

Article

Dynamics of Ice Nucleation along the Central Himalaya, Skardu, Pakistan

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ABSTRACT: Freshwater resources are precipitation and ice nucleation, which are vulnerable in environmental density with increasing human population and anthropogenic inclined indulgence, leading to the threat of complete disappearance of ice cover. Regarding global prospects, the Himalayas have grave significance regarding ice resources, and Pakistan's dependency upon fresh water from ice covers is more than 70%. Investigation of ice nucleation in the central Himalayas and Skardu regions in Pakistan indicates that abiotic factors like pH and temperature and biotic factors like bacterial presence in sediments or soil, ice meltwater, stream water and plants play a vital role in ice nucleation. Vegetation is the best habitat for bacteria with movement of 50 to 500 culturable cells/m²/sec (Suzuki et al. 1993, Morris et al. 2004 and Goodnow et al. 1990). Application of Vali's formulation $c(T) = \{\ln(N_0) - \ln(N_0 - N(T))\}/A$ for ice nucleation per bacterial cell shows biotic factors are more significant than abiotic factors. Trees like Cedrus deodara (Roxb.ex D. Don) (common name Diar) family name is Pinaceae showed the most significant tendency among the studied variables for ice nucleation, with $\ln c(T) = -14.84$ as compared to Pinus roxburghii Sargent (botanical name) Chir (common name) Pinaceae (family name) $\ln c(T) = -15.9$. Ice nucleation per cell ($\ln c(T)$) in ice meltwater, stream water and sediment were -16, -15 and -15.14, respectively. P.H.PH range was near to neutral value (7) with the highest 8.4 in deposits and the lowest 7-making range of 1.4. The lowest mean monthly temperature in January from 2000 to 2022 is -12.6 °C (2013), while the warmest mean low temperature is -6.2 °C (2022), as per PMD. These temperature ranges are ideal for heterogeneous ice nucleation, as 50% of drops were frozen in the sample taken from the plants at a temperature of -10 °C. In comparison, stream water showed 35% frozen drops in this study. The research methodology following bacterial tests (H₂S and Gram tests) and evaluation of abiotic factors like pH, temperature, wind direction indicates more impactful are biotic factors and low temperature for heterogeneous ice nucleation.

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Introduction

Ice nucleation has a significant role in the water cycle [1]. More precisely, 0°C is the temperature ice coexists with water [2]. In homogenous ice nucleation, water freezes at -39°C or -40°C, and clustering needs 70 water molecules, while at -5°C, 4500 water molecules are required to provide energy requisition [1, 3].

Ice nucleation requires some surfaces and temperatures to occur below 0°C even this kind of ice nucleation can happen only at a few degrees below 0°C [3]. It's been observed by meteorologists and plant pathologists since 1971 that plants are vital in the rain-making and icing process. The atmospheric conditions are closely linked to plant growth and their health. Plants have direct and profound impacts from hailing, air pollution and rain [3]. It is concluded that all plants, whether healthy or diseased,

are surrounded by bacteria with catalysing ice formation capacity at comparatively warmer temperatures like -3°C to -5°C [3]. In all seasons, plants face the pressure of temperature limits and numerous plants have unique responses against freezing conditions. Plants face dehydrative and mechanical stress due to cytolysis. Plant leaves have 10^7 bacteria per cm^2 . Also, bacteria have 10^{30} total inhabitants, while 10^{24-26} are present on plants, leave the surface and become part of atmospheric aerosol. For bacteria, airborne cells originate principally from plants and soil [4]. Environmental factors, including temperature, aerosols, wind direction, U.V, and pH, can impact the growth of a bacteria called *Pseudomonas syringae*, which can catalyse the rate of ice nucleation [5, 6]. For the first time, a French meteorologist, Soulage, studied and concluded the presence of bacterial cells in ice crystals. The study of ice nucleation activity, the pathogenic impact of *pseudomonas syringae* on plants and their interconnection with the ice nucleation rate have grave importance in microbiology and ecology. Emerging climatic trends are leading to variation in atmospheric variables including precipitation and ice nucleation which is one of the largest sources of water in Pakistan. This indicates and urges to conduct more research to investigate factors impacting ice nucleation to maintain consistent supply of water and find sustainable solutions.

The present study's goals are to evaluate present scenario of ice nucleation and its connection with biotic and abiotic factors to figure out its future trends at the study area.

LITERATURE REVIEW

Ice nucleation is divided into two significant parts biological and non-biological. The latest research in ice nucleation indicates that biological factors can play an important role in ice nucleation for both natural and commercial sectors.

Homogeneous ice nucleation occurs at -39°C or -40°C , where only 70 water molecules are required, compared to ice nucleation at -5°C , where 4500 water molecules share energy [1, 3]. A few substances are used as a catalyst for heterogeneous ice nucleation, like silver iodide, amino acid crystals, and monolayers of long-chain alcohols [6]. In 1990, it was noticed that aliphatic alcohol monolayers whose lattice dimensions are close to that of ice could support ice nucleation [7]. At sub-zero temperatures, the plant faces the stress of shallow temperature leading to dehydrative stress [1]. Ice nucleation active *pseudomonas syringae*, a bacterium, is an extrinsic agent, while the plants prepare an intrinsic agent. Infrared thermography helps to determine ice nucleation active agents. Successful experiments have been conducted on tomatoes (*Lycopersicon esculentum*) to estimate ice nucleation at -3.2°C . Antifreeze proteins exist in plants which depress the freezing point by only 0.2°C to 0.4°C . Also, complex plant species can survive cooling to -196°C in liquid nitrogen. *Pseudomonas syringae* contains a protein formed by a gene called *inaZ* which plays a vital role in ice nucleation. Similarly, other organic and inorganic materials like silver iodide, kaolinite, cholesterol and amino acids can also play a role in ice nucleation [8]. *P. syringae* is a member of the *pseudomonas* genus and is based on 16S rRNA analysis [9].

