Geopersia 2022, 12(1): 23-37
DOI: 10.22059/GEOPE.2021.323407.648616

RESEARCH PAPER

# Quantify karstic aquifer potential recharge zones by integrated hydrogeology and GIS approaches, Northern Iran

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Received: 07 May 2021, Revised: 20 June 2021, Accepted: 11 July 2021


**Abstract**

One of the main challenges facing the sustainable development of the Kalaleh region is the need for better management of this limited karstic fresh water resources. Kalaleh mountainous region in the north of Iran is drained by several karstic springs such as Zaw, Aghsoo and Yal-cheshme, having discharge rate of 20 to 2500 lit/s. The springs are mainly discharged from Lar, Tirgan, Mozduran karstic formations. In this research, the potential recharge mapping and catchment area of the major karstic springs have been investigated based on the groundwater balance and a GIS approach considering geological factors. In another part of the study, the determined primary catchment area by hydrogeological budget method was reconfirmed with geological method and the results were acceptable. Eight potential recharge contributing factors were evaluated using the GIS including drainage and slope, karstic features, lithology, land cover, precipitation and lineaments. The weights and the score of the factors were assigned based on aerial photos, geological maps, land use database and field verification. The different prepared layers were overlaid in GIS environment, and finally, the mean annual recharge rate of the karstic springs was determined which are in the range of 39-44% of the precipitation. Understanding the groundwater potential recharge zone of the Kalaleh watershed is important for management, proper utilization and future planning of water resources for sustainable management. The Lar formation has higher potential for karst development and infiltration than others. The spring's discharge rates have also confirmed these results. The effective karstic formations in recharging process in order of significance are Lar, Mozduran and Tirgan

**Keywords:** Karst spring, Catchment area, Hydrogeology, Groundwater recharge, Kalaleh.

## Introduction

Groundwater is one of the most valuable natural resources, which supports human health, economic development and ecological diversity. About 25% of the world's groundwater resources are stored in karst systems (Todd & Mays, 2005). Karst system is an extremely heterogeneous porosity with complex characteristics which contains important aquifers especially in arid and semi-arid regions, where the surface waters are not permanently available (Freeze & Cherry, 1979; Fetter, 1999; Bakalowicz, 2005; Todd & Mays, 2005; Ford & Williams, 2007). Investigation of the karstic aquifers is one of the most important management programs in water resources for conservation and sustainable development (Bonacci & Andric, 2015). It is one of the most complex problems to determine the source of catchment area in karst areas due to the heterogeneity of the system (Bonacci et al., 2006). One of the important water cycle components is groundwater percolation recharge. The amount of recharge in karstic

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aquifer is normally determined based on hydrogeological, geological, topographical and meteorological parameters (Freeze & Cherry, 1979; Shaban, 2003). It is controlled by many factors including the rainfall, surface slope, lineament density; land cover, lithology, drainage density and etc. These indicators have been employed more frequently by groundwater researchers as the guides to groundwater investigation in the karstic systems (Shahid et al., 2002; Dar et al., 2010; Dibi et al., 2010; Ramaiah et al., 2012; Dadgar et al., 2017; Ghorbani et al., 2017; Nair et al., 2017; Nayak et al., 2017; Rashid et al., 2017; Al-Shabeeb et al., 2018; Savita et al., 2018; Prasad & John, 2018). Groundwater recharge potential zone are mainly assessed using remote sensing (RS) techniques and geophysical, geological, hydrogeological data. The geographic information system (GIS) has been widely used over the last decade as an effective tool in exploring, evaluating, and managing vital groundwater resources (Meresa & Taye, 2018; Yeh et al., 2009; Krishnamurthy et al., 1996; Saraf & Choudhury, 1998; Murthy, 2000; Yeh et al., 2016; Bierwirth & Welsh, 2000; Leblanc et al., 2003; Shaban et al., 2006; Tweed et al., 2007; Senanayake et al., 2016; Elbeih, 2015; Rwanga & Ndambuki, 2017). The present study deals with assessing groundwater recharge potential zones and catchment area of the karstic springs of Kalaleh region in the east of the Golestan province, northern Iran. This area comprises important karstic groundwater resources which are mainly utilized by the local inhabitants for domestic and agricultural uses. In addition, the area is a major source of groundwater recharging the surrounding alluvial aquifers. The existence of the karstic rocks and the relatively high rate of precipitation mainly as the snow lead to the emergence of large karst springs in the region. The main aims of this study were to determine the spring catchment areas, potential recharge rate and its recharge potential (RP) map in the study area. Future development of karstic resources in this region needs mapping of the groundwater catchment area and potential recharge zones. However, due to limited information, assessing groundwater resources in Kalaleh region is challenging. Therefore, it is important to systematically study the karstic resources to efficiently manage and utilize these valuable water resources.

