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MACRO- AND MICRO-ANATOMICAL DIVERSITY IN THE *ALNUS NITIDA* (SPACH) ENDL. GROWING IN VARYING CLIMATIC CONDITIONS OF SINO JAPANESE REGION OF PAKISTAN

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Abstract

Alnus nitida (Spach) Endl. belong to the family Betulaceae. It is distributed in the western Himalayas and the Hindu Kush regions of the Sino-Japanese belt of northern Pakistan. This study was designed to investigate the morpho-anatomical variation in different populations of *Alnus nitida* (Himalayan Alder). Preliminary, a variation was observed in the size of both male and female catkins. Similar variations were observed in pollen morphology in different populations of the species. The micronutrients in soil and environmental conditions are the main drivers of these variations. These palynological, anatomical, soil characteristics and environmental conditions were analyzed through multivariate statistical software i.e., PCORD and CANOCO. It was observed an efficient accumulator of all heavy metals as revealed by the values (>1) of Biological Accumulation Coefficient (BAC) in shoots, BCF in roots and the Translocation Factor (TF). Detailed morpho-anatomical and palynological variations in *Alnus nitida* were enlisted. The palynological and anatomical variations were not enough to define the taxonomic status of different populations. However, further molecular studies will be essential to clear the genetic diversity in these populations.

Key words: *Alnus nitida;* Anatomy; Palynology; Light Microscopy; Scanning Electron Microscopy.

Introduction

Alnus nitida (Spach) Endl belongs to the family Betulaceae and is distributed in the temperate and sub-alpine zones of Asia, Europe, North and South America. The genera of Alnus and Betula represent Betulaceae in Pakistan (Perveen *et al*., 1999; Haq *et al*., 2020a). Genus *Alnus* has thirty-five species all over the world, of which eighteen species occur in Asia (Taleshi *et al*., 2019), eight in North America (Hurd *et al*., 2001), four in Europe (Chen & Li, 2004), two species in Mexico and one species in the Andes mountains of South America. Seeds of *Alnus nitida* are wingless or smaller in length as compared to other species of *Alnus* genus (Navarro *et al*., 2003; Haq *et al*., 2020b). Close geographic distribution and relationships among *Alnus* species led to taxonomic confusion worldwide (Vidaković *et al*., 2018). Taxonomically, the genus *Alnus* is quite ambiguous and complex due to its overlapping morphological characters, making it hard to distinguish the species within the genus. Several species of *Alnus* have been divided based on palynology, morphology and molecular studies. Leopold and his co-worker in 2012, divided genera and subgenera based on geographic distribution and gross morphology. Few species were reclassified based on nr-DNA and ITS sequence data (Chen & Li, 2004). Based on molecular studies, two subgenera are recognized in *Alnus* i.e., *Gymnothysus* and *Alnobetula* (Navarro *et al*., 2003). Few taxonomists recognize *Alnus* as a monophyletic group, divided it into three sub-species i.e., *Clethropsis, Alnobetula* and *Alnus* subgenera. Chen & Li (2004) divided the species into two complexes (1) *A. viridis* and (2) *A. incana* complex. *Alnus dolichocarpa* was further divided into *subcordata* variety, while *Alnus subcordata* was further divided into *villosa* and *viscversa* types. *Alnus glutinosa* was further divided into three subspecies based on DNA barcoding (May & Lacourse, 2012). Furthermore, three subspecies of *Alnus maritime* have been introduced based on anatomical and palynological characters (Schrader & Graves, 2003). Li *et al*., (2004) mentioned distribution maps for three subgenera of *Alnus, Clethropsis* and *Alnobetula*. *Clethropsis* was limited to Japan, Asia and the USA (Haq *et al*., 2021). Foliar epidermal characters are helpful in identifying and delimiting the taxon (Khan *et al*., 2011; Rahman *et al*., 2020a). The anatomical studies also provide information regarding the plant's chemical composition, which plays a crucial role in modern taxonomy (Hussain *et al*., 2010; Shah *et al*., 2019). Leaves provide important identification characters i.e., shape and size of stomata, stomatal index, trichomes type, epidermal cells, and orientation (Olowokudejo *et al*., 2008). The stomatal features show us the arrangement and distribution of stomata, subsidiary cells, stomatal pores arrangement, shape and size of guard cells (Wilkinson *et al*., 2001). The pollen studies are very significant in the identification and classification of plant species. Light microscopy and scanning electron microscopy have been contributing to the field of palynology for the identification of taxon (Walker & Doyle, 1975).

This study was designed to investigate the morpho-anatomical variation in different populations of *Alnus nitida*. What are the drivers for these variations (either macro or micronutrients in the soil and environmental factors)? Keeping in mind this research gap and hypothesis, we have carried out the morphological and anatomical evaluation of *Alnus nitida* across the Sino-Japanese belt of Pakistan.

Materials and Methods

Samples collection, identification and preservation: Fresh leaves, catkins (male & female) and fruit specimens were collected from various areas of Northern Pakistan. The areas covered during research work were Kashmir, Hazara, Swat, Buner, Dir and Bajaur (Fig. 1). These areas were visited several times to collect the maximum number of samples (Nazakat *et al*., 2020; Kamran *et al*., 2020). Measuring tape and stainless-steel ruler (6 inch/15 cm) was used for measurement of the diameter at breast height and different morphological characteristics. All the associated plant species of *Alnus nitida* were also recorded. The collected species were identified with the help of expert taxonomists and Flora of Pakistan (Ali, 1980).

Sample collection from different herbaria: *Alnus nitida* has been distributed in different habitats of the Sino-Japanese belt. Several researchers have been collected *Alnus nitida* specimens from different areas of Northern Pakistan and deposited them in various herbaria across the country. We have collected herbarium specimens from various herbaria to study the morphological variation in different parts of the species to clarify the taxonomic ambiguities, i.e. prominent differences in its male and female catkins (Fig. 2).

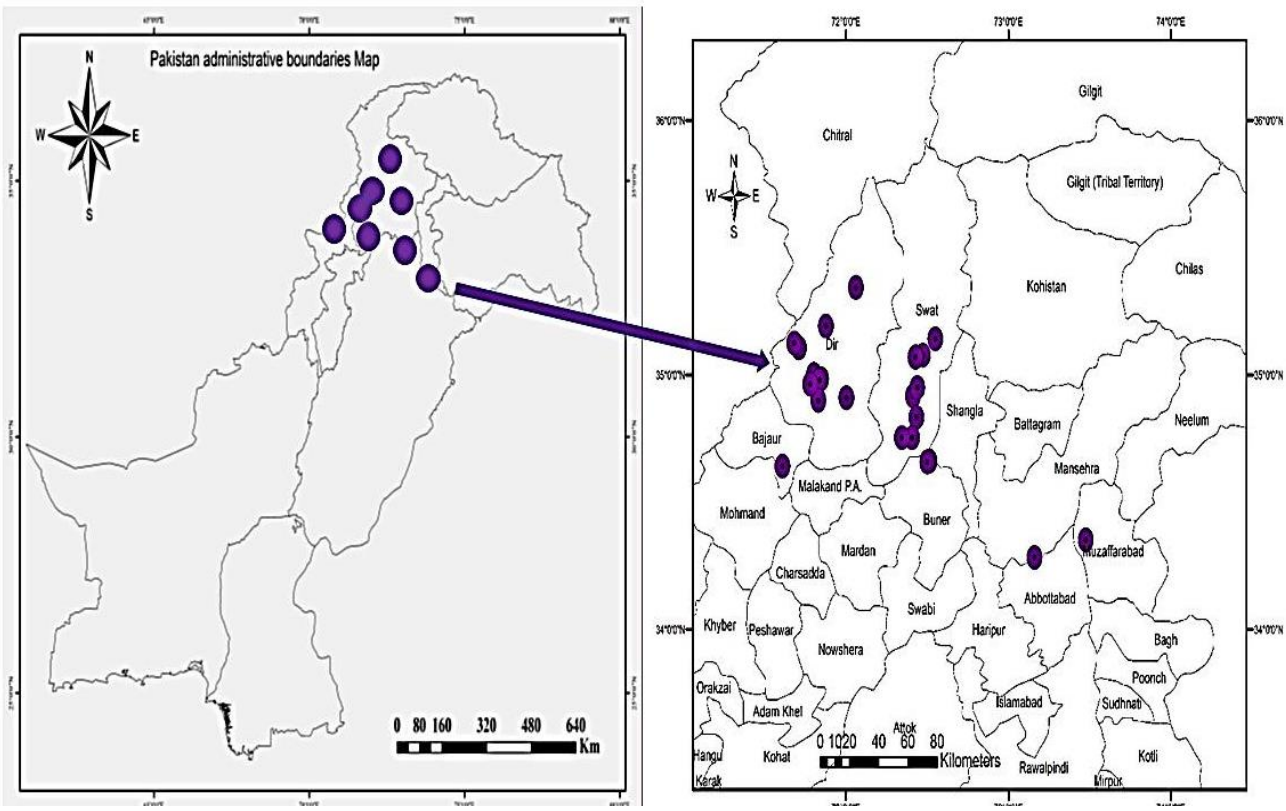

Fig. 1. Map of the study area showing different localities where the samples were collected.

Fig. 2. Differences in length size of Male catkins and Female inflorescence were collected from the different field areas.

Sampling techniques

Heavy metals and soil analyses: Soil samples were collected from each zone and their physicochemical properties, i.e., soil pH, organic matter, electrical conductivity (EC), soil texture, phosphorous, saturation and potassium, were measured in the Agricultural Department, Government of Punjab, Pakistan (Rahman *et al*., 2019; Rahman *et al*., 2020b; Abbas *et al*., 2021). A modified protocol of below scientists was used for the soil pH, EC (Rhoades, 1996; Bano *et al*., 2018; Rasheed *et al*., 2021), organic matter (Nelson & Sommers, 1982; Iqbal *et al*., 2018; Iqbal *et al*., 2021), soil texture (Nazif *et al*., 2006; Khan *et al*., 2011; Ahmad *et al*., 2016; Khan *et al*., 2016; Khan *et al*., 2020), phosphorus (Gradowski & Thomas, 2006; Manan *et al*., 2020) and potassium (Graham, 1959; Abbas *et al*., 2020) measurements. The *Alnus nitida* shoot, leaves, bark and root along with soil and water samples were collected to find out the phytoremediation ability and its impact on morphological characteristics of the species across different areas (Ahmad *et al*., 2021; Anum *et al*., 2019). Plant metal uptake was documented by the wet acid digestion technique (Luo *et al*., 2011; Ahmad *et al*., 2019). The samples were then used to find the concentrations of desired metals and nutrients i.e. Ni, Cu, Mn, Co, Zn, Cd, Cr, Pb, Mg, Ca, Na, K via atomic absorption spectrometry (Varian AAS-240, Triad Scientific, New Jersy, USA) (Noreen *et al*., 2019; Zeb *et al*., 2021). The Bioaccumulation factor provides an index of the ability of the roots and shoots to accumulate the heavy metal concerning the metal concentration in the soil (Malik *et al*., 2010). Biological Concentration Factor (BCF), Translocation Factor (TF) and Biological Accumulation Coefficient (BAC) were determined through the method of (Yu *et al*., 2011; Ahmad *et al*., 2019).

Macro-morphology: The macro-morphological i.e., internode length, petiole length, lamina length & width, male inflorescence length, male inflorescence width, inflorescence per branch, peduncle length, stalk length, female inflorescence catkin length & width, number of scales per column, scale length, scale width stalk and the size of fruit, nut length, as well as width were determined.

Leaf Epidermal anatomy and pollen morphology: Scanning Electron Microscopy (SEM) studies were conducted to get clear images for best comparison (Gul *et al*., 2020). All the pollens and leaf samples were mounted with double adhesive tape on stubs, sputter-coated with gold-palladium and observed under SEM. The observation was conducted via scanning electron microscope (JEOL JSM-5910), and the snapshots were taken with polaroid P/N 665 film housed at the Central resource laboratory, Department of Physics, University of Peshawar, Pakistan. The quantitative characters are represented in table 1 denoted by 10-17 (14.25) ±1.49 minimum − maximum = mean + standard error. The quantitative data were processed using Statistical Package for the Social Sciences (SPSS 16.0) software to determine minimum, maximum, average means and standard error for each parameter of the five different traits. Pollens collected from different localities were observed under SEM and then Pollen size, equatorial and polar diameter, pore length and width, the thickness of exine, pore length and width were studied. Qualitative characters, including the shape of pollen, pollen ornamentation, pore ornamentation and the number of pores, were also observed.

Data analyses: The multivariate statistical techniques i.e., PCORD and CANOCO were used to analyze the impact of various environmental factors on *Alnus nitida* micro and macro morphology (Khan *et al*., 2011; Mumshad *et al*., 2021).

Results

The variation was observed in both male and female inflorescence at different localities. The maximum length in male inflorescence was found in Marghuzar, Swat i.e., 13.5cm while the minimum was 7.8cm in the Manyal region. In female inflorescence maximum length was 3 cm recorded in Shinkyari (Hazara) (Table 1). These two floristic characters compelled us to measures the rest of the parameters of anatomy and palynology for the possible report of new species or subspecies.

Leaf cuticle morphology: The leaf epidermal anatomical characteristics were studied under the microscope. The cuticle morphology shows variations in size, shape, stomatal indexes, length and width of epidermal cells, stomata and stomatal pores and subsidiary cells. Measurements of foliar epidermal anatomy shows that epidermal cell width (10-17=4.25 ± 1.49µm), stomatal length (4-10= 6.8654 ± 0.409µm), stomatal width (6-17=11.50 ± 0.52µm), length of guard cell (2-10=4.880 ± 0.3908µm), width of guard cell (2-15=8.67 ± 0.59µm), length of subsidiary cells (1.5-11=3.13 ± 0.54µm), width of subsidiary cells (2-16=10.25 ± 0.69µm), length of stomatal pore (5-12 =6.87 ± 0.343µm), width of stomatal pore (1-6 =2.12 ± 0.202µm) and number of pores (4-5= 4.14 ± 0.63µm) (Fig. 3). These are the average values of specimens collected from different sites of the studied regions (Table 2).

Palynology: Palynology characters of *Alnus nitida* were observed with light and scanning electron microscopy. The characteristics of the quantitatively and qualitatively (pollen ornamentation, number of pores, exine thickness and pore diameter) are analyzed and summarized in Fig. 4. Morphological characters show that *Alnus* pollens are tetra and pentaporate. The pollen parameters were pollen length (20.12-29.22 = 23.57) ± 0.544), equatorial diameter (18.18-26.23 = 21.21 ± 0.37), number of pores in polar axis (2-3=2.42 ± 0.098), exine thickness (1.12-3.33 = 2.55 ± 0.116) and number of pores in equatorial axis (3-4 = 3.7 ± 0.452) (Table 3).

Table 1. Macro-Morphological features of *Alnus nitida* collected from different areas of Pakistan.

	MD	RH	NK	AT	M	BD	CB	KK	MZ	PD	BA	AY	DK	KL	GR	WK	ST	TG	MN	PT	GK	BK	SK	CG	MA	BR
Internode length	3.5	4.05	5	1.74	1.74	3.2	3.35	2.4	2	1.94	3.05	2.6	2.64	2.77	2.5	2.6	2.64	2.6	2.7	2.6	2.57	3	1.64	2	3.25	2.9
Petiole length	2.77	3.94	3.17		2.5	5.38	3.78	2.24	2.03	3.14	2.37	2.37	2.44	2.44	2.34	2	2.44	2.34	2.34	2.37	2.27		2.06	4.03	3.27	2.96
Lamina length	12.67	14.75	13	11	7.5	16.44	12.56	12.56	13.64	16.5	13.67	13.34	13.5	14.03	12.97	8.5	13	13.06	13.37	13.03	13.03	15.3	10.34	12	12.56	9
Lamina width	4.84	6.5	13	4.5	3	7.97	7.78	7.4	6.37	9.1	7.57	7.4	7.4	7.4	7.5	5	7.4	7.4	7.4	7.5	7.5	7.2	5.7	8	6.05	5.66
Male inflorescence length	8.67	8.5	10.67	8	9	13	8.25	10.64	11.5	13.5	11.38	10.34	11	11	11	8.74	11.67	11.67	11	10.67	11	10.1	10.4	9	7.8	7.9
Male Inflorescence width	0.1	0.1	0.2	0.1	2.1	0.2	0.2	0.15	0.1	0.21	0.2	0.1	0.25	0.15	0.15	0.2	0.15	0.15	0.15	0.15	0.15	0.1	0.1	0.2	0.9	1.5
Inflorescence per peduncle/branch	4	3.5	5	6	4.5	4	4.5	4	5.5	7	4	5	4	5	4	4	4	4	4	5	5	4	3.5	5	5	1.27
Peduncle length	1.45	2	1.9	1	0.4	1.27	1.73	4	1.5	2.1	1.56	5	5	5	5	2.4	5	5	5	5	5	1.5	0.57	0.47	2.1	1.5
Stalk length	1.25	2.1	1.1	1	0.5	0.25	1.25	0.97	0.5	2.1	0.5	1.03	1.06	1.3	1.06	2	1.03	1.06	1.1	1.06	1.03	1	1	1.9	2	2
Female inflorescence/ Mature cone length	3.07	1.85	2.2	1.75	2.1	1.8	1.4	2.2	3.3	3.98	3.64	1.9	2.1	1.9	1.9	3.35	2.3	2.2	2.2	1.7	1.9	1.4	0.47	3	1.3	1
Female inflorescence/ Mature cone width	1	0.7	0.5	0.5	1.3	0.55	4	1.1	1.1	0.67	0.44	1	1.1	1.1	1.2	0.5	1.5	1.4	1.3	1.2	1.4	0.2	0.35	0.6	0.7	1
No. of Scales per column/length	14.34	9.5		11	13	12	21	14	19	21	13.67	14	11		13	14	11	12	14	15	14	15	13	13	6	4
Scale length	0.5	0.3	0.5	0.3	3.5	0.3	0.3	0.6	0.4	1	0.37	0.6	0.71	0.56	0.66	0.6	0.67	0.71	0.64	0.66	0.56	0.4	0.45	0.6	0.4	1
Scale width	0.4	0.2	0.4	0.2	4.5	0.3	0.3	0.6	0.35	0.7	0.3	0.45	0.5	0.34	0.4	0.4	0.44	0.45	0.41	0.43	0.44	0.3	0.45	0.4	0.4	0.3
Stalk length of fruit	1.1	1.3	1.5	0.8	0.8	0.9	1.44	1.1	0.5	2.1	0.5	1.4	1.5	0.9	0.9	0.6	1.7	1.8	1.7	2.1	1.5	1.9	1	0.3	2	1.4
Nut length	0.3	0.3	0.3	0.3	0.2	0.2	0.2	0.3	0.3	0.5	0.3	0.3	0.2	0.3	0.3	0.4	0.4	0.3	0.3	0.2	0.31	0.2	0.3	0.6	0.3	0.3
Nut width	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.4	0.3	0.2	0.2	0.2	0.3	0.3	0.4	0.2	0.3	0.2	0.3	0.2	0.2	0.4	0.4	0.3

Table 2. Anatomical measurements parameters collected from different Quadrats (All the measurements mentioned in the table are in centimeter)

	MD	RH	NK	AT	M	BD	CB	KK	MZ	PD	BA	AY	DK	KL	GR	WK	ST	TG	MN	PT	GK	BK	SK	CG	MA	BR	MIN-MAX (Mean) ± standard Error
ECL	15	17	9	12	17	8	10	19	15	17.5	13	7	10	17	10	17	16	14	11	12	11	18	16	13	12	17	10-17 (14.25) ±1.49
ECW	5	7	5	6	10	4.5	5	9	5	8	7	4	6	5	6	9	10	9	7	5	5	10	10	5	6	10	4-10 (6.8654) ± 0.40
SL	11	17	11	8	14	9	11	8	12	15	11	14	10	11	11	10	12	10	17	11	12	6	12	14	8	14	6-17 (11.500) ± 0.52
SW	3	5	2.5	2.5	3	2	2	5	5	6.5	6	6	6	5	5	6	7	6	10	6	6	4	7	6	2.5	3	2-10 (4.880) ± 0.39
LGC	9	2	2	8	7	10	6	6	9	11.5	7	7	11	9	9	15	13	12	9	7	9	8	13	11	8	7	2-15 (8.673) ± 0.59
WGC	9	11	11	2	2	3	1.5	2	2	4	1.5	2	2	1.5	1.5	2	2.5	1.5	1.5	1.5	2	2.5	2.5	4	2	2	1.5-11 (3.13) ± 0.54
LSC	5	15	2	7	11	10	9	7	8	12.5	7	8	9	11	16	13	16	13	9	8	13	11	16	12	7	11	2-16 (10.25) ± 0.69
WSC	3	3	10	6	3	5	3	3	6	3.3	4	3	6	4	4	2	3	3	3	2	3	5	3	3	6	3	2-10 (3.94) ± 0.34
LSP	5	7	5	5	7	6	6	5	7	6.5	7	9	7	12	5	8	8	7	9	5	10	6	8	6	5	7	5-12 (6.87) ± 0.34
WSP	2	2	2	2	2	2	2	2	1.5	2	1.5	2	2	6	1.5	1.5	4	3	1.5	1	2	2.5	4	1.5	2	2	1-6 (2.12) ± 0.20
NP	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	5	5	5	4	4	4	4	4	4	4	4-5 (4.14) ± 0.63

Table 3. Palynological parameters collected from different areas of Pakistan.

Madyan	MD	RH	NK	AT	M	BD	CB	KK	MZ	PD	BA	AY	DK	KL	GR	WK	ST	TG	MN	PT	GK	BK	SK	CG	MA	Minimum – Maximum = mean ± standard error
Pollen length	21.23	23.25	26.25	27.22	21.22	26.22	25.23	21.22	29.25	28.26	22.23	20.24	27.21	27.23	22.23	20.22	23.25	27.23	25.20	22.23	22.23	21.26	25.26	21.26	20.24	20.12-29.22 (23.57) ± 0.544
Equatorial diameter	20.20	22.21	21.25	20.18	19.20	22.23	22.24	19.20	26.23	21.24	20.18	21.22	25.26	21.20	18.20	18.18	22.23	21.20	20.21	20.18	20.18	23.25	21.2022	23.24	21.22	18.18-26.23 (21.21) ± 0.37
No. of pores in pollar axis	3	2	3	3	3	3	2	3	2	4	4	3	2	2	3	2	2	3	3	4	4	4	3	4	4	2-3 (2.42) ± 0.098
Exine thickness	2.22	2.19	3.22	2.22	2.12	2.22	1.12	2.12	2.21	2.91	3.23	2.32	2.15	3.32	2.21	2.22	2.21	3.32	2.22	3.23	3.23	3.33	3.23	3.33	2.32	1.12-3.33 (2.55) ± 0.116
No. of pores in equatorial axis	4	4	4	4	4	4	4	4	4	3	3	4	4	4	4	4	4	4	3	3	3	3	4	3	4	3-4 (3.73) ± 0.452
P/E raio	1.05	1.04	1.23	1.34	1.105	1.17	1.13	1.10	1.11	1.33	1.10	0.95	1.07	1.28	1.22	1.11	1.04	1.284	1.24	1.10	1.101	0.914	1.19	0.91	0.95	0.91- 1.35 (1.134) ± 0.118
No of Aperture	3	4	4	4	4	4	4	4	4	4	3	3	3	3	3	3	3	3	4	4	4	4	4	3	3	3.00-4.00 (3.807) ± 0.4019

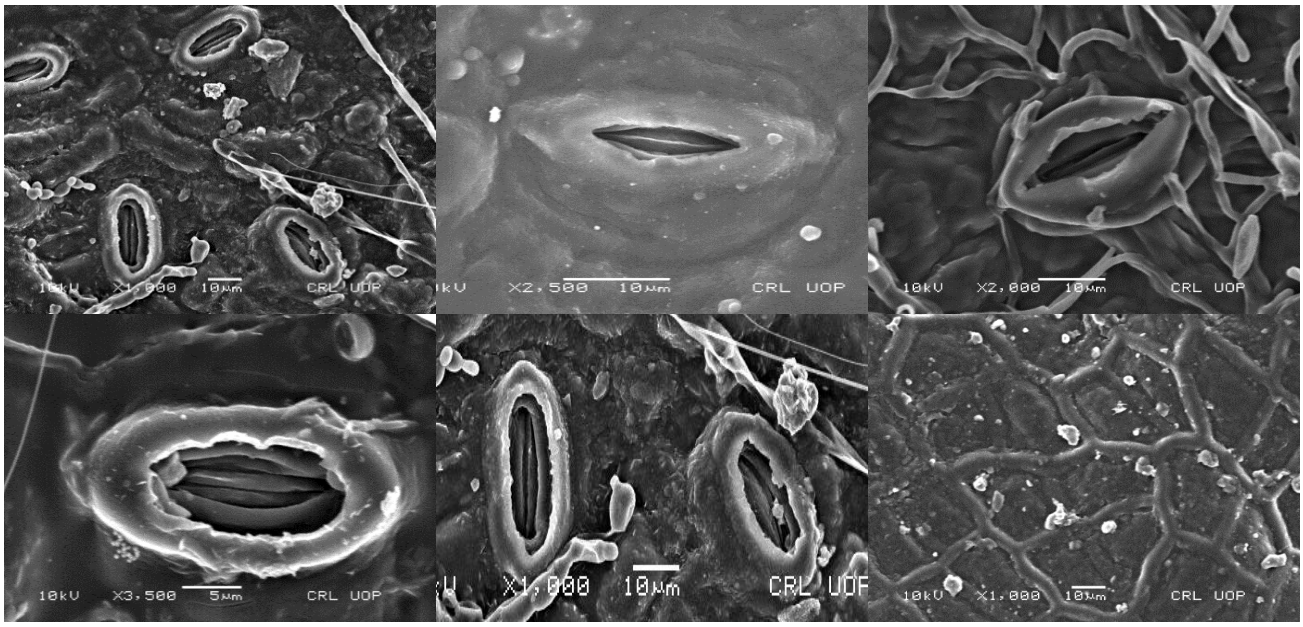

Fig. 3. Anatomy of Scanning electron and Light Micrographs a) Ghor (GH) b) Gokand (GK) c) Chatti Gatti (CG) Murghuzar (MA) Muzzafarabad (MZ).

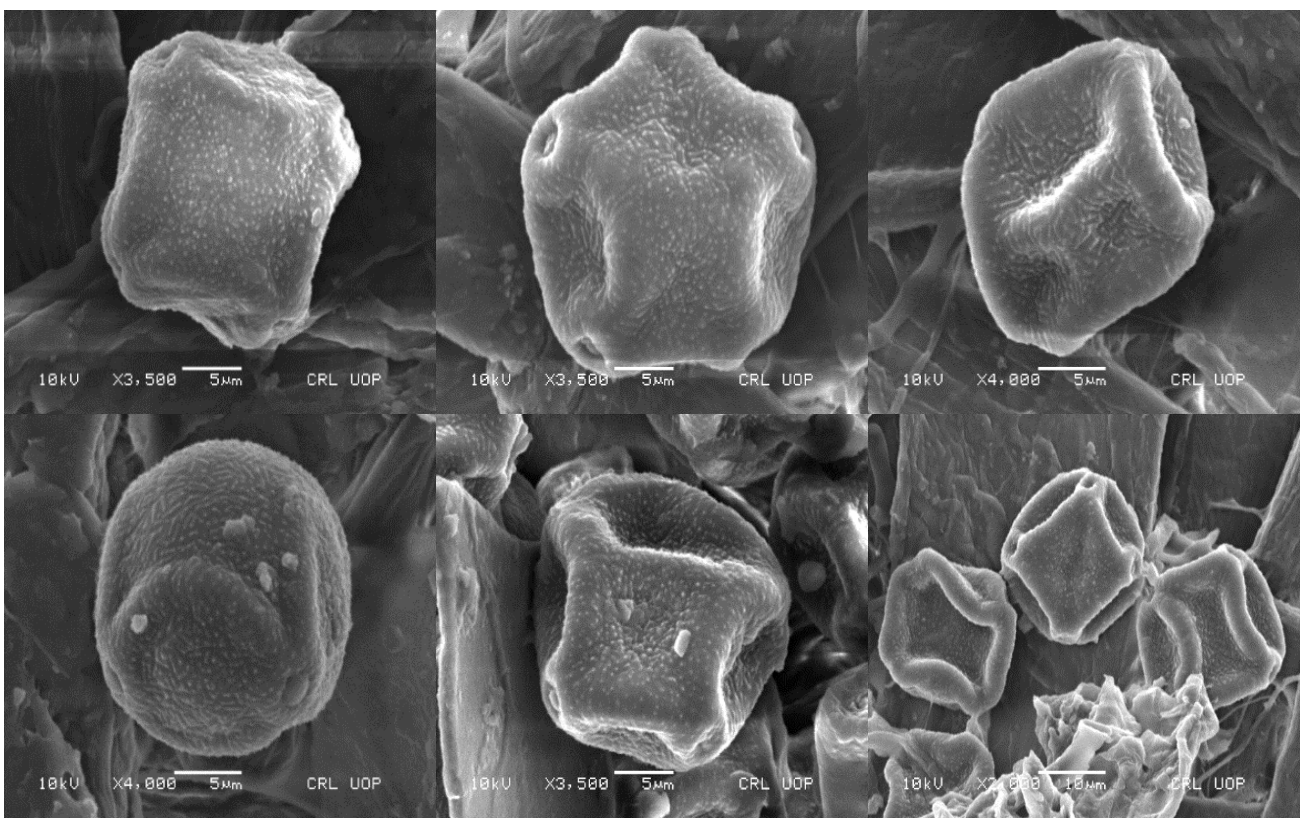

Fig. 4. Pollens Scanning electron and Light Micrographs a) Ghor (GH) b) Gokand (GK) c) Chatti Gatti (CG) Murghuzar (MA) Muzzafarabad (MZ).

Heavy metals accumulation: The amount of heavy metal accumulation varies in different parts of the plant. Maximum heavy metal accumulation was observed in the leaves, while the lowest accumulation was in the root (leaves > shoot > bark > root). The plant was collected from different habitats disturbed by various pollutants i.e. domestic, industrial pollutants and many other heavy metals producing bodies. In water samples, the highest Ni contamination was observed in the samples collected from Chinarkot (404.5µ/L). At the same time, the lowest accumulation was observed in the samples of Gokand (164.65 µ/g). The maximum concentration of Cu was observed in the sample collected from Rahat Kot (105.2 µ/L), while the lowest was observed in samples collected from Manyal (18.45 µ/g). The highest Cu accumulation was observed in leaves (78.9). In root

samples, the highest Mn concentration was observed in the sample collected from Afreen tang (269.1 µ/L), while the lowest concentration was in the samples of Muzzafarabad (8.7 µ/g). In root samples, the highest Zn contamination was observed in the samples collected from Afreen tang (324.45 µ/L), while the lowest was in the samples of Chinarkot (40µ/g). The highest Cr concentration was found in the samples collected from Pandh (2194.1 µ/L) followed by Nokharai (77.65µ/L). In root samples, the highest Pb contamination was observed in the sample collected from Rahat Kot (321.9 µ/L) followed by Gokand (131.85µ/L). In root samples, the highest Mg concentration was present in the sample collected from Rahat Kot (841µ/L) followed by Manyal (142.2 µ/L). In root samples, the highest Ca concentration was present in the sample collected from Nokharai (2363.18 µ/L) followed by Pandh (2100 µ/L). In root samples, the highest Na concentration was present in the samples collected from Rahat Kot (6614.1 µ/L) followed by Muzzafarabad (3786.6 µ/L). In root samples, the highest K concentration was present in the sample collected from Rahat Kot (163.075 µ/L) followed by Muzzafarabad (187.75 µ/L). The plant was observed as an efficient accumulator of Ni as revealed by the values (>1) of BCF in shoots, BCF in roots and also the TF. From current sampling examinations, we revealed that *Alnus* has the highest Phyto-extraction capability of heavy metals. The reason behind this is the presence of increased heavy metals contents in the soil sample compared to others. The heavy metal accumulation pattern in different station was as PD> KK > SK > RH > DK > AT > NK > MZ and so on.

Effects of environmental variables and heavy metals on *Alnus nitida*: There is a significant impact of environmental variables on macro-morphology, palynology and anatomy of *Alnus nitida* and its associated flora. The environmental variables considered were aspect, pH, EC, organic matter, calcium carbonate, phosphorous, potassium and textural class. It reflects a significant impact of these factors on each other at the first canonical axis. The environmental variables and soil data were co-related with anatomical, palynological and macro-morphological parameters. The first quadrant revealed that secondary vein, internode length, equatorial diameter, pores per equator, stalk length, the width of guard cells, stomatal length are tremendously affected by excessive grazing pressure. The third quadrant showed that pore axis, peduncle length inflorescence per branch pollen length, male inflorescence and scale per column are affected by the high concentration of organic matters and texture. The fourth quadrant clustered exine thickness, No of pores, width of secondary subsidiary cells and Scale width, which are affected by the high amount of pH, Saturation, Potassium, Electrical conductivity and Phosphorous (Fig. 5).

The relation of heavy metals with taxonomic parameters was also determined (Fig. 6). The first quadrant revealed that internode length, Stalk length, the width of guard cells, stomatal length, stomatal pore width, peduncle length, and equatorial diameter are affected by the high concentration of Cobalt, Zinc and Lead. The second quadrant shows that Chromium highly affects the stomatal width, length of the stomatal pore, secondary vein, epidermal cell length, epidermal cell width, length of the subsidiary stomatal cell, length of guard cell and male inflorescence width. The third quadrant shows the cluster of pore axis, peduncle length, scale per column and male inflorescence size are highly affected by Manganese, Copper, Sodium, Nickel, Cadmium, Potassium and Calcium. The fourth quadrant revealed that the number of pores, exine thickness, pollen length, scale width, inflorescence per branch and width of the subsidiary cell are highly affected by Magnesium concentration.

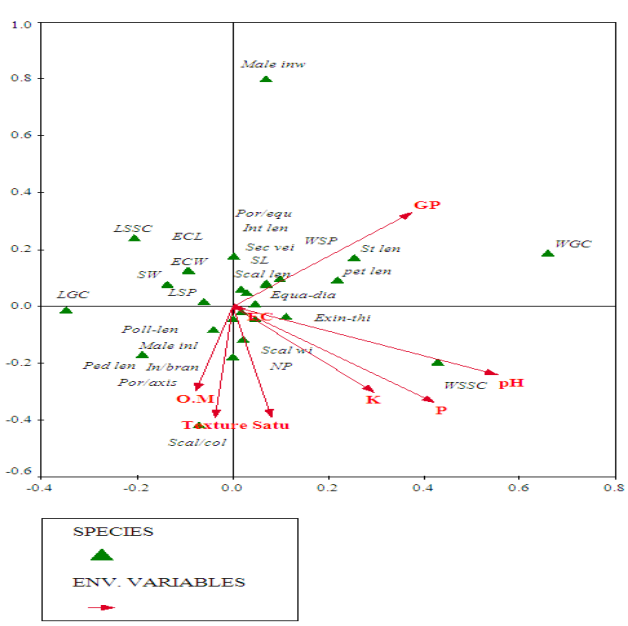

Fig. 5. Environmental variables affecting the distribution of various taxonomical features.

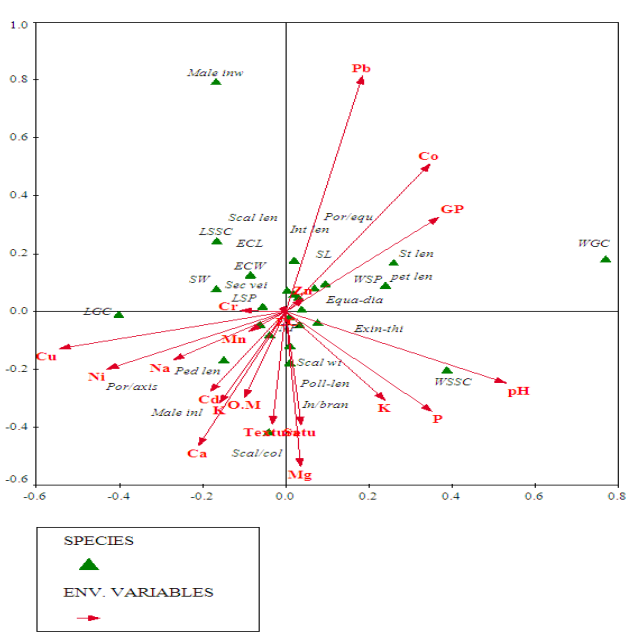

Fig. 6. Effect of heavy metals and environmental variables on anatomy, Macro-Morphology and palynology of *Alnus nitida* plant.

The pore per equator is affected by grazing pressure, Copper, Lead and Zinc by relating it with heavy metals and Soil data, while it is affected by Chromium in combined co-relation of soil with heavy metals. The secondary vein is highly affected by Chromium by relating it with heavy metals. The high grazing pressure effect the scale length by relating it to soil data, while in the case of heavy metal, it is affected by Chromium. The pollen length in soil data is affected by organic matter and texture and in the case of nutrients, it is affected by Potassium, Phosphorous, and Magnesium saturation while co-relating the pollen length with soil data plus heavy metal it is affected by Magnesium. The macro-morphological character Inflorescence per branch by co-relating with soil data shows that grazing pressure effect it while in heavy metal correlation, it is affected by Potassium, Phosphorous Magnesium and saturation while combining effect of soil plus heavy metal show that only magnesium is affecting the number of plants**.**

Discussion

The current study emphasizes the macro and micro-morphological factors of *Alnus nitida* and their co-relation with abiotic and biotic factors. The maximum male inflorescence length was observed in the Marghuzar (Swat) i.e., 13.5 cm while the minimum was 7.8 cm in Manyal (Dir) region. In female inflorescence maximum length was 3 cm reported in Shinkyari (Hazara). Our results are in close harmony with (Zare *et al*., 2012) who differentiated two species of *Alnus* i.e. *Alnus orientalis* and *Alnus subcordata* based on morphological characters, their findings showed that *Alnus orientalis* male catkins are thick and relatively short from 4 to 6.5 cm long and 0.8 to 1.2 cm broad, usually 2 or 4 together on last year twigs; female catkins green and more or less sticky. The same kind of studies was carried out by (James & William, 2002; Mozaffarian, 2002; Leopold *et al*., 2012; Chen *et al*., 2004; Furlow, 1979) who recorded species and subspecies in the genus *Alnus* based on palynology, anatomy, DNA barcoding and macro morphology, respectively. The impact of heavy metals, edaphic and environmental variables on the morpho-anatomical characters of *Alnus nitida* were examined. The order for heavy metals accumulation was different in different organs such as leaves>shoot>bark>root>soil, respectively. The localities tested for heavy metals availability revealed a sequence as Pandh> Kokarai > Sakhra > Rahat Kot> Dir Khas > Afreen Tang > Nokharai > Muzaffarabad. The possible effect of heavy metals was tested via co-relating the abiotic data with macro morphological, anatomical and palynological observations in the region. The current result showed that the pore per equator size and diameter were affected by grazing pressure, Copper, Lead and Zinc. The secondary veins size of the leaf is highly affected by Chromium and grazing pressure. The pollen length was affected by organic matter, Potassium, Phosphorous, Magnesium and the saturation state of soil condition. The macro-morphological character inflorescence per branch by co-relating it with soil data showed that the grazing pressure effect it.

At the same time, in heavy metal co-relation it is affected by Potassium, Phosphorous, Magnesium and saturation while combining effect of soil plus heavy metal shows that only magnesium was affecting the number of inflorescences per branch. The same co-relation between floral parts of *Becium hoblei* and heavy metal was performed by (Howard-Williams, 1970), where the flower size and seed weight were prominently affected by the soil where the heavy metals (Copper, Nickel and Silver) were present while the results were different in the soil with no heavy metal. In our findings, the pore/ equator was affected by grazing pressure, Copper, Lead and Zinc, Inflorescence per branch, while pollen length size is affected by Potassium, Phosphorous, magnesium, while Secondary veins size of the leaf is highly affected by Chromium and grazing pressure. Ali, (1980) studied internode, petiole and lamina length, width, male inflorescence length and width, inflorescence per branch, peduncle length, stalk length, female inflorescence catkin length and width, number of scales per column, scale length, scale width, stalk the length of fruit nut length and nut width, pollen ornamentation, pore number, exine thickness and pore diameter of *Alnus nitida* plant. The morphological characters showed that the *Alnus* spore was tetra and pentaporate. However, the internode length, inflorescence per branch, leaf epidermal anatomical characteristics were studied under light and scanning electron microscope which was rarely present in the available literature.

The leaf epidermal anatomical characteristics were studied under a light microscope. The data collected from various stations were with the minute variations in each character. Cuticular studies show variations in size, shape, stomatal indexes, length and width of epidermal cells, stomata and stomatal pores and subsidiary cells. Different parameters for foliar epidermal anatomy were epidermal cell width (10-17 (14.25) +1.49), stomatal length (4-10 (6.8654) + 0.409), stomatal width (6-17 (11.500)+ 0.52697), length of guard cell (2-10 (4.880) + 0.3908), width of guard cell (2-15 (8.673) + 0.5928), length of subsidiary cells (1.5-11 (3.13) + 0.5445), width of subsidiary cells (2-16 (10.25) + 0.6935), length of stomatal pore (5-12 (6.87) + 0.343), width of stomatal pore (1-6 (2.12) + 0.202) and no of pores (4-5 (4.14) + 0.63). Palynological characters of *Alnus nitida* from different regions were observed with light and scanning electron microscopy techniques, pollen ornamentation, pore number, exine thickness and pore diameter, etc. The pollen parameters were pollen length (20.12-29.22 (23.57) + 0.544), equatorial diameter (18.18-26.23 (21.21) + 0.37), number of pores in polar axis (2-3 (2.42) + 0.098), exine thickness (1.12-3.33 (2.55) + 0.116) and number of pores in equatorial axis (3-4 (3.73) + 0.452) in the abaxial surface, the average length was 18.28 µm and the average width was 17.42 µm so the stomatal index was indicated. It was found that the pollen morphology of this species studied is triangular on the polar side, elliptical on the equatorial side, the polar side size 12.5 (10.9-13.9) µm and the equatorial side 14.6 (13.8-16.8) µm. The apertures were tricolporate, also the pollen grain narrow colpi, the equatorial view refer to the size of the polar side is 11.0 µm and the equatorial side is 15.6 µm.

Conclusion

Prominent variation was recorded in the catkin of *Alnus nitida* due to edaphic, heavy metal accumulation and other measured climatic variables. However, further work on its genetic studies is recommended to address the factors responsible for such type of variation.

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