After studies, it is established that bacteria play a significant role in precipitation for rain and ice nucleation and atmosphere and clouds at certain altitudes. *Pseudomonas syringae* causes diseases in the foliage of plants as well [10]. These bacteria interact with other species of insects and bacteria, leading to ice nucleation yielding frost injury to plants [5, 6]. The ice-nucleating activity depends on an outer membrane lipoglyco protein. This discovery paved a new orientation in ice nucleation, spray ice technology and biotechnological processes.

Heterogenous and homogeneous crystallization have been studied, and many organic and inorganic substances like silver iodide, kaolinite, cholesterol, and amino acids also play a role in ice nucleation. Besides *P. Syringae*, temperature also profoundly impacts the induction of ice nucleation. Parallel to that, high relative humidity has importance in growing *P. syringae* in plants. *P. Syringae* may be successfully applied for supper cooling reduction, high freezing temperature, crystallization control, freezing time reduction, improved stability, and decreasing energy cost suggested the application of *P. syringae* in snowmaking, which led to its commercialization in 1985 [8].

Pseudomonas syringae has the role of the plant pathogen and provides information to understand the link between pathogenicity and the molecular mechanism of plant interaction in biological cycles. It can cause stomatal closure, which leads to the effect of immunity of the plant. Atmospheric conditions like humidity, dew, fog and rain favour an abundant population of *P. Syringae*.

It can also act as a decomposer and primary producer with body dimensions 1-3 μm long to 0.3-0.5 μm wide [5, 6]. These microorganisms originate from seeds during germination and later become part of atmospheric aerosols. It is observed that the upward movement of bacteria from plant bodies and leave the surface is on the order of 50 to 500 culturable cells/m²/sec suggesting bacteria are flying up into the atmosphere than falling back to Earth's surface [5, 11]. *P. syringae* is a non-toxic microorganism if ingested by humans; therefore, frequently used in foodstuff but has pathogenic effects for plants due to a protein having an optimum temperature of 28 °C. This bacterium can cause diseases in tomatoes, potatoes, cherries, plums, peaches and apricot [4, 5, 10]. In Pakistan, nearly 8 to 16 percent of vascular plants, including wild weeds, occur in the Northern Mountain regions where over 5700 species of floral resources are registered. In Naran, major vegetation types are forests, crops and weeds [12].

LOCATION

One of the most famous and beautiful regions of the central Himalayas is Skardu (35°16'44" N 75°36'48" E, altitude is 2225m). Skardu district has the highest peak in Pakistan K2 with an altitude of 8611m, following Broad Peak (8,047), Gasherbrum (8,000+m) and Masherbrum (7,821m). For sampling at the study area, the following three regions were selected i. Skardu khas ii. Shagri Kalan iii. Katpana desert or cold desert.

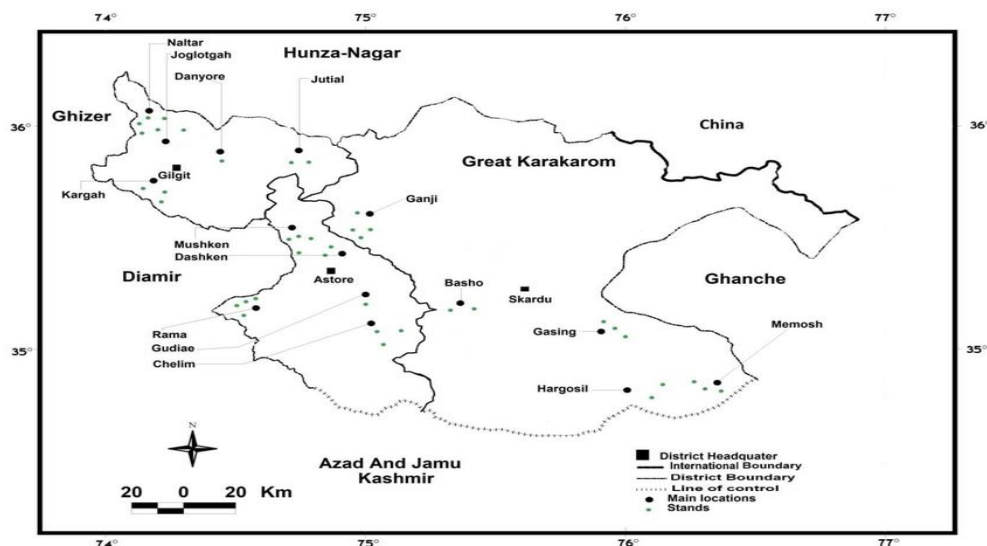


Fig.1: (a) Sampling areas of Skardu

METHODS AND MATERIALS

1. Sampling

Sampling regions Skardu was divided into three areas, i.e., Skardu khas, shagri kalan and katpatana desert or cold desert. From each marked point, ten samples from each source like sediments, ice meltwater, soil, river or stream water and from each marked point, two trees, i.e., plant one which *Cedrus deodara* (Roxb.ex D. Don) (common name Diar) family name is Pinaceae and plant two which *Pinus roxburghii* Sargent (botanical name) Chir (common name) Pinaceae (family name) were collected. One hundred fifty samples (50 samples from each point) were collected from 21st April to 25th April 2023 from three marked points of the study area Skardu. Each step of this research work was repeated five times for more accurate and precise results.

