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AN EVALUATION OF CONSERVATION STATUS AND ECOLOGICAL ZONATION OF *ALNUS NITIDA*; A MONOPHYLETIC SPECIES OF THE SINO-JAPANESE REGION

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ABSTRACT

Alnus nitida is a monophyletic species of the Sino-Japanese belt i.e., western Himalayas and Hindu Kush region. The current study was aimed to explore vegetation structure, role of edaphic and climatic factors on distribution, plant zonation and conservation status of this Alder species in the western Himalayas and Hindu Kush parts of the Sino-Japanese region. The standard quadrat method was used for vegetation sampling. All the collected data were analyzed through Two-way Cluster Analysis, Cluster Analysis, Indicator Species Analysis and Canonical Correspondence Analysis using PC-ORD and CANOCO software's. Google Earth Path software (V 1.4.6) was used for the calculation of Extent of Occurrence (EOO) and Area Of Occupancy (AOO) in conjunction with IUCN red list criteria for evaluation of conservation status of *Alnus nitida.* A total of 146 plant species associated with *Alnus nitida* were reported belonging to 106 genera and 47 families from the region which clustered in 3 major zones. It was concluded that electrical conductivity, phosphorus concentration along with sandy loam soil condition and grazing pressure were the main environmental variables that play a significant role in vegetation structure, associated flora and distinct co-indicators of the *Alnus nitida* in the Sino-Japanese belt. The current study will provide a baseline for further comprehensive studies on *Alnus nitida* to explore its sustainable use and conservation priorities.

Keywords: *Alnus nitida*; Plants zone; Conservation Status; IUCN; Sino-Japanese; Multivariate statistics.

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INTRODUCTION

Vegetation is a constitutive segment of an ecosystem that shows all the outcomes of an environment (Gray *et al.,* 2019). Vegetation is an important part of governing the shape of an ecosystem. It reflects plant species that are in association with various environmental factors like soil, atmosphere, anthropogenic activities and other crude resources of a region. It is the outcome of habit, environmental conditions and floral composition. Vegetation shows a complex variety in a cyclic way. It starts with one season to the next and further uncovers the change in a successional path throughout the years. These changes in the vegetation complex propose a particular reaction of each plant species to the predominant natural conditions (Abbas *et al.,* 2019; Pirini *et al.,* 2014; Anjum *et al*., 2020). The vegetation of any area represents potential future changes in an area. On another hand, the sample size affects vegetation quantification (Iqbal *et al.,* 2015). The change in sample plot size may also result in a change in vegetation i.e. appearance or disappearance of vegetation. Due to the change in sample plot size, the vegetation may transfer from positive to negative or from negative to positive. Change of sampling size and sampling procedures may result in various classifications. Likewise, vegetation shows an impact on relationship among the species aggregation or outcome.

Vegetation structure of an area is controlled by its environmental conditions like temperature, water and soil (Kumar *et al.,* 2019). Climatic variation has a great influence on the ecosystem, especially an area which has a fragile contact between climate and ecosystem. Climatic fluctuation has a significant effect on vegetation dynamics and ecosystems at a global and local level as well (Wang *et al*., 2018). It provides a base for the evolutionary behavior of flora. Natural events like a pro-genitive cycle, the effect of abiotic factors and seedling growth (Gungor *et al.,* 2019; Rahman *et al*., 2020; Shah *et al.,* 2020), has also a great influence on vegetation structure. These changes occur during soil-plant and plant-plant interaction. It also plays a major role in territory/habitat formation for fauna. It is considered to be a system that is structured according to space, function and timing features. In the modern era, different statistical techniques are applying to analyze the vegetation dynamics (Ahmad *et al*., 2016). Multivariate techniques are one of them, for the classification and ordination of vegetation of an area/ecosystem. Indicator species of an area or community were identified while correlating plant species with environmental variables such as soil texture, EC, pH, Phosphorous, Potassium, saturation, Grazing pressure, altitude slope through these statistical packages (Ahmad *et al*., 2019).

A total of 5220 plant species has been documented for the flora of Pakistan (Ali, 2008; Haq, 2019). Out of which numerous plants are facing a high risk of extinction due to anthropogenic disturbances. According to (Jan and Ali, 2009) very little research documentation has been available on the threatened flora of Pakistan. However, 500 species were claimed to be threatened and needs evaluation via IUCN red list categories and criteria. The conservation status of any plant species is assessed by the IUCN red list category and criteria which predict the future and present situation of any plant in its geographical range. *Alnus nitida* (Himalaya Alder) is monophyletic species of the Holarctic kingdom belong to family Betulaceae. It is riparian and sometimes considers as a successional tree to initiate flora in rivers coast. *Alnus nitida* population is reducing in different ranges of Sino Japanese belt due to Water Scarcity Stress that leads to leaf fall and mitigate Chlorophyll contents (Davies and Zhang, 1991). The Sino-Japanese belt is bestowed with many endemic and monophyletic species; numbers of scientists are working on different aspects of such plants such as ecology, edaphology, Red listing and associations.

Alnus nitida is a monophyletic species of the Sino-Japanese region facing huge threats. Keep in view, different factors responsible for the reduction of *A. nitida* population in Sino Japanese belt the current study was designed. The main focus of this research work was to document, classify and quantify plant species into various associations and analyzed the influence of different environmental variables on vegetation structure and its composition. It also evaluates the conservation status of *Alnus nitida* in the Sino-Japanese region via IUCN Red List categories and criteria. It was hypothesized that the edaphic and climatic factors are responsible for the formation of different plant zonation associated with *Alnus nitida* each with distinct co-indicators. This study will provide a baseline for further research in the field of ecology especially in terms identification of ecological indicators via robust multivariate statistical approaches. It could also be used to determine the conservation status of any species in general and endemic in particular via standard IUCN Red List categories and criteria.

MATERIALS AND METHODS

The Sino-Japanese Belt: There are four Phyto-geographical regions i.e., Sino-Japanese Region, Irano-Turanian Region, Indian Region and Saharo-Sindian Region in Pakistan (Takhtajan, 1980). Each of the regions has been defined by certain aid of category and criteria. The western boundary of the Sino-Japanese region is up to western Nepal and spreads up to the west limit of Himachal Pradesh (Takhtajan, 1980) (Fig. 1). It spreads through Pakistan into Afghanistan in their western limit, supported by the interruption of the high rainfall (180 cm) regions. Before glaciation, the present Sino-Japanese flora has relatively little changed from the vegetation of the north temperate regions that surrounded the whole of the northern hemisphere (Haq *et al*., 2016). Flora of the northern temperate zone is one of the richest, especially in trees. Sino-Japanese elements represent 10% of the total flora of Pakistan.

Vegetation Sampling: A total of six stations (Bajaur, Dir, Swat, Buner, Hazara, and Kashmir) from Sino-Japanese Region were surveyed for vegetation sampling during the years of 2017-18. Each station was further divided into different numbers of transects. At each transect, 2-5 numbers of quadrat were taken place having a size of 10×10 m, 5×5 m and 1×1 m for trees, shrubs and herbs, respectively according to the availability of *Alnus nitida* species (Khan *et al.,* 2013). The phytosociological attribute was measured for each plant species at each quadrat. All the associated plant species were identified with the help of Flora of Pakistan, skillful taxonomist and other available literature (Ali, 2008).

Soil Collection and Analyses: Soil samples were collected from each quadrat and its physiochemical properties i.e., soil pH, organic matter, electrical conductivity (EC), soil texture, phosphorous, saturation and potassium were measured. The extracts were prepared by taking 20 gm of soil and dissolved it in 100 ml of distilled water, placed on a shaker for 30-40 min at 70 rpm and filtered into another flask through filter paper. The soil pH was analyzed by using 1:5 water suspensions/solution by pH meter. EC was measured by using 1:5 soil suspensions through conductivity meter. Organic matter was measured by the Walkley black procedure (Khan *et al*., 2016). The soil texture was analyzed by the hydrometer method. Available phosphorus was determined by taking a 2.5 g soil sample in 0.5 M $NaHCO_3$ solutions (Iqbal *et al.,* 2018). Potassium was determined by graph readings taken from the flame photometer.

Data Analyses: The multivariate statistical techniques were used to analyze the effect of various measured environmental factors on the distribution of associated species (Digby *et al.,* 2012) All the data of plant species and environmental variables were put in MS EXCEL 2010 and prepared 1, 0 data for CA and TWCA of PCORD version 5 for classification of associated plant species into various association (Lepš and Šmilauer, 2003). Co-indicator species also found out using Indicator species analysis (ISA) for each zone. Canonical Correspondence Analysis and Detrended Correspondence Analysis of CANOCO software version 4.5 was used for ordination analysis to know about the impact of a various environmental variables on associated plant species composition, distribution pattern and abundance (Anwar *et al.,* 2019). The treated environmental variables were aspects, altitude, grazing pressure, phosphorous, organic matter, pH, loamy soil, sandy soil and clay soil.

Evaluation of *Alnus nitida* in Sino-Japanese belt: The IUCN Red List criterion (2014) was used to asses' conservation status of *A. nitida* in the Sino-Japanese belt. First of all, the area was spotted where the *A. nitida* species were present. Area covered by the species and Extinct of occurrence was measured. The local community was interviewed for the confirmation of EOO and other environmental variables affecting *Alnus* population in its vicinity. Initially, the GPS data was inserted in Google Earth Software and a caption was drawn from extreme points of a specific region. The Global Positioning System (GPS) data was dragged from Madyan to Marghuzar regions and AOO was recorded. The same procedure was repeated for the Dir region by dragging a line from Patrak to Timergara regions, in Buner a line from Gokand to Bekand and in Hazara from Shinkyarai to Chatti Gatti. In Kashmir and Bajaur single areas of Muzzafarabad and Barang were spotted respectively.

RESULTS

A total of 146 associated plant species of *A. nitida* were recorded belongs to 105 genera and 53 families, of which 40 were trees, 25 shrubs and 104 herbs. Floristically, family Asteraceae and Poaceae were the topmost leading families along with 13 species (9.09 % of the total vegetation) each followed by Rosaceae with 11 species (7.69 %). The dominant trees associated with *A. nitida* were *Ailanthus latissimus, Platanus orientalis* and *Ficus carica* with maximum Important Value Index (IVI) in the region. The rare tree species were *Parrotiopsis jacquemontiana, Pyrus persica* and *Nanorrophs ritchiana*. *Arundo donax*, *Rubus fruiticosus* and *Hedra nephalensis* were dominant shrubs and *Berberis uliciana*, *Capparis spinosa* and *Lantana cammara* were the rare shrubs associated with *A. nitida* species. Among 84 herbs, *Dichanthium annulatum*, *Dicliptera bupleuroides* and *Cannabis sativa* were dominant and *Sorghum halepense*, *Lespedeza juncea* and *Aspidium molle* were rare herb species with minimum IVI in the region.

Result of Cluster Analysis and Two-way Cluster Analysis: Clustering analysis of PCORD V5 clustered all the stations and associated flora of *A. nitida* into three different habitat types/zone/associations (Fig. 2). Two-way Cluster Analysis further comprehended the distribution of plant species in the region. The white dot shows the absence of species while the black dot shows the presence of species (Fig. 3).

Zone 1: A total of 8 stations were clustered in this zone, comprised mainly of district Swat. Top co-indicators of this zone were *Celtis caucasica*, *Rubus fruticosus* and *Chenpodium murale* (Table 1). These were the indicators of higher electrical conductivity and maximum phosphorus concentration along with sandy loam soil conditions. The EC ranged from 1.5-5.9 um/cm, pH from 6.3-7.9, Phosphorous from 4.6-10.2 ppm, potassium from 65-151 ppm, saturation from 28-52 while the soil texture was from loamy to clay loamy.

Zone 2: A total of 13 stations comprised this zone. The top 3 indicators were *Eucalyptus camaldulensis*, *Arundo donax* and *Mentha arvensis* under the influence of low grazing pressure, moderate phosphorus and saturated environmental variables. The EC of this zone ranged from 1.7-3 um/cm, pH from 6.5-8.2, Phosphorous from 4.8-10.1 ppm, potassium from 79-154ppm, saturation from 29-52 while the soil texture was from loamy, sandy loam and clay loam (Table 2).

Zone 3: Three stations were grouped in this zone. The topmost 3 co-indicators of this zone were *Platanus orientalis*, *Saccharum munja* and *Oxalis corniculata* (Table 1). These were the indicators of clay loam and low electrical conductivity environmental factors. The EC ranged from 1.7-2.1 um/cm, pH from 7.5-7.9, Phosphorous from 6.7-9.4 ppm, potassium from 135-145 ppm, saturation from 36-39 while the soil texture was from loamy (Table 2).

Ecological gradient through Canonical Correspondence Analysis: A significant effect of environmental data was found on species distribution pattern, composition and abundance (Table 3). The CCA (bi-plot diagram) of the first quadrant indicated that most of the plants were under the influence of phosphorous and potassium concentration, whereas the second quadrant revealed high organic matter, pH and saturation while in the third quadrant majority of the plants were assembled under the influence of grazing pressure, electrical conductivity and soil texture (Fig. 4).

The CCA ordination bi-plot revealed that first quadrant stations (i.e., BK-Buner, BD-Swat) are under the influence of P and K concentration, whereas the second quadrant (Br-Bajaur, TG-Dir, MZ-Kashmir, DK-Dir, BA-Dir) clustered under the high OM, pH and saturation, and few stations (AY-(Amneya) Swat, RH-(Rahat kot) Swat, PD-(Pandh) Swat, KK-(Kokarai) Swat, ST-(Shoor kot) Dir, AT-(Afreen Tang) Swat, KL-(Kohistan) Dir) were influenced by higher grazing pressure, EC and texture class of soil.

Population estimation: The population of mature individuals in different valleys and regions was done.

Total estimated mature individuals were present in swat (100 in MD, 190 in RK, 170 in NK, and 120 in PD), Dir (145 in BR, 111 in GH, 50 in TG and 310 in PT), Buner (133 in GK, 15 in BK), Hazara (310 in SK, 510 in CG), Kashmir (150 in MZ) and Bajaur (245 in BR). According to local inhabitant of the area decline was observed in the thick forests of *Alnus* from the last few years due to its consumption as fuel, fodder and deforestation, along with that habitat destruction for the construction of hotels, fish farms and restaurants. Its occurrence was observed in a majority of the places of tourist spots that attract peoples. This decline satisfies the IUCN Red List Category and criteria A1 (Nature 2001)

Status summation: *Alnus nitida* was found in six localities i.e. Bajaur, Dir, Swat, Buner, Hazara and Kashmir. After assessing it through IUCN criteria we found it as Critically Endangered (CE) in Bajaur and Buner according to A2 (a, c, d) + B1B2 (b (i, ii, iii, iv), c (ii), Endangered in Dir according to A2 (a, c, d) + B1B2 (b (i,ii,iii), c(ii) and in Kashmir according to A2 (a, d) + B1B2 (b (i, ii, iii), c(ii), Vulnerable in Swat and Hazara according to A2 (a, d) and in Hazara according to A2 (a, d) + B1B2(b (i, ii, iii, iv), c (ii) respectively. The limiting factors for the species were Dryness of rivers/Nallahs, deforestation and habitat loss. *Alnus nitida* is facing critically endangered status in extreme regions of its occurrence.

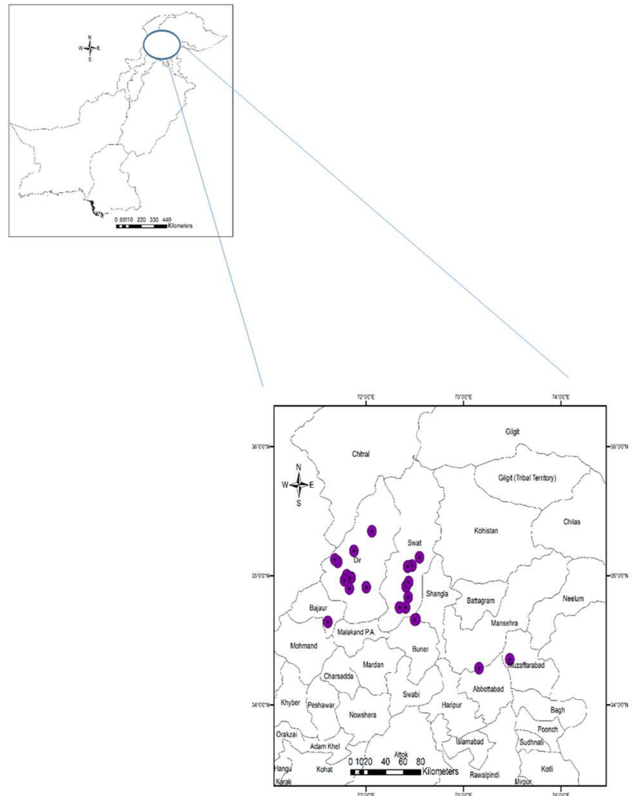

Fig. 1. The research area spotted as a blue circle on the map of Pakistan.

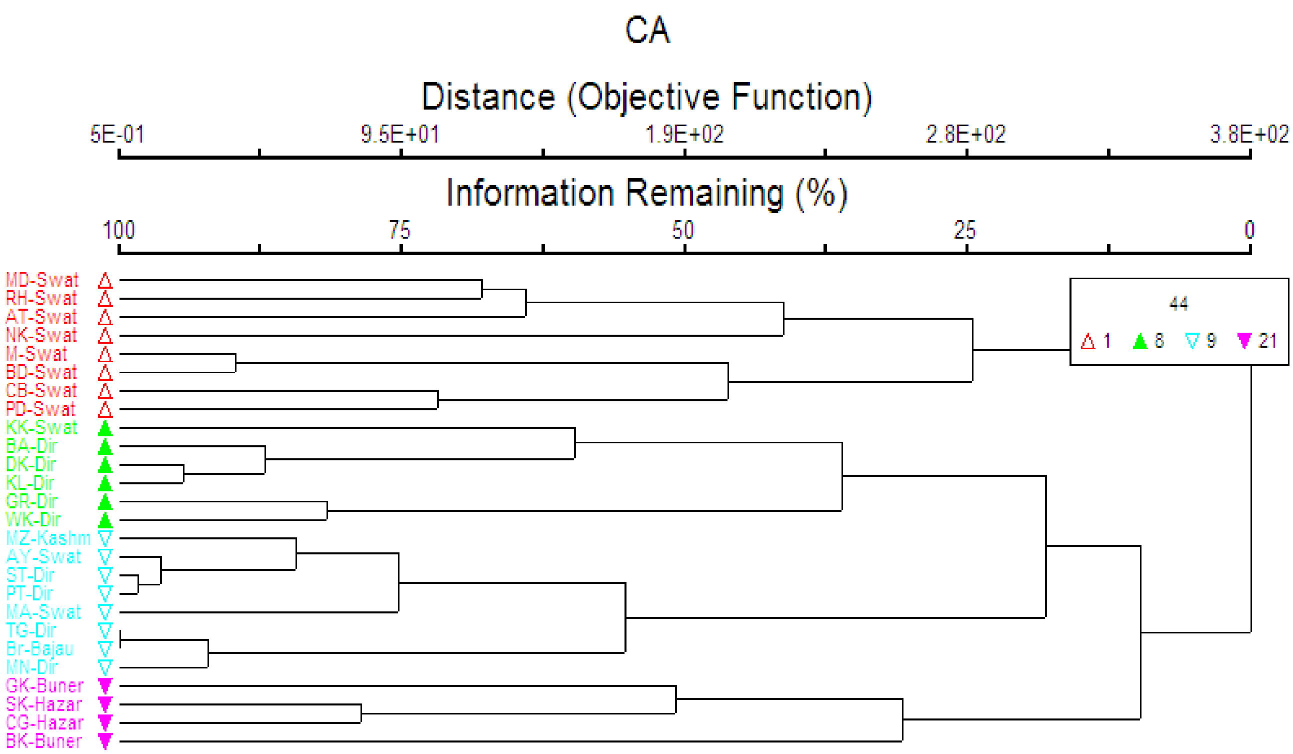

Fig. 2 Cluster dendrogram of 26 Quadrats which resulted in 3 Zones based on Euclidean measures.

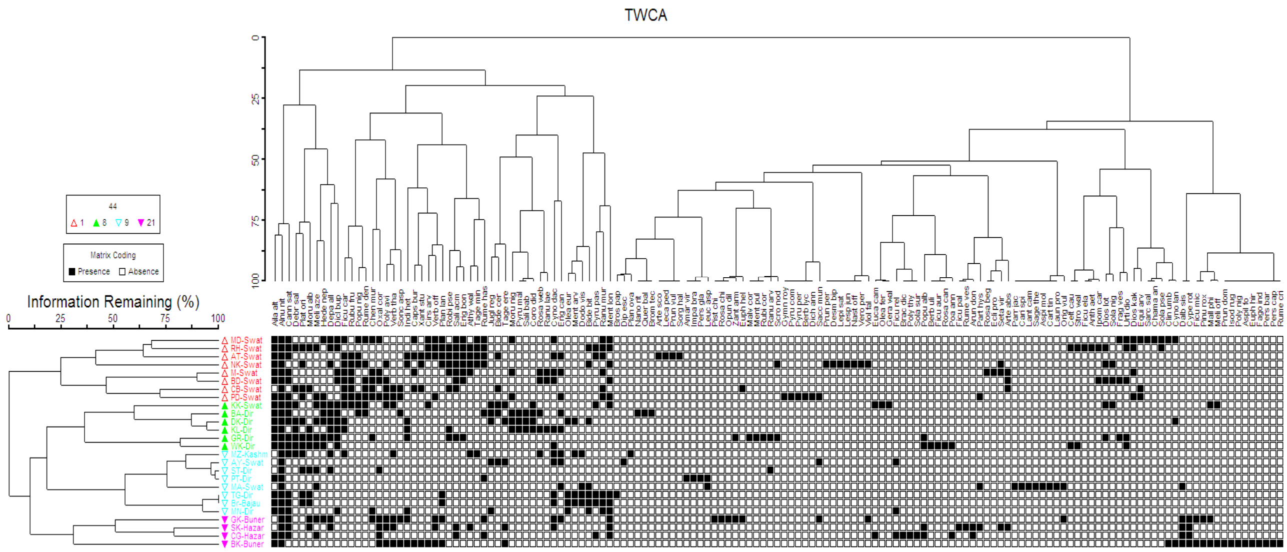

Fig. 3. The dendrogram (Two-way cluster) showing the distribution of 146 different species in the region.

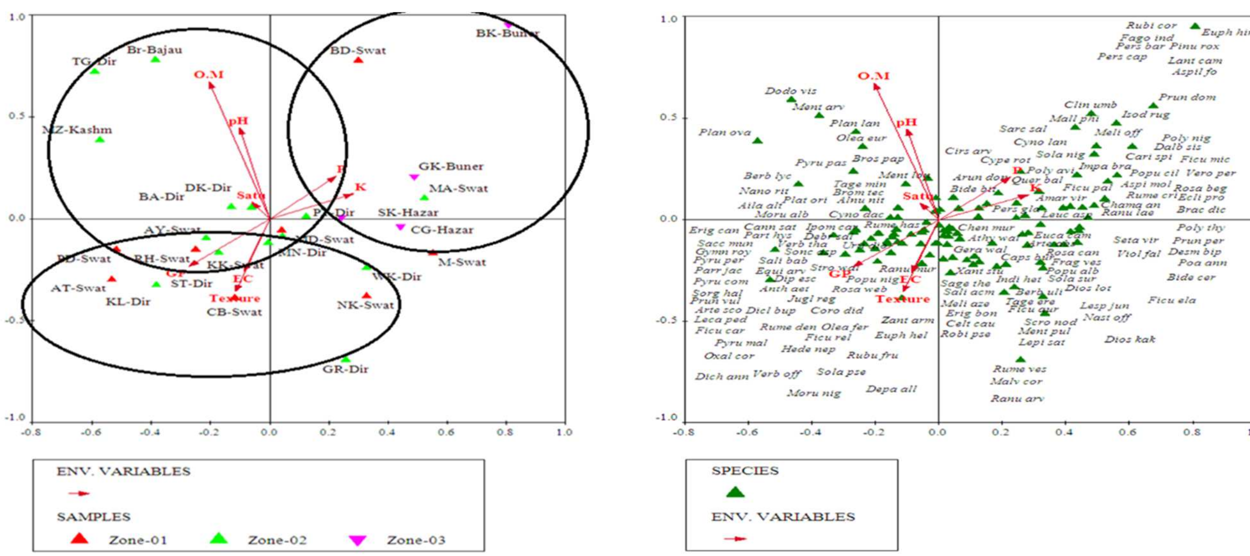

Fig.4. CCA data attribute plot shows 146 species correlation to environmental variables, where P= Phosphorous, K= Potassium, Satu=Saturation EC= Electric Conductivity, GP= Grazing pressure, OM= Organic matter and Soil tex= Soil texture.

Table 1. Data summary table of 146 associated plant species in relation to all the environmental variables after CCA of CANOCO Software.

Axes	1	2	3	4	Total inertia
Eigenvalues	0.342	0.268	0.259	0.230	4.893
Species-environment correlations	0.923	0.917	0.931	0.926	
Cumulative percentage variance of species data	7.0	12.5	17.8	22.4	
species-environment relation	19.7	35.2	50.2	63.4	
SMC test					
TSFCA (Test of significance of first canonical axis)		Test significance of all canonical axis			
Test of significance of first canonical axis: eigenvalue =	0.342				
F-ratio	1.127	F-ratio	1.027		
P-value	0.4440	p-value	0.3320		

Table 2. Detail information of indicator species of all association along with significant Environmental variables.

S. no.	Indicator species	Variables	IV	P* value
Zone 1	*Celtis caucasica,*	Sandy Loam	40	0.045
	Rubus fruticosus	EC	66	0.049
	Chenpodium murale	P	78	0.015
Zone 2	*Eucalyptus camaldulensis*	GP	67	0.01
	Arundo donex	P	51	0.003
	Mentha arvensis	P	60	0.03
Zone 3	*Platanus orientalis,*	Clay loam	66	0.024
	Saccharum munja	Clay loam	42	0.034
	Oxalis corniculate	EC	61	0.046

Table 3. Soil physicochemical analysis of each association in the studied region of Dir, Hindu Kush Mountains.

Locality	GP	EC d/Sm	pH	OM %	P mg/kg	K mg/kg	Satu	Texture
Zone 1								
MD	1	1.5	7.3	0.69	5.4	150	38	Clay soil
RH	2	2	7.6	0.48	4.8	79	28	Loamy soil
NK	1	3	6.3	0.34	6.3	91	32	Clay soil
AT	2	2.7	7.4	0.69	4.6	65	29	Loamy soil
M	2	1.7	7.1	0.55	6.3	80	28	Loamy soil
BD	1	2.1	7.9	2.84	8.6	151	42	Clay soil
CB	3	5.9	7.9	3.1	10.2	131	52	Sandy soil
PD	1	1.6	6.8	0.55	5.4	85	32	Clay soil
Zone 2								
M	1	1.7	7.1	0.55	6.3	80	28	Clay soil
KK	1	1.9	7.1	0.98	5.8	84	32	Clay soil
MZ	1	2.7	7.5	4.13	8.4	154	52	Clay soil
BA	1	1.9	7	1.03	7.1	79	32	Clay soil
AY	1	3	7.9	0.77	9.1	104	38	Clay soil
DK	3	2.1	8	0.77	8.7	137	40	Sandy soil
KL	2	2	6.5	0.62	4.8	80	32	Loamy soil
GR	1	2.6	7.4	0.77	8.9	174	49	Clay soil
WK	1	2.5	7.3	0.55	5.7	85	29	Clay soil
ST	2	1.7	7.7	0.62	3.5	143	36	Loamy soil
TG	1	2.2	8.2	2.84	8.4	94	38	Clay soil
PT	1	2.6	7.3	0.93	7.5	131	38	Clay soil
MA	1	2.1	7.7	0.82	10.1	124	38	Clay soil
Zone 3								
GK	1	2.1	7.9	0.87	9.4	145	39	Clay soil
BK	1	1.9	7.4	3.1	6.7	141	36	Clay soil
SK	1	1.7	7.5	0.82	7.5	135	38	Clay soil

GP, Grazing pressure. EC, Electric conductivity. OM, Organic matter. P, Phosphorous. K. Potassium. Satu, Saturation. MD; Madyan., RH; Rahat Kot., NK; Nokhara., AT;Afreen Tang., M;Matta., BD; Bedara., PD; Pandh., KK; Kokarai., MZ; Muzafarabad., BA; Bedara., AY; Ameya., GR, Gorh., WK; Wrsakay TG;Timergara., PT; Patrak., MA; Murghazar., GK; Gokand., BK; Bekand.,

Table 4. Botanical names, Family and Habit of associated with *Alnus nitida.*

S. NO	Family	Botanical Names	Habit
1	Acanthaceae	*Strobilanthes wallichii* Nees	Herb
2	Amaranthaceae	*Achyranthes aspera* L.	Herb
3		*Amaranthus viridis* L.	Herb
4	Apocyanacae	*Carissa spinarum* L.	Shrub
5		*Vinca rosea* L.	Herb
6	Araliaceae	*Hedera nepalensis* K. Koch	Shrub

7	Arecaceae	*Nanorrophs ritchiana* L.	Tree
8	Araceae	*Pistacia chinensis* Bunge	Tree
9	Aspleniaceae	*Aspidium molle* Sw.	Herb
10	Asteraceae	*Artemisia absinthium* L.	Herb
11		*Artemisia scoparia* Waldst. and Kitam.	Herb
12		*Bidens cernua* L.	Herb
13		*Bidens pilosa* L.	Herb
14		*Carthamus tinctorius* var. albus Alef.	Herb
15		*Cirsium arvense* (L.) Scop.	Herb
16		*Eclipta prostrata* (L.) L.	Herb
17		*Erigeron bonariensis* L.	Herb
18		*Erigeron canadensis* L.	Herb
19		*Parthenium hysterophorus* L.	Herb
20		*Tagetes erecta* L.	Herb
21		*Tagetes minuta* L.	Herb
22		*Xanthium strumarium* L.	Herb
23	Balsaminaceae	*Impatiens balsamina* L.	Herb
24		*Impatiens bicolor* Royle	Herb
25	Berberidaceae	*Berberis lycium* L.	Shrub
26		*Berberis ulicina* Hook, f. and Thoms	Shrub
27	Betulaceae	*Alnu nitida* L.	Tree
28	Brassicaceae	*Capsella bursa-pastoris* (L.) Medik.	Herb
29		*Coronopus didymus* (L.) Sm.	Herb
30		*Lepidium sativum* L.	Herb
31		*Nasturtium officinale* R.Br.	Herb
32	Buxaceae	*Sarcococca saligna* Müll. Arg.	Shrub
33	Cactaceae	*Opuntia dillenii* (Ker Gawl.) Haw.	Herb
34	Cannabaceae	*Celtis caucasica* Willd.	Tree
35		*Cannabis sativa* L.	Herb
36	Celastraceae	*Gymnosporia royleana* Wall.	Tree
37	Chenopodiaceae	*Chenopodium murale* L.	Herb
38	Convolvulaceae	*Ipomoea carnea* Jacq.	Shrub
39	Cyperaceae	*Cyperus rotundus* L.	Shrub
40	Ebenaceae	*Diospyros kaki* L.f.	Tree
41		*Diospyrus lotus* L.	Tree
42	Equisetaceae	*Equisetum arvense* L.	Herb
43	Euphorbiaceae	*Euphorbia helioscopia* L	Herb
44		*Euphorbia hirta* L.	Herb
45	Fabaceae	*Dalbergia sissoo* DC.	Tree
46		*Indigofera heterantha* Wall. ex Baker	Tree
47		*Mallotus philippensis* (Lam.) Müll.Arg.	Tree
48		*Melilotus officinalis* (L.) Pall.	Tree
49		*Robinia pseudoacacia* L.	Tree
50		*Lespedeza juncea* (L.f.) Pers.	Herb
51	Fagaceae	*Quercus baloot* Griff.	Tree
52	Gereniaceae	*Geranium wallichianum* D.Don ex Sweet	Herb
53	Hamamelidaceae	*Parrotiopsis jacquemontiana* (Decne.) Rehder	Tree
54	Juglandaceae	*Juglans regia* L.	Tree
55	Lamiacae	*Isodon rugosus* (Wall. ex Benth.) Codd	Shrub
56		*Clinopodium umbrosum* (M.Bieb.) Kuntze	Herb
57		*Leucas aspera* (Willd.) Link	Herb
58		*Mentha longifolia* L.	Herb
59		*Mentha pulegium* L.	Herb
60		*Mentha arvensis* L.	Herb
61		*Origanum vulgare* L.	Herb
62		*Plectranthus amboinicus* (Lour.) Spreng.	Herb

63		*Prunella vulgaris* L.	Herb
64	Malvaceae	*Malvastrum coromendelianum* Linn.	Herb
65	Meliaceae	*Melia azedarach* L.	Tree
66	Moraceae	*Broussonetia papyrifera* (L.)vent	Tree
67		*Ficus carica* L.	Tree
68		*Ficus elastica* Roxb. ex Hornem.	Tree
69		*Ficus microcarpa* L.f.	Tree
70		*Ficus palmata* Forssk.	Tree
71		*Ficus religiosa* L.	Tree
72		*Morus alba* L.	Tree
73		*Morus nigra* L.	Tree
74		*Ficus auriculata* Lour.	Shrub
75	Myrtaceae	*Eucalyptus camaldulensis* Dehnh.	Tree
76	Oleaceae	*Olea ferruginea* Linn.	Tree
77		*Olea europaea* L.	Tree
78	Onagaraceae	*Chamaenerion angustifolium* (L.) Scop.	Herb
79	Oxalidaceae	*Oxalis corniculate* L.	Herb
80		*Oxalis corymbosa* DC.	Herb
81		*Oxalis globosa* Poepp. ex R.Knuth	Herb
82	Pinaceae	*Pinus roxburghii* Sarg.	Tree
83	Plantaginaceae	*Plantago lanceolata* L.	Herb
84		*Plantago ovata* Forssk.	Herb
85		*Veronica persica* Poir.	Herb
86	Platanaceae	*Platanus orientalis* L.	Tree
87	Poaceae	*Arundo donax* L.	Shrub
88		*Saccharum munja* Roxb.	Shrub
89		*Anthoxanthum aethiopicum* I.Hedberg	Herb
90		*Brachiaria dictyoneura* (Fig. and De Not.) Stapf	Herb
91		*Bromus tectorum* L.	Herb
92		*Cynodon dactylon* (Linn.) Pers	Herb
93		*Cynoglossum lanceolatum* Forssk.	Herb
94		*Desmostachya bipinnata* (L.) Stapf	Herb
95		*Dichanthium annulatum* Forssk.	Herb
96		*Dicliptera bupleuroides* Nees	Herb
97		*Setaria viridis* (L.) P.Beauv.	Herb
98		*Sonchus asper* (L.) Hill	Herb
99		*Sorghum halepense* (L.) Pers.	Herb
100	Polygonaceae	*Persicaria barbata* (L.) H.Hara	Herb
101		*Polygonum aviculare* L.	Herb
102		*Polygonum thymifolium* Jaub.	Herb
103		*Rumex dentatus* L.	Herb
104		*Rumex vesicarius* L.	Herb
105		*Rumex crispus* L.	Herb
106		*Rumex hastatus* D. Don	Herb
107	Pteridaceae	*Adiantum capillus-veneris* L.	Herb
108		*Adiantum caudatum* Klotzsch	Herb
109		*Adiantum abscissum* Schrad.	Herb
110		*Adiantum raddianum* C. Presl	Herb
111	Rannaculaceae	*Ranunculus arvensis* L.	Herb
112		*Ranunculus muricatus* L.	Herb
113		*Ranunculus laetus* Wall. ex Hook. f. and J.W. Thomson	Herb
114	Rhamnaceae	*Sageretia thea* (Osbeck) M.C. Johnst.	Shrub
115	Rosaceae	*Prunus domestica* L.	Tree
116		*Prunus persica* (L.) Batsch	Tree

117		*Pyrus communis* L.	Tree
118		*Pyrus persica* L.	Tree
119		*Pyrus malus* L.	Tree
120		*Pyrus pashia* Buch.-Ham. ex D.Don	Tree
121		*Rosa beggeriana* Schrenk	Shrub
122		*Rosa canina* L.	Shrub
123		*Rosa chinensis* Jacq.	Shrub
124		*Rosa webbiana* Wall. ex Royle	Shrub
125		*Rubus fruticosus* L.	Shrub
126	Rubiaceae	*Rubia cordifolia* L.	Herb
127	Rutaceae	*Zanthoxylum armatum* DC.	Shrub
128	Salicaceae	*Populus alba* L.	Tree
129		*Populus nigra* L.	Tree
130		*Salix acmophylla* Boiss	Tree
131		*Salix babylonica* L.	Tree
132	Sapinadaceae	*Dodonaea viscosa* (L.) Jacq	Shrub
133	Scrophulariaceae	*Scrophularia nodosa* L.	Herb
134		*Verbascum thapsus* L.	Herb
135	Simaroubaceae	*Ailanthus latissimus* (Mill.) Swingle	Tree
136	Solanaceae	*Solanum nigrum* L.	Herb
137		*Solanum pseudo-capsicum* L	Herb
138		*Solanum surattense* Burm. f., Fl.	Herb
139	Urticaceae	*Lecanthus peduncularis* (Wall. ex Royle) Wedd.	Herb
140		*Debregeasia salicifolia* (D.Don)	Tree
141		*Urtica dioica* L.	Herb
142	Verbanaceae	*Verbena officinalis* L.	Herb
143		*Lantana camara* L.	Shrub
144	Zygophyllaceae	*Fagonia cretica* L.	Herb
145		*Fragaria vesca* L.	Herb
146		*Tribulus terrestris* L.	Herb

DISCUSSION

Environmental variables influence the vegetation structure of an area. A total of 146 associated plants belonging to 105 genera and 53 families were recorded from 6 stations of Sino-Japanese belt (Table 4). The highest percentage of species was recorded by family Asteraceae and Poaceae having 13 species with 9.09% share followed by Rosaceae with 11 species and 7.69% share. Our result was in close harmony with (Khan *et al.,* 2014) who recorded 132 plant species belonging to 104 genera and 47 families. Where, they reported Asteraceae and Poaceae as dominant families. Asteraceae is one of the successful plant family with more than 23000 species distributed all over the world (Gibson, 2009). One of the significant characters of the family is its specialized inflorescence capitulum. It attracts insects towards himself (Broholm *et al.,* 2008). Another important character of the family is the production of seeds with lightweight, which can migrate easily from one ecological zone to another. The same pattern studies were performed by (Iqbal *et al.,* 2018) who recorded 203 plant species belong from 70 families with Asteraceae, Rosaceae and Lamiaceae leading families. Iqbal *et al*. (2015) also reported Asteraceae with 18 species and 17 genera from the adjacent Malakand region. Similar (Tesfaye, 2015) worked on *Quercus glauca* associated trees. Where, they reported *Dalbergia sisso*, *Bauhania variegata*, *Machilus duthiei*, *Myrica esculentus*, *Pyrus pashia, Pspalum corypahaeum, Digitaria cruciata, Thalictrum foliolosum, Myrsine africana, Lonicera quinquelocularis, Caryopteris wallichiana* and *Deutzia staminea* associated plants. The same results were also recorded by (Pagano *et al*., 2012) recorded 6 trees, 14 shrubs and 32 herbs in associated flora of *Cedrus deodara* from District Shimala. To find the floristic groups and other aspects of ecology different modern software i.e. PCORD (McCune and Mefford, 1999) CANOCO (Ter Braak and Smilauer, 2002) were used by different ecologist (Andersen *et al*., 2006; Pirini, 2014). The Cluster analysis, Two-way Cluster Analysis, Indicator species analysis was carried out via PCORD software to classify the vegetation into various zonation/association and its associated indicators. While CCA and DCA of CANOCO software were used for ordination analysis to know the role of different environmental variables on plant species distribution and formation of zones. A number of researchers used these software's for various types of ecological analysis under the influence of different abiotic and biotic factors (McCune and Mefford, 1999). During community formation, via PCORD the identical floristic communities come close enough to one another while the least identical communities are bunched as separate communities (Khan *et al.,* 2013). All the stations and associated species were classified into three zones. The first zone clustered 8 stations with indicators species of *Celtis caucasica, Rubus fruticosus* and *Chenpodium murale* under the influence of high electrical conductivity and maximum phosphorus concentration with sandy loam soil condition. The EC ranged from 1.5-5.9 um/cm, pH from 6.3-7.9, Phosphorous from 4.6-10.2 ppm, potassium from 65-151 ppm, saturation from 28-52 while the soil texture was from loamy to clay loamy. Zone 2 clustered 13 stations with indicator species of *Eucalyptus camaldulensis, Arundo donax* and *Mentha arvensis* under the influence of low grazing pressure, moderate phosphorus and saturated environmental variables. EC of this zone ranged from 1.7-3 um/cm, pH from 6.5-8.2, phosphorous from 4.8-10.1 ppm, potassium from 79-154ppm, saturation from 29-52 while the soil texture was from loamy, sandy loam and clay loam. The Zone 3 group based on 3 stations having indicators of *Platanus orientalis, Saccharum munja* and *Oxalis corniculate*. These were the indicators of clay loam and low electrical conductivity environmental factors. The EC ranged from 1.7-2.1 um/cm, pH from 7.5-7.9, phosphorous from 6.7-9.4ppm, potassium from 135-145ppm, saturation from 36-39 while the soil texture was from loamy. There are different reasons behind the association of species, first one was temperature harshness and secondly, different factors like EC, pH, organic matter, potassium and phosphorus (Khan *et al.,* 2017). The Alder species have nodules to fix nitrogen from the air which enhance the growth of plant itself as well as associated species (Kamran *et al*., 2018). The IUCN red list categories and criteria are the most authentic and acceptable method worldwide to find the conservation status of any species. This criterion has been updated for decades and in the present study, we applied the IUCN Red list criteria 2014 to assess the conservation status of *Alnus nitida.* The IUCN criteria were applied to *A. nitida* species in Sino-Japanese range of Pakistan. The species was assessed as Vulnerable according to VU, A2 (a, c) A3 (c, d) +B1B2 (b (ii, iii)). Whereas VU means Vulnerable and A taxon is Vulnerable when the best available evidence indicates that it meets any of the criteria A to E for Vulnerable, and it is therefore considered to be facing a high risk of extinction in the wild. Our findings were in close harmony to Khan *et al.* (2013) who recorded 64 species from Naran valley where 12 species were vulnerable, 20 species were critically endangered and 7 species were near threatened by assessing it through IUCN red list criteria (A, B, C and D). Alam and Ali (2010) declared *Astragalus clarkeanus* as critically endangered (CR) in Hindu Kush ranges by IUCN criteria CRB1ab (iii) +2ab (iii); C2 (ii). The same IUCN criteria were applied by (Khan *et al.,* 2016) who declared the endemic species of the area *Berberis pseudumbellata subsp. gilgitica* as critically endangered in Karakorum mountains according to criteria:'CRA1acd; B1b (i, ii, iii) is also supporting our findings. Local inhabitants can only fully utilize the benefits of native flora if they own it and take practical steps for its conservation (Negi, 2010). The recent terminology introduced in 90's Ethno-Ecology is a famous approach for the conservation of Plant important for the ecosystem (Abdullah *et al.,* 2019; Shah *et al.,* 2019).

Conclusion: It was concluded that electrical conductivity, phosphorus concentration along with sandy loam soil condition and grazing pressure were important environmental variables that play a significant role in vegetation structure, associated flora of *A. nitida* and its distinct co-indicators in the Sino-Japanese belt. The Alnus species is recorded in various regions of the Sino-Japanese belt of Pakistan but no comprehensive study was ever conducted on its associated species, this study will provide help to future researchers working on various aspects of *Alnus nitida.*

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