ENVIS BULLETIN
HIMALAYAN ECOLOGY

Volume 31, **2023**

Bioprospecting Mountain Ecosystems
Himalayan Environment and Ecology

ISSN: 0971-7447 (Print)
ISSN: 2455-6815 (Online)

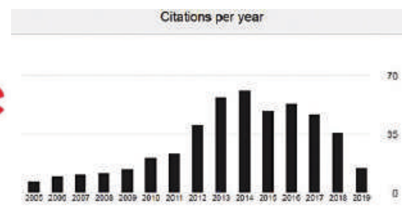
EIACP **CENTRE ON HIMALAYAN ECOLOGY**
G.B. Pant National Institute of Himalayan Environment
(An Autonomous Institute of Ministry of Environment, Forest and Climate Change, Government of India)
Kosi-Katarmal, Almora-263 643, Uttarakhand, India

Vol. 31, 2023
ENVIS Bulletin
HIMALAYAN ECOLOGY

Kosi-Katarmal, Almora, Uttarakhand

Coverage and Indexing of ENVIS Bulletin

Citation Indices	All	Since 2014
Citation	1137	524
h-index	16	11
i10-index	37	13



Global Impact and Quality Factor* : 0.643
Research Impact Factor** : 0.091
Sources: *GIF Database (2014)
**ICI Database (2010)

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HIMALAYAN ECOLOGY

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Volume 31, 2023

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G.B. Pant National Institute of Himalayan Environment
(*An Autonomous Institute of Ministry of Environment, Forest and Climate Change, Government of India*)
Kosi-Katarmal, Almora-263 643, Uttarakhand, India

Guest Editor’s Note

Mountains, with 24% of the global terrestrial area, occur in all the continents, in all the latitudinal zones, and within all the principal biomes. As one of the most fragile ecosystems, the mountains represent the unique areas for detecting climate change and its impacts. The mountain ecosystems and mountain communities are increasingly receiving attention in international debates due to its rich biodiversity, warming climate, and the cascading effects to humanity. The yearly organized Conferences under United Nations Framework Convention on Climate Change (UNFCCC) serve as the formal meeting of the Conference of the Parties to assess the progress in dealing with climate change. Importance of resilience and adaptations acquired by the mountain communities using biodiversity to cope up in harsh climatic conditions has been realized in the forums like- the United Nations Conference on Environment and Development in Rio de Janeiro 1992, the Sendai Framework for Disaster Risk Reduction 2015, the Paris Agreement 2015 of the UNFCCC, the Sustainable Development Goals and Targets 2030,including the recently held Fifteen Conference of Parties meeting of the Convention on Biological Diversity in Kunming and Montreal 2022 and the Conference of Parties 28meeting of the UNFCCC in United Arab Emirates 2023.

Considering the intricate link between climate change and biodiversity loss and its impact on human wellbeing, Graphic Era (Deemed to be University), Dehradun, India organized an International Conference entitled “**Mountain ecosystems: Biodiversity and adaptations under Climate Change scenario**” in collaboration with **International Centre for Integrated Mountain Development (ICIMOD), Kathmandu, Nepal** during **22-24 March 2023**(Pandey and Chettri 2023). The conference witnessed some of the leading research groups around the world actively working on various aspects of mountain and extreme ecosystems, mainly Himalaya, Antarctica, and Andean ecosystems. The major themes addressed in the conference included- Biodiversity assessment and adaptations; socio-economic effects on biodiversity, including sustainable food systems, managing agroecosystems and adapting to climate change; and consideration of specificities of mountain regions for technology development and policy formulations. Selected papers, presented by the leading scientists, on the state of biodiversity prevailing climate change scenarios, have been considered for publication in this special issue of ENVIS Bulletin.

The issue begins with an Opinion paper authored by Prof AN Purohitreferring climate in two distinct categories- natural (long term weather patterns) and social (global meta trends). He emphasizes that responding to these changes to mitigate the adverse impact of climate change on any mountain ecosystem is crucial. It is followed by the Views of Dr. Eklabya Sharma from Ashoka Trust for Research in Ecology and the Environment (ATREE), Bengaluru, India on Call for Cooperation and Action. Dr. Sharma discusses this aspect considering Governments, Private sectors, Research institutions, civil societies and communities for an urgent call to SAVE this GLOBAL ASSET by proactively cooperating for developing resilient and sustainable Hindu Kush Himalaya. In third article, Dr. Nakul Chettri (ICIMOD, Kathmandu, Nepal) discusses the insights of the relevance of Kunming-Montreal Global Biodiversity Framework with narratives on achievements and missing opportunities for mountains with strategic way forward. Then, in the next five articles, Dr. Vaneet Jishtu (Himalayan Forest Research Institute, Shimla, India), Dr. Amit Chawla (CSIR-Institute of Himalayan Bioresource Technology, Palampur, India), Dr SR Joshi (North Eastern Hill University, Shillong, India), Dr RK Maikhuri (HNB Garhwal Central University, Srinagar, India) and Dr. Rajesh Joshi (GBP-NIHE, Sikkim, India), discuss the trends, patterns, impacts, elevation, and community response depending on warming in terrestrial as well as aquatic ecosystem biodiversity and its importance at global discourses.

Diversity of microbial communities with reference to their adaptations and applications was one of the major attractions of this conference. Experts working on Andean, Antarctica, and Himalayan regions discussed interesting research aspects of these ecosystems. Andes, the longest continental mountain range in the world, extend over 7000 km along the West border of South America, from Southern Chile to Venezuela. Dr Luis Andrés Yarzábal Rodríguez (Universidad Católica de Cuenca, Cuenca, Ecuador) provides information concerning the available data relating to Tropical Andes glaciers' microbes and comments on their importance as friends and foes to humans, animals, plants and, in general, to entire ecosystems. Dr. María Sofía Urbieta (Universidad Nacional de La Plata, Argentina) discusses the rich biodiversity and complex community structure of the microbial species, bacteria and archaea that inhabit the Copahue geothermal area correlating with the geology and physicochemical characteristics. Antarctica represents the extreme cold environment with respect to coldest part of the Earth- highly attractive for research projects focusing on biodiversity, life strategies, and adaptation mechanisms. Dr. Avinash Sharma (National Centre for Cell Science, Pune, India) explains the cold-adapted microbial diversity along with the sampling methods, adaptation strategies, and biotechnological applications of Antarctica samples while Dr. Sergio Leiva Poveda (Universidad Austral de Chile, Valdivia, Chile) discusses epiphytic bacteria associated with macroalgae in Antarctica. The Himalaya, referred as the Asia's Water Tower, is well known for its heritage value enshrined in cultural diversity and sacredness. The region has been recognized amongst the 36 global biodiversity hotspots. The region has a discrete geographic and ecological entity that produces a distinct climate of its own and influences the climate of much of Asia. Highlighting the actinobacterial diversity of actinobacteria in Himalayan glacier soil, Dr M Radhakrishnan (Sathyabama Institute of Science &Technology, Chennai, India) provides information on the anti-infective properties of these cold adapted organisms.

The focus of the next two articles is on the socio-economic aspects and development and policy issues relevant to mountain ecosystems, particularly the Indian Himalayan region. Development approach and citizen centric innovations in mountain perspective, discussed by Prof N Farooquee (Indira Gandhi National Open University-New Delhi, India) and Prof BS Bisht (National Centre for Good Governance-Mussoorie, India) are important articles at policy level.

Finally, success stories about the economically important cultivars relating to their contribution in livelihood and food security are presented. Conservation strategies and sustainable utilization of Himalayan medicinal plants for livelihood security in the face of changing climate is discussed by Dr ID Bhatt (GBP-NIHE, Almora, India). Prof Jyoti Vakhlu (University of Jammu, Jammu, India) provides information onhigh altitude locations in Jammu and Kashmir regions suitable for saffron cultivation with intent to provide alternative income to the farmers. Dr. Deepika Saraswat (Defence Institute of Physiology and Allied Sciences, DRDO, India) presents information on nanocurcumin based formulation for combating cardiac and skeletal muscle damages in high altitude mountain regions. Prof Anil Kumar (Thapar Institute of Engineering & Technology, Patiala, India) describes the modern methods for potato seed production in sustainable hill economy viewpoint.

Anita Pandey, Graphic Era, Dehradun
Nakul Chettri, ICIMOD, Nepal
Indra D Bhatt, GBP-NIHE, Almora
Vishal Tripathi, Graphic Era Dehradun
Rakesh K Sharma, Graphic Era Dehradun

About the Bulletin

ENVIS Bulletin on Himalayan Ecology is an annual non-priced publication of the EICAP previously known as ENVIS Centre, which was established at the headquarters of the G.B. Pant National Institute of Himalayan Environment (GBPNIHE) in the financial year 1992-93 with the fiscal support from the Ministry of Environment, Forest & Climate Change (MoEF&CC), Government of India, New Delhi. The present volume of the ENVIS Bulletin is 31st in the series of its annual publication and contains papers on Bioprospecting Mountain Ecosystems and Himalayan Environment and Ecology The news and views offered in the papers in this publication are the views of the concerned authors. Therefore, they do not necessarily reflect the views of the editors, EIACP Centre and Institute. The content of the Bulletin may be quoted or reproduced for non-commercial use provided the source is duly acknowledged.

The contributions to the next issue of the Bulletin in the form of a research paper, popular article, news item and technical report, etc., related to Himalayan ecology, are always welcome. However, the matter supplied by the individual/organization may be edited for length and clarity as per EIACP publication guidelines. Request for institutional subscription of the Bulletin may be sent to the coordinator of the EIACP Centre. The comments/suggestions for further improvement of the Bulletin are welcome.

Er. M.S Lodhi
Executive Editor - ENVIS Bulletin,
EIACP Centre on Himalayan Ecology,
G.B. Pant National Institute of Himalayan Environment
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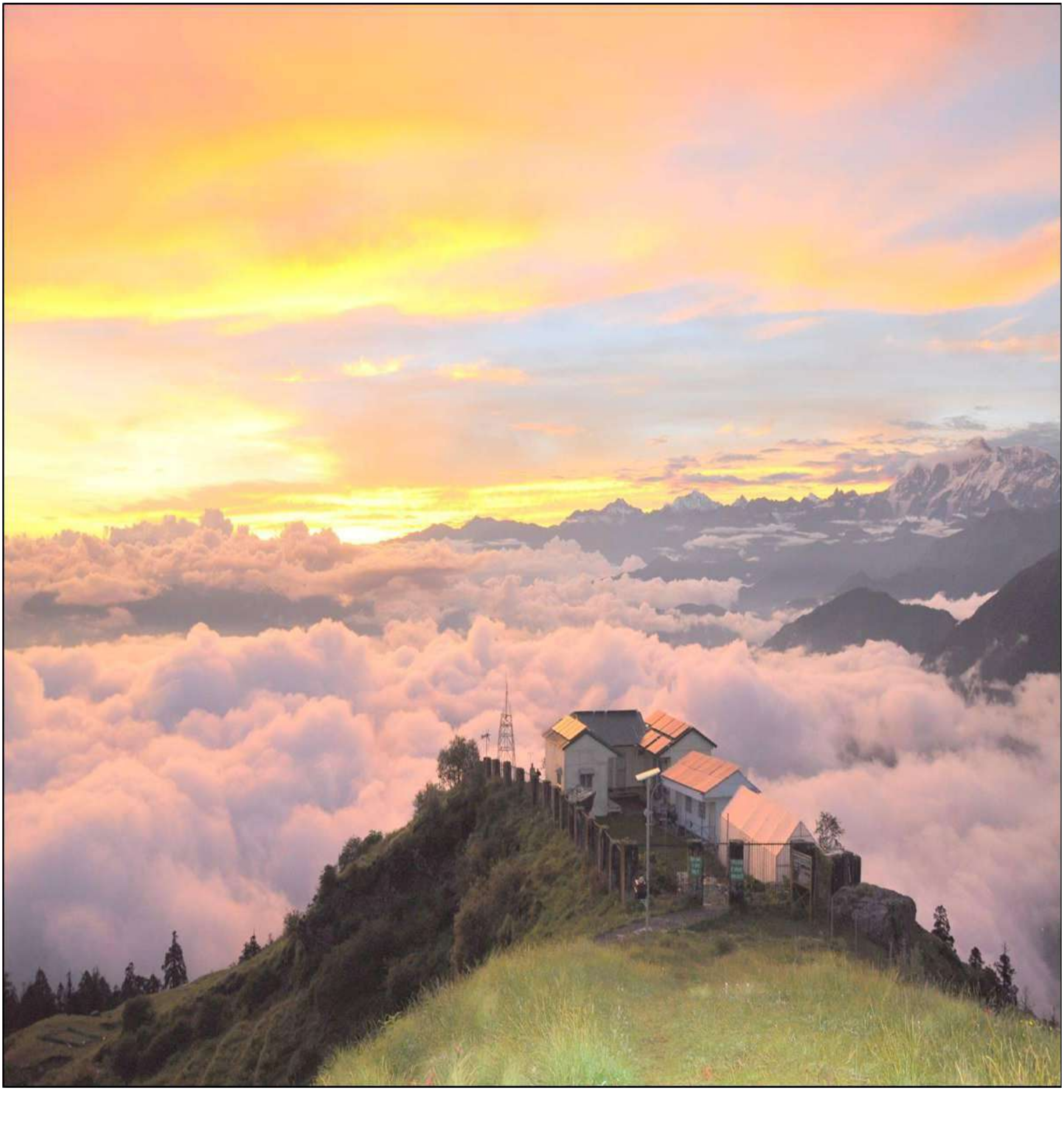
CONTENTS

