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Case Report

Impact of Inundations on Morphological and Anatomical Characteristics of *Dalbergia sissoo* Roxb. ex DC. – A Case Study

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Abstract

The living organisms acclimatize themselves according to the environment in which they live. The plants show modifications with climate and physiographic conditions. Some of the events of history such as major floods and droughts are conserved within plants with specific characteristics, they develop onset of these adverse events. Flood stress lowers biomass production and carbon sequestration by affecting tree stand growth yield. This synthesis focuses on impacts of flood on morphological and anatomical characteristics of *Dalbergia sissoo* Roxb. ex DC in Punjab, India. We assessed the impact of floods on young plantations on morpho- anatomical characteristics by comparing means for eight variables through paired t-test. The features; height, diameter at breast height, collar diameter, canopy shape index, leaf area, leaf specific area, leaf dry matter content, leaf color, bark color and wood color, vessel shape, vessel frequency and vessel diameter, fibre length, fibre diameter, fibre lumen diameter, fibre wall thickness were taken under consideration. The consequences of t-test were significant for all morphological variables however, not all anatomical variables were found significantly different. The study reveals the severeness of flood impacts and climate tracks that remain conserved in history of woodlands. To lessen the negative consequences of climate change, research initiatives must be included, sustainable land management techniques must be used, and the restoration ecosystems shall be designed. There is an urge to monitor forest environmental conditions and create future plans by studying the morpho-anatomical characteristics of plants.

Keywords

Dalbergia Sissoo, Water Stress, Plant Functional Traits, Wall Thickness, Flooded and Non-flooded Plantation

1. Introduction

Plants undergo diverse stressful conditions when subjected to flooding, and the nature of these challenges is dependent upon the depth and duration of inundation. Due to the rising incidence of climate-related floods, millions of trees are perishing as a result of flooding and water accumulation [2]. Before dying or acclimatizing, the alterations brought on by natural calamities could be revealed through changes taking place within plant characteristics. The imposition of flooding stress results in a decrease in leaf nitrogen content, adversely affecting the leaf's water potential, CO_2 assimilation, and photosynthetic processes. Moreover, it hastens the occurrence of leaf chlorosis, with the possibility of observing senescence [12].

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The solubility of O_2 and diffusion rates of gases in water compared with in air decreases substantially [5, 1]. Additionally, under conditions of flooding stress, there is an elevation in the generation of reactive oxygen species (ROS). This heightened ROS production leads to oxidative harm, resulting in the deterioration of cellular membranes, proteins, and lipids [8]. The production of ROS induced by waterlogging specifically triggers lipid peroxidation, leading to membrane impairment, enzyme deactivation, and ultimately culminating in cell death [4]. The effect of all of these internal changes first begin to appear phenotypically in vegetations. The hormonal production is also uttered; however, this synthesis limits it scope to impacts of flood on fifteen morphological and anatomical features.

India faces significant susceptibility to floods with over 40 million hectares out of a total geographical area of 329 million hectares being prone to flooding [7]. The nation has a long-standing history of grappling with floods owing to its intense monsoon seasons, riverine overflow, and coastal inundation. Flash floods, landslides, and floodplain inundation are recurrent challenges. Particularly vulnerable regions include the Northeastern area, Bihar, Uttar Pradesh, and West Bengal. In 2023, an unprecedented surge in monsoon activity, compounded by the impact of a western disturbance, brought about the most substantial rainfall in decades in certain locales of the region. This deluge led to the overflow of adjacent rivers, precipitating floods and landslides. The fields were inundated for several months in various parts of the country which must have brought momentary or everlasting changes in vegetations. In this synthesis, we attempted to study the changes brought on three years young plantations of *D. sissoo* in a small town of Punjab, a northwestern state of India (Figure 1). The forest plantation was carried out in July, 2020 by Punjab Forest Department. In a plain terrain of elevation range from 243-247 m, it was observed that a part of *Dalbergia sissoo* plantation was submerged by floods (243 m) during monsoon season in 2023 whereas in another part of plantation with elevation 247 m; water has not entered. These two plots are just adjacent to each other with approximately similar latitudes and longitudes and were planted at the same time of the year. The saplings used were also same and grown up in similar environmental conditions. Therefore, the two plots provided an ideal situation for comparison. The one part of the plantation remained submerged for four-five months (from July, 2023 – November, 2023). During the beginning of December, 2023, we observed that two plantation plots appear distinct morphologically. We were intrigued with the distinctness in two plantations; therefore, we attempted to assess the impact of flood conditions on morphological and anatomical features of *D. sissoo*. Fifteen traits (Table 1) i.e., The features; height, diameter at breast height, collar diameter, canopy shape index, leaf area, leaf specific area, leaf dry matter content, leaf color, bark color and wood color, vessel shape, vessel frequency and vessel diameter were taken under consideration to study the difference between non-flooded (NF) and flooded (F) plantations. The impact of floods on fibre morphology has also been studied through microscopic examination of Fibre Length, Fibre Diameter, Fibre Lumen Diameter and Fibre Wall Thickness.

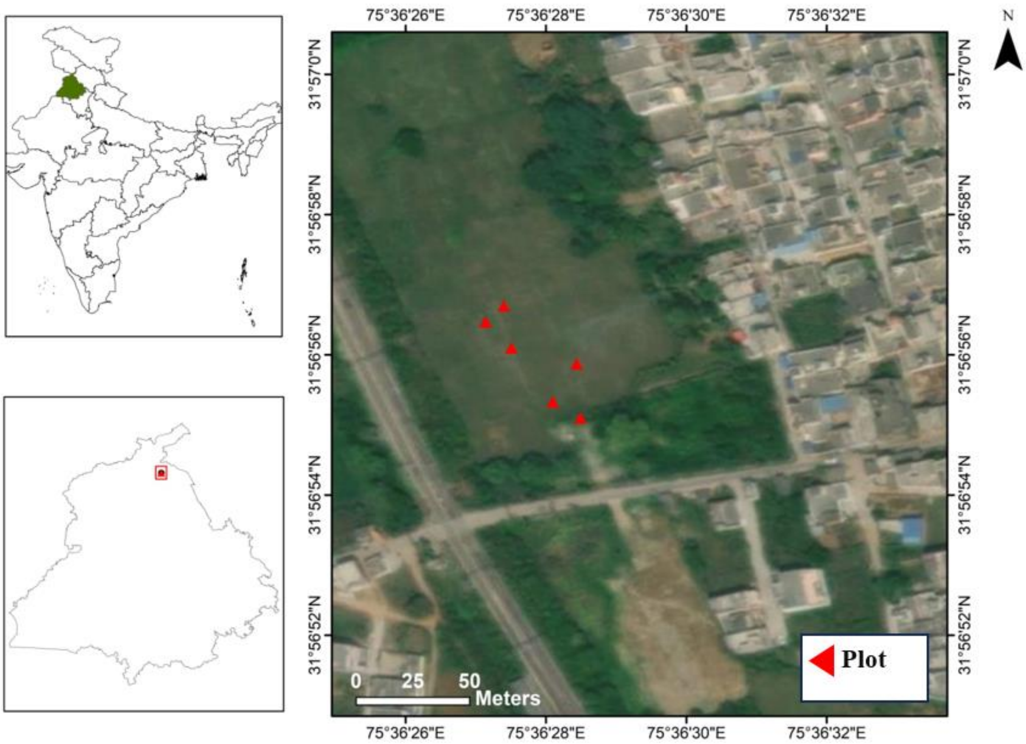

Figure 1. *Location map of study area (Mukerian, Punjab, India).*

Table 1. *List of variables studied to compare the impact of floods.*

Sr.no.	Variables	Units
1	Height (H)	(m)
2	Diameter at Breast Height (DBH)	(cm)
3	Collar Diameter (CD)	(cm)
4	Canopy Shape Index (CSI)	-
5	Leaf Dry Matter Content (LDMC)	(mg/g)
6	Leaf Area (LA)	(cm^2)
7	Specific Leaf Area (SLA)	(g/cm^2)
8	Vessel Frequency (VF)	(n/mm^2)
9	Vessel Diameter (VD)	(µm)
10	Vessel Shape (VS)	-
11	Fibre Length (FL)	(µm)
12	Fibre Diameter (FD)	(µm)
13	Fibre Wall Thickness (FWT)	(µm)
14	Leaf Colour (LC)	-
15	Bark and Wood Colour (B/WC)	-