**Geological and hydrogeological settings**

The study area is located in the Kalaleh karstic region at the east of Golestan province, north of Iran. It is situated between the two eastern Alborz and Kopet Dagh geological zones. The highest elevation in the study area is 3000 m.a.s.l. The anticlines of this area have mostly northeast-southwest trends, being mainly composed of limestone and dolomite of Lar, Tirgan and Chaman Bid karstic Formations (Fig. 1). The main geological formations, in decreasing order of age, are Mozduran limestone Formation (Jurassic), Chaman Bid limestone Formation (Jurassic), Lar limestone Formation (Jurassic), Tirgan limestone-dolomitic Formation (Cretaceous), Sarcheshmeh shale and marl Formation (Cretaceous) and recent quaternary sediments and losses. In terms of tectonics, several faults with the northeast-southwest trend have been determined in the study area. Due to the activity of these fault systems with related joints, fissures and fractures, an environment for the water cycle in carbonate rocks is developed enhancing subsequent karstification in the area. The structural and hydro-geomorphological functions of some thrust faults in the area have played an important role in the emergence of the springs, especially the Zaw spring. They act as a hydraulic barrier thus controlling the groundwater flow direction in the basin. The suitable lithology and snow falling in most of the winter months, provides good environments for karst development as well as recharge to the groundwaters. The study area consists of a developed karstic aquifer discharged by several important karstic springs including Yal-cheshme, Aghsoo and Zaw. The amount of oscillations in the springs shows a good dependence on the amount of rainfall. The discharge rate fluctuates at 20-170 lit/s in Yal-cheshme, 50–500 lit/s in Aghsoo and 100-2500 lit/s in Zaw springs. The annual rainfall and temperature vary between 500 to 1000 mm and 5 to 28 $^{o}$C, respectively.

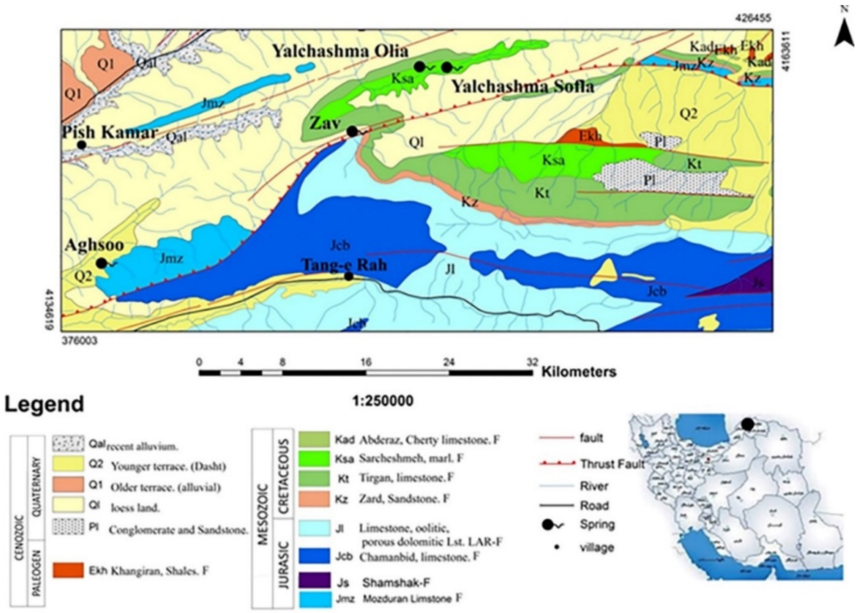


**Figure 1.** Geological map of the study area

## Methods of study

In this research, catchment area of the karstic springs was originally plotted and then its surface area was calculated using a combination of geology, geomorphology and hydrogeology methods. The initial map of the catchment areas was obtained or drawn based on direct on-site observation of major karstic facies such as sinkhole, karen, voids, dry valley and faults, the major geological formations, existence of permeable layers and precise analysis of lineation and aspect. The catchment area (A) of the major karstic springs was calculated based on hydrogeological methods using the water balance formula (Yeh et al., 2016; Meresa & Taye, 2018):

$$A = (Q \cdot t) / (P \cdot I) \quad (1)$$

Where P is the annual precipitation amount, Q is the annual discharge rate of the spring, t is time and I is the rate of recharge to the spring catchment area. The recharge potential map of the area was determined using the GIS tools. Tectonic structure, topography, geology, hydrology and hydrogeology are known as the main factors significantly controlling movement and storage of groundwater in a karstic aquifer. Their roles in groundwater conditions were assessed in the form of different thematic layers including lithology layers, slope, aspect, precipitation, fracture density, drainage density, land cover (vegetation cover) and karstic domains using GIS techniques. The methodology of the study is presented in Figure 2. In order to generate thematic layer of the rainfall, the altitude effects on geo-spatial distribution of precipitation on the area was firstly determined and then, the equation was utilized in the ArcGIS platform to convert DEM into the rainfall map. The thematic maps were discretized into some arbitrary classes and then the same weight was assigned to all values in each class depending on their ability to store and transmit groundwater.

## Results and discussion

The potential recharge map and karstic spring catchment areas in the Kalaleh karstic region were determined based on the remote sensing, geology and hydrogeological methods.

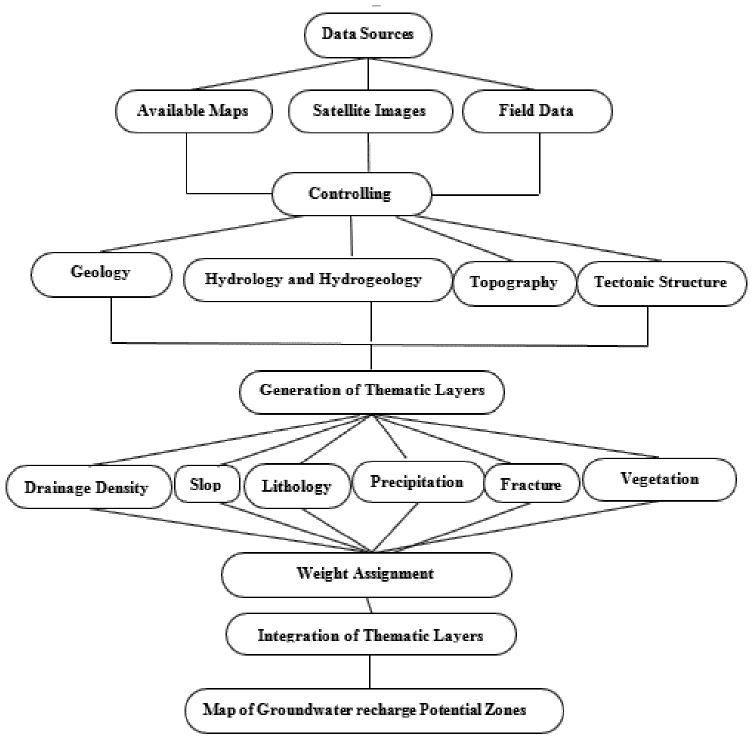


**Figure 2.** Flow diagram showing methodology of the research

*Determining recharge rate in the area*

The most important factors affecting the annual recharge rate in the studied area are lithology, slope, aspect, precipitation, fracture density, drainage density, karstic domains and vegetation cover. In order to determine the annual recharge rate in the studied area, the information layers are provided in the GIS environment. Then, by applying the appropriate weights for each of these data layers and the classification of each parameter from the recharge point, the average annual recharge percentages in different parts of the region are estimated. Table 1 shows the classifications and rating of the most important factors affecting the annual recharge rate in the study area. The field verification and geology maps were used to derive the weights of each recharge potential factor (Table 2). According to the analysis and the expert opinion, the factors affecting the recharge of groundwater in the Kalaleh region in descending order include the karstic domains, slope, rainfall, vegetation, lineament, aspect and drainage density.

## Factors influencing recharge rates

*Lithology*

The lithology factor is associated with the water permeability and the ability of the formations to host groundwater (Oikonomidis et al., 2015). The rock units that are highly fractured are more prone to high runoff resistance and high infiltration, therefore, in the groundwater potential zone assessment the highest rank is assigned to lithology (Shaban et al., 2006; Arnous, 2016). Lithology plays an important role in the occurrence and distribution of groundwater and also significantly affects groundwater recharge by controlling the percolation of water flow. The lithology factor can be divided and scored according to different types of lithology, their hydrogeological significance and effect on recharge rate. The distribution of the lithologic formations was depicted on geological maps (1:50000 scale). The limestone, dolomitic limestone, marly limestone, shale, conglomerate and recent alluvium are the major exposed formations as six major groups in the study area. Then, according to the degree of permeability of each category, they can be classified in terms of the effect on recharge rate. Table 1 and Figure 3 show the classification and rating of various lithologies in the region. In this layer, the areas with karstified carbonate units are most valuable, and the shale and marl regions have the lowest value on the basin groundwater recharge potential.

**Table 1.** Categorization and rating of the most important factors influencing recharge potential in the area

| factor | Range of effect (a-b) | score | Wight (%) | factor | Range of effect (a-b) | score | Wight (%) |
|---|---|---|---|---|---|---|---|
| | north | 9 | | | 400-500 | 3 | |
| | northeastern | 9 | | | 500-600 | 4 | |
| | northwestern | 5 | | | 600-700 | 5 | |
| | Southern | 1 | | precipitation | 700-800 | 6 | 13 |
| **Slope direction** | southwestern | 2 | 8 | | 800-900 | 7 | |
| | southeastern | 3 | | | 900-1000 | 8 | |
| | west | 4 | | | 1000-1100 | 9 | |
| | east | 4 | | | 0-5.51 | 9 | |
| | flat | 6 | | | 5.51-11.69 | 8 | |
| | limestone | 9 | | | 11.69-17.53 | 7 | |
| | Dolomitic limestone | 7 | | Slope value (%) | 17.53-23.54 | 6 | 14 |
| | Marbly limestone | 4 | | | 23.54-30.57 | 5 | |
| **lithology** | conglomerate | 2 | 9 | | 30.57-66.17 | 3 | |
| | Marl and salt | 2 | | | 0-25 | 2 | |
| | shale | 2 | | Drainage (%) | 25-50 | 4 | 4 |
| | alluvium | 1 | | | 50-75 | 7 | |
| | Plain | 1 | | | >75 | 9 | |
| | Very highly developed karst | 9 | | | 0-20 | 2 | |
| | Highly developed karst | 8 | | | 20-40 | 4 | |
| | Moderately developed karst | 6 | | | 40-60 | 6 | |
| **Karstic domains** | Low development of karst | 4 | 28 | Fracture density (%) | 60-80 | 8 | 11 |
| | Non-indicative development of karst | 2 | | | >80 | 9 | |
| | Very high | 9 | | | | | |
| | high | 8 | | | | | |
| **Vegetation cover** | moderate | 6 | 13 | | | | |
| | low | 4 | | | | | |
| | Very low | 2 | | | | | |

**Table 2.** Effective weight of each recharge potential factor in the area

| | **Factors** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Karstic domains | Lithology | Slope | Fracture density | Precipitation | Vegetation cover | Drainage density map | Slope direction |
| **weight** | 28% | 9% | 14% | 11% | 13% | 13% | 4% | 9% |

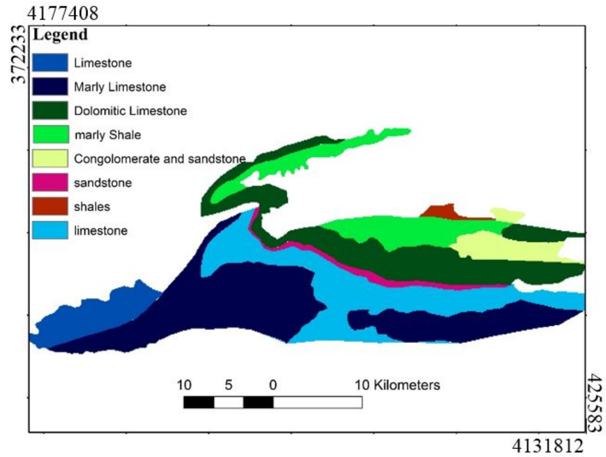


**Figure 3.** Map of the lithology distribution in the study area

*Slope*

The topography slope is one of the geomorphologic factors that influence the amount of infiltration and recharge rate. Topography of ground surface is effective in changing the temperature and precipitation value, vegetation type as well plays a role by creating different slopes and its effect on the amount of generated runoff, the recharge rate and, finally, the development of karst regions. The topographic slope directly influences the recharge rate. With increasing the topography slope gradient, the recharge rate to the aquifer decreases due to the rapid formation of surface runoff in the steep slopes and the lack of sufficient time to recharge to the saturated zone. The slope factor has an inverse relationship with the groundwater recharge. The data from the digital terrain model (DTM) database are used to prepare map of variation and distribution of topographic slope values in the study area (Fig. 4).

*Aspect*

The sun's angle at the north and northeast of the area is different from that of the south and southwest. The snow fall is the main precipitation in this area. The snowpack remains on the northern flank of the area for longer periods and melts over time, creating a favorable environment for more recharge. The layer of aspect was prepared using the regional digital model and classified in the GIS environment. So, north facing slopes score higher than the others (Table 1 and Fig. 4).

*Fracture (lineament) density*

Lineaments and faults facilitate the movement and storage of groundwater. They increase infiltration to the subsurface layers. Lineaments are generally referred to fault and linear zones in the RS analysis of the fractures or structures. Lineaments are the simple and complex linear properties of geological structures (Sener et al., 2005). Faults and fractures are weaknesses in each region that can increase the infiltration rate, and due to the dissolution of subsurface channel, groundwater easily flows along them. The role of the fault in controlling and transferring groundwater, depending on the fault condition and the region, can be neutral, positive or negative. The most accurate method of plotting linear paths is field approach, but this is not possible due to the limited view and the problems caused by field operations. In satellite imagery, large lines that go beyond the limits of aerial image cannot be revealed due to their large scale but using satellite imagery with a wide field of view can provide reliable maps of the lineament. To identify fractures and faults in the study area, geological maps, satellite imagery and Google Earth software was used. The density map was prepared using the ENVI software (Fig. 5).

*Drainage density map*

Another influential factor in recharge rate is a surface phenomenon known as drainage system. Drainage density was determined by dividing the total rivers to the total area of the drainage basin. The extraction and analysis of the drainage network was conducted based on information from field data, topographic maps, aerial photographs and satellite images. The quality of a drainage network depends on lithology, which provides an important index of the infiltration rate. In order to calculate the drainage frequency, the layers of drainage density were mapped using ArcGIS digital elevation model (Fig. 6).

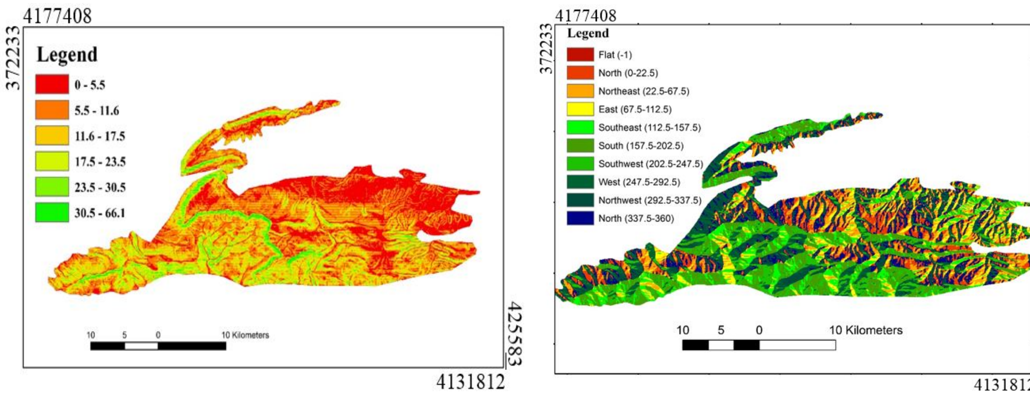


**Figure 4.** Spatial distribution map of (a) slope gradient (left) and (b) aspect (right) in the study area

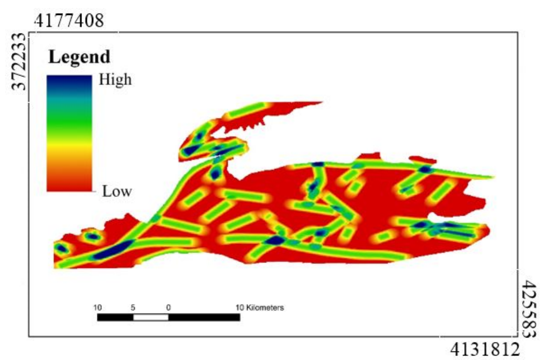


**Figure 5.** Fracture (lineament) density map of the Kalaleh basin

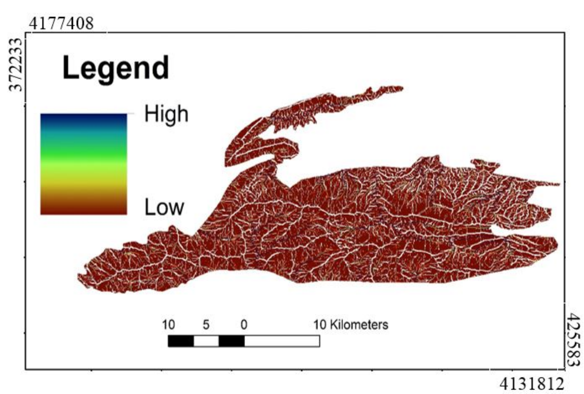


**Figure 6.** Drainage map of the study area

Flow directions are more important in areas with low slopes or at the intersection of faults, because water has more time to recharge. The higher score is proposed for drainage systems that reserve more volumes of water over longer periods of time.

*Precipitation*

Precipitation is another important factor affecting the infiltration of waters to a vulnerable reservoir (Sener et al., 2005). The climate condition and altitude factor effect the precipitation amount in the area. The type of precipitation and its intensity affect the rate of recharge. Recharge rate to the karstic aquifer increases during the snow-fall and slow rainfall events which have higher scores in comparison with the storm rainfall. The precipitation layer of the study area was prepared and shown in Figure 7.

*Karstic domains*

The variation of superficial karst features in carbonate formations suggests a good solubility of these formations and it creates a suitable environment for the development of karst landforms, more recharge rate and subsequent storage of groundwater. The dissolution process often begins in and extends from existing geologic structures. Based on geologic and topographic maps and field investigations, various landforms of superficial karst were observed. The major types of developed karst features in the area are sinkholes, Karen and dry valleys. The areas with non-apparent karst which significantly affect the recharge rate are covered by thick soil in the study region. Areas with major and non-apparent karstic domains have been pointed out as a thematic layer in ArcGIS (Fig. 8).

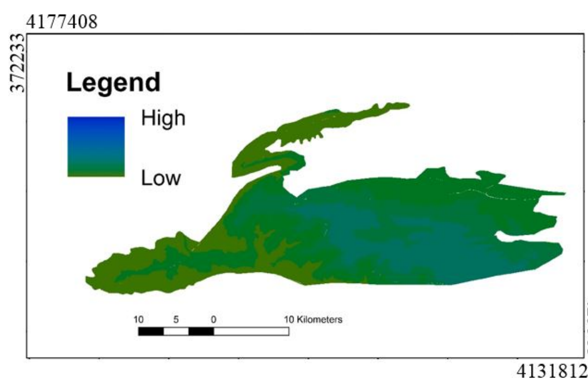


**Figure 7.** The layer of precipitation prepared using GIS software. Precipitation is mainly in the form of snow. It has a direct relation with elevation.

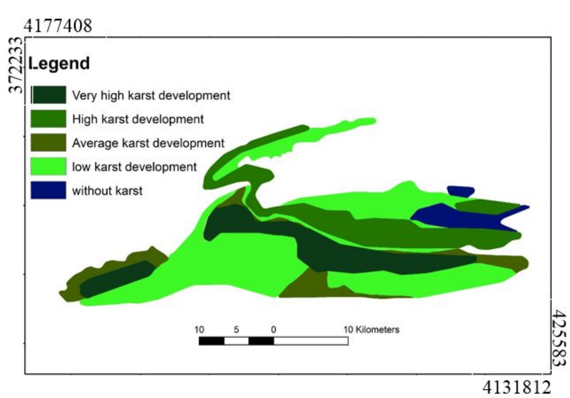


**Figure 8.** Map of karstic zones in the study area

*Land cover*

The vegetation cover and soil deposits are the main components of the land cover factor. Vegetation has both positive and negative effects on karst development. The roots pressure of the plants causes the destruction and fracture of the rocks which creates a permeable space next to them. The presence of plant leaves increases the humus content of the soils, and the increase in the $CO_2$ resulted from root nutrition reduces the acidity of the moving water and then increases the dissolution of carbonate rocks. In the event of a low rainfall rate in dense and massive vegetation area, the arrival of atmospheric waters to the ground surface is reduced and these waters evaporate directly from the surface of the leaves. The recharge rate and evapotranspiration are mainly affected by land use and land cover factors. According to the study area, which has a high slope and high rainfall, a positive factor is considered. The density of different vegetation can be determined based on the degree of influence on percolation. Thus, the high density of vegetation, takes higher score in comparison to lower vegetation density (Table 1 and Fig. 9).

*Weighted overlay analysis*

Weighted overlay methods were used to integrate and analyze influential factors to obtain a recharge potential (RP) map (Senanayake et al., 2016; Elbeih, 2015; Rwanga & Ndambuki, 2017). The weight of each layer was determined based on expertise judgment. In order to determine the recharge rate of the area, the thematic layers including the drainage density, the density of lineaments, lithology, slope, aspect, precipitation, karstic domains and vegetation cover were reclassified in the range of 1 to 9. The values given to each class of the thematic layers are presented in Table 1. The score increases as the significant influence on recharge rate enhances. To obtain groundwater potential zones, after assigning the above weights and scores, the weighted overlying approach was used. Finally, the five classes were determined on final map of RP (Fig. 10). The annual recharge percentages and area of each class are presented in Table 3.

**Table 3.** Recharge percentages and area of each order in the study area

| Area ($Km^2$) | Annual recharge (%) |
|---|---|
| 80 | <38 |
| 160 | 38-40 |
| 120 | 40-42 |
| 130 | 42-44 |
| 90 | >44 |

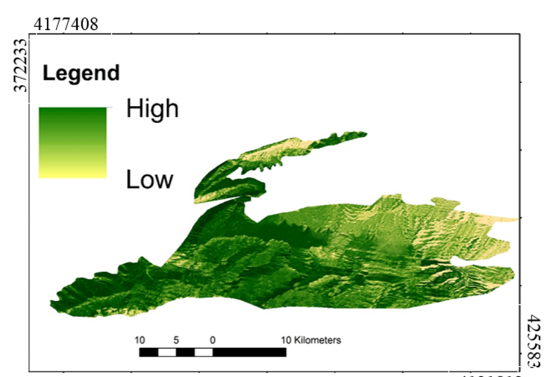


**Figure 9.** The density map of different vegetation based on the degree of influence on infiltration. The high density of vegetation, take higher score in comparison to lower vegetation density

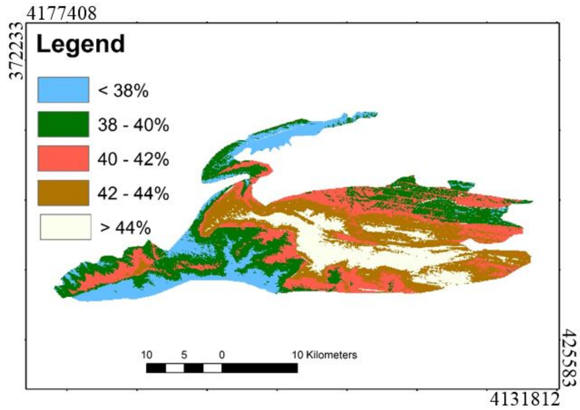

**Figure 10.** Map of recharge potential zones in the study area

*Hydrogeological budget of karstic area*

A hydrological budget calculates the difference between the input and output waters to the aquifer and resulted changes in the volume of reservoir storage during a particular period (Yeh et al., 2016; Meresa & Taye, 2018). The main objective of the water budget calculation in the karstic formations is determining the total volume of recharged and drained groundwater and consequently the catchment area of the main karstic springs (Zaw, Aghsoo & Yal-cheshme) in the studied area. In order to calculate and estimate the water budget, the area of the karst formations, average annual rainfall and recharge percentage were determined. The total karst area of the study region is approximately 290 km$^2$. The average rainfall in this area is 829 mm and the percentage of recharge to the karstic formation based on the pervious section, is about 42%. The volume of annual recharge in this region is about 100 Mm$^3$. The total amount of annual discharge via the springs is about 65.5 Mm$^3$. Therefore, it can be concluded that the karstic reservoir of the region has more storage in comparison to the groundwater discharge by the springs and that karstic mountains totally encompass the catchment area of the mentioned karst springs.

*Determining catchments area of the karst springs*

The initial boundary of catchment area of the important springs has been determined using the geological method which includes the stratigraphically state, geomorphology and tectonics of the region (Fig. 11). Using the hydrological budget method (equation 1), the catchment area of the spring was corrected, and then the amount of recharge obtained in the catchment area of springs was compared. At this stage, if there was no significant difference between the annual discharge of the springs and the amount of recharge obtained, and the relative error rate between them is small, then the first drawn catchment area is confirmed.

*Zaw spring catchment area*

The geological and hydrogeological methods have been used to determine the probable catchment area of the Zaw spring. According to the stratigraphic sequence tectonic and morphological conditions of the area, the initial boundary of the catchment area of the spring was determined in an area of about 85 km$^2$ (Fig. 12). This spring, with annual discharge of 23.3 Mm$^3$, is emerging from Lar limestone formation in the northern part of the area which is mostly covered by vegetation and trees. The hydrogeological water budget method was also used to confirm the calculated geological boundary of catchment area for the Zaw spring. Based the water balance calculation and annual discharge of the spring (23.3 Mm$^3$) and annual recharge of 29.4 $Mm^3$, about 83.5 $km^2$ of catchment area belongs to this spring which has little difference with initial geological catchment area.

*Aghsoo spring catchment area*

To determine the probable boundary of the catchment area of the Aghsoo spring, the geological and hydrological water budget methods were used. According to the stratigraphic sequence, the tectonic condition and morphology, the probable area of the basin for this spring was determined (Fig. 13) which was about 32 $km^2$. This spring is emerging from the Mozduran limestone formation and along shear fractures. The groundwater flow direction and karst development are mainly affected by joints and faults in the area.

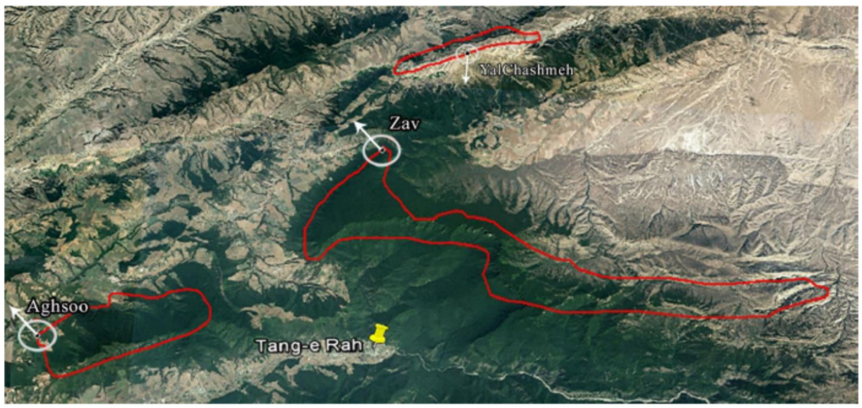


**Figure 11.** The initial boundary of catchment area of the important springs in the region using the geological method which includes the stratigraphically state, geomorphology and tectonics conditions of the region

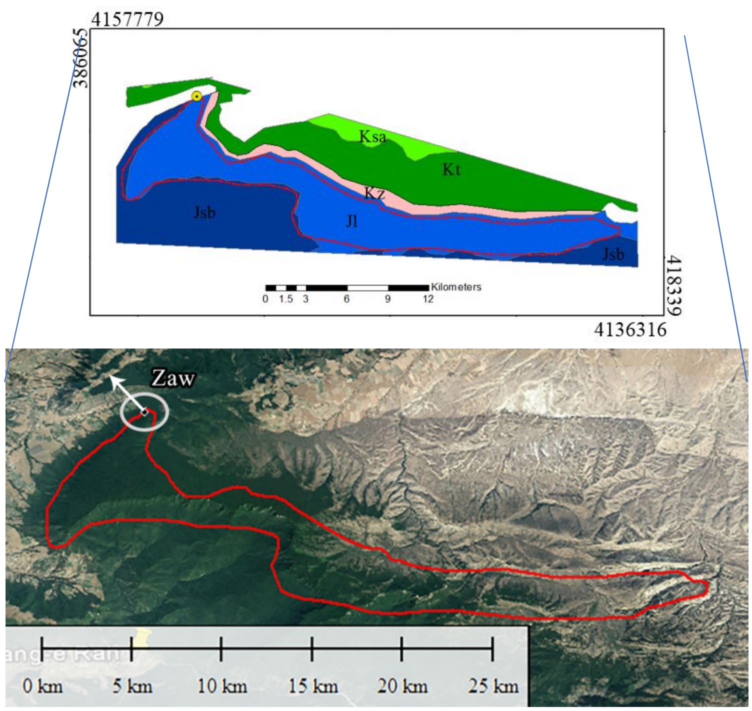


**Figure 12.** Catchment area of the Zaw spring in both geological map and Google Earth map

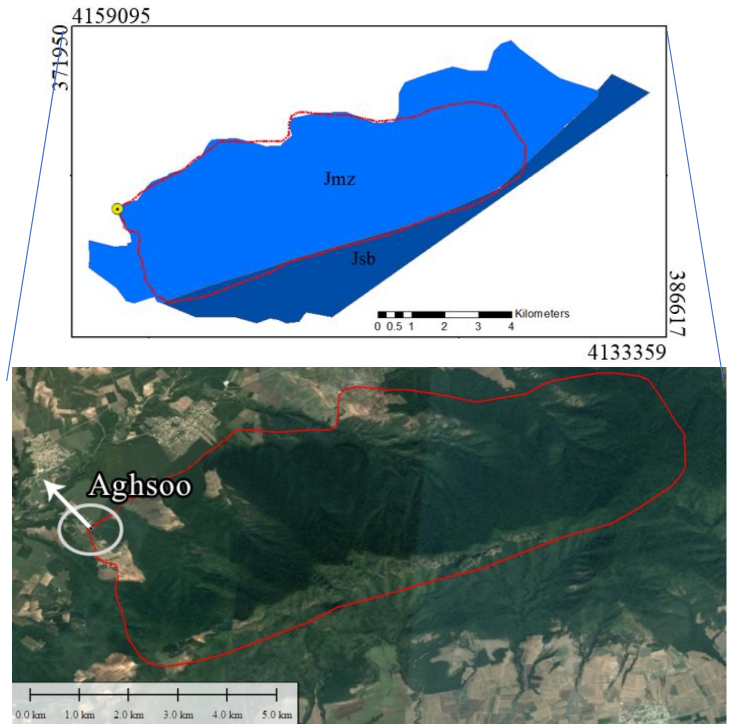


**Figure 13.** Catchment area of the Aghsoo spring

In the south and southeast borders of catchment area, the impermeable layers of Chaman-Bid marl and shale Formation act as a hydrogeological non-karst barrier and thus prevent the groundwater movement toward the recent adjacent alluvial deposits. The center and near northern border of the catchment area is limited to the fault as a conduit, which transfers water from melting snow in the mountains to the spring. The hydrogeological water budget method has also been used to confirm the calculated geological boundary of catchment area for the Aghsoo spring. Based the water balance calculation and annual discharge of the spring (7.4 $Mm^3$) and annual recharge of 9.4 $Mm^3$, about 31.8 $km^2$ of catchment area (Mozduran limestone formation) belongs to this spring which has little difference with the initial catchment area determined by geological method (32 $km^2$).

*Yal-cheshme spring catchment area*

The Yal-cheshme spring emerges between the contact of Tirgan and Sarcheshmeh limestone Formations. The joints and faults act as a feeder boundary in the development and flow direction of groundwater in the catchment area of this karst spring which transmits water from melting snow in the mountains to the spring. Based on sequence stratigraphy, the catchment area predominantly includes Tirgan limestone Formations with an area of 9 $km^2$. Based the water balance calculation and annual discharge of the spring (2.14 $Mm^3$) and annual recharge of 2.66 $Mm^3$ with recharge rate of 39%, about 9.1 $km^2$ of the catchment area (Tirgan limestone Formation) belongs to this spring which has little difference in comparison to primary determined geological catchment area (9 $km^2$) (Fig. 14).

## Conclusions

The demand for fresh water in Kalaleh region is rapidly increasing. Determining the recharge potential zone and catchment area is needed in order to maintain the long term sustainability of water resources.

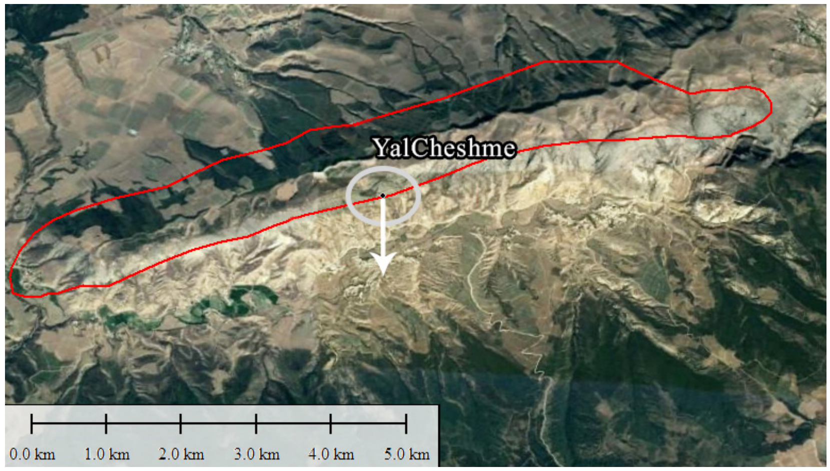


**Figure 14.** Catchment area of Yal-cheshme spring

The main karstic springs in the Kalaleh area mainly discharge from Lar, Tirgan, and Mozduran karstic formations. The two adjacent Lar and Tirgan karstic formations have the potential to form the catchment area of Zaw spring. Different karst features such as closed depression, sinkhole, joint and fractures were observed in the Lar formation during field investigation which led to conclude that Zaw spring is recharged from Lar karstic formation. The layers of karstic domains, slope, aspect, precipitation, fracture density, lithology, drainage density and land cover map were integrated on a GIS platform employing the weighted linear combination method. The results indicated that the most effective groundwater recharge potential zone was located in the central and north east of the area. In this region, the karstic formations have high infiltration capacity. Also, the results indicate that the annual recharge in Zaw, Aghsoo and Yal-cheshme spring's catchment area is 44, 41, and 39%, respectively. Based on the hydrological water budget and geology methods, the catchment area of Zaw, Aghsoo and Yal-cheshme springs are determined as 83, 32 and 10 km$^2$, respectively. In comparison, the effective karstic formations in recharging process in order of significance are Lar, Mozduran and Tirgan. Therefore, it can be concluded that the Lar formation has higher potential for karst development and infiltration than others. These results can be applied in other similar regions. It also provides good baseline information for water resources experts and policymakers in order to efficiently manage water resources in future development planning.

**Acknowledgments**
The authors thank the Research Council of Shahrood University of Technology for continuous support during this investigation.

**Authors' contributions:**
All authors contributed to the study conception. Material preparation, data collection and analysis were performed by R. Bagheri and GH. Mirhasani. The first draft of the manuscript was written by R. Bagheri and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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