitrite changes showed that wells located in residential areas had more suitable conditions for increasing concentration of the considered pollutants due to the possibility of permeation of pollutants to the wells [2].

According to field studies on land use status, agriculture is the main land use in this area, which includes dry farming, irrigated agriculture, gardens and tree complexes, and central parts as well as some parts in the east, south and southwest have agriculture applications. The amount of nitrate in areas with agricultural use is higher. The application of agriculture lands and the use of agricultural fertilizers cause a large part of the consumed nitrate to enter the groundwater [27].

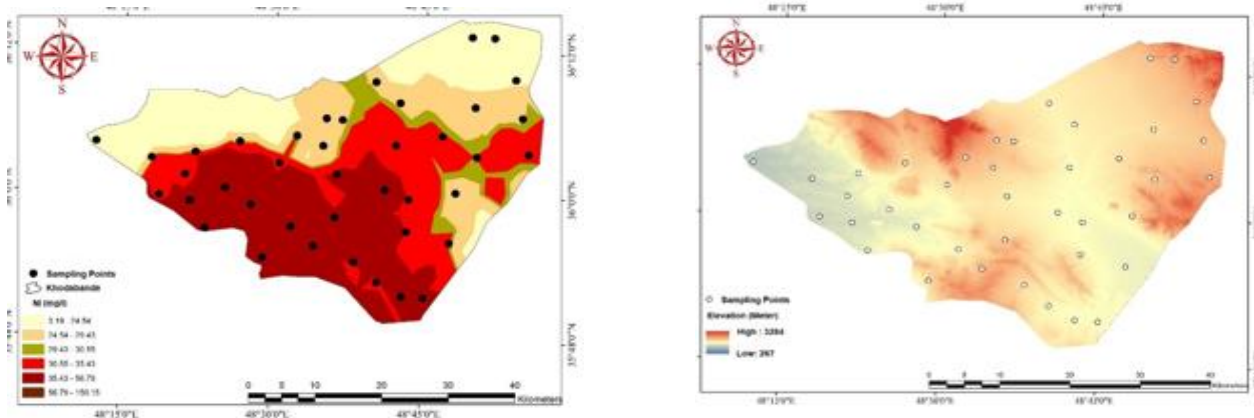


Figure. 2: Digital Elevation Model Map and Spatial Distribution of Nitrate in Ground Waters of Central District in Khodabandeh County

In areas with natural vegetation cover and no agricultural activity, like in northwestern and northeastern regions of the study area, the amount of nitrate is low. However, we should not ignore the kind of rock units in the area that have low capability for

increasing nitrate concentration in ground waters and surface waters.

The amount of nitrate at Dughanlu (110.56) and Dalayer-e Sofla (151.15) stations are much higher than the national nitrate limit (Figure 2).

This region could act as a pollution center and nitrate spreads by ground water. Considering the area's geology, Dughanlu and Dalayer-e Sofla are located in an area composed of lichen, marl, gypsum marl, gypsum and sandstone. Based on this, high concentration of nitrate in samples from the area can be attributed to the geological factor. In addition, the DEM map shows that the height of elevations decreases from north to south. There is also a low arête in the middle section of the region, with the north-south trend, which has formed two eastern and western watersheds in the region. Figure 3 shows the direction of groundwater stream in the central district of Khodabandeh County. In the study area, there are two watersheds that have given two stream directions to area waters. In the direction of water stream in the

western half of the area, which is north west to south west, nitrate is washed from the highlands and brought into the lower lands and increases the amount of nitrate in these parts. The two stations of Dughanlu and Dalayer-e Sofla with high nitrate concentration are located in this basin. The rest of this flow goes to Afshar and Bizineh Rud districts and there is a possibility of nitrate increase in these two districts. In western district, water flows to west and southwest. This water flow could transmit water from high concentration areas to ground waters and surface waters. Similar findings were reported by Ousati and Salajeghe (2000) reported high permeability and low slope in salt marshes and agricultural applications in Dasht-e Kordan [4, 6].