2. Materials and Methods

2.1. Study Area

The samples were collected from Mukerian (Figure 1) which is situated in the northeastern part of Punjab, India and is known for its scenic surroundings. One of the five forest ranges of Dasuya Forest Division, it is located at an average elevation of 245 m (804 ft) above sea level with most of its area under plain terrain. The mean annual temperature is 28.02 ^{0}C. The most common soil types found in Mukerian are loamy, clay, and sandy loam. Tall vegetation is comprised majorly of *Albizia procera, Azadirachta indica, Bauhinia variegata, Dalbergia sissoo, Holoptelea integrifolia, Kigelia pinnata, Melia azedarach, Morus alba, Phyllanthus emblica, Pongamia pinnata, Psidium guajava, Syzygium cumini and Terminalia arjuna* etc. The cultivated tree species of *Eucalyptus* and *Populus* are also frequently seen. The small vegetation is dominated by herbs and shrubs such as *Achyranthes aspera, Argemone mexicana, Cannabis sativa, Calotropis procera, Parthenium hysterophorus, Ricinus communis and Saccharum spontaneum* etc.

2.2. Data Collection

The data for defined variables were collected individually for non-flooded and flooded conditions. Three plants from each plot, therefore, nine plants for each non-flooded and flooded conditions were sampled. Height (H), diameter at breast height (DBH) and lateral spread of the plantation were assessed using measuring inch tape. Collar diameter was evaluated using vernier caliper. Canopy shape index (CSI) was calculated by dividing lateral spread to the height and is dimensionless. As categorical variables, Leaf color (LC), bark color (BC) and wood color (WC) were observed with naked eyes in natural light. Leaf dry-matter content (LDMC) was calculated as the oven-dry mass (mg) of a leaf divided by its water saturated fresh mass (g). Leaf area (LA) was measured using graph paper by drawing leaves and counting and adding full and half squares covered under leaf area. Individually, thirty leaves each for non-flooded and flooded plantations were measured for leaf parameters. Specific leaf area (SLA) is one sided area of a fresh leaf divided by its oven dry mass. We also intended to work out the impact of floods on anatomical features. Therefore, samples of first ramus from both viz., non-flooded and flooded conditions were collected randomly. Wood samples were washed and put into 1:1 solution of glycerin and water to soften the tissue. For microscopic examination, 30-35 micrometer thick transverse section, tangential longitudinal section and radial longitudinal sections were cut on Reichert microtome. Dehydration of these sections was carried by passing through different alcoholic grades and they were mounted with DPX and examined under light microscope. Vessels falling within 1 mm × 1 mm were counted to evaluate the vessel frequency. The tangential vessel diameter was also evaluated by measuring vessel lumina, excluding the wall. Both, vessel frequency and vessel diameter were measured in transverse section and twenty-five readings were taken for each sample. The maceration using Schultz's method (HNO_3 and $KCLO_3$) was also carried out to study the impact of flood on fibre dimensions of *D. sissoo*. Twenty-five readings have been taken for each sample and their averages have been evaluated and analyzed. The photomicrographs of the sections were snapped using Carl Zeiss compound light microscope (Scope. A1. Axio) equipped with Carl Zeiss camera.

2.3. Data Analysis

The data collected was analyzed using SPSS 16.0 software. We hypothesized that the floods have transformed plantations morphologically as well as anatomically. The means of defined variables were evaluated and compared using paired t-test. The means for anatomical variables were also compared in SPSS 16.0 software.

3. Results

Among the fifteen plant functional traits, four traits i.e., vessel shape, leaf color, bark color and wood color observed were categorical. We observed a clear distinction for all four variables. The leaf color has turned lime green from dark green; wood and bark has turned lightish brown in flood impacted plantations. Vessel shape was found balloon or lunar shaped in flood impacted plantations, however, change in vessel shape was infrequently seen (Figure 2).

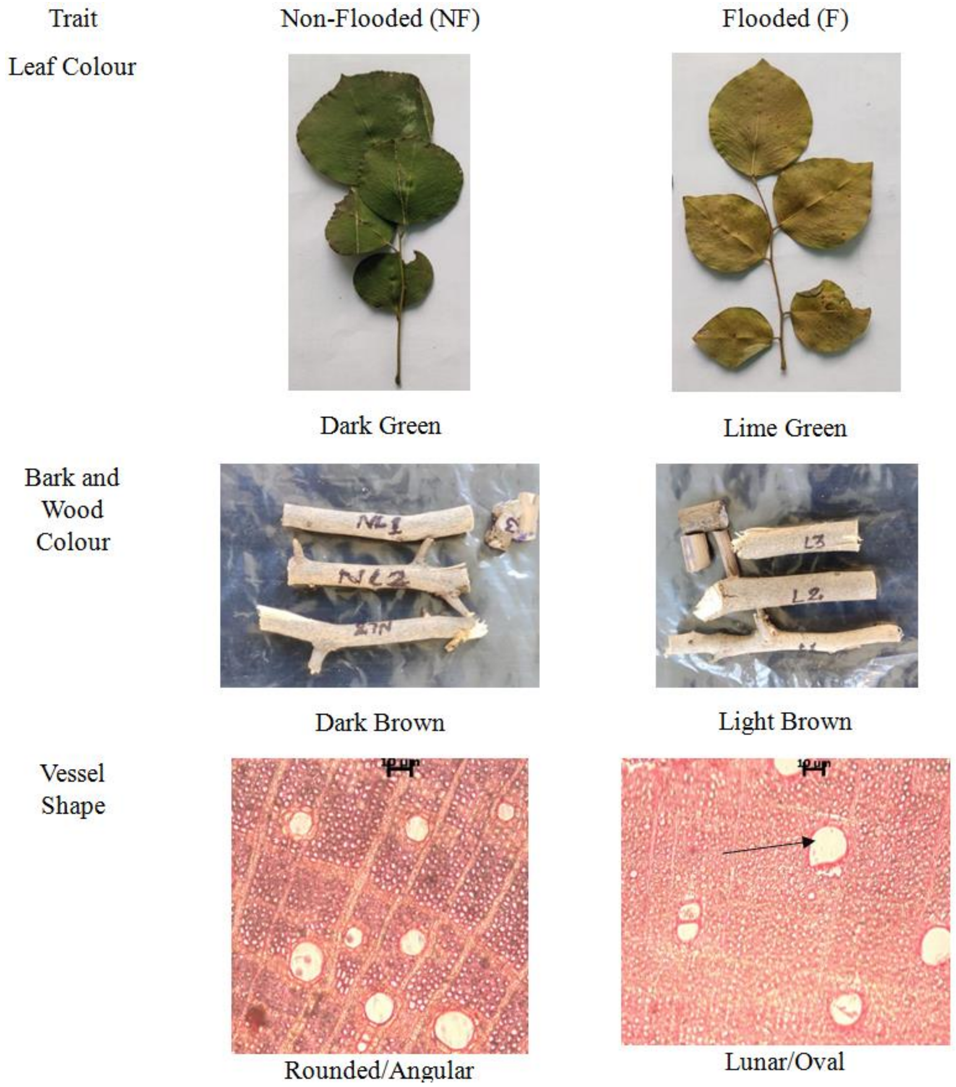

Figure 2. *The difference in categorical variables for non-flooded and flooded plantations.*

Vessel shapes were rounded or oval shaped for non-flooded plantations. The most of quantitative variables were also found transformed in water-logged conditions (Table 2 & Figure 3). The results of paired t – test revealed that growth parameters i.e., average height, diameter and collar diameter were reduced in flooded plantations. Leaf area, canopy shape index and specific leaf area were also found reduced and significantly different for flood impacted plantations, however, among the morphological features; leaf dry matter content was not found statistically significant. Among anatomical variables, vessel frequency was significantly different in two plantation and no difference was found in vessel diameter of two plantations. The number of vessel frequency has reduced nearly half of non-flooded plantations in flood impacted plantations. We compared means for every anatomical variable viz., fibre length, fibre diameter, fibre lumen diameter and wall thickness with non-flooded samples. Among three replicates of flood impacted samples, one sample each in second and third replicate; mean fibre length was significantly different. Similarly, mean fibre diameter and mean fibre lumen diameter were found with mixed observations. In first and second replicate (one in each) two samples were observed with significantly different mean fibre diameter whereas in third replicate, the impact of flood was evidently recorded as all three replicates were found significantly different from non-flooded plantations. No rigid observations were found for mean fibre lumen diameter. Moreover, wall thickness was also observed to be evidently impacted and thickened in flooded plantations (Table 3 & Figure 4).

Table 2. Mean ±S.E. for different morpho-physiological traits in affected and non-affected plantations.

Sr.no.	Variables	Mean ±S.E. Non-Flooded	Mean ±S.E. Flooded
1	H (m)	2.62±0.099	2.36±0.0988
2	DBH (cm)	3.46±0.256	2.67±0.245
3	CD (cm)	3.55±0.26	2.71±0.2
4	LA (cm^2)	7.64±0.7	5.42±0.16
5	CSI	0.93±0.01	0.72±0.054
6	SLA (g/cm^2)	0.63±0.051	0.53±0.016
7	LDMC (mg/g)	0.31±0.25	0.27±0.003
8	Vessel Frequency	34.66±1.89	19.5±2.32

Table 3. Comparison of anatomical variables for flooded and non-flooded plantations.

			Flooded 1	Flooded 2	Flooded 3
Non-Flooded 1	Fibre Length	MFL	570.43±21.18	902.5±24	611.25±19.55
		F – Value	0.951	1.270	2.692
		S/NS	NS	NS	NS
	Fibre Diameter	MFD	15.83±0.77	15±0.65	17.50±0.35
		F – Value	1.02	0.54	1.37
		S/NS	NS	S	NS
	Fibre Lumen Diameter	MFLD	13.02±0.82	11.04±0.59	11.04±0.59
		F – Value	2.38	2.62	0.47
		S/NS	NS	NS	S
	Wall Thickness	MWT	2.8±0.27	6.04±0.40	10.58±0.59
		F – Value	0.61	1.33	.004
		S/NS	NS	NS	S
	Vessel Diameter	MVD	65.37±2.82	71.29±3.49	80.76±2.58
		F – Value	1.47	1.06	1.13
		S/NS	NS	NS	NS
Non-Flooded 2	Fibre Length	MFL	570.43±21.18	898.40±24.93	611.25±19.55
		F – Value	1.25	1.15	0.409
		S/NS	NS	NS	S
	Fibre Diameter	MFD	16.10±0.60	15.17±0.58	16.71±0.32
		F – Value	0.26	1.93	0.92
		S/NS	S	NS	NS
	Fibre Lumen Diameter	MFLD	13.02±0.82	11.04±0.59	11.04±0.59
		F – Value	2.38	2.62	0.47
		S/NS	NS	NS	S

			Flooded 1	Flooded 2	Flooded 3
	Wall Thickness	MWT	2.72±0.19	5.78±0.33	10.67±0.52
		F – Value	0.20	3.90	4.92
		S/NS	S	NS	NS
	Vessel Diameter	MVD	72.27±5.15	70.00±5.99	80.00±3.50
		F – Value	0.99	1.41	2.46
		S/NS	NS	NS	NS
	Fibre Length	MFL	570.43±21.18	896.95±95	607.39±20.02
		F – Value	0.617	1.05	6.312
		S/NS	S	NS	NS
	Fibre Diameter	MFD	16.10±0.60	15.17±0.58	16.71±0.32
		F – Value	0.27	0.51	0.40
		S/NS	S	S	S
Non-Flooded 3	Fibre Lumen Diameter	MFLD	13.38±0.64	10.93±0.38	11.25±0.52
		F – Value	2.35	1.91	0.699
		S/NS	NS	NS	NS
	Wall Thickness	MWT	2.72±0.19	5.78±0.33	10.67±0.52
		F – Value	.037	0.39	0.008
		S/NS	S	S	S
	Vessel Diameter	MVD	65.57±2.92	71.34±3.63	80.76±2.58
		F – Value	1.16	0.82	2.67
		S/NS	NS	NS	NS

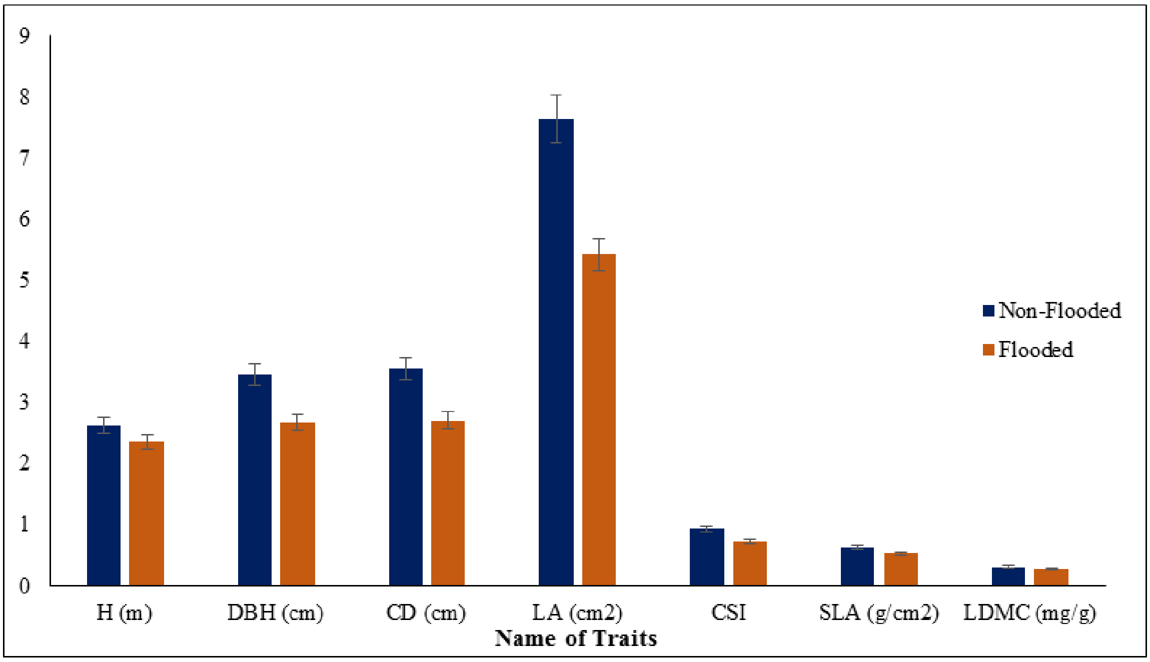

Figure 3. *Comparison of morphological traits for non-flooded and flooded plantations.*

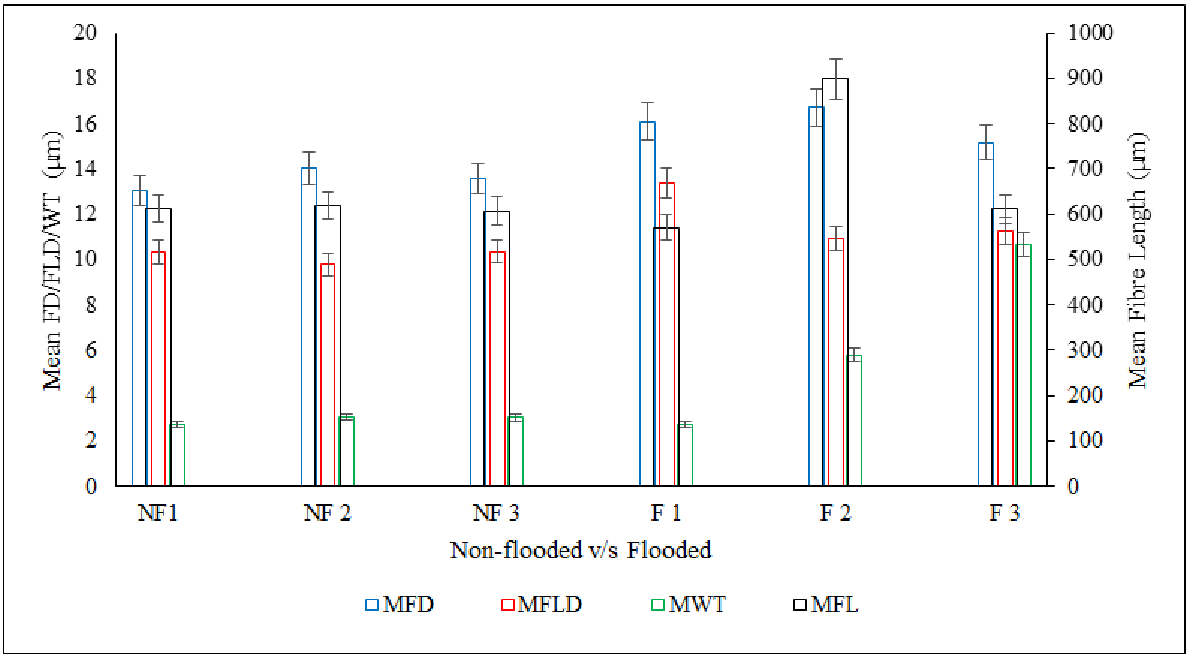

Figure 4. *Comparison of quantitative anatomical traits (mean fibre length, fibre diameter, fibre lumen diameter and wall thickness) for non-flooded and flooded plantations.*

4. Discussion

The categorical variables i.e., leaf color, bark color and wood color, vessel shape were distinct in two plantations. Leaves in water logged conditions were found lime green than dark green in non-water-logged plantations (Figure 2). On high water stress conditions, plant leaves tend to loss chlorophyll; a process called as chlorosis. The chlorophyll synthesis and enzyme activity are also reduced in persistent flooding [11]. The phenomenon of chlorosis leads to reduced photosynthesis and eventually carbon storage. Loss of extractives from wood and bark through lenticels must have resulted in lightish colors for these traits in flooded plantations. Vessel shape was altered in water logged conditions however, the trait was not observed frequently. Only a small number of vessels were found balloon shaped. The observation is consistent with a study of Brauning et al, 2016 [3] who reportedly believed that early wood vessels adopt lunar shape and expand latewood vessels in ring porous oaks when subjected to high water-logged conditions. The growth parameters i.e., Height, Diameter at Breast Height and Collar Diameter were also reduced in flood impacted plantations. Excessive flooding causes a notable decrease in the stomatal conductance, photosynthesis rate, nitrogen fixation, shoot growth, root development, and nutrient uptake in plants, ultimately resulting in considerable reductions in crop yields [6]. We also observed a substantial decrease in leaf area, specific leaf area and canopy shape index for flooded plantations. Leaf size is a response to the environment and an adaptation [10], and it can be used as a gauge for the environmental circumstances that plants grow in, therefore, reflects the severeness of flood impacts on vegetations and could also be helpful in reconstruction of climate. A certain plant's reproductive strategy can be inferred from its specific leaf area depending on a variety of parameters, including light and moisture levels [9]. A reduced specific leaf area of 0.53±0.016 from 0.63±0.051 indicates that plant reproductive strategies must have been altered by high and long-lasting water logging in studied plantations. One of the most intriguing outcomes of the study is the notable difference between the vessel frequency (Table 2) in two plantations. In water logged conditions, vessel frequency decreased to 19.5±2.32 from 34.66±1.89. This reflects the stress management adaptability in trees. The reduction in vessel frequency must have aided plantations to reduce water absorption during high water stress. Whereas in another part of plantations with non-water-logged conditions, higher vessel frequency assisted in maintaining required water absorption. We did not record any significant difference between vessel diameter of two plantations. The alterations in reducing vessel frequency and not vessels diameter infers that vessel diameter requires more time to acclimatize than vessel frequency which might have not been passed during observations were taken for the current study. Also, increase in vessel diameter make plants prone to embolism which eventually leads plant death. We came across a very limited literature on impact of floods on anatomical characteristics such as fibre morphology and vessel dimensions and majorly impact is assessed on aerenchyma formation [11] which is meant for gaseous exchange during flooding. In this study, we have observed a significant thicker fibre wall formation in inundated plantations (Table 3 & Figure 4). The adaptation of thick fibre wall formation during water logging must have aided plantations to resist other plant tissues damages. Furthermore, it provided mechanical strength to the shoot tissue system to stand out against high water stress conditions.

The cohort (a group of individuals of the same age) of *D. sissoo* plantations was the major advantage which facilitated us with standard comparison conditions. Moreover, the juvenility (of three years) of the plantation was of utmost importance for the study as all plant functional traits are more susceptible to environment-based changes in its earlier phase than mature phase. Due to juvenility of the plantations, we were able to note features such as vessel frequency and wall thickness in a short-impacted plantation of four to five months period.

A mix of observations i.e., significant and non-significant for fibre morphology specifically for fibre diameter and fibre lumen diameter highlights the major drawbacks of current study. We regret to be not able to collect large number of samples which would have been emphasized the impact of floods on fibre morphology and could have helped us to arrive a robust decision. A preliminary approach of arriving conclusive results derived on the basis of distinct morphological observations deteriorated our quality results for fibre morphology. Also, comprehensive dependency on the concept of at least three replicates played a role in diluting results. Therefore, we highly recommend the concept of repeating observations particularly for carrying out studies which are based on fresh climate impacts. We further endorse to study impacts of climate change, draught and floods on molecular, biochemical and physiological changes brought on vegetations. This shall help in preparing strategies for future prospect and planning large area afforestation and reforestation.

5. Conclusions

Here, we found noticeable changes in flooded plantations and the prevalence of the different plant traits and life strategies that characterize the post-flood recovery in D. sissoo plantations. The flooding stress impacts growth yield in tree plantations and hence reduction in biomass production and carbon sequestration. The challenges put forth by these natural calamities are needed to be addressed. It shall require a comprehensive approach, incorporating research efforts, implementing sustainable land management practices, restoring ecosystems, and developing policies aimed at climate change mitigation. Through the adoption and implementation of these strategies, it is possible to mitigate the adverse impacts of flooding in the face of evolving environmental conditions. Moreover, research on plant morphophysiological features developed during natural calamities could help us to trace the environmental conditions taking place in the history of woodlands and develop strategies for future in the present scenario of climate change.

Abbreviations

H	Height
DBH	Diameter at Breast Height
CD	Collar Diameter
CSI	Canopy Shape Index
LDMC	Leaf Dry Matter Content
LA	Leaf Area
SLA	Specific Leaf Area
VF	Vessel Frequency
VD	Vessel Diameter
VS	Vessel Shape
FL	Fibre Length
FD	Fibre Diameter
FWT	Fibre Wall Thickness
LC	Leaf Colour
B/WC	Bark and Wood Colour

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Author Contributions

Upasna Sharma: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resource, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing

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Dheeraj Kumar: Conceptualization, Supervision, Validation, Visualization, Writing – review & editing

Sangeeta Gupta: Conceptualization, Data curation, Methodology, Resources Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing

Conflicts of Interest

The authors declare no conflicts of interest.

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