VOLUME 31 YEAR 2023 ISSN: 0971-7447 (Print)
ISSN: 2455-6815 (Online)

Section -I
Bioprospecting Mountain Ecosystems



INTERNATIONAL CONFERENCE ON MOUNTAIN ECOSYSTEMS: AN OPINION

Aditya Narayan Purohit

ABOUT THE AUTHOR

Prof. Aditya Narayan Purohit is a well-known Indian Scientist and Professor who has mainly worked on eco-physiology of tree species and high altitude medicinal plants. He established a prestigious High Altitude Plant Physiology Research Centre at H N B Garhwal University Srinagar Garhwal, Uttarakhand, India and acted as its founder Director from 1985-1990and again from 1995- 2002. He was appointed Director of GB Pant National Institute of Himalayan Environment, Kosi-Katarmal, Almora, Uttarakhand, India during 1990-95. Subsequent to his superannuation from H. N. N. Garhwal University, he was offered "ML Bhartiya Chair" by Uttarakhand Govt. during which he helped Uttarakhand Govt to establish a Centre for Aromatic Plants. Prof. Purohit is honoured with a galaxy of National and International awards including the prestigious Fellowships of Indian National Science Academy, National Academy of Sciences, National Academy of Agricultural Sciences and Prestigious civilian award of Padmashri by Govt. of India.

Probably during the course of this seminar you will be discussing three important buzzwords: climate change, biodiversity, and adaption in mountain ecosystems. I believe that understanding these issues is crucial for everyone concerned about the environment and our future in the mountains.

For this seminar conference, I asked myself four questions: what is climate change? What is biodiversity and how it responds to climate change? And how do these issues relate to mountain ecosystems? I turned to the ChatGPT for answers and I share with you what I learned.

WHAT IS CLIMATE CHANGE?

My understanding of climate change is that it can be divided into two distinct categories: natural and social. The concept of natural climate refers to long-term weather patterns and conditions that exist in a particular region or ecosystem, and is determined by PHYSICAL, GEOLOGICAL and ASTROLOGICAL positioning of the earth. These control the factors such as temperature, precipitation, humidity, wind, and other meteorological variables. Social climate, on the other hand pertains to the social and cultural environment in which people live, encompassing social norms, attitudes, values, and beliefs that shape the way people interact with one another and their environment. This is influenced by the Global META TRENDS, such as communication, personal interactions, tourism and patterns of modernization.

Although natural and social climates are separate concepts, they are often interconnected, and changes in one can significantly impact the other. In mountainous regions, human activities such as deforestation, agriculture, and urbanization can lead to changes in the natural climate, for example, through a reduction in the number of trees that absorb carbon dioxide from the atmosphere, changes in the land use, and alteration in soil moisture, runoff, and albedo. These activities can also indirectly affect the natural climate by disrupting ecosystems and decreasing biodiversity.

Therefore, it is important to take into account the social climate and human activities when studying the natural climate in mountainous regions. Understanding the complex interactions between the two can help to mitigate the negative impacts of human activities on the natural environment.

HOW MANY TYPES OF BIODIVERSITIES ARE THERE IN NATURE?

Based on my knowledge there are two types of biodiversity: microbial and macrobial biodiversity. Microbial biodiversity, including bacteria, fungi, and protists are the primary contributors to microbiodiversity. These organisms are often overlooked but play critical roles in larger ecosystem processes such as nutrient cycling, decomposition, and biogeochemical processes. Although the term "macrobial" is not widely recognized and used, it is used here to refer to the diversity of species of higher taxonomic groups within an ecosystem or biogeographic region. Macrobiodiversity encompasses a wide range of organisms, including plants, animals, and fungi, in both terrestrial and aquatic ecosystems. The number of species present in a given area, such as national park or a country, is often used is to measure macrobiodiversity. Biodiversity can be used assessing various metrics, such as species richness, species evenness, and phylogenetic diversity.

HOW WILL BIODIVERSITY RESPOND TO CLIMATE CHANGE IN MOUNTAINS?

Climate change is anticipated to have significant impacts on the biodiversity of mountain ecosystems. The rise in temperature is expected to cause changes in snow cover, soil moisture, and other crucial environmental variables in these ecosystems, which will have cascading effects on biodiversity.

There are several anticipated impacts of climate change on biodiversity in mountain ecosystems. Firstly, as temperatures increase, species that are adapted to cooler temperatures may move to higher elevations in order to maintain their preferred temperature range. This movement could lead to the loss of species from lower elevations and the emergence of new species in higher elevations. Secondly, the timing of biological events, such as flowering or migration, may change due to the warming temperatures. This shift in phenology could result in mismatches between species that depend on each other for pollination and other interactions. Thirdly, the composition of mountain communities may change as species shift their ranges and phenology changes. Some species may be better adapted to the new conditions, while others may struggle to adapt and decline. Finally, climate change is expected to increase the risk of extinction for many species, particularly those that are already rare or restricted to small ranges. Mountain species may be particularly vulnerable, as they often have limited options for moving to new locations.

Overall, the impacts of climate change on biodiversity in mountain ecosystems are likely to be complex and varied. The extent of the impacts will depend on a variety of factors, including the rate and magnitude of change, the specific characteristics of the mountain ecosystem in question, the interactions between different species, and altitude as well as latitude and longitude.

CONCLUSION

Mountain ecosystems are unique and complex environments that provide vital ecosystem services to millions of people worldwide. They are home to diverse range of species, many of which are adapted to extreme climatic conditions. However, these ecosystems are facing significant challenges due to both natural as well as social climate change. These changes are affecting the biodiversity of mountain ecosystems and the adaptations of species that inhabit them. For example, as temperatures increase, some plant and animal species are moving further up mountains to find cooler habitats. This can lead to changes in species composition and distribution, with some species being displaced or becoming locally extinct.

At the same time, some species are adapting to changing conditions by altering their behavior, physiology, or morphology. For example, some mountain plants are flowering earlier in response to warming temperatures, and some birds are changing their migration patterns to adjust to changes in food availability.

To mitigate the impacts of climate change in mountain ecosystems, it is important to conserve biodiversity and support species adaptation. This can be done through actions such as protecting habitats, reducing habitat fragmentation, and promoting sustainable land-use practices.

In conclusion, the shape of a mountain ecosystem is determined by various factors, such as, altitude, climate, geology, astrological positioning of the earth and human activity. Mountain ecosystems are generally characterized by distinct elevational zones that exhibit unique flora and fauna. The base of the mountain ecosystem may include forests or the grasslands, while higher elevations may contain different plant and animal species. At the top of the mountain, the ecosystem may feature alpine tundra with harsh weather-conditions and low-growing plants. Human activities such as land use changes, resource extraction, and pollution can disrupt natural processes and alter the distribution and abundance of species within the ecosystem. Despite their complexity, it is important to take action to mitigate climate change and protect biodiversity in mountain ecosystems.

STATEMENT

In my opinion, climate change is essential for the survival of all living being and to blame CO_2 for all the wrongs on this planet is unfortunate. I strongly believe that 'CO_2 as a main cause of global warming' is a simple political gimmick.

While NATURAL climate is prone to fluctuations, with some of its components beyond our immediate control, changes in the SOCIAL climate often occur linearly and rapidly. As such, it is crucial that we actively attend and respond to these changes to mitigate the adverse impact of climate change on any mountain ecosystem. I strongly believe that climate change will cause an increase in microbial biodiversity and a decrease in macrobial biodiversity. In Himalayan ecosystems, fast changes in social climate are causing stronger adverse impact than in the natural climate.

CALL FOR COOPERATION AND ACTION

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An International Conference on "Mountain Ecosystems: Biodiversity and Adaptations Under Climate Change Scenario" was organized in Graphic Era Deemed to be University, Dehradun in March 2023. Deliberations clearly indicated that there is a need for cross sectoral and cross scale cooperation and actions in meeting the challenges of climate change and sustainable development in the Hindu Kush Himalaya (HKH). Mountains occupy more than one-fifth of the world's land area and are home to about one-eighth of the world's population. Mountains support 25% of the world's terrestrial biodiversity and include nearly half of the world's biodiversity 'hotspots'. The HKH region spans over 4.2 million sq km including Bhutan and Nepal in their entirety and parts of six other countries, namely Afghanistan, Bangladesh, China, India, Myanmar, and Pakistan with widely varying geographical terrain, ecosystems, and a plethora of cultures. It is often referred to as the "Third Pole" and the "Water Tower of Asia," as it stores a large volume of water, particularly in the form of ice and snow, while regulating the flow of the 10 major river systems in the region. The region provides ecosystem services that directly sustain the livelihoods of 240 million people in the mountains and hills of the HKH. Nearly 1.9 billion people living in the river basins also benefit directly and indirectly from its resources, while more than 3 billion people enjoy the food produced in its river basins. The region is also home to some of the most diverse cultures, languages, religions, and traditional knowledge systems in the world.

The HKH Region is one of the most important mountain ecosystems of the world providing immense goods and services to humanity. The world is changing and things are changing fast in the HKH. The HKH has been witnessing rapid biodiversity loss, ecosystem degradation, climate change, increased disaster risk, and rising vulnerabilities of people both in the mountains and downstream areas.

Scenarios from now to the end of the 21st Century for the HKH region can be running downhill, or muddling through, or it may advance toward prosperity. The third scenario of moving towards prosperity is required for transformative change if we want to see resilience and sustainability in the HKH region. Evidence-based actions to maintain biodiversity for enhanced ecosystem services, to reduce disaster risk, to mitigate and adapt to climate change, to protect cryosphere and manage water, and to adopt good governance, are central to ensuring resilience and sustainability in the HKH, as well as collaboration among government and non-government actors. Regional cooperation, transboundary sharing of information and knowledge, and capacity building among the eight HKH countries will be necessary to meet the challenges such as drivers of change including climate change, sustaining biodiversity and ecosystem services, future energy needs, understanding and acting on cryospheric change, water security, food and nutrition security, disaster risk reduction and management, reducing poverty and vulnerability, adaptation to climate change, gender and social inclusion, migration and governance. Community resilience can be achieved by applying approaches that lead to integrated contours for sustainability. Sustainable solutions should be targeted for integrating three dimensions of development namely, research, policy and practice. All these have to be connected, overlapped and overlaid for results on the ground. The roles of scientists and policy makers on think tanking; informed policy and decision making by using research findings and knowledge; and practitioners and communities adopting good practices and policies could be the ways of collaboration for sustainable solutions.

Governments, private sectors, research institutions, civil societies and communities should be aware of the rising challenges in the HKH. Sustainable solutions on issues related to the challenges demand strong will to work together and connecting all spheres of development. For example, contemporary knowledge should be linked with traditional knowledge; data and information sharing among the institutions and the countries; moving from disciplinary to multi-disciplinary to inter-disciplinary to transdisciplinary approaches; linking science with policy and practice; designing and planning for linking people with nature; linking upstream with downstream ecosystems and communities in river basins for reducing risks and vulnerabilities; linking mountain economies with outside markets for growth and sustainability; linking and integrating different types of ecosystems towards developing, restoring and maintaining multi-functional landscapes; and cooperating among the countries on transboundary issues. The urgent call to all the players mentioned above is to SAVE this GLOBAL ASSET by proactively cooperating for developing resilient and sustainable HKH.

KUNMING-MONTREAL GLOBAL BIODIVERSITY FRAMEWORK – A MISSED OPPORTUNITY AND A WINDOW OF HOPE FOR REWILDING ECOSYSTEMS IN THE HINDU KUSH HIMALAYA

Nakul Chettri

International Centre for Integrated Mountain Development, Kathmandu, Nepal

ABSTRACT

Mountains have been projected as a pivotal ecosystem maintaining habitat heterogeneity, global biodiversity, ecosystem functions and services to humans. With over 24% of the global terrestrial areas and half of the Global Biodiversity Hotspots, mountain are also repository of rich culture and laboratory of good practices demonstrating social-ecological resilience. The prevailing triple planetary crises of climate change, biodiversity loss and pollution, are bringing visible challenges in the mountain ecosystem. The Hindu Kush Himalaya, the highest, most fragile, and critically important for one third of the humanity through its diverse services is witnessing the above average warming with wide array of consequences from melting glaciers, increasing incidents of disaster to water crises, change in phenology and productivity to biodiversity loss. The global community recognised the importance of mountains at the Rio Conference on Environment and Development in 1992, which led to the inclusion of Agenda 21 in Chapter 13, relates to sustainable mountain development. Similarly, the World Summit on Sustainable Development (WSSD) also dealt on mountain ecosystems and advocated that mountains should be considered as special places in the global sustainable development agenda. Consequently, the Conference of the Parties to the Convention on Biological Diversity (CBD) adopted 'Mountain Biodiversity' as Decision VII/27 at its 7th meeting, held in Kuala Lumpur in February 2004. However, in the recent times, the global significance of mountain ecosystem and biodiversity has been underrated and shadowed deepening the crises and missing opportunities for mitigation and adaptation. Even the recent Kunming-Montreal Global Biodiversity Framework agreed in Montreal, Canada could not garner due recognition for the mountains. The mountain communities were forced to read between the lines and look for its relevance largely due to lack of coordinated, collaborative and collective voice and negotiations. This paper presents the insights of the relevance of Kunming-Montreal Global Biodiversity Framework with narratives on achievements and missing opportunities with strategic way forward.

Keywords: Mountain agenda, Planetary crises, Global biodiversity framework, Underrated mountains, Opportunity, Rewilding

INTRODUCTION

Mountains are complex and fragile ecosystems that cover almost a quarter of the earth's land surface and host 12 percent of its people (Waster *et al*., 2023). Due to the extreme heterogeneity of environments (climates and soils), rapid elevational changes (altitudinal vegetation belts), and variable directional orientation (aspect), the mountains have diverse vegetation and varied microclimatic and ecological conditions (Payne *et al.,* 2020). Consequently, mountains exhibit high biodiversity, often with sharp transitions (ecotones) in vegetation sequences, and equally rapid changes from vegetation and soil to snow and ice. In addition, mountain ecosystems are often rich in endemics, because many species remain isolated at high elevations compared to lowland vegetation communities that can occupy climatic niches spread over wider latitudinal belts (Mittermeier *et al.,* 2011). Thus, they are the last bastions of wild nature "islands" in a sea of transformed lowlands and provide several very important ecological functions. These functions contribute to half of the humanity for their wellbeing well beyond the immediate vicinity, benefiting entire river basins (Reader *et al.,* 2023). In addition, natural and semi-natural vegetation cover on mountains helps to stabilize headwaters, preventing flooding, and maintain steady year-round flows by facilitating the seepage of rainwater into underwater aquifers. As a result, mountains have often been referred to as 'natural water towers' because they contain the headwaters of rivers, which are vital for maintaining human life in the densely populated areas downstream (Viviroli *et al.,* 2020; Wester *et al*., 2022). Mountains also represent unique areas for the detection of climatic change and the assessment of climate-related impacts (Wester *et al.,* 2019). One reason for this is that, as climate changes rapidly with height over relatively short horizontal distances, so does vegetation and hydrology (Xu *et al.,*2019).

THE HINDU KUSH HIMALAYA UNDER CRISES

The Hindu Kush Himalaya (HKH), the highest mountain ecosystem in the world with world's ten highest mountain peaks, considered as a Third Pole and water tower of Asia, is a global asset (Wester *et al.,* 2019). Stretched over 3,500 kilometers (Km) and covering more than four million square kilometers, the HKH includes all of Bhutan and Nepal and parts of Afghanistan, Bangladesh, China, India, Myanmar, and Pakistan. It is one of the most diverse ecosystems among the global mountain biomes with extreme variations in vegetation, climate and ecosystems, resulted from altitudinal and latitudinal gradients (Xu *et al.,* 2019). Owing to these enabling ecological conditions, the region is among the richest with two of its member countries– India and China, being 'Mega Diversity Countries' (Brooks *et al.,* 2006). Blessed with diverse plants and animals of global significance and unique ecosystems, the HKH is also among the Global 200 Ecoregions (Olson and Dinerstein, 2002). This rich biodiversity has also nurtured culture and traditions of more than 1000 ethnic groups (Turin, 2005). In addition, the region is source of ten major river systems as the source of a wide range of ecosystem services supporting 240 million people in the region and benefit some 2 billion people in the mountains and downstream river basin areas (Wester *et al.,*2023).

However, in the course of human civilization, the HKH lost more than 70% of its original ecosystems, which resulted the HKH to be considered as parts of 'Crisis Ecoregions' and 'Biodiversity Hotspots' (Mittermeier *et al.,* 2011); hotspot region for crossing the planetary boundaries (Gerten *et al.,* 2020; Rechardson *et al.,* 2023) and region indicating clear range shift of species to higher elevation (Titley *et al.,* 2021). Environmental degradation has been identified as a major threat to the functioning of HKH ecosystems and flow of ecosystem services (Xu *et al.,* 2019). Among others– climate change, habitat change, over-exploitation, pollution, and invasive alien species – are the major drivers of changes (Chettri and Sharma 2016, Chettri *et al.,* 2023). Adapting to and mitigating the effects of these changes and sustaining ecosystem services in the context of a burgeoning human population and climate change is a major challenge in the HKH as elsewhere (Wester *et al.,* 2023). Advocated as the 'Third Pole' and 'Water Tower of Asia' due to the largest mass of glaciers outside the two poles, the HKH region is in the limelight for both climate change and biodiversity (Wester *et al.,* 2023; Chaudhary *et al.,* 2023). It is evident that, due to elevation dependent warming, the HKH is witnessing a higher rise in temperature compared to the other mountain ranges and higher than the global average (Krishnan *et al.,* 2019). Warming has shown cascading consequences on biodiversity, water, people's livelihood, and food security among others (Wester *et al.,* 2023).

MOUNTAINS IN GLOBAL DISCOURSES

The global community recognised the importance of mountains at the Rio Conference on Environment and Development in 1992, which led to the inclusion of Agenda 21 in Chapter 13, relates to sustainable mountain development. The chapter sets the scene by stating the role of mountains within the global ecosystem, and expresses serious concerns related to the decline in the general environmental quality of many mountains. A summarized version (UNEP 1992) of Agenda 21/13 reads: "Mountains are important sources of water, energy, minerals, forest and agricultural products and areas of recreation. They are storehouses of biological diversity, home to endangered species and an essential part of the global ecosystem. From the Andes to the Himalayas, and from Southeast Asia to East and Central Africa, there is serious ecological deterioration. Most mountain areas are experiencing environmental degradation." Similarly, the World Summit on Sustainable Development (WSSD) also dealt on mountain ecosystems and advocated that mountains should be considered as special places in the global sustainable development agenda. Paragraph 42 of the Plan of Implementation of the WSSD focuses on mountains, stating: "Mountain ecosystems support particular livelihoods, and include significant watershed resources, biological diversity and unique flora and fauna. Many are particularly fragile and vulnerable to the adverse effects of climate change and need specific protection." Consequently, the Conference of the Parties (COP) to the Convention on Biological Diversity (CBD) adopted Programme of Work on Mountain Biodiversity (PoWMB) as Decision VII/27 at its 7th meeting, held in Kuala Lumpur in February 2004 (Sharma and Acharya, 2004). The PoWMD invites the parties to the CBD to adopt outcome-oriented targets for mountain biodiversity. Getting mountains on the global agenda was not an easy task. Introducing the mountains and getting it accepted in the Agenda 21 preparation and finalization process was a very uphill task. First a great deal of convincing was needed on integrated mountain development being a high priority global issue and that it concerned a fairly sizable number of people in all the major continents of the world. The crowded agenda meant that a lot of hard work was needed. Second the key countries and organization involved in such global exercises outside the formal processes also needed to agree about the value of introducing the mountain global agenda. Third, there was the question about the support for driving through the arduous and labyrinthine road of UN reviews and committees? It was only because of the commitment and dedication of some key organizations that Chapter 13 finally came to fruition. However, inspite of the recognition and realisation of mountain ecosystem for human wellbeing, climate change mitigation and biodiversity conservation have been realised ((IPBES, 2019), due attention and investments are not reflected as per the need for mountains and biodiversity (WEF, 2022; Legagneux *et al.,* 2018).

CONFERENCE OF PARTY MEETING OF THE CONVENTION ON BIOLOGICAL DIVERSITY

In the mid night of 19th December 2022, the chilling weather of Montreal, Canada, could not stop the Parties to the Convention on Biological Diversity (CBD) to make a 'historic' agreement on biodiversity– the Kunming-Montreal Global Biodiversity Framework widely known as GBF (Secretariat to CBD, 2022) that includes ambitious commitments to "halt and reverse biodiversity loss" by 2030 and sets out an ambitious pathway to reach the global vision of "a world living in harmony with nature by 2050". The Framework with four goals and 23 targets emphasizes action and results-oriented implementation by revisiting national biodiversity strategies and action plans, and to facilitate the monitoring and review of progress at all levels in a more transparent and responsible manner. The notion of 'historic' agreement was brought forward by many considering the framework as 'ambitious;' that will 'galvanize urgent and transformative action' to reduce biodiversity; addresses prevailing challenges such as climate change and pollution more explicitly and rationalizes contributions towards the achievement of the 'Sustainable Development Goals.' It builds on the Convention's previous Strategic Plans, and the realization that none of the Aichi Targets 2020 set during the Tenth Conference of Party (CoP-10) meeting in Nagoya, Japan, were met in full by 2020, though considerable progress was made on some of them, and gave opportunities to look back and identify the gaps. It was realized that targets were missed due to the mismatch between global and national priorities set, the lack of measurable indicators and weak support mechanism including inadequate financial resources mobilizationamong others (Legagneux *et al.,* 2018). The slow but positive progress on global biodiversity indices (Hu *et al.,* 2022) often related to complexity involved and less focused compared to other Conventions such as Paris Agreement under the United Nations Framework Convention on Climate Change (UNFCCC). Ironically, a vast and one of the most vulnerable and biodiversity rich areas – the mountains with over 24% of the global terrestrial areas and home to half of the 36 Global Biodiversity Hotspots was missed in the priority setting and negotiation processes while finalizing the framework (Secretariat to CBD 2022). Despite receiving global attention in the past at the Rio Conference, World Summit on Sustainable Development (WSSD), Conference of the Parties to the CBD and recent resolution of the UN General Assembly that proclaimed 2023–2027 "Five Years of Action for the Development of Mountain Regions" (UNGA 2022), the Parties having mountains could not hold the word– mountains– in the final document– though Nepal made its honest effort. This was indeed a missed opportunity for the vulnerable and fragile mountains and the people living and dependent on them from downstream. However, this should not be considered as barriers for efforts towards conserving biodiversity and implementing the targets relevant to the mountains to reach the 'ambition' raised by the 'historic agreement'.

WINDOW OF HOPE FOR REWILDING ECOSYSTEMS

Our decadal reviews on progress towards the CBD commitments by the HKH member countries namely 2010 Biodiversity Targets (Desai *et al.,* 2011) and Aichi Target 2020 (Chaudhary *et al.,* 2022) showed progressive but different levels of achievements. Considering the progress on Aichi Targets 2020 in the HKH, about 11 per cent of the targets have been "exceeded", more than half (55 per cent) of the targets was "on track"; on about 29 per cent of the targets, "progress has been made but at an insufficient rate"; 3 per cent of the targets, "no significant change" has been reported. This is far better than the Global Progress (Secretariat of the CBD 2020). The targets on which the best progress has been made are- Target 7 (sustainable agriculture, forestry); Target 11 (protected areas); Target 15 (ecosystem resilience); and Target 16 (Nagoya Protocol). The degree to which each country has been able to comply with CBD targets reflects their national capacities, level of development, and specific limitations in articulating mountain specific national or sub-national targets and indicators as well as availability of funding.

The HKH is known for its rich biodiversity and 41.17 per cent of its area is already covered by the protected area network. However, a recent study revealed that 67 per cent of Ecoregions, 39 per cent of Global Biodiversity Hotspots, 69 per cent of Key Biodiversity Areas, and 76 per cent of Important Bird and Biodiversity Areas are still outside the existing protected area network (Chaudhary *et al.,* 2022). At the national level, different countries are at various stages of commitments towards Aichi target 11 (17 per cent of the land) and Target 3 of the GBF (30X30). For example, Bhutan have already reach to over 50 per cent of its land are under protected area coverage and Nepal over 23 per cent. However, countries like Afghanistan (3.6 per cent), Bangladesh (4.6 per cent), Myanmar (6.6 per cent) and India (7.5 per cent) coverage of protected areas till 2020 were below the committed 17 per cent. Pakistan (12.3 per cent) and China (15.3 per cent) progressed with marginal shortfall. Though mountains are not prioritized in the GBF, it gives ample opportunities for the HKH member countries and mountains of the world a reason to make more concreate efforts to conserve biodiversity and contribute to the 2030 targets and 2050 goals– especially on rewilding the degraded areas through ecosystem restoration. To make the implementation of the GBF results oriented, the CBD Secretariat has already initiated to form different technical teams and decentralized support systems to cater the regional and sub-regional priorities that guides developing indicators, facilitate resources mobilization and develop monitoring mechanism, among others.

As indicated, the HKH is the most vulnerable region for three planetary crises - climate change, biodiversity loss and pollution. The GBF opens a window of opportunity to rewild the lost ecosystems through ecosystem restoration using afforestation or natural growth of degraded areas. This could be a promising action on climate change supporting carbon sequester. The emerging restoration practices in countries like Pakistan (Khan *et al.,* 2019), China (Liu *et al.,* 2023) and others are showing promising results. Similarly, the option for Other Effective Conservation Measures (OECM) provisioned in target 3 could encourage other countries to look for opportunities to contribute towards global target of 30% of protected are by 2030 – without making the target as national obligation. Interestingly, countries like India and Nepal and even Bhutan are already exploring opportunities for OECMs considering community forests, indigenous protected lands and establishment of transboundary biosphere reserve or corridors between existing protected areas. International Centre for Integrated Mountain Development (ICIMOD), with a regional mandate and with an observer status in the Convention on Biological Diversity, is committed to support its regional member countries on these twin targets of restoration and expansion of protected areas. ICIMOD, in its new Strategy 2030– Moving Mountains (ICIMOD 2023) have already included biodiversity as a cross-cutting themes (Fig. 1) and Restoring and Regenerating Landscapes as dedicated programme to contribute the implementation of GBF along with Strengthening global leadership in sustainable mountain development to support global advocacy and resources mobilization. ICIMOD envisaged to contribute directly or indirectly to 17 out of 23 targets (Fig. 2).

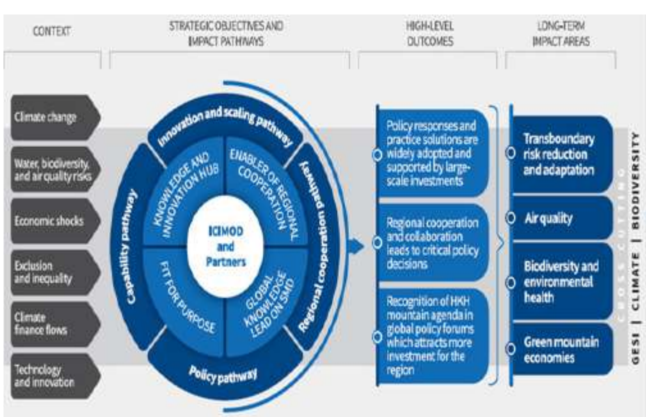

Fig. 1. Schematic diagrams showing biodiversity as a cross cutting theme and long-term impact area.

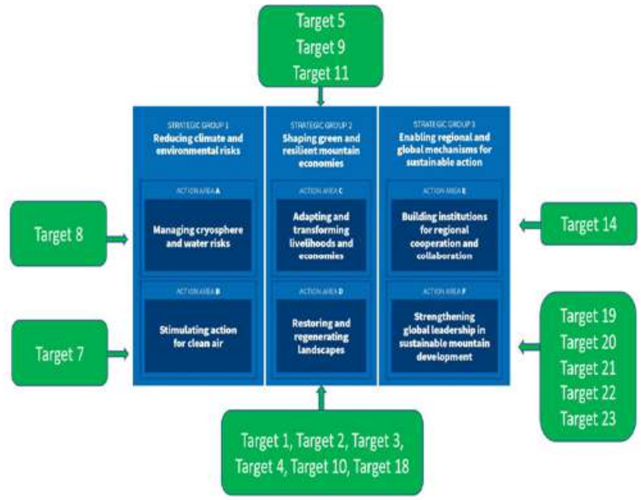

Fig 2. Schematic diagrams showing three strategic groups, six action areas and potential link to GBF Targets.

As we celebrate the Decade of Ecosystem Restoration, we call on everyone to support the global campaign for the healing of nature through restoration of our ecosystems. We must do our part as responsible stewards of our planet before it is too late. It is, after all, the only planet we have gotten.

ACKNOWLEDGMENT

The author is thankful to ICIMOD for its encouragement and support. This study was supported by core funds of ICIMOD contributed by the governments of Afghanistan, Australia, Austria, Bangladesh, Bhutan, China, India, Myanmar, Nepal, Norway, Pakistan, and Switzerland. The views and interpretations in this publication are those of the author and they are not attributable to ICIMOD.

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PROMOTING CONSERVATION AND SUSTAINABLE UTILIZATION OF HIMALAYAN MEDICINAL PLANT FOR LIVELIHOOD SECURITY IN THE FACE OF CHANGING CLIMATE

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ABSTRACT

The escalating demand for herbal medicines threatens Himalayan medicinal plants, known for their high concentration of bioactive molecules. As a result, indiscriminate harvesting from their natural habitats affects the availability issue and endangers their existence. Therefore, approaches for promoting cultivation must be pursued to conserve these species and to uplift the socio-economic dynamics of the local communities. Currently, the practices adopted are inadequate and unlikely to stop ongoing species loss; therefore, more emphasis on species-specific intervention programmes is needed.Further, future land-use and climate change impacts must be considered together when devising conservation and management plans. This is so because the conservation of species can be thwarted by poor governance; hence,community-based conservation initiatives might provide unique opportunities to improve governance, sustain livelihoods, and conserve biodiversity. Doing this can negate the contradictions that exist between economic development and biodiversity conservation.

INTRODUCTION

Over the past five decades, we have witnessed a major advancement in our understanding of conservation and its interrelationship with the elusive goal of sustainable development. Two antagonistic developments have also marked this period. These include, (i) the rise in the establishment of conservation-orientated institutions, negotiation and implementation of a series of treaties (e.g. United Nations Convention on Convention of Biodiversity Diversity, 1992) that address the conservationism issues, and (ii) the scale and intensity of human interactions with the environment have led to increased habitat loss/degradation and fragmentation, with subsequent loss of species and genetic variability. As a consequence, in the twenty-first century, we are still entrapped in these issues (a) how to address the conservation and management of species, (b) how to curtail habitat and species loss, (c) how to enhance species recovery programmes, and (d) what new and innovative approaches be framed in decision making so that it safeguards our unique biodiversity as a whole. This is so because the push factors contributing to its degradation are far more complex and powerful than those working to protect it. For example, a recent study reports that an average of 2.3 species have become extinct each year for the past 2.5 centuries (Humphreys *et al.,* 2019). The study further estimates the rate of ongoing extinction [18–26 extinctions per million species years (E/MSY)] is up to 500 times the background extinction rate for plants (0.05–0.35 E/MSY) (De Vos *et al.,* 2015). This sounds astounding because plant extinctions endanger other organisms, ecosystems, and human well-being, and must be understood for effective conservation planning.

With climate change being embarked as one of the dominant stressors in the twenty-first century, these estimates might see a several-fold increase at the end of this century. This is so because climate change will alter environmental conditions such that they are no longer ideal for some species that inhabit them. While other species will benefit from increases in their possible range (e.g. over-dominance spread of alien invasive species), it is anticipated that the acceptable range for many species, including medicinal plants, would shrink or shift significantly in response to anticipated climate changes (Singh *et al.,* 2022). Several studies (Table 1) have now reported the distributions of many organisms are already shifting rapidly towards higher altitudes, which increases competitive pressure on existing species in these ranges. Thus, a question arises, are they reaching towards no return point? As per the report of the International Union for Conservation of Nature (IUCN) about 13.49% (~40,468) of the flowering plant species, a subgroup of vascular plants, are under varying degrees of threat (IUCN, 2021).

In the Indian Himalayan region (IHR), 85 species representing 41 trees, 34 herbs, 3 shrubs, 3 mosses, 2 vines, and 2 bryophytes belonging to 56 genera under 41 families are presented in various IUCN threat categories (Mehta *et al.,* 2022). All these estimates suggest that there is an urgent need to develop strategies for the conservation and mainstreaming of biological wealth. Doing this can help reap maximum benefits and equivocally sustain the Himalayan bioresources.

Table 1. Projected Himalayan species are likely to perceive habitat alteration and upward migration in the face of climate change scenarios.

S. No	Species	Outcomes	References
1	*Betula utilis*	Highly suitable area for B. utilis is predicted to shift towards the eastern parts of the Himalayas in the future, with suitability declining towards the western part of the Himalayas	Hamid *et al.,* 2019
2	*Taxus wallichiana*	The suitable climatic niche of the species area is projected to shrink by 28% (RCP 4.5) and 31% (RCP 8.5).	Rathore *et al.,* 2019
3	*Hippophae salicifolia*	A suitable area is likely to shrink by 87% in all climate change scenarios, making H. salicifolia highly vulnerable in its actual habitats. Further, an upward shift in the habitat of the species by 1700 m asl (2800-4500 m amsl) is also predicted.	Dhyani *et al.,* 2018
4	*Rheum webbianum*	The current habitat suitability for the species was predicted to be 103760 km2. Under future scenarios, a significant reduction in the habitat was reported, i.e., from -78531.34 Km2 (RCP 4.5 for 2050) to -77325.81 Km2 under RCP 8.5 for 2070.	Wani *et al.,* 2021
5	*Aconitum heterophyllum, A. violaceum, Jurineadolomiaea, Sinopodophyllum-hexandrum*	These species were reported to have a narrow niche width, and as a result, they may face an extinction risk due to the upward shift of non-native species.	Ahmad *et al.,* 2021
6	*Swertia bimaculata*	A significant decrease in the habitat from 869.48 (current distribution) to 0 km2 under RCP 8.5 for the year 2070 was reported. Further, the results predicted a significant upward shifting of the species along the altitudinal gradient.	Boral and Moktan, 2021
7	*Dactylorhizahatagirea*	Compared to the present distribution, habitat suitability under future projections, i.e., RCP 4.5 and RCP 8.5 (2050 and 2070), was reported to shift to higher elevation towards the north-west direction, while lower altitudes will invariably be less suitable.	Singh *et al.,* 2022
8	*Valerianawallichii*	The suitable habitat of V. wallichii could be reduced by about 57.231% (under RCP4.5, 2050), to about 97.878% under RCP 8.5 for the year 2070.	Kumari *et al.,* 2022
9	*Aconitum heterophyllum*	The suitable habitat was reported to reduce by 33.35% (under RCP4.5 2050), to 56.59% under RCP8.5 for the year 2070.	Wani *et al.,* 2022
10	*Cypripedium cordigerum, C. elegans, and C. himalaicum*	The study predicts a trivial increase in suitable habitat areas with a shift toward the northwest	Chandra *et al.,* 2023

Of the several resources, medicinal and aromatic plants (MAPs) form an important component of alternate livelihood options in the IHR. This is especially true in places like the high alpine regions, where the window for agriculture is very short and has low outputs; thus, the options to generate revenue mostly depend on natural resources. Over the years, stimulated by high population growth rates and the shift in social preference towards herbal medicine, the use and trade of plants for medicine is no longer confined to traditional healers but has entered both the informal and formal entrepreneurial Sectors. However, the gap between demand and supply is widening, which has affected pharmaceutical companies on the one hand and the livelihoods of several people on the other. In response, introducing these species into farming systems is increasingly advocated. Further, MAPs are projected to help small-scale farmers strengthen their livelihoods directly through income generation from their trade as well as health care provision, thereby reducing vulnerability to poverty in the longer term.

Medicinal plant resource base in the IHR

In India, the Himalaya harbors a rich plant diversity of over 8000 angiosperms, 44 gymnosperms, 600 pteridophytes, 1737 bryophytes, 1159 lichens, etc. Of these, in the IHR, the presence of over 1748 plant species (1685 angiosperms, 12 gymnosperms, and 51 pteridophytes), including 1020 herbs, 335 shrubs, and 330 trees of medicinal value, is reported (Samant *et al.,* 1998). Among these, the diversity of MAPs varied across the different biogeographic provinces of IHR. About 133 species are reported from Jammu and Kashmir (http://www.bioinfoku.org/db/medsearch.php), 643 species are reported from Himachal Pradesh (Samant *et al.,* 2007), 964 species from Uttarakhand (Kala, 2015) and 400 species from Sikkim region (Rai *et al.,* 2000). A high nativity of 31%, endemism of 15.5% and threatened status (14% of total Red Data plant species of IHR) manifest the region's unique and sensitive medicinal plant diversity (Badola and Aitken, 2003). Among several species, few have gained considerable importance (Fig. 1) include *Arnebiabenthamii*, *Aconitum heterophyllum, Angelica glauca, Dactylorhizahatagirea, Jurineadolomiaea, Nardostachysjatamansi, Paris polyphylla, Picrorhizakurrooa, Sinopodophyllumhexandrum, Malaxismuscifera, Taxus wallichiana, Habenariaedgeworthii, Rheum emodi, Swertia chirayita, Trillium govanianum, Valeriana jatamansi*, etc. These species are popular sources of active constituents for various medicine systems. The detailed ethnobotanical knowledge of these high-value medicinal species and their parts used, principal active constituent, and threat status is highlighted in Table 2.

Table 2. Some of the high-value medicinal plants from IHR, their main constituents, and applications potential

S. No.	Species	Altitudinal Range (m)	Parts used	Active constituent	Medicinal value/use	IUCN Status
1.	*Aconitumheterophyllum* (Ranunculaceae)	3200-4500	Tuber	Aconitine, Heteratisine, Heterophyllidine, heterophylline	Fever, abdominal pain, reproductive disorders, Anti-periodic, Anti-flatulent	EN
2.	*Angelica glauca* (Apiaceae)	2000-3800	Rhizome, Root	(Z)-Ligustilide, (Z)- Butylidinenephathalie, Ascorbic acid	Dysentery, vomiting agent, bronchitis, spices and condiments	EN
3.	*Arnebiabenthamii* (Boraginaceae)	3000-3900	Root	Shikonin and β, β-dimethyllacrylshikonin	Arthritis, throat, fever, cardiac disorder, wound healing	DD
4.	*Dactylorhizahatagirea* (Orchidaceae)	2500-4500	Tuber	Dactylorhin (A, B, C, D, E); dactyloses A and B, loroglossin, and millitarine	Chronic fever, cough and cold, stomachache, cuts and wounds healing, spermatorrhoea	EN
5.	*Fritillaria cirrhsa* (Liliaceace)	2800-4000	Bulb	Peimine and peiminine	Healing wounds, asthma, rheumatism	VU
6.	*Habenariaintermedia* (Orchidaceae)	2000-3300	Tuber	Habenariol, starch, minerals, taxol	Intellect promotion, aphrodisiac, depurative, anthelmintic	-
7.	*Malaxis muscifera* (Orchidaceae)	2400-3600	Pseudo bulb	β-sitosterol, glucose, rhamnose	Seminal weakness, fever, dysentery, burning sensation, emaciation	VU
8.	*Nardostachysjatamansi* (Valerianaceae)	3300-5000	Rhizome/ Root	Nardostachone, valeranol, angelicin, nardal	Heart pain, fits, urination, menstruation, digestion	CR
9.	*Paris polyphylla* (Liliaceae)	1800-3500	Rhizome	Polyphyllin D, diosgenin, dioscin, saponins	Anti-phlogistic, analgesis, burn, cut or injury, depurative, diarrhea, dysentery	VU
10.	*Picrorhizakurroa* (Scrophulariaceae)	3000-4000	Rhizome/ Root	Picroside I and picroside II, tinidazole	Anaemia, asthma, diarrhoea, jaundice, stop bleeding	EN
11.	*Sinopodophyllum hexandrum* (Phodophylaceae)	2600-3800	Fruits, Rhizomes	Podophyllotoxin, α-peltatin, β-peltatin	Purgative, tuberculosis, cough, joint pains, arthritis, asthma	-
12.	*Rheum emodi* (Polygonaceae)	2800-3800	Root, Rhizome	Chysophanic acid, emodine, rhein and physcion	Laxative, tonic, diuretic ,fever, cough, indigestion	DD
13.	*Swertia chirayita* (Gentianaceae)	1500-3000	Whole plant	Swertia chiralatone, swertiachoside, swertiachirdiol A and B	Anti-fungal, anti-bacterial, stomachic, laxative, skin diseases, ulcers, diabetes	-
14.	*Taxus wallichiana* (Taxaceae)	1000-4400	Leaves, Bark	Taxol, taxicin-I and taxicin-II	Anticancer, cold, cough, fever, and pain	EN
15.	*Trillium govanianum* (Trilliaceae)	2400-3200	Rhizome	Borassoside E, pennogenin, diosgenin, govanoside A	Analgesic, Anti-cancer, Anti-fungal and wound healing	EN
16.	*Jurineadolomiaea* (Asteraceae)	3000-4000	Root	Albicolide, jurineolide, juricanolide	Colic, puerperal fever, incense, skin eruptions, cuts	-
17.	*Valerianajatamansi* (Valerianaceae)	3500-3600	Whole plant	Valerenic acid, valeriandoidsA-C, Jatamanins N-P	Stomach disorder, epilepsy, hysteria, skin diseases, hepatic tonic	-

Note: Threat status as per IUCN 2024: CR-critically endangered, EN-endangered, VU-vulnerable, LC-least concern, DD- data deficient.

Fig. 1. Threatened Himalayan species in trade (a) *Aconitum heterophyllum*, (b) *Polygonatum cirrhifolium*, (c) *Rheum australe*, (d) *Paris polyphylla*, (e) *Meconopsis aculeata*, (f) *Nardostachys jatamansi*, (g), *Sinopodophyllum hexandrum*, (h) *Picrorhiza kurroa* and (i) *Trillium govanianum*.

Medicinal plants and trade volume

Given the growing popularity of botanical remedies and the very little cultivation of medicinal herbs, it is sensible to conclude that plant collecting is frequently unsustainable. The worldwide demand for herbal products was estimated at US $1.2 billion in 1990 and rose to US $29.4 billion in 2017. This is expected to hit US $39.6 billion in 2022, with a compound annual growth rate of 6.1 percent between 2017 and 2022 (BCC, 2019). By 2050, the trade in herbal products is expected to surpass US $ 5 trillion (Booker *et al.,* 2012; Negi *et al.,* 2018). Considering the economic prosperity, the trade of these commodities has led to the development of vital resource enterprises, particularly in developing nations. However, the backdrop of surplus demand and limited resource availability (raw material) under cultivation has put extra pressure on wild populations and consequently encouraged illegal harvesting (Table 3). Illicit and rampant medicinal plant collection is recognized as one of the major threats to their wild population in the western Himalaya. As a result, population decline has been quite dramatic over the years. To an estimate, about 70-90% of MAPs under trade are harvested from the wild (Bhattacharya *et al.,* 2008). The abrupt harvesting practices are also in full swing in the IHR, wherein approximately 90% of MAPs are harvested directly from the wild (MOEF, 2014). The region (i.e., IHR), although exceptionally rich in biodiversity, is amongst the world's most fragile and vulnerable ecosystems. In addition to considerable anthropogenic disturbances, environmental perturbations have seriously affected the structural integrity of this pristine ecosystem.

Table 3. Estimated annual consumption/ trade of high-value medicinal plants from the Indian Himalayan Region

Species	Trade name	Traded form	IUCN Status	Quantity (MT)	Rate (Rs./kg)	Raw material source
Aconitum heterophyllum	Atis	Root	EN	100-200	3500-10500	W
Aconitumlethale	Bish	Root	-	<10	-	W
Angelica glauca	Choru	Rhizome	EN	<10	-	W
Arnebiabenthamii	Gauzaban	Root	DD	100-200	150-220	W
Saussureacostus	Kuth	Root	CR	100-200	250-350	C
Dactylorhizahatagirea	Salampanja	Tuber	EN	10-50	5000-6000	W
Polygonatumcirrhifolium	Meda	Tuber	-	100-200	250-350	W
Habenaria intermedia	Vriddhi	Tuber	-	10-50	-	W
Malaxis muscifera	Risbak	Tuber	VU	<10	-	W
Nardostachysjatamansi	Jatamansi	Root and Rhizome	CR	500-1000	850-900	W
Paeonia emodi	Himalayan peony	Rhizome	LC	<10	-	W
Paris polyphylla	Satua	Rhizome	VU	10-50	-	W
Picrorhizakurrooa	Kutki	Root	EN	1000-2000	800-900	W
Sinopodophyllum hexandrum	Bankakri	Fruit, Root	-	10-50	-	W
Rheum emodi	Dolu	Rhizome	DD	100-200	-	W
Swertia chirayita	Chiraiyata	Whole Plant	-	500-1000	300-325	
Taxus wallichiana	Yew	Leaf	EN	100-200	-	W/C
Trillium govanianum	Nagchatri	Rhizome	EN	200-500	-	W
Chlorophytum borivilianum	Safed musali		CR	100-200	700-1800	w/C
Valerianajatamansi	Tagarganth	Root	-	1000-2000	370-425	w/C

Note: Trade/annual consumption data source, Goraya and Ved (2017); Threat status as per IUCN 2024: CR-critically endangered, EN-endangered, VU-vulnerable, LC-least concern, DD- data deficient; Raw material source: W- wild, C – cultivated, MT – metric ton, kg – kilogram.

Propagation and multiplication strategies

Various propagation protocols involving macro-propagation and micro-propagation techniques have been used for high-value endemic and threatened MAPs in the region. These techniques have significantly uplifted plant propagation strategy, mainly multiplication and large-scale availability of planting materials. Some of the propagation milestones achieved in this direction include Sinopodophyllum hexandrum (Nadeem *et al.,* 2000), Saussureaobvallata (Joshi and Dhar, 2003), Aconitum balfourii (Pandey *et al.,* 2004), Arnebiabenthamii (Manjkhola et al. 2005), Angelica glauca (Bisht *et al.,* 2008), Hedychium spicatum (Giri and Tamta, 2011), Habenariaedgeworthii (Giri *et al.,* 2012), Lilium polyphyllum (Dhyani *et al.,* 2014), Dactylorhiza hatagirea (Warghat *et al.,* 2014), Valeriana jatamansi (Purohit *et al.,* 2015), Paris polyphylla (Raomai *et al.,* 2015), Nardostachys jatamansi (Bose e*t al.,* 2016), Zanthoxylum armatum (Purohit *et al.,* 2020), Trillium govanianum (Chauhan *et al.,* 2020) and Berberis asiatica (Bisht *et al.,* 2021).

Community-based cultivation of commercially used medicinal plants

There is a need to channelize production and promote a sustainable supply of medicinal plants through capacity building of Van Panchayat/ local SHGs/ BMCs about the medicinal plants & aromatic species of medicinal value that are locally available for encouraging sustainable harvest, adoption of good collection practices, proper post-harvest handling, marketing and regeneration of Non-Timber Forest Produces (NTFPs), etc. (Fig. 2) . This activity will provide livelihood augmentation to local and forest fringe communities.

Fig. 2. Commercially cultivated selected Himalayan species (a) Allium stracheyi, (b) Hedychium spicatum, (c) Amomum subulatum, and (d) Picrorhiza kurroa.

Box 1. NIHE success stories of promotion of large-scale cultivation of MAPs for livelihood enhancement

Himachal Pradesh

• Cultivation of Picrorhiza kurroaand Swertia chirayita for livelihood development: A success story: Shri Jahan Singh Puniaa prospective farmer fromShangarh village of Sainj valley, Kullu district, Himachal Pradesh. Mr. Punia, with the support of NIHE, established a farm, known as the Chanayara farm for the cultivation of Himalayan medicinal plants, i.e., Picrorhiza kurroa, Podophyllum hexandrum, and Angelica glauca. Among these, Picrorhiza kurroa was cultivated around 15 bighas (3 acres) of land that produced a total of around 1500 kg dry material of Picrorhiza kurroa.The herb was processed and sold in herbal Mandis located in Amritsar and Delhi at the rate of Rs. 1300/kg, and generated around Rs. 19.50 lakhs from its sale.

Uttarakhand

• A cluster-based approach (funded by NMHS) for the cultivation of medicinal and aromatic plants (MAPs) was initiated by NIHE in the Chaudas Valley, district Pithoragarh, Uttarakhand. Under this initiative, the Institute succeeded in cultivating seven (i.e., Allium stracheyi, Angelica glauca, Cinnamomum tamala, Hedychium spicatum, Picrorrhizakurrooa, Saussureacostus, and Valeriana jatamansi) species in 11 villages. Here, a total of 172 motivated farmers participated and were registered with the Herbal Research Development Institute (HRDI), Gopeshwar, Govt. of Uttarakhand. During the programme, a total of 125 Nali (~6.2 acres) of left land over land was brought under MAPs cultivation, which generated a total revenue of Rs. 10.49 lakhs after 2-3 years of cultivation.

• Similarly, under the project 'Mainstreaming Himalayan Biodiversity for Sustainable Development' cultivation of medicinal plants (Hedychium spicatum, Salvia Rosmarinus, Cinnamomum tamala, and Zanthoxylum armatum) in the Bamnigarh cluster of Almora district is being carried out. In the year 2024, a total of 2250 kg Hedychium spicatum was harvested from which the villagers earned worth Rs. 1.35 lakhs in total.

Towards sustainable integration of medicinal plants into the supply chain and marketing

Economic growth and sustainability issues have been a long discussion in ensuring the balance of profit-making and minimizing the depletion of natural resources. Many agree that management principles should value conservation more than profit-making (Timonen *et al.,* 2021). However, ecological risks are still present. Therefore, we need to strike the right balance to sustainably supply a reasonable volume of medicinal plants into the supply chain. A long-term, integrated, scientifically oriented action plan is required for the conservation and sustainable use of medicinal plants (Fig. 3). It is worth noting that biotechnology has opened up new avenues for the conservation of medicinal plants, enabling the speedy propagation, mainstreaming of commercially important species into the environment, and identifying potential new gene product uses. Besides these, the challenges that might be of concern generally include improving the value chain, quality assurance, appropriate market canals, and supply chain. For any herbal medicinal product to enter the global market, it must adhere to three sets of quality standards: the International Standard for Sustainable Wild Collection of Medicinal and Aromatic Plants (ISSC-MAP), Good Agricultural Practices (GAP), and Good Manufacturing Practices (GMP) (WHO 2003 & 2013).

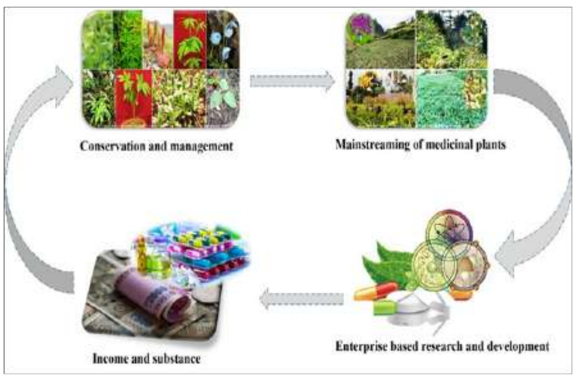

Fig. 3. A conceptual framework for integration and mainstreaming of Himalayan bioresources into a circular economic model

Policies and regulatory frameworks

In India, there are no distinct policies or laws for saving wild herbal plants. They are conserved according to the existing laws pertaining to forestry. The laws for the conservation of forests formulated by the Indian government directly or indirectly protect the natural flora of medicinal importance. These include the Forest Act, 1927,Wildlife (Protection) Act, 1972; Environment Protection Act, 1986; Forest (Conservation) Act, 1980; National Forest Policy, 1988; National Biodiversity Amendment Act, 2023; Wildlife (Protection) Amendment Act1991, and the Scheduled Tribes and Other Traditional Forest Dwellers Act, 2006, etc.

Box 2. Legal Protection of Medicinal Plant and IntellectualProperty Rights

Medicinal plants are protected under several Acts and regulations such as the Biological Diversity Act 2002 (Amendment, 2023), The Drugs and Cosmetics Act, 1940 (Amendment 2005), and Forest Act. These Acts have been implemented to protect the use of traditional medicinal plants.

National Biodiversity Authority (NBA): Astatutory body that performs the regulatory and advisory function of conservation, sustainable use of biological resources, and equitable sharing of benefits of use for the Government of India.

Biological Diversity Amendment Act 2023: Advises the Central Government on the conservation of biodiversity, sustainable use of its components, and equal sharing benefits of utilizing biological resources through a decentralized system. Further, it seeks to (i) reduce the pressure on wild medicinal plants by encouraging the cultivation of medicinal plants; (ii) encourage the Indian system of medicine; (iii) facilitate fast-tracking of research, the patent application process, and transfer of research results while utilizing the biological resources available in India without compromising the objectives of United Nation Convention on Biological Diversity and its Nagoya Protocol; (iv) decriminalize certain provisions and attract more foreign investments in the chain of biological resources, without compromising the national interest.

(Please refer https://egazette.gov.in/WriteReadData/2023/247815.pdf for detail about the Act)

State Biodiversity Board (SBBs): Advises the State Government on guidelines issued by the Central Government relating to biodiversity conservation, sustainable utilization, and equal share benefits for utilizing biological resources. Regulated by granting approvals for commercial utilization of any biological resource by people.

Biodiversity Management Committees (BMCs): responsible for promoting conservation, sustainable use, and documentation of biological diversity, including preservation of habitats, conservation of land races, folk varieties and cultivators, domesticated stocks, and breeds of animals and microorganisms.

Geographical Indication (GI): It is another type of intellectual property right that identifies the origin of goods. Also, can be used for the protection of products, which are based on traditional medical knowledge.

Major supply issues in the medicinal plants sector

Medicinal plants will always be at the core of meeting healthcare needs. The limitation of plant materials due to over-harvesting, incompetency of regulation monitoring, lack of an established global supply chain, and imbalance of sustainable production strategies are some of the major issues in this sector. In brief, these include:

- Lack of quality planting materials
- Technical knowledge of good agriculture practices and post-harvest processing
- Issues related to the quality of the produce
- Un-attractive market price.
- There was a lack of organized support for producers and also for other players in the supply chain
- Producers complained that technical guidance, financial resources, and marketing information were not promptly available.

9. Conclusion and Recommendations

An exhaustive understanding of demand and production processes and their value chain is needed to commercialize medicinal products effectively. Despite the discussed challenges, there are promising opportunities for the conservation and mainstreaming of biodiversity (Fig. 4). A holistic approach such as Institutional collaborations, integration of traditional knowledge, sustainable harvesting practices, and conservation education programs can contribute significantly to the conservation of medicinal plants. Further, engaging local communities and stakeholders in decision-making processes and implementing legal and policy frameworks are essential for sustainable conservation. Prioritizing research and developmental activities to bridge information gaps and improve conservation planning is critical. The following are some of the action points for effective conservation and economic dimensions:

- Sustainable wild harvest management schemes supported by governments and authorities can be implemented.
- Cluster-based cultivation approaches need to be strengthened
- Gene banks need to be developed, particularly for habitat-specific, slow-growing species with high susceptibility to being over-harvested
- Mainstreaming into the cultivation of commercially important species needs to be promoted
- User rights over the resource and access to it need to be clarified. This is particularly the case where MAPs are considered common property.
- Intellectual property rights must be acknowledged so local users or other entities can adequately compensate.

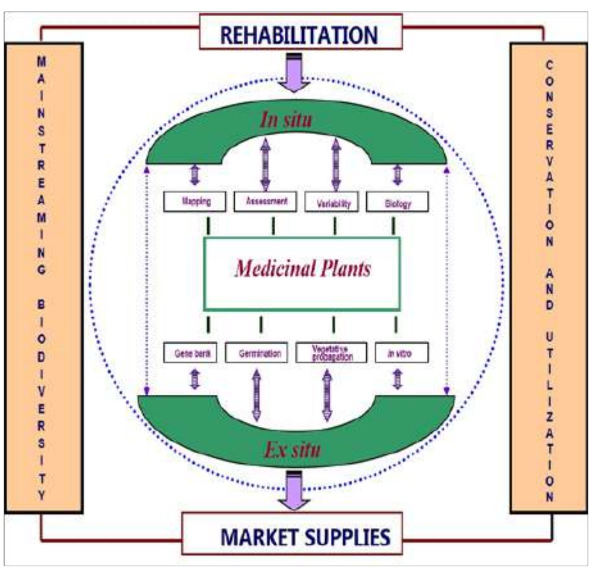

Fig. 4. Future strategies envisaged for rehabilitation, mainstreaming, conservations and sustainable utilization of Himalayan medicinal plants.

ACKNOWLEDGMENTS

The author thanks the director of G. B. Pant National Institute of Himalayan Environment, Kosi-Katarmal, Almora, Uttarakhand, India, for providing necessary facilities and encouragement. Members of the Centre for Biodiversity Conservation and Management are greatly acknowledged for their support and cooperation during the study. Special support from Drs. Laxman Singh, Amit Bahukhandi, HimansuBargali, and other colleagues of the centre are greatly acknowledged. Support from Inhouse Project4 is greatly acknowledged.

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HIGH ALTITUDE TRANSITION ZONES IN HIMACHAL PRADESH: LONG-TERM STUDIES TO ASSESS THE EFFECTS OF GLOBAL WARMING

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ABSTRACT

High mountain systems across the world are undergoing hostile effects of global warming, but it is more prominent and visible across the Indian Himalayan Region (IHR). Evidence of global temperature change in many studies have brought in focus, the fact that high altitude ecosystems are very susceptible to climate change. The amalgamation of natural hazards and anthropogenic activities are putting pressure on these sensitive regions. Glacial melt, invasion of exotic species, upward shift in timberline, change in precipitation pattern, erratic phenology, etc., are some evident effects of global warming across the IHR. These effects can modify the structure and function of the ecosystem, and thus hamper the important ecosystem services provided by them. Although this mountainous region has been crowned as one among the world's biological hotspots, adequate attention with rigorous and comprehensive studies, regarding the real impact of global warming and climate change, on its fragile ecosystem is still wanting. The high-altitude transition zone (HATZ) pertains to the timberline that marks the merging of temperate tree lands with the alpine pastures through sub-alpine scrub. Thus, representing an ecotone between the sub-alpine and alpine zones, and marks a gradual recession from close canopy forests to stunted shrubby formations. The general altitude of this zone in Himachal Pradesh varies from about 3300 to 3900 m asl, depending upon the aspect and location. The transition zones provide important ecosystem services and increased habitat heterogeneity, and are often described as important contributors to species diversity, though this contribution has rarely been quantified. The project as titled above, is a novel initiative by the research wing of the Himachal Pradesh Forest Department in collaboration with the Himalayan Forest Research Institute (HFRI), Shimla. The project initiated during 2012, is a long-term project, with the basic concept being, to study the floristic composition in carefully selected and established five plots, and monitor any changes in this composition over a period of time, to arrive at meaningful conclusions about the impact of global warming in the upward shift of vegetation. Besides, the environmental data is also being recorded with data loggers for temperature and humidity monitoring. The main activities under the study were taken up in the HATZ falling in Sutlej catchment in parts of Kinnaur, and the activities were further extended to the high-altitude areas falling in Beas and Ravi catchments to have a broader perspective and comparative information on the subject. The paper throws light on the aims and long-term objectives of the project, briefly discussing the trends that have come up during this study.

Key words: Himalaya, global warming, climate change, ecosystem services, phenology, tree line shift, high altitude transition zone

INTRODUCTION

Global warming leading to climate change have today widely become the greatest threat to all life forms on earth. The changes in the climate are more evident and happening, and is expected to bring warmer temperatures and increased hydrologic extremes with more frequent droughts and floods (Pachauri *et al.,* 2014). On a global average, the response to climate change by organisms, population, and ecological communities is minimalincomparison to the regional changes, because of the highly spatially heterogeneity, it thus becomes more relevant in terms of ecological response to climate change (Walther *et al.,* 2002). The heterogeneity in ecological dynamics across systems is certainly due to asymmetry warming in many regions with climate being one major dominant controllingfactor for the distribution of ecosystems. According to fossil records (Morley, 2011), various studies and observed trends (Hughes, 2000; Walther *et al.,* 2002) have helped in concluding that vegetation distribution is highly influenced by the changing climate (Beniston, 2003; Liu and Rasul, 2007). It is now more or less accepted that by the end of the 21st century the projected climate change will have a major impact on the vegetation distribution at a global level with species range and the distribution of its ecosystem boundaries being predominantly controlled by temperature and precipitation (Walther *et al.,* 2002; IPCC, 2014; Osland *et al.,* 2017). As stated by numerous authors, the biodiversity is closely interrelated with climate; thereby its primary and immediate effect would bring changes inbiodiversity, affectingits composition, abundance besides itsdistribution. In the mountain ecosystem for alpine plant life, low temperature is the main controlling factor (Telwala *et al.,* 2013). At high altitude zones, the climate-related ecological factors are dominant, so the slight change in climate affects the montane plant diversity and its distribution. Thus,the sensitivity of the mountain flora to slight climate changes makes mountain ecosystems more suitable studies sites for tracking the impacts of climate change(Körner, 2003; Diaz *et al.,* 2003).

Himalaya being the youngest mountain system of the world, with variable climatic conditions is well known for the highest elevational limits of plant species (Arenaria bryophylla, 6100- 6200m.; Miehe, 1991). It represents the longest bioclimatic elevational gradient with aesthetic, geo-hydrological and cultural values. Frequent orographic changes, melting of glaciers, erratic and unpredictable weather conditions, changing rainfall pattern, the rapid increase in temperature and extensions of growing seasons, etc., have greatly influenced the biodiversity of the montane ecosystem (Singh and Rawat, 2000). Global warming and climate change is of major concern across the Himalaya because the rate of temperature increase here is significantly higher than the global average (IPCC, 2007). The Himalaya has been experiencing unprecedented warming (Shrestha *et al*., 2015), resulting in rapid snow melts (Ballesteros-Canovas *et al.,* 2018). The shrinking of snow not only resulted in climate variability (Bajracharya *et al*., 2015), but also led to catastrophic snow avalanches, flash foods, landslides, and soil erosions (Arora and Taulavuori, 2016) causing significant natural and socio-economic losses (Ballesteros-Canovas *et al*., 2018). This unusual monsoon rains of this year in the NW Himalayan state of Himachal Pradesh, a stark example of the devastation that the global warming and climate change can bring.

Number of studies support the fact that mountain ecosystemsare most sensitive to climate change and global warming and,is being affected at a faster rate than other ecosystems (Dash *et al.,* 2007; Egan and Price, 2017; Negi *et al.,* 2012). The mountains, with only about 24% of overall land surface area, are ecologically very important, as it represents almost half of the world's biodiversity hotspots and support 12% of total global population (Sharma *et al.,* 2010). These high montane ecosystems show high sensitivity and vulnerability towards climate change, playing a vital role in global early warning system (Anonymous, 1&2). Thus, global warming and climate change pose a major risk to the pristine biodiversity of mountains and, to the essential ecosystem services of the vulnerable population that depends on them. Himalayan region represents the highest and most diverse treeline over the world. This alpine HATZ has attracted the interest of researchers for many decades, especially across Europe. However, in the Himalaya, this region is poorly researched in comparison with the European counterparts (Shi *et al.,* 2020), largely due to the remoteness and difficult terrain. Though some attempts to study the timberline have been made in the recent past (Schickhoff *et al.,* 2015; Singh and Gumber, 2018; Hamid *et al.,* 2020; Shi *et al.,* 2020; Singh *et al.,* 2021), but long-termmonitoring (LTM) studies are still wanting.

HIGH-ALTITUDE TRANSITION ZONES (HATZ)

The high-altitude transition zones (HATZ) in the state pertain to the timberline that marks the merging of temperate tree lands with the alpine pastures through sub-alpine scrub. This transition zone, thus, represents an ecotone between the sub-alpine and alpine zones and marks a gradual recession from close canopy forests to stunted forests or the German Krumholtz or the English Elfin Woods, along increasing gradient of altitude. In general, the altitude of this zone in Himachal Pradesh, varies between 3200m asl to 3900m asl depending upon the site aspect and location.

This zone is broadly characterised by a treeline that is formed towards the upper limits of this zone by fir-birch (*Abies spectabilis-Betula utilis*) formation with a mixture of maple (*Acer* sp.), birdcherry (*Prunus cornuta*) and kharsu oak (*Quercus semecarpifolia*) and spruce (*Picea smithiana*) and kail (*Pinus wallichiana*) towards its lower limits. The treeline in this zone, at higher elevations, merges first with sub-alpine scrub formed by various species of Sorbus, Rhododendron, Salix, Rosa, Lonicera, etc., gradually moving to the alpines, reduc-

Fig.1: The alpine vegetation here corresponds to desertic, mesophytic and moist

ing only to a mixed scrub with rich occurrence of medicinal herbs. This zone plays an important role in the region's ecology, as it traps the snow mass for a longer period and, thus, helps recharging of the aquifers in addition to saving the soil from erosion. This zone also forms home to a variety of floral and faunal species, many of them having been assessed as 'endangered'. The alpine vegetation here corresponds to desertic, mesophytic and moist (Fig. 1).

Recent studies on global warming, receding glaciers, change in phenological behaviour of plants, etc. have brought this crucial timberline zone in focus. This zone has come to be believed as an excellent indicator of global warming due to its narrow altitudinal band and exacting conditions. The general limit of timberline is marked by a mean temperature that does not exceed 6oC, thusplaying a vital role in species formation. The species of this zone are likely to respond much faster to any change in the ambient environmental temperature and prominently exhibiting shifts in the ecosystem boundaries. Today, the transition zones in the state are, however, under tremendous biotic pressure. These fall in the path of migratory graziers who shepherd their flocks of sheep and goat to and from alpine meadows each year, resulting in degradation of many of these areas. Many of these areas also attract uncontrolled tourist flow that has made areas vulnerable to damage, while many other areas are facing developmental pressure on account of road construction, hydro-power projects, etc.

GOAL AND OBJECTIVES

The long-term goal of this study is to -

(i)Assess and monitor the impact of global warming through study of the vegetation formations and dynamics in permanent plots laid across high altitude transition zone in temperate Himalaya,

The immediate objectives to achieve the long-term goals and to test the above-mentioned hypothesis included -

- Reconnaissance of the high-altitude transition zone in Kinnaur, Shimla, Kullu and Chamba districts, to identify appropriate locations and set up permanent plots for generating baseline ecological and floristic data.
- Undertaking ecological and floristic studies, with special focus on the status, structural and functional aspects of timberline vegetation.
- Assessing the spatio-temporal changes in the timberline (contiguity, vertical and horizontal extent, and interspersion) using remote sensing and GIS.
- Analysis of the ecological and floristic data towards development of models for predicting future scenario along timberline ecotone due to climate change and continued anthropogenic pressures, and evolving mitigation strategies.
- Developing and putting in place protocols for periodic assessment of floristic data.

HYPOTHESIS

This study, with an aim to investigate the various aspects related to the subject in a comprehensive manner, has been developed around the following hypothesis:

i. Natural and human influenced timberlines differ significantly in terms of structure and composition and they represent inherent and induced edges respectively,

ii. Increase in mean annual temperature due to global warming will lead to upward shift of timberline as indicated by better regeneration of tree species, especially with anthropogenic pressures,

iii. The taxa obligate to timberline zone would be affected the most in the event of increased anthropogenic pressures and climate change.

METHODOLOGY

Reconnaissance of the Transition Zones in the State

Surveys were conducted across the timber line covering the entire state of Himachal Pradesh. Several sites in this zone were surveyed and, finally 5 sites were shortlisted for laying the permanent plots for ecological and floristic studies. The major focus of the project activities/studieswas on the HATZs falling in the Sutlej catchment forming parts of Kinnaur where main research under the project is beingcarried. Thestudieswere extended to the HATZs areas falling in Beas valley (UNESCO Heritage GHNP) and Ravi valley (Sach pass area) to have a broader perspective and, for comparative information about global warming and climate change. During the reconnaissance, the following basic information in respect of the sites visited was taken into consideration and recorded -

- Broad floristic composition, especially with respect to the occurrence of keystone treeline species
- Occurrence of any RET plant species and their population status
- Incidence of biotic pressure and degradation status
- GPS coordinates for mapping purpose

Mapping of the Transition Zones using GIS

The sites have been mapped and these maps would be very useful during monitoring of the vegetation dynamics in the zone over a specified period.

Laying Permanent Plots and Preparation of Baseline Data

Based on the rapid surveys/mapping exercise, as mentioned above, 5 sites were identified across the HATZs and permanent plots of approximately 5 hac laid at each site. These plots are being subjected to detailed floristic studies over different seasons to prepare a baseline of species diversity, species enrichment and phenological behaviour. Data with respect to ambient temperature and humidity at these sites is also being recorded using digital 'data loggers' on a 6-hourly basis. The floristic documentation is being backed up with photographic images and standard herbarium specimens for future references (Fig. 2).

Fig. 2. The floristic documentation is being backed up with photographic images and standard herbarium specimens for future references

DISCUSSION

Ambient Temperature and Relative Humidity

The data on ambient temperature and humidity is being recorded for the past over eight years now, which will be analysed on the completion of 10 years, during 2025. The data is being recorded on Onset U23-001A – HOBO U23 Pro v2 internal temperature/relative humidity loggers fixed at each of the marked plots (sites). The data from each plotis downloaded annually and is being archived for final analysis. For looking at the trends, the temperature and humidity data of one plot/site for three years (from 2019 to 2020) is considered (Fig.3).

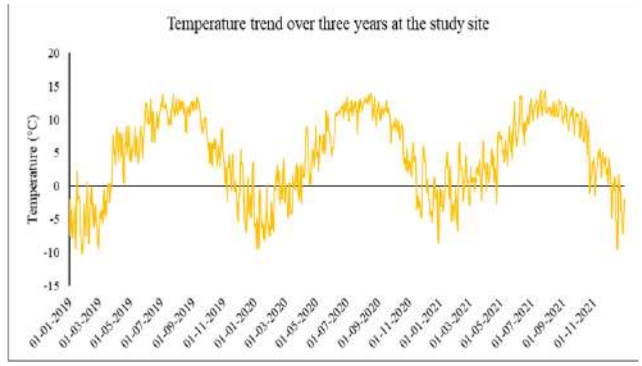

Fig. 3. The temperature and humidity data of one plot/site for three years (from 2019 to 2020) is considered.

Looking at the trends emerging from the figure, it is noticed that the mean temperature has shown an upward trend over the years. The values increased from 4.29°C in 2019 to 4.52°C in 2020, and further to 5.21°C in 2021. This is indicative of the fact that, on an average, the temperature has been rising in the study site during this time lapse. The minimum and maximum temperatures also increased between 2019 to 2021. The minimum temperature rose from -10.18°C in 2019 to -9.54°C in 2021, and the maximum temperature increased from 13.87°C in 2019 to 14.40°C in 2021. This indicates that both the lower and higher temperatures experienced in the study site have been on a steady rise. The analysis of relative humidity (RH) data for the study site over three different years (2019, 2020, and 2021) (Fig.4), revealed that the mean RH decreased from 67.30% in 2019 to 64.50% in 2020 and further to 62.77% in 2021, indicating a gradual decline in humidity levels over the period.

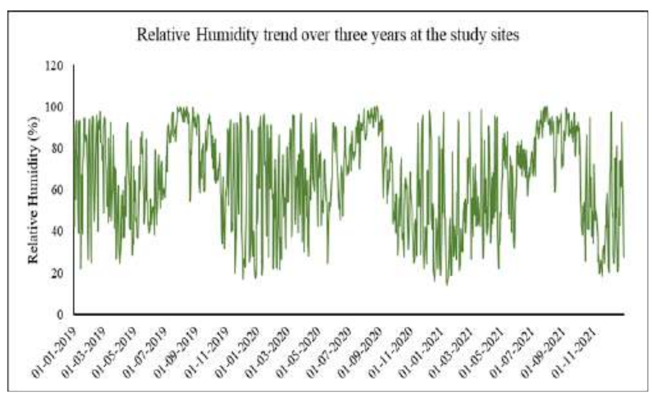

Fig.4. The analysis of relative humidity (RH) data for the study site over three different years (2019, 2020, and 2021)

From the above data, it is evident that the maximum RH remains constant at 100% during theperiod under consideration. This indicates that the study site experienced periods of high humidity throughout the observed period, with air reaching its saturation point. On the other hand, the minimum RH values does show some variation across the years. In 2019, the minimum RH was 16.74%, which decreased slightly to 16.19% in 2020, and then further to 14.41% in

2021. This variation in minimum values suggests that the study site experienced variable degrees of dry periods or low humidity at several points during the three years.

IMPACT ON ALPINE FLORA

Impacts of global warming and climate change on native flora are increasingly evident across a wide range of ecosystems, affecting phenology as well as the range shifts towards higher elevations have been observed in numerous studies (Parmesan and Yohe 2003; Neilson *et al.,* 2005; Schwartz *et al.*, 2006; Wolkovich *et al*., 2012; De Frenne et al., 2013). It is also expected that climate change would increase the dominance of plant species adapted to lower warmer altitudes at the expense of the cold-adapted species (termed as thermophilization) (Bertrand *et al.,* 2011; Gottfried *et al.,* 2012; De Frenne *et al.,* 2013; Vanneste, 2017).The HATZ in the Himalaya, is providing necessary and valuable ecosystem functions, as well as a critical source of biodiversity, sheltering the high number of endemic and endangered species (Samant *et al.,* 2007; Katel *et al.,* 2015; Jishtu *et al.,* 2021). Results of climate change is observed in these fragile and climate sensitiveregions by the increase in shrub cover. Main factors responsible are the ambient temperature and humidity, along with the retreating snow cover, is creating favorable conditions for shrub encroachment (Hallinger *et al.,* 2010; Venn *et al.,* 2021).The upward movement of tree and shrub line isresulting in the shrinking area of alpine meadows, resulting in its flora facing constant competition from fast growing lower elevation plants, raisingconcerns for the protection and conservation of endemic flora (Salick *et al.,* 2014). Recent study conducted in Kashmir Himalaya (Hamid *et al.,* 2020) found significant increase in the number of vascular plant species between 2014– 2018, which they acknowledged as response to climate change resulting from global warming. As alpine zone acts as a sensitive and suitable indicator site, it thus provides a perfect platform to evaluate potentials of impacts (biotic pressure, climate change, global warming, etc.) and to establish a baseline information for predicting future changes in species distribution (Walther *et al.,* 2002; Körner, 2003); plant responses to changes like community composition, phenology, precipitation and climate changes (Root *et al.,* 2003; Menzel *et al.,* 2006; Cleland *et al.,* 2007; Primack *et al.,* 2009).

PHENOLOGY

Spring refers to the temperature transition between winter and summer – a season of rebirth, rejuvenation, resurrection, and regrowth. Spring phenology is assumed to be temperature dependent processes; the chillingrequired to complete endo-dormancy and,the heat required for bud formations. Abnormal increase in spring temperature can strongly influence plant ontogeny (Badeck *et al.,* 2004; Molau, 2005; Luedeling *et al.,* 2013; Yang *et al.,* 2015). From mid-1970s to the end of the century, spring season arrived earlier by 2.3 to 5.2 days per decade (IPCC, 2007). With global warming increasing gradually, the current trends of advancing phenology might slow or delay for an increased number of temperate species (Qin *et al.,* 2009). Some studies reveal snow melt as an important variable for early spring phenophases. Snowmelt timing being influenced by the effect of global warming, brings different changes in early spring phenophases (Inouye *et al.,* 2003; Bertin, 2008). Some studies supported the fact that advance in early spring phenology is more strongly influenced than those in later spring phenology (Polito and Pinney, 1997; Ahas *et al.,* 2002; Miller-Rushing *et al.,* 2007; Molau *et al.,* 2005). Rhododendron species in the Himalaya are now flowering a month earlier than normal (Xu *et al.,* 2009; Suonan *et al.,* 2019).

EFFECT ON TREE LINE SHIFTS

The highest and noticeable treeline occurs is in the north west and western part of the Himalaya, where it generally lies in the altitudinal range of 3200m to 3600m. Here, forest treeline represents the transition zone between low land and high-altitude ecosystems (the broad area of 50-100m below the treeline skirting the complete forest) and, acts as the most conspicuous physiognomic boundaries for vegetation besides acting as a key environmental limit for the ecological boundaries (Körner and Paulsen, 2004; Schickhoff, 2005). Change in treeline dynamics signifies continuous change of ecological conditions with increasing elevation, expressed by sudden unexpected alterations of dominating life forms and,has come to be used as an observatory of landscape response to climatic change (Körner, 2003; Miehe *et al.,* 2007; Gaire, 2014; Tiwari *et al.,* 2017). It is pertinent, that relationships of Himalayan timberline positions must be seen in affinity with climate change and global warming. The observations in the present studies indicates that there aredistinct formations in the HATZ treeline along the east to north eastern slopes at Dhel (GHNP). Here, the upper timberline shows a sharp transfer from the *Abies spectabilis, Betula utilis* tree formation to the Krumholtz R*hododendron campanulatum*shrubby formation. Shrub growth has earlier been correlated with warm summers and winter snow cover, advocating for ecosystem changes if global warming and climate change continues as projected (Perfors, 2004; Hallinger, 2010). It has been observed that Juniperus (*J. communis, J. indica* and *J. squamata*) acts as a primary timberline shrub species across the experimental plots. Over the years, these clumps of shrubby Junipers have been showing a gradual increase in their spread, validating an ongoing shrub expansion.It was also observed that the Satluj valley sites (Kinnaur)with arid habitatsshow little higher elevations in the natural timberline as compared to the Ravi and Beas catchment slopes which are more moist. These timberline elevational differencesmay be due to exposure of solar radiations and human impacts across the mountains. Observations across all plots shows higher shifting rates and sapling establishmentfor conifers (*Abies spectabilis* and *Pinus wallichiana*), while for *Betula utilis* the sapling establishment trend is negligible. In case of *Quercus semecarpifolia* below Sach pass in the Ravi catchment, the establishment of saplings is dense with distorted multi-stemmed growth. Researched evidence supports the facts that the treeline shift in the Himalaya in response to climate change fluctuates significant upwards (Gaire *et al.,* 2014, 2017; Tiwari *et al.,* 2017; Singh *et al.,* 2018; Sidgel *et al.,* 2018), moderately upward (Chhetri and Cairns, 2016), or almost stationary with no visible fluctuation (Gaire *et al.,* 2011; Liang *et al.,* 2011; Schickhoff *et al.,* 2015; Shrestha *et al.,* 2015). Some studies do indicate probability of treeline shift to upper elevation in the near future (Shrestha *et al.,* 2007; Harsch *et al.,* 2009; Tiwari *et al.,* 2017). The data generated would also help in determining the timberline change detection, using topographic map and satellite imagery as suggested in earlier studies (Panigrahy *et al.,* 2010).

CONCLUSION

The unique Himalayan ecosystem is notable for its endemic, heterogeneous, and fragile nature, which is also bestowed in resources richness and their complexity. This mountainsystem being fragile,is being marred by several factors, like deforestation, land use change because of varied development activities, livestock overgrazing, NTFP collections, etc. These factors are widely acknowledged to have a rapid and potential extreme effect on its biosphere resources such as water supply, soil and native biodiversity. Recent studies conducted has emphasized the complexity in determining the exactprediction and extend of climate change specially in high altitude regions, like the HATZ. Thus, tree line shift and plant phenology; two sensitive bio-factors can be monitored to evaluate, not only the present effects of climate change but also the past and future. Remote sensing and GIS, ecological niche modelling etc., have emerged as advanced monitoring tool for tree line shift studies over other conventional methods. The unique and endangered biodiversity of highland region of Himalaya is under constant threat due to global warming and changing climate patterns. Hence anthropogenic factors contributing to global warming ought to be identified and appropriate mitigation steps should be followed in order to minimize the effects of climate change on floral diversity. In the present study, floristic studies coupled with ambient temperature and humidity data is being documented. This data along with NDVI can be accessed for plant changes, incorporating spatial and temporal aspects of satellite data. There is an urgent need to conduct more in-depth studies to see the real effects of climate warming on plants.The study is expected to provide the baseline information on the status, structure and composition of timberline ecotone (HATZ) in the western Himalaya. The population status of selected indicator taxa which are sensitive to climate change and anthropogenic pressures would be valuable for further conservation planning and LTM. The study will also provide information on the recent trends in the location of timberline over 10 years, which would be useful for developing models for predicting future scenario along timberline in the event of global warming due to climate change. The study will be of direct relevance to conservation of high-altitude ecosystems in the North West and Western Himalaya.

ACKNOWLEDGEMENTS

The funding provided by the office of the Additional Principal Chief Conservator of Forests (Research & Training), Karnody, Sundernagar, Himachal Pradesh is gratefully acknowledged. The authors are extremely grateful to the forest officials and staff of Kinnaur and Chamba Forest Divisions and, GHNP, Shamshi, for the logistics and field assistance.

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AFTER MATH OF DAMAGE: IMPACT OF SHORT-TERM CLIMATIC EXTREME EVENT ON ALPINE PLANT COMMUNITIES

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ABSTRACT

There is aprojected intensification of extreme climