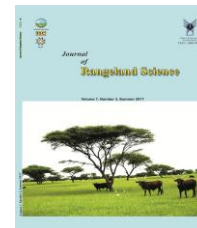


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Research and Full Length Article:

Recognizing Ecological Species Groups and their Relationships with Environmental Factors at Chamanbid-Jozak Protected Area, North Khorasan Province, Iran

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Abstract. Classification of Ecological Species Groups (ESG) in plant community analysis is one method to describe vegetation and relating them to environmental factors. This study was conducted to recognize ESG and their relationships with some environmental factors in Chamanbid-Jozak protected area from 2014 to 2016. This area is located in the eastern part of North Khorasan province, Iran. The study area contains steppe vegetation with some woodlands and shrubs of *Paliurus spina-christi*, *Cerasus pseudoprostrata*, and *Cerasus microcarpa*. To recognize ESG, a systematic-random sampling, by using 1 m² Sampling Unit (SU), was carried out to provide a matrix of 74 sampling units and 42 species. Canopy cover percentage of different species was recorded in each SU. Physiographical, physical, and chemical factors, including altitude, slope, soil texture, gravel percentages, Organic Carbon (OC), Nitrogen (N), Potassium (K), lime, soil acidity (pH), Phosphorus (P), and Electrical Conductivity (EC) were measured. Euclidean distance and Ward's method of clustering were used to classify the plant species. Six ESG were detected in clustering and indicator species analyses. The relationships among these ESG and environmental factors were analyzed using Canonical Correspondence Analysis (CCA). The first ESG consisting four indicator species was influenced by organic carbon and K of almost f.0 fertile soils. ESG2 and ESG3 consisting four and one, indicator species, respectively. These ESG were only slightly affected by phosphorus and less by environmental factors evaluated in this study. ESG4, including eight indicator species was mostly affected by environmental factors such as altitude, slope, pH, and N. Both ESG5 and ESG6 were affected by gravel percentages. ESG classification of vegetation for sound and proper resource management in future via using long-term projects is recommended.

Keywords: Euclidean distance, Ward's method of clustering, CCA, Indicator species

Introduction

Numerous methods are available to describe the vegetation on the basis of classification into vegetation units (Daubenmire, 1968; Kershaw and Looney, 1985; Mueller-Dombois and Ellenberg, 1974; Whittaker, 1962). Two-way indicator species analysis (TWINSPAN) as a divisive hierarchical method is one of the most famous methods of grouping indicator plant species (Hill, 1979; Gauch and Whittaker, 1981) and has been widely used in numerical classification (Greig-Smith, 1983; Kent, 2012). In recent years, this method has also become one of the most criticized (e.g. McCune and Grace, 2002). Legendre and Legendre (1998) also criticized the complexity of the method and the artificial nature of pseudo-species employed in this method.

Ecological species groups (ESG) consist of groups of species that simultaneously occupying homogeneous habitat with similar environmental affinities (Spires and Barnes, 1985; Godart, 1989; Grabher *et al.*, 2003). In combination with analysis of communities, classifying ESG is one method to describe vegetation and relating them to environmental factors (Dufrêne and Legendre, 1997).

ESG is achieved by using cluster analysis and like TWINSPAN is agglomerative, hierarchical, and the ultimate results are presented as a dendrogram (Kent, 2012; Borcard *et al.*, 2011; Legendre and Legendre, 1998). Agglomerative Clustering proceeds by taking many separate observations and grouping them into successively larger clusters until one cluster are obtained (Greig-Smith, 1983; Gotelli and Ellison, 2013). In hierarchical method, clusters with few observations should be embedded within higher-order clusters with more observations among different methods of clustering. Ward's method (minimum variance) with Euclidean

distance is a common strategy in cluster analysis (Gotelli and Ellison, 2013).

In canonical correspondence analysis (CCA), composite gradients are linear combinations of environmental variables, giving a much simpler analysis that provides a summary of the species–environment relationships. In vector diagrams, vector lengths show the efficacy of each factor, with a longer length indicating a stronger relationship. Also, closeness of species or sampling unit to a vector shows its responsiveness to that factor. An occurrence of species or sampling unit near a vector indicates a positive relationship with that factor and its diminished relationship with the other factors (Kent, 2012; Tatian, 2009; Jangman *et al.*, 1987; Ghorbani *et al.*, 2003).

Using these methods in different studies have defined associations of plant species that have been used to create ecological groups in response to a complex collection of soil characteristics or a mixture of physiographic, climatic, and edaphic factors. Interactions between plant groups and environmental parameters provide a useful opportunity to alter management to improve rangeland ecosystems (Tatian, 2009). Eshaghi Rad and Banj Shafiei (2010) investigated the distribution of ESG in *Fagetum* communities of Caspian forest, Iran. Cluster analysis was used for the classification of vegetation samples. Their results showed that the distribution of the four ecological groups, recognized in the study area was better associated with an aspect, content of clay, total nitrogen, organic matter, and phosphorus. There was no direct relationship between ecological groups and elevation, slope, percentage of sand and silt, C/N ratio, and pH in the study area (Eshaghi Rad and Banj Shafiei, 2010). Zereen *et al.* (2015) studied natural vegetation of Lahore district, Pakistan. Vegetation data were evaluated using multivariate analysis method, i.e., TWINSPAN and

CCA. They found that the distribution of plant species with respect to environmental variables indicated that water pH, soil EC, water content, and water table had a great influence upon species distribution. Akbarluo and Nodehi (2016) investigated the effect of some environmental factors with distribution of medicinal plants using principal component analysis (PCA) at Ghorkhud protected area in North Khorasan Province, Iran. The results showed that the most important factors affecting the distribution of medicinal plants were organic matter, Nitrogen, and pH.

The results of vegetation classification can be used for ecological studies and practical monitoring of vegetation cover (Woldewahid *et al.*, 2007). Determination of vegetation types has been the subject of numerous studies in a wide range of environments (Orloci, 1968; Cowlishaw and Davies, 1997; Dias *et al.*, 2004). The aims of this study were:

1. Determination of floristic composition of steppe vegetation of Chamanbid-Jozak protected area;
2. Detection of ESP in the study area; and

3. Determination of the major environmental factors affecting ESG in these habitats.

Material and Methods

Study area

The Jozak-Chamanbid, a protected site with an area of 1129 ha, is located in Maine and Semelghan Counties in the western part of North Khorasan province, Iran, between latitudes of 37° 25' 59" to 37° 26' 5" and longitudes of 56° 36' 13" to 56° 40' 45" with an elevation of 1094 to 1798 m.a.s.l (Fig.1). Based on the Emberger classification, the climate is semi humid. According to the nearest meteorological station the mean annual precipitation is 560 mm. The annual mean maximum and minimum temperature have been recorded in July (22.1°C) and in February (0.0°C), respectively (Anonymous, 2015).

The soil of study area is mostly silt loam with an average pH of 7.36. The vegetation includes mostly herbaceous species with some woodlands and shrubs of *Paliurus spina-Christi* Miller, *Cerasus microcarpe*, C. A. Mey—Boiss. and *Cerasus pseudoprostrata* Pojark.

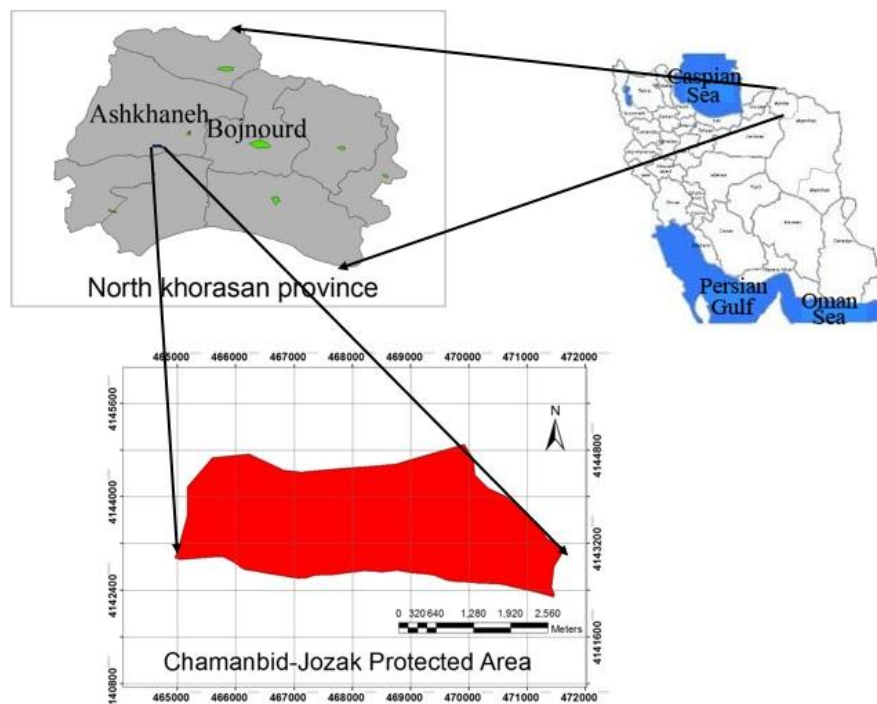


Fig. 1. Study area and its situation in relation to North Khorasan Province, Iran.

Sampling Method

In this study, we used systematically-random method for sampling. Plot with sizes of 1, 25, and 100 m² were selected for sampling herbs, shrubs, and trees, respectively (Kent, 2012). In each of 74 sampling units, we recorded plant species list, canopy cover percentages of herbaceous species, slope, and altitude. Due to the relationships among ESG and edaphic factors, soil samples were taken close to the plots from a depth of 0-30 cm (Northup *et al.*, 1996). Oven air-dried of soil samples were prepared for physical and chemical soil analysis.

The soil properties such as total nitrogen (N) were analyzed by the Kjeldahl method (Bremner, 1996). Available phosphorus (P) was measured by a colorimeter according to the Bray-II method (Bray and Kurtz, 1945). Organic carbon (OC) was determined by Walkley Black (1934) method. Soil electrical conductivity (EC) and acidity (pH) were determined by using pH and EC meters. Soil texture was determined by the hydrometric method (Bouyoucos, 1962). Total potassium (K) was analyzed by Flame atomic absorption spectrophotometer (MAPA, 1994). The lime was estimated by titration (Bundy and Bremner 1972).

Data analysis

Before analyzing, species with less than 5% frequency were removed from the data matrix as recommended by Van der Maarel (1979). To classify the SU by Q-mode (Ludwing and Reynolds, 1988), we

have used Euclidean distance and Ward's method of clustering to group sampling units. ESG was considered as indicator species in each group of SU. Based on combined information on the concentration of species abundances in a particular sampling group, indicator values were tested for statistical significance by using Monte Carlo randomization test (Dufrêne and Legendre, 1997).

The relationship between ESG and environmental variables were identified by using CCA. This method shows simultaneously the environmental variables and distribution of plant species (Ter Braak, 1986). The detail of graphic interpretation of CCA was explained by Kent (2012).

In ordination graph, environmental factors which were correlated with other factors such as soil texture and EC have been excluded to prevent collinearity (Ozkan *et al.*, 2010).

Multivariate data analysis and indicator species analyses were accomplished by R freeware (<http://cran.r-project.org/>) and PC-ORD version 5.

Results

List of species

A total of 134 species and 100 genera were recorded in 74 SU (Table 1). List of species and growth form of the 42 species of more than 5% frequency used the analysis is presented in the Table 1.

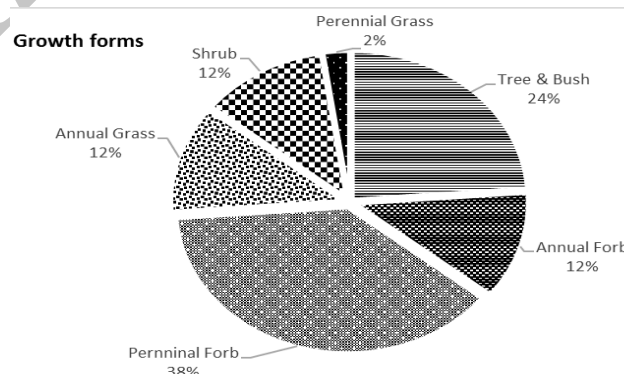
Table 1. List of species recorded in 74 sampling units at study area.

Scientific name	Abbr.	Growth form
<i>Acer monspessulanum</i> L.	Acmo	Tree
<i>Alyssum desertorum</i> Stapf	Alde	Annual Forb
<i>Androsace maxima</i> L.	Anma	Annual Forb
<i>Artemisia kopetdaghensis</i> Krasch., M.Pop. & Lincz. ex Poljak.	Arko	Bush
<i>Asperula glomerata</i> (M.B.) Griseb.subsp <i>turcomanica</i> (Pobed.)shonb-tem	Asgl	Perennial Forb
<i>Astragalus jolderensis</i> B.Fedtsch.	Asjo	Bush
<i>Astragalus verus</i> Olivier	Asve	Bush
<i>Avena sativa</i> L.	Avsa	Annual Grass
<i>Boissiera squarrosa</i> (Banks & Sol.) Nevski	Bosq	Annual Grass
<i>Bromus danthonia</i> Trin	Brda	Annual Grass
<i>Centaurea behen</i> L.	Cebe	Perennial Forb
<i>Cerasus microcarpa</i> (C.A.Mey.)Boiss.	Cemi	Shrub
<i>Cerasus pseudoprostrata</i> Pojark.	Ceps	Shrub
<i>Codonocephalum peacockianum</i> Aitch. & Hemsl.	Cope	Perennial Forb
<i>Colutea porphyrogramma</i> Rech.f.	Copo	Shrub
<i>Convolvulus calvertii</i> Boiss.	Coca	Bush
<i>Convolvulus dorycnium</i> L.	Codo	Bush
<i>Cousinia decipiens</i> Boiss.& Buhse	Code	Perennial Forb
<i>Dianthus crinitus</i> Sm.	Dicr	Perennial Forb
<i>Echinaria capitata</i> (L.)Desf.	Ecca	Annual Grass
<i>Ephedra intermedia</i> Schrenk et C.A.Mey.	Epin	Shrub
<i>Eremurus spectabilis</i> M.Bieb.	Ersf	Perennial Forb
<i>Gaillonia oliveri</i> A.Rich	Gaol	Bush
<i>Galium verum</i> L.	Gave	Perennial Forb
<i>Haplophyllum perforatum</i> (M.B.) Kar. & Kir.	Hape	Perennial Forb
<i>Hymenocrater bituminosus</i> Fisch. & C.A.Mey.	Hybi	Bush
<i>Juniperus exelsa</i> M.B	Juex	Tree
<i>Lagochilus cabulicus</i> Benth.	Laca	Perennial Forb
<i>Paliurus spina-christi</i> Miller	Pasp	Shrub
<i>Phlomis cancellata</i> Bunge	Phca	Perennial Forb
<i>Poa bulbosa</i> L.	Pobi	Perennial Grass
<i>Prangos latiloba</i> Korov.	Prla	Perennial Forb
<i>Scabiosa rotata</i> M.B.	Scro	Annual Forb
<i>Scandix stellata</i> Banks & Soland.	Scst	Annual Forb
<i>Serratula latifolia</i> Boiss.	Sela	Perennial Forb
<i>Steptorrhampus tuberosus</i> (Jacq.)Grossh.	Sttu	Perennial Forb
<i>Taeniatherum crinitum</i> (Schreb)Nevski	Tacr	Annual Grass
<i>Teucrium polium</i> L.	Tepo	Perennial Forb
<i>Tulipa micheliana</i> Hoog	Tumi	Perennial Forb
<i>Tulipa montana</i> Lindl.	Tumo	Perennial Forb
<i>Verbascum cheiranthifolium</i> Boiss.	Vech	Bush
<i>Viola tricolor</i> L.	Vitr	Annual Forb

Abbr. = abbreviation

The pie chart of growth forms is shown in Fig. 2. As in Fig. 2, herbaceous species dominant in the study area which are

mostly perennial forbs, with some bushes and few trees were also seen in wet meadows.

**Fig.2.** Composition of growth forms in the study area.

Clustering and indicator species analysis

By clustering of 74 SU, 6 groups of sampling units were detected (Fig. 3).

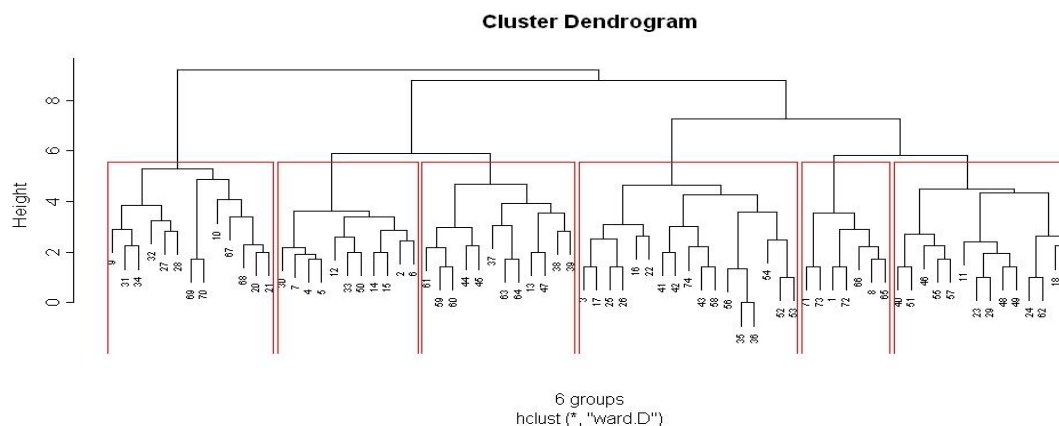


Fig. 3. A dendrogram of partition 74 sampling units into six clustering groups (CG).

Accompanied method of indicator species analysis (Dufrêne and Legendre, 1997) with Monte Carlo test (Table 2) were led to identify the indicator species

of the 6 different ESG. Species with significant p-values designated to its ESG (Table 3).

Table 2. Introducing indicator species in each ecological species group (ESG) based on Monte Carlo test.

Species name	P-value	ESG	Species name	P-value	ESG
<i>Acer monspessulanum</i>	0.004	6	<i>Dianthus crinitus</i>	0.010	1
<i>Androsace maxima</i>	0.040	4	<i>Haplophyllum perforatum</i>	0.005	5
<i>Artemisia kopetdaghensis</i>	0.016	3	<i>Juniperus exelsa</i>	0.004	6
<i>Astragalus jolderensis</i>	0.011	2	<i>Paliurus spina-christi</i>	0.004	4
<i>Astragalus verus</i>	0.013	2	<i>Phlomis cancellata</i>	0.002	1
<i>Avena sativa</i>	0.002	1	<i>Prangos latiloba</i>	0.018	6
<i>Boissiera squarrosa</i>	0.002	4	<i>Scandix stellata</i>	0.014	4
<i>Bromus danthonia</i>	0.028	4	<i>Taeniatherum crinitum</i>	0.006	1
<i>Cerasus pseudoprostrata</i>	0.002	4	<i>Teucrium polium</i>	0.002	6
<i>Colutea porphyrogramma</i>	0.008	4	<i>Tulipa micheliana</i>	0.002	2
<i>Convolvulus calvertii</i>	0.002	1	<i>Tulipa montana</i>	0.043	4

Table 3. The ecological species groups (ESG), numbers of sampling units (SU) and indicator species (IS).

ESG	SU No.	IS No.	Species name
1	7	4	<i>Avena sativa</i> , <i>Dianthus crinitus</i> , <i>Phlomis cancellata</i> , and <i>Taeniatherum crinitum</i>
2	11	4	<i>Astragalus jolderensis</i> , <i>Astragalus verus</i> , <i>Convolvulus calvertii</i> , and <i>Tulipa micheliana</i>
3	17	1	<i>Artemisia kopetdaghensis</i>
4	13	8	<i>Androsace maxima</i> , <i>Boissiera squarrosa</i> , <i>Bromus danthonia</i> , <i>Cerasus pseudoprostrata</i> , <i>Colutea porphyrogramma</i> , <i>Paliurus spina-christi</i> , <i>Scandix stellata</i> , and <i>Tulipa montana</i>
5	14	1	<i>Haplophyllum perforatum</i>
6	12	4	<i>Acer monspessulanum</i> , <i>Juniperus exelsa</i> , <i>Prangos latiloba</i> , and <i>Teucrium polium</i>

Canonical corresponding analysis (CCA)

The CCA ordination of the data is shown in Fig. 4. Eigenvalues of 3 axes were 0.57, 0.46, and 0.44, respectively. The results of CCA indicated that OC, lime, and K were the most important factors in the first ESG separation with four indicator species. ESG2 and ESG3 consisting four and one indicator species, respectively. These ESG were only

slightly affected by P and less by environmental factors evaluated in this study. The ESG4 including eight species was mostly affected by altitude, slop, pH, and N contents of soil. Both ESG5 and ESG6 were affected by gravel percentages. Correlations between axes, environmental factors, and ESG were shown in Table 4.

Table 4. Results of Canonical Correspondence Analysis (CCA) of environmental factors, soil properties, and Ecological Species Groups (ESG)

Environmental factor	CCA1	CCA2	ESG	CCA1	CCA2
Altitude (Alt)	0.192	-0.009	ESG 1	0.327	0.453
Slope (SL)	0.566	-0.054	ESG 2	-0.475	0.044
Organic carbon (OC)	0.012	0.012	ESG 3	-0.207	0.567
Lime (LM)	0.246	0.202	ESG 4	0.768	-0.401
Soil acidity (pH)	0.206	0.007	ESG 5	-0.207	-0.185
Gravel % (GP)	-0.250	-0.222	ESG 6	-0.401	-0.367
Potassium (K)	0.055	0.092			
Phosphorus (P)	-0.041	0.046			
Nitrogen (N)	0.076	-0.101			

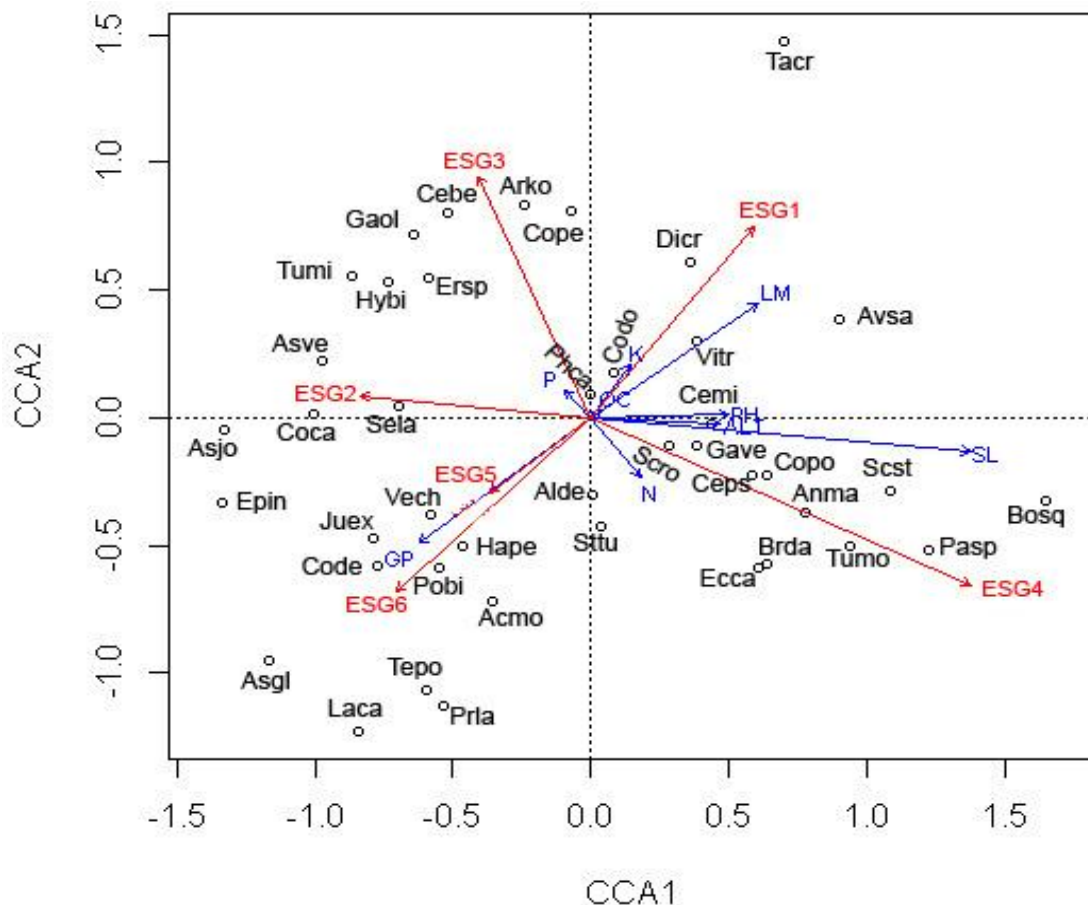


Fig. 4. Ordination diagram of CCA in Jozak-Chamanbid data. Environmental factors and ESG represented by arrows. Species show their positions in relation to environmental factors. Two first letters of genus and two first letters of the species are used to show the species in the graph. For abbreviations of species and environmental factors, see Tables 1 and 4 respectively.

Discussion

The CCA was conducted to determine the most effective environmental factors (Fig. 4) in discrimination of ESG (Fig. 3). The most important factor in the separation of the first group (ESG1) was lime (Fig. 4). Jafari *et al.* (2009) categorized vegetation in Zirkouh rangelands of Qaen. They found that lime was one of the effective factors in

separation of vegetation in the study area. Aliakbari *et al.* (2011) showed that lime affects *Astragalus verus* and *Agropyron trichophorum* distribution.

Another effective factor on characterizing ESG1 was OC% (Fig. 4). Arekhi *et al.* (2010) studied vegetation–environmental relationships of Ilam Oak forest landscape. Five ESG were specified in the study area. They

concluded that OC was the important factor in community separation. When the OC is high, biological activities are promoted.

K is important in characterizing the ESG1 too. Adel *et al.* (2014) have reported on the role of K in the separating ESG in non-harvested beech forests in the north of Iran. They expressed that K plays a role in regulation of photosynthesis, carbohydrate transport, protein synthesis, and other important physiological processes.

ESG2 and 3 were only slightly affected by phosphorus and less by environmental factors which were evaluated in this study (Fig. 4). Mataji *et al.* (2010) investigated the relationships between understory vegetation and some environmental factors in natural forest ecosystem. Results showed a significant relation between distribution of plant types and environmental factors. Environmental factors were soil acidity (pH), slope, and phosphorus (Mataji *et al.*, 2010). Aghaii *et al.* (2012) and Pourbabai and Adel (2015) have also reported the role of phosphorus in the distribution of plant species.

The CCA graph shows the situation of IS for every ESG (Fig. 4). Based on Table 3, the species *Avena sativa* L., *Dianthus crinitus* Sm., *Phlomis cancellata* Bunge, and *Taeniatherum crinitum* (Schreb) Nevski were the indicator species that were close to the vector of ESG1. Therefore, these species were occurred on fertile soils. Adjacency of indicator species to lime vector represent this species spread in lime soil. The two ESG2 and ESG3 include four indicator species (*Astragalus jolderensis* B.Fedtsch., *Astragalus verus* Olivier, *Convolvulus calvertii* Boiss., and *Tulipa micheliana* Hoog) and one species (*Artemisia kopetdaghensis* Krasch., M.Pop. & Lincz. ex Poljak.), respectively (Table. 3) and as these species belong to ESG2 and ESG3, they

are less affected by environmental factors in this research.

As shown in Table. 3, ESG4 including eight IS, was most affected by environmental factors, including altitude, slope, pH, and N contents of soil (Fig. 4). In separation of ESG4, the chemical contents of soil have an important role. The indicator species of this ESG (*Androsace maxima* L., *Boissiera squarrosa* (Banks & Sol.) Nevski, *Bromus danthoniae* Trin, *Cerasus pseudoprostrata* Pojark., *Colutea porphyrogramma* Rech.f., *Paliurus spina-christi* Miller, *Scandix stellata* Banks & Soland., and *Tulipa montana* Lindl. (Table. 3), were occurred in high altitude and steep slopes. On the other hand, pH and N in the ESG4 were higher than those in other ESG. High N caused fertile soil, thus ESG4 had 8 indicator species which was the largest in comparing to the other ESG.

Pourbabai *et al.* (2015) and Aghaii *et al.* (2012) have reported that altitude and slope affect the distribution of plant species. Basiri and Mahmodi Sarab (2012) showed that pH is the important factor in separating indicator in Kolzar Izeh area.

ESG5 and 6 were affected by gravel percentages (Fig. 4). Therefore, the distribution of the IS (Table 3) in ESG5 (*Haplophyllum perforatum* (M.B.) Kar. & Kir.) and ESG6 (*Acer monspessulanum* L., *Juniperus exelsa* M.B., *Prangos latiloba* Korov., *Teucrium polium* L.) were affected by gravel percentages. Nosrati *et al.* (2008) and Asadian *et al.* (2017) have also shown the same results in Darab and Gonbad, Hamadan area, respectively.

Conclusion

Each plant species has a specific relation to environmental variables and is affected by habitat conditions, ecological requirements, and its tolerance range. Understanding the important environmental factors of a given area

helps us to perform the conservation measures needed; and to recommend adaptable species for restoration and reclamation of similar areas (Jafari *et al.*, 2004).

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چکیده. این مطالعه برای شناسایی گروه گونه‌های اکولوژیک و ارتباط آن‌ها با بعضی از عوامل محیطی در مراتع حفاظت شده چمن‌بید- جوزک در شرق استان خراسان شمالی طی سال‌های ۱۳۹۳ تا ۱۳۹۵ انجام شده است. منطقه مورد مطالعه دارای پوشش گیاهی استپی است که در آن بعضی گونه‌های بوته‌ای مراتع مشجر مانند تلو و آلبالوی دانه‌ریز و آلبالوی پاکوتاه نیز مشاهده می‌شود. برای تفکیک گروه‌های اکولوژیک، از روش نمونه‌گیری تصادفی- سیستماتیک با پلات‌های یک مترمربعی استفاده شد که به ماتریسی از گونه‌ها و واحدهای نمونه‌گیری با ابعاد ۷۴×۴۲ ختم گردید. در هر واحد نمونه‌گیری معیار درصد پوشش تاجی ثبت گردید و عوامل محیطی شامل شیب، جهت، ارتفاع، عمق، بافت، pH و کربن آلی خاک همراه با عناصر شیمیایی K، N و P اندازه‌گیری شدند. برای طبقه‌بندی ماتریس گونه‌ها در واحدهای نمونه‌گیری از استراتژی خوشه‌بندی وارد (Ward) و شاخص عدم تشابه اقلیدوسی استفاده شد. روابط بین ۶ گروه اکولوژیک تفکیک‌شده با عوامل محیطی با استفاده از روش آنالیز تطبیق متعارفی (CCA) بررسی شد. اولین گروه شامل ۴ گونه شاخص بود که روی خاک‌های نسبتاً حاصلخیز رخ نموده و تحت تأثیر کربن آلی و پتاسیم خاک قرار داشت. دومین و سومین گروه‌های اکولوژیک هر کدام به ترتیب با ۴ و ۱ گونه شاخص تحت تأثیر فسفات محلول و غیرمتأثر از سایر عوامل محیطی بودند. چهارمین گروه شامل ۸ گونه شاخص بود که بیشتر تحت تأثیر عوامل محیطی نظیر ارتفاع، شیب، pH و نیتروژن موجود در خاک بودند و بالاخره پنجمین و ششمین گروه‌های اکولوژیک بیشتر بر روی خاک‌های سخت و صخره‌ای با درصد بالای سنگ دیده شدند. برای مدیریت صحیح و احیای پوشش گیاهی زیستگاه‌های طبیعی دست خورده، استفاده از گروه گونه‌های اکولوژیک توصیه می‌شود.

کلمات کلیدی: کلمات کلیدی: فاصله اقلیدوسی، گروه گونه‌های اکولوژیک، آنالیز خوشه‌ای، آنالیز تطبیقی متعارفی، گونه‌های شاخص