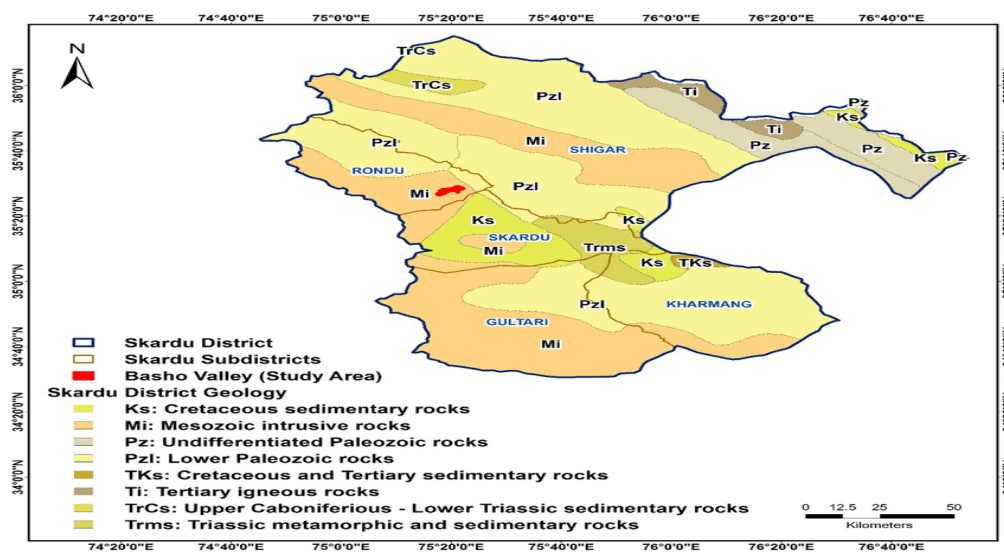


Fig. 1: (b) Study areas of Skardu

2. Bacterial strains

Following sampling methods to isolate *P. syringae* were used. Bacterial strains were cultured for two days at 16 °C using King's medium B. At 4°C and 1-hour incubation, bacterial suspension with distilled water was taken 5×10^6 to 5×10^8 cells/mL.

3. Gram test.

The gram method was used to find the percentage of Gram-positive bacteria to assess the potential probability of ice nucleation agents.

4. H₂S test.

Ice meltwater and stream water were examined for bacterial presence by the H₂S test pH of ice, stream water, ice meltwater, sediments and two said plants were assessed with P.H meter to evaluate its impact on ice nucleating. These samples were collected in five days; two samples were collected each day to determine uniform impact after taking the mean and average of the results.

5. Pakistan Met Department.

The climate data from the Pakistan Meteorological Department for the temperature, wind direction and precipitation variability were analyzed to find future trends and correlations of ice nucleation in the study area.

6. Vali method for ice nucleation.

Following Vali method, 50 drops of 25 microliters with dilution suspension of 5×10^6 to 5×10^8 cells/mL were spread on a sterile aluminium sheet and floated on a cooling bath. The initial temperature was at -1°C followed by a reduction of 1°C till -10°C with incubation of 5 minutes per 1 °C change in temperature followed by visual counting of frozen drops. Ice nucleation per bacterial cell was computed according to Vali (1971) proposed procedure, and equation $c(T) = \{\ln(N_0) - \ln(N_0 - N(T))\} / A$ here $c(T)$ is ice nuclei per bacterial cell at temperature T, N_0 is several drops tested, N is a total number of frozen drops at temperature T, and A is bacterial cell concentration per drop. For example, the degree of INA was computed with an unlimited number of ice nuclei per cell, and the highest temperature at freezing occurred.

RESULTS ANALYSIS

Table 1: H₂S test for Bacterial presence in the samples

Region	Ice	Stream Water	Ice Melt Water	Sediment	Plant 1	Plant 2
Skardu	Less Brownish	No Change	Less Brownish	Less Change	More Brownish	More Brownish
Shagri Kalan	No Change	Less Change	Less Brownish	No Change	More Brownish	More Brownish
Cold Desert	Less Brownish	Less Change	Less Brownish	Less Change	More Brownish	More Brownish

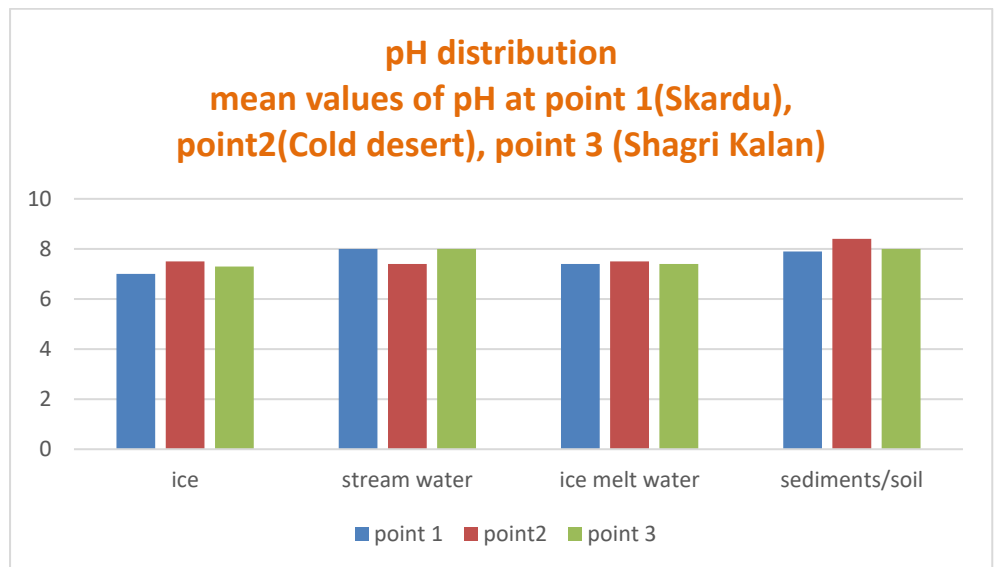
The above-tabulated data shows the presence of bacteria which plays a role in ice nucleation. In the study areas, ice samples collected were tested with H₂S,

indicating the presence of bacteria as the colour changed to brownish, but lesser intensity was observed. Stream water and sediment samples were tested for bacteria. Still, less colour change was observed after the H₂S test, indicating less culture of bacteria, while ice melt changed the colour, indicating the presence of bacteria. On the other hand, plant one and plant two leaves were tested with H₂S and showed positive results. The colour of the solution changed to a greater degree indicating the presence of bacteria with higher concentration than other tested samples taken from the study area.

Table 2: Gram Test for Bacterial Presence in the Samples

	Ice		Stream Water	Ice Melt Water		Sediment		Plant 1		Plant 2	
	Gram +	%		Gram +	%	Gram +	%	Gram +	%		
Skardu	12		9		13		3		27		25
Cold Desert	11		7		20		2		26		23
Shagri Kalan	10		9		21		3		26		26

Gram test of the samples collected from the study area was conducted, and bacterial culture was examined to find the percentage of gram-positive bacteria. The bacterial cultures were counted visually to find their percentages, and the results are tabulated in table 2. This shows ice samples from three points of the study area have less variation, and overall gram-positive bacterial concentration is higher than stream water and sediments but less than the plants and ice meltwater. Stream water and sediment analysis for bacterial concentration show the lowest percentage in the study area. The highest concentration level of gram-positive bacteria was observed on the tree considered in the research, with very less variation with each other but a significantly higher level of gram-positive percentage was observed. It is established that per cm² area of a plant carries 10⁸ bacteria. The results indicate that ice and plants have more potential to find gram-positive bacteria. As *Pseudomonas syringae* is one of the gram-positive bacteria, its higher percentage makes it more suitable for ice nucleation. The study area has vegetation with 50 types of plants from 25 families that can be used as a cure for 33 diseases. The most commonly used are leaves (41%), and roots (26%) treat different ailments. Biotic factors indicated in fig.1 and fig.2 shows higher concentration of bacteria in the samples collected from the plants at study area. This also signifies stronger role of bacteria and other biotic factors in ice nucleation.



Graph 1 (pH distribution and evaluation)

The graph shows pH and its variability in the study area. Its range is seven, with the lowest and highest value being 8.4. It has already been established that pH at 4.1 hinder ice nucleation [7]. The data in the graph reflects that pH values are safe, leaving no direct negative impacts on ice nucleation. But if environmental conditions change due to urbanisation or industrialisation and tourism, it can decrease pH value, hindering ice nucleation. Atmospheric effects on ice nucleation from acidic pH, NO₂, O₃ and UV [1].

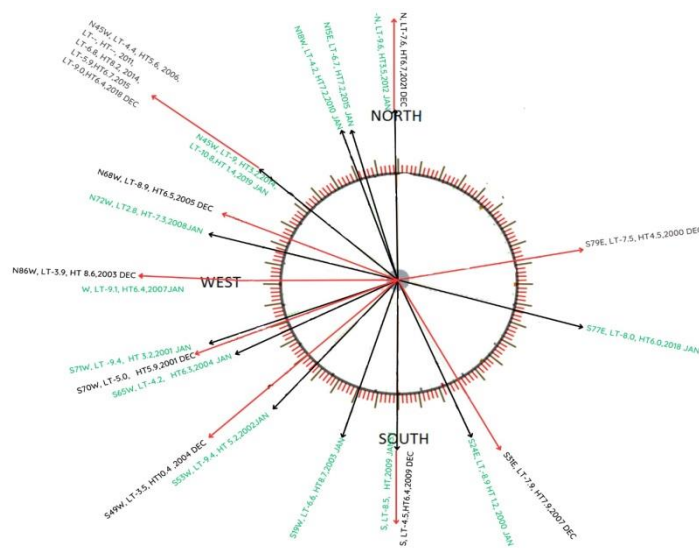


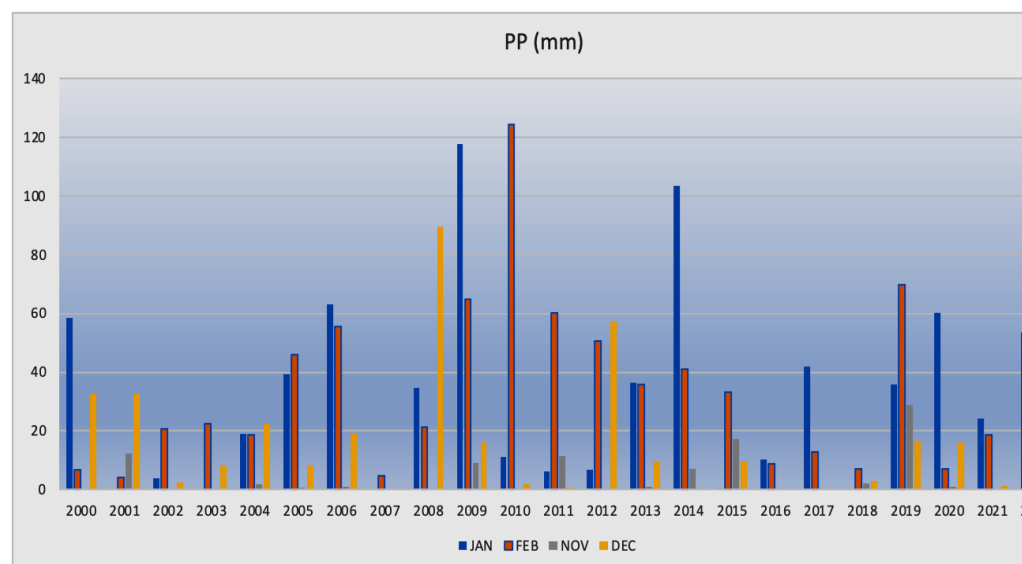
Fig. 2: Wind Direction at the study area

The wind wheel indicates the wind direction with the highest temperature and lowest temperature from 2000 to 2022 in December and January, which are the coldest months and more suitable for snowfall. The data indicates orthodox observations that in 22 years, only two times wind direction was from west to east in December 2000 (northeast) and December 2007(southeast), with temperature variation from -7 °C to 9 °C. Similarly, for January 2000 and 2018, wind direction was east-south and south-east respectively, with temperature variation from -8 °C to 12 °C. In 22 years, the most frequent direction of the wind in December is northwest. These changes could be due

to changes in atmospheric pressure followed by snowfall and precipitation, and temperature in the surrounding bigger populations like Kashmir and Gilgit.

PRECIPITATION

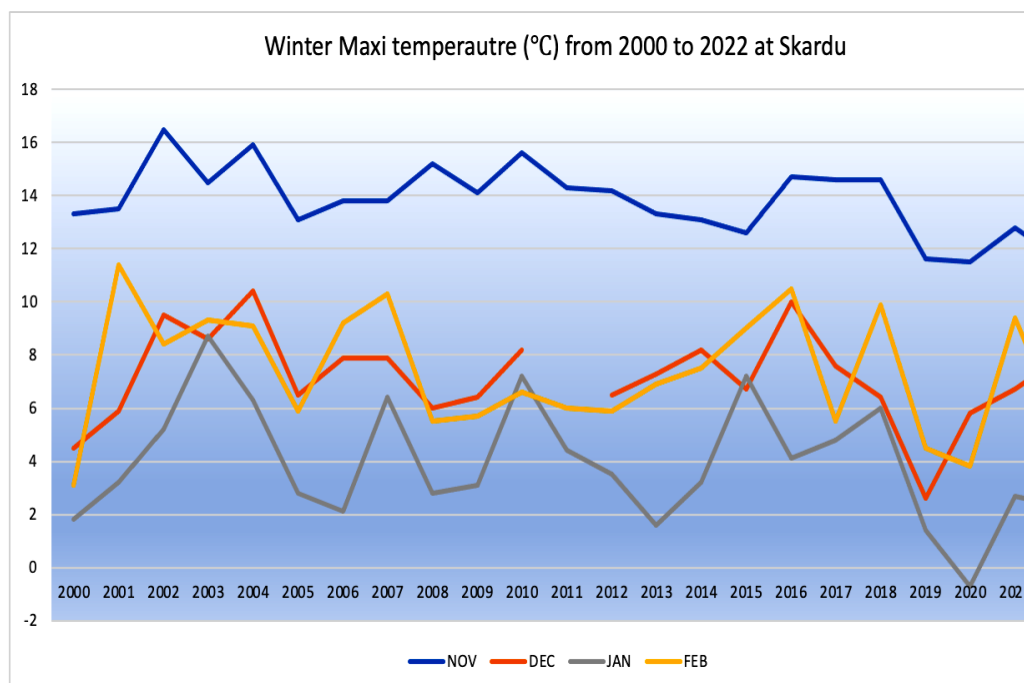
As per PMD, in the coldest months of January and December from 2000 to 2022, the highest mean precipitation recorded was 117.6 mm in January 2009, followed by 103.5 mm in January 2014. In 2002, 2007 and 2017, no precipitation was observed, which reveals a significant potential risk to understanding the parameters of new strategies. On the other hand, the mean highest level of precipitation in December was registered at 89 mm and 57.2 mm in 2008 and 2012, respectively. It is pertinent to mention that no precipitation was observed in 2007, 2014, 2016 and 2017.



Graph. 2 (Precipitation at Skardu from 2000 to 2020)

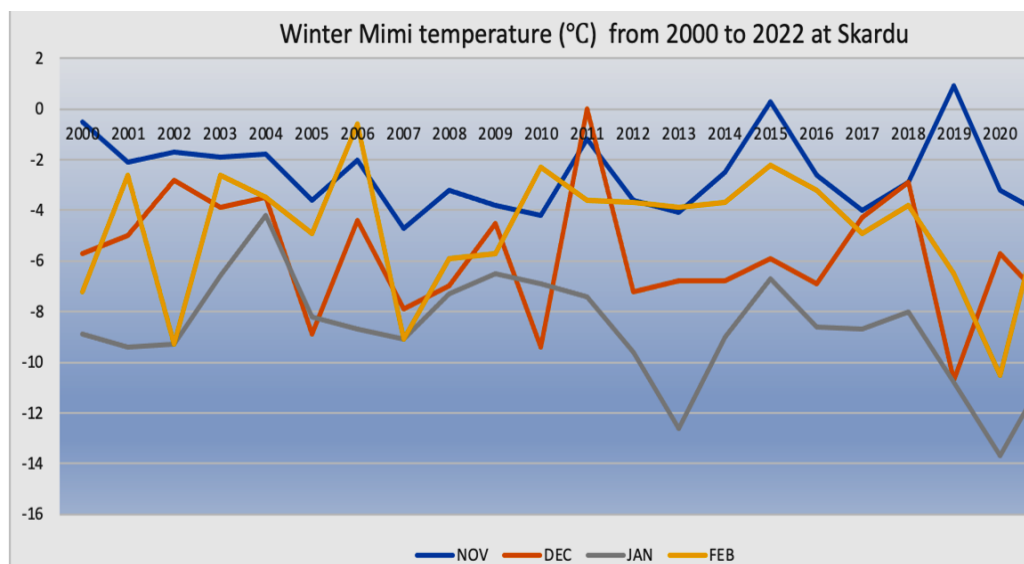
TEMPERATURE

Temperature variance of the study area from 2000 to 2022 shows that in the coldest months (December and January) of a year, the maximum mean temperature recorded in January 2003 was 8.7 °C, and in December 2004 was 10.4 °C. The similarly lowest mean temperature recorded in January 2013 was -12.6 °C, and in December 2010 lowest mean temperature recorded was -9 °C. In the coldest months, at the study area of Skardu, the lowest mean temperature was -12 °C from 2000 to 2022. This shows a potential opportunity for INA in the study area of the central Himalayas. Experimental results of INA also show the lower temperature is more suitable for INA. Graph 2 for precipitation shows analogy with high level of precipitation and temperature variation shown in graph 3 and graph 4.



Graph 3 (Winter maxi temperature from 2000 to 2020)

Data represented in the graph (winter maxi temperature) shows that from higher temperature measurement ranges, the lowest temperature recorded in November is 12 °C, in February is 11 °C, in December is 3°C, and in January is -1 °C from 2000 to 2022. This is a consistent decline of lower temperatures from November to January.



Graph 4 (Winter mini temperature from 2000 to 2020)

Winter minimum temperature measurement range indicates that the lowest temperature in November is -5 °C, December -11 °C, January is -14 °C and February -11 °C. These temperature-lower extremes indicate suitability for ice nucleation has the potential to address the biotic and abiotic factors to enhance the ice nucleation.

ICE NUCLEATION ACTIVITY

As stated in methodology ice nucleation activity, $C(T) = \frac{\ln(N_0) - \ln(N(T))}{A}$ described by Vali in 1971, was applied and \ln of final reading is shown in the following table.

Table 3: Here IMW = Ice Melt Water, ST = Stream Water

ln C(T) OF THE SAMPLES					
T/°C	IMW	ST	SEDIMENT	PLANT1	PLANT2
-1	0	0	0	0	0
-2	0	0	0	-18.66	0
-3	0	-18.26	-18.26	-17.6	-18.26
-4	0	-17.33	-17.33	-17.6	-16.96
-5	-17.77	-17.6	-17.6	-15.79	-16.05
-6	-17.6	-15.66	-15.66	-15.54	-15.66
-7	-16.82	-15.3	-15.3	-15.3	-15.4
-8	-16.13	-15.15	-15.15	-15.04	-15.06
-9	-16.11	-15	-15.14	-14.93	-14.99
-10	-16	-15	-15.14	-14.84	-15.9

Surface Ice and glaciers are a very sensitive indicator of global warming. The first decay of twenty-first century (2000-2010) is recorded as the warmest decade, with an average rise in temperature is +0.53°C while the average recorded rise in temperature of the mountainous range is 1.5 °C facing extraordinarily depleting environmental threats, including the intensity of ultraviolet electromagnetic radiation in HKH (IPCC 2007) Cryosphere under the stress of global warming are shrinking reducing snow time duration [13, 14].

It is assessed that more than 30% of planet Earth's surface is covered with periodic seasonal snow while 10% is covered permanently, becoming a source of 80% fresh water [15, 16]. Abiotic factors like silver iodine and aerosols have contributed to ice nucleation. Alcohol monolayer contribution was confirmed by Gavish in 1990. Aliphatic alcohol monolayers have similarities with ice lattices. The series of $C_nH_{2n+1}-OH$ for $n=16$ to 31 after sprinkling over water drops introduce double nucleation with an increasing number of carbons in alcohol. If the carbon number reaches more than 22, ice nucleation reaches -8 °C; when the Carbon number reaches 31, the ice nucleation temperature registered is -1 °C. Similar observations were recorded with carboxylic acid [7]. This precipitation and low temperature in winter months are supporting factors for INA. A study of other bio factors and correlation with INA at the study area would be helpful to understand and predict the dynamics of ice nucleation. Biotic factors could be vegetation and bacterial presence, including *Pseudomonas syringae*. The data with lower temperatures from -1 °C to -10 °C indicates nucleation more in biotic factors than abiotic factors. Plant 1 tends to nucleate in biotic factors as plant leaves are a better bacterial habitat. Ice nucleation, experimentally proved, has inclined trends with lowering temperature as shown in the table 3 while data in the graph 4 shows lowest temperatures in the months of January and February making these two months the most suitable time range of ice nucleation.

Table 4: Percentage of Number of Frozen Drops of the samples

Temperature /oC	Stream Water	Ice Melt Water	Sediments	Plant 1	Plant 2
-1	0	0	0	0	0
-2	0	0	0	2	0
-3	2	0	0	4	2
-4	4	0	0	8	6
-5	6	2	0	20	14
-6	20	2	0	24	20
-7	30	4	2	30	26
-8	32	8	4	42	40
-9	35	14	6	46	42
-10	35	18	10	50	48

In this investigation shown in table 4, the bacterial percentage was calculated by providing bacterial culture and test for gram-positive and gram-negative tests. Counting visually percentages of gram-positive and gram negative were calculated. These samples were tested at different temperatures to find several frozen drops. These results indicate a comparison of biotic and abiotic factors of the study area for ice nucleation activity. Biotics factors, the samples collected from plant leaves have more bacterial presence as 50% of drops were frozen at -10 °C.

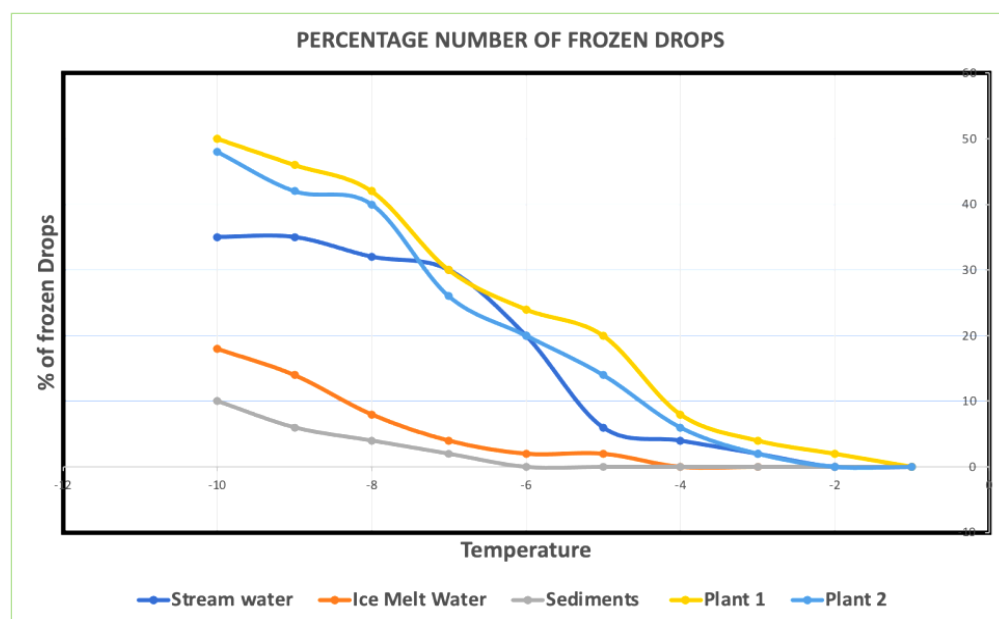
At warmer temperatures, more frozen drops were observed in biotic factors (plants) compared to abiotic factors (water samples). For plant1 (*Cedrus deodara* (Roxb.ex D. Don) (common name Diar) family name is Pinaceae) at -2 °C, 2% drops were frozen, which indicates an ice nucleation trend at a warmer temperature. As compared to biotic factors, plant 2 which is *Pinus roxburghii* Sargent (botanical name) Chir (common name) Pinaceae (family name), also showed better ice nucleation trends showing 2% frozen drops at -3 °C. From abiotic factors, stream water showed better freezing trends; further study is needed to investigate the reason behind it.

Multiple R	0.991					
R Square	0.981					
Adjusted R Square	0.958					
Standard Error	0.619					
Observations	10					
ANOVA						
	df	SS	MS	F	Significance F	
Regression	5	80.970	16.194	42.327	0.001	
Residual	4	1.530	0.382			
Total	9	82.5				
	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%

Intercept	-1.518	0.457	-3.315	0.030	-2.799	-0.247
Stream water	-0.032	0.061	-0.522	0.629	-0.202	0.138
Ice Melt Water	0.255	0.283	0.902	0.417	-0.530	1.041
Sediments	-0.536	0.487	-1.101	0.333	-1.889	0.816
Plant 1	-0.413	0.187	-2.201	0.093	-0.935	0.108
Plant 2	0.294	0.219	1.344	0.249	-0.313	0.903

Table 5: Summary of statistical analysis of data for R square and standard error

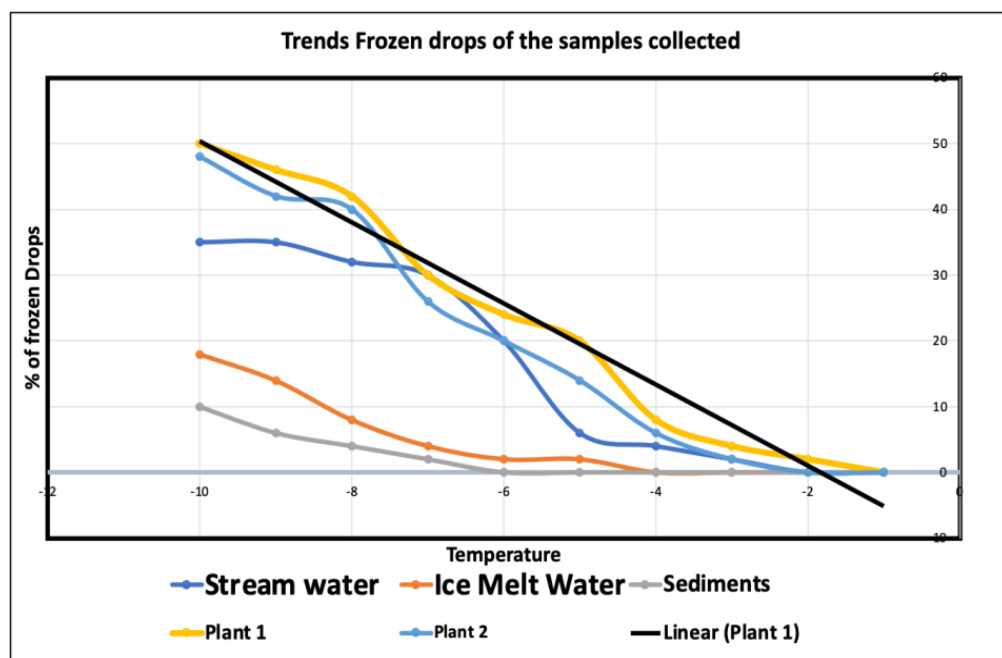
Regression results for the data (negative temperature vs percentage of frozen drops) indicate acceptance of our research question as P-values are in the best range, indicating that control of biotic and abiotic factors in the study area can play a significant role in ice nucleation. At a lower temperature, bacterial presence on the leaves of the plants can result in an exponential increase in ice nucleation. Abiotic factors are interlinked with biotic factors and can have additional and constructive roles in ice nucleation in the study area in the central Himalayas, especially at Skardu, Pakistan. The R-square value is more than 0.9, indicating a strong relation between temperature changes and environmental factors for ice nucleation. This is established to focus on biotic factors to be added to the environment to yield more ice nucleation in the study area.



Graph 5 (% of frozen drops vs temperature)

The graph above shows the number of frozen drops with a temperature change. These are average results of the study area. All biotic and Abiotic factors have similar trends

for ice nucleation, but biotic factors have increasing trends of ice nucleation. According to U.S. US Environmental Agency,2000, glaciers will disappear completely in the next 30 years. In Europe, half of the glaciers, and in Africa, 8% of ice disappeared [16]. This highlights the significance of glaciers, and the study of biotic and abiotic factors for ice nucleation shows how the depletion of glaciers can be mitigated. Biotic factors in ice nucleation revealed that monolayer and a specific protein can directly and deeply impact freezing nuclei at comparatively warmer temperatures [7, 17]. Bacterial presence is assured as ice nucleation is with increasing trends in biotic factors of the study area. Considering the background of ice nucleation, it is reinforced that organic involvement in ice nucleation in the study area shows the same increasing trends. Abiotic factors have increasing trends, as shown in Graph 5. However, the collaborative role of all environmental factors can provide the best variable for ice nucleation.



Graph 6 (frozen drops vs temperature trends)

The graph shows future trends for the highest degree of ice nucleation. The trends for plant 1 and plant 2 show a linear increase, which is a positive sign for ice nucleation, signifying the need for vegetation. Abiotic factors impact ice nucleation, but these are directly or indirectly associated with biotic factors. Biotic factors for ice nucleation were found in the 1970s after the discovery of bacterial ice nucleation at -2°C . a similar role was found in the 1980s from fungus in ice nucleation. It was established that DNA segments play an important role in ice nucleation and bacterial outer membrane containing a protein [7, 17]. Abiotic factors like silver iodine and aerosols have contributed to ice nucleation. Alcohol monolayer contribution was confirmed by Gavish in 1990. Aliphatic alcohol monolayers have similarities with ice lattices. The series of $\text{C}_n\text{H}_{2n+1}\text{-OH}$ for $n=16$ to 31 after sprinkle over water drops introduce double nucleation with increasing number of carbons in alcohol. If the carbon number reaches more than 22, ice nucleation reaches -8°C , and when the Carbon number reaches 31, the ice nucleation temperature registered is -1°C . Similar observations were registered with carboxylic acid [7, 18]. These are all established factors for ice nucleation in other parts of the world. Similarities are observed with the published results making it easy to find more potential initiatives needed for ice nucleation in the study area.

Conclusion

Pakistan has approximately 13700km² glacial covered area making 13% of the mountains of the upper Indus Basin with the fastest snow reduction rate as compared to the world. With this current reduction rate, glacial cover may disappear in 2035, which is the source of 70% of fresh water in Pakistan. Climate data from 1971-2000 indicates the highest winter temperature increased while the lowest winter temperatures slight decline. These rising temperature trends speed up snow melting. In the study area, the pH evaluation shown in graph 1 is mostly in the normal range, a positive sign for environmental density. The study area has an ideal temperature for precipitation and snowfall as shown in Graph 3 and Graph 4. H₂S and Gram test results prove the presence of ice nucleation active bacteria *Pseudomonas syringae*. Application of Vali formula for ice nucleation $c(T) = \{\ln(N_0) - \ln(N_0 - N(T))\}/A$ shows results shown in table 3, shows heterogeneous ice nucleation at a warmer temperature. Bacteria in water, sediments, or soil samples is an additional supporting factor for ice nucleation. These results concludes that abiotic and biotic factors collectively are contributing for ice nucleation in the study area seeking for sustainability of all parameters and variables.

Author's Contribution: A.A.k, Conceived the idea; A.A.K, Designed the simulated work or acquisition of data; A.A.K, executed simulated work, data analysis or analysis and interpretation of data and wrote the basic draft; A.A.K, Did the language and grammatical edits or Critical revision.

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