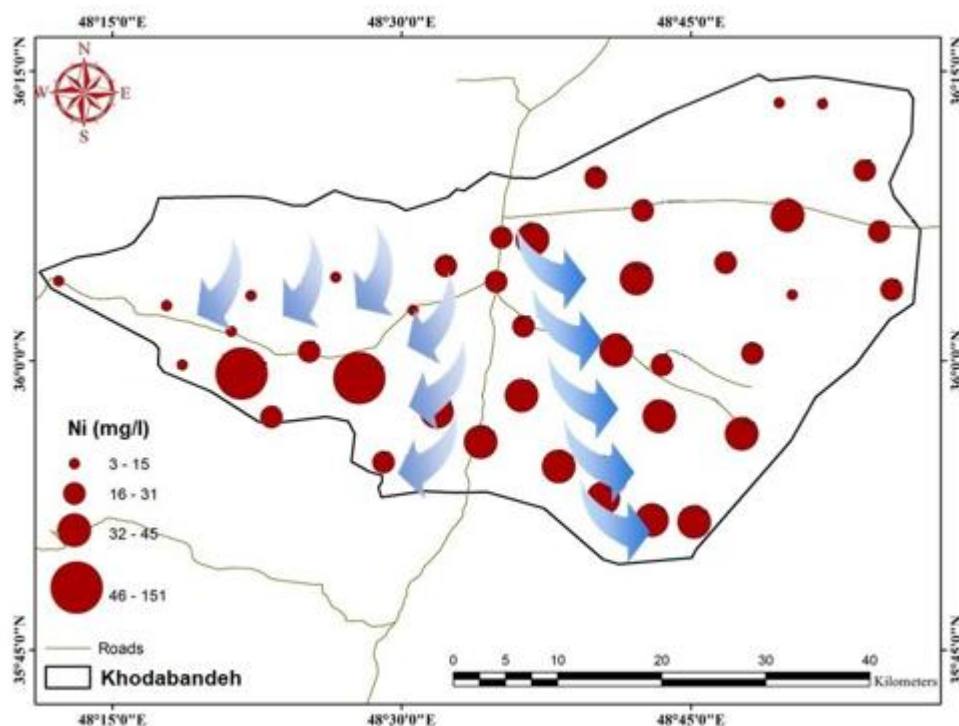


Figure 3: Map of Ground Water Flows in Central District of Khodabandeh County in Zanjan

4. Conclusion

Nitrate concentration distribution in the ground waters of central district in Khodabandeh County was observed to be heterogeneous. Most amounts of nitrate were observable in southwest, south central and some eastern parts of the region. The least amount of nitrate was related to the area's north west and north east. The comparison of nitrate zoning, area geology and DEM indicated that places with higher elevation have lower amounts of nitrate and places with lower elevation have higher concentrations of nitrate. A survey of the

land use map also showed that land uses such as agriculture, which increases nitrate, are located in the lower lands. According to the region's geological map, the southern and southwestern parts are covered with lichen, gypsum, marl, gypsum marl, sandstones, and sometimes salt. These geological units are capable of high concentration of nitrate, and so a high amount of water nitrate in these areas has natural and geological reasons. In addition, the flow direction of surface waters and ground water in the central district of Khodabandeh County indicates the presence of two

watersheds in the area, and these two watersheds have given two water flow directions to area waters. In the western part of the region, nitrate is washed from the southern elevations containing rock units that are susceptible to nitrate concentration by surface water and groundwater, and is transported to the lower regions, which is one of the important reasons for the high amount of nitrate in the southwestern part of the region. However, in the southwestern part, the amount of nitrate is relatively high, and the natural factor cannot alone increase it to such an extent. So the waters in this area are affected by agricultural activities and human waste in water as well. High population density and residential and agricultural applications in these areas boost the effect of human factors in increasing nitrate. The comparison of land use and nitrate zoning maps showed that areas with agricultural use also have higher nitrate. Therefore, human activities including agriculture can be considered as important reasons for increasing nitrate in the area's waters. The application of geostatistics showed that although there were limited numbers of sampling stations, but the cokriging method along with nitrate dependent characteristics can be used to analyze nitrate distribution in an area with high pollution, notwithstanding the limited number of stations.

Conflict of Interest

The authors declare no conflict of interest.

References

- Masetti M, Poli S, Sterlacchini S, Beretta GP, Facchi A. Spatial and Statistical Assessment of Factors Influencing Nitrate Contamination in Groundwater. *J Environ Manage*. 2008; 86(1): 272–81.
- Trivedi HB, VEDIYA SD. Assessment of Nitrate Contamination of the Groundwater Samples in Bhiloda Taluka of Sabarkantha District, Gujarat. *IJPLS*. 2012; 3(11): 2103-06.
- Chatterjee S, Han Woo S. The Removal of Nitrate from Aqueous Solutions by Chitosan Hydrogen Beads. *J Hazard Mater*. 2009; 164: 1012-18.
- Ousati Kh, Salajeghe A, Arekhi S. Spatial Variations of Nitrate in Groundwater Using GIS (Case Study: Kurdan Plain). *6th National Conference on Watershed Management Science and Engineering in Iran. Tehran*. 2010; 29-30.
- WHO (World Health Organization). Nitrate and Nitrite in Drinking Water—Background, Document for Development of WHO Guidelines for Drinking-Water Quality. *Geneva*; 2011.
- Obeidat MM, Ahmad FY, Hamouri NA, Massadeh AM, Athamneh FS. Assessment of Nitrate Contamination of Karst Springs, Bani Kanana, Northern Jordan. *Revista Mexicana de Ciencias Geológicas*. 2008; 25: 426-37.
- Uyan M, Cay T. Geostatistical Methods for Mapping Groundwater Nitrate Concentration. *3rd International Conference on Cartography and GIS*. 2010: 15-20.
- Tamaddoni Kenari S, Hesam M, Dehghani A, Leftah Hlghi M. Investigation of Temporal Variation of Drought and its Impacts on Groundwater Nitrate Concentrations in Drinking Water Wells (Case Study: Gorgan city and vicinity). *J Water Soil Conserv*. 2014; 21(1): 85-103.
- Hasani Pak AA. Geostatistics. *Tehran Univ Press*. 1998.
- Dagostino V, Greene EA, Passarella B, Vurro G. Spatial and Temporal Study of Nitrate Concentration in Groundwater by Means of Coregionalization. *Environ Geology*. 1998; 36: 285-95.
- Barca E, Passarella G. Spatial Evaluation of the Risk of Groundwater Quality Degradation. A Comparison between Disjunctive Kriging and Geostatistical Simulation. *Environ Monit Assess*. 2008; 137(1-3): 261-73.
- Baalousha H. Assessment of a Groundwater Quality Monitoring Network Using Vulnerability Mapping and Geostatistics: A Case Study from Heretaunga Plains, New Zealand. *Agric Water Manag*. 2110; 97: 240-6.
- Ghahreman B, Hosseini SM, Asgari HR. Geostatistics Application in Investigation of Network Monitoring Groundwater Quality. *J Amirkabir*. 2003; 14(55): 971-81.
- Zazouli, MA, Belarak D, Karimnezhad F, Khosravi F. Removal of Fluoride from Aqueous Solution by Using of Adsorption onto Modified Lemna Minor: Adsorption Isotherm and Kinetics Study. *J Mazandaran Univ Med Sci*. 2014; 23 (109): 195-204.
- Setare S, Rezaei M, Hassani AH, Zinatyzadeh AA. Distribution of Groundwater Nitrate Contamination in GIS Environment: A Case Study, Sonqor plain. *J Kermanshah Univ Med Sci*. 2014; 18(3): 157-64.
- Khosravi Y, Jabbari MK. Principles of Geographic Information System and ArcGIS 10.3 Tutorial. *Azarkelk Press*. 2016.
- Lausch A, Herzog F. Applicability of Landscape Metrics for the Monitoring of Landscape Change: Issues of Scale, Resolution and Interpretability. *Ecol Indic*. 2002; 2: 3-15.

18. Momeni Damaneh J, Joulaei F, Alidadi H, Peiravi R. Evaluation of Interpolation Methods to Determine Spatial Variations of Groundwater Qualitative Parameters (Case study: Gonabad Plain). *J Res Environ Health*. 2015; 1(3): 165-76.
19. Rezaei M, Davatgar N, Tajdari K, Abolpour B. Investigation the Spatial Variability of Some Important Groundwater Quality Factors in Guilan, Iran. *J Water Soil*. 2010; 24(5): 932-41.
20. Zehtabian GhR, Janfaza A, Mohammad Askari H, Nematollahi M. Distribution Modelling of some Groundwater Quality Indices (Case Study: Garmsar Basin). *J Range Desert*. 2010; 17(1): 61-73.
21. Zakariapour H, Mousav M, Amiri A, Hosseinzadeh S. Estimation and Interpolation of Porosity and Permeability in Oil Reservoirs. *J Appl Geology*. 2011; 7(2): 107-20.
22. Jahanshahi A, Rouhi Moghaddam E, Dehvari A. Investigating Groundwater Quality Parameters Using GIS and Geostatistics (Case Study: Shahr-Babak Plain Aquifer). *Water Soil Sci*. 2014; 24(3): 183-97.
23. Khosravi Y, Zamani AA, Parizanganeh AH, Yaftian MR. Assessment of Spatial Distribution Pattern of Heavy Metals Surrounding a Lead and Zinc Production Plant in Zanjan Province, Iran. *Geoderma Regional*. 2018; 12: 10-7.
24. Callis JB, Illman DL, Kowalski BR. Process Analytical Chemistry. *Analytical Chemistry*. 1987; 59(9): 624A-37A.
25. Marques R, Goncalves PZCA, Castro SE, Maria BA, Arruda D, Evangelista S, et al. Correlation Analyze between pH Values, Nitrate Concentration, Conductivity Electric, Precipitation Volume and Wind Direction in Cuiaba City Rain, Mato Grosso State, Brazil. *ICSHMO*. 2006; 8: 131-7.
26. Najjari J, Mojarrad A, Sobohi Saboni M. Simulation of Agricultural Drought and its Impact on the Level of Underground Water Sources in Zanjan Province, Case Study: khodabandeh. *4th Iran Water Resources Management Conference, Tehran*. 2011; 3-4.
27. Zhao BQ, Li XY, Liu H, Wang BR, Zhu P, Huang SM, et al. Results from Long-Term Fertilizer Experiments in China: The Risk of Groundwater Pollution by Nitrate, NJAS-Wageningen. *J Life Sci*. 2011; 58: 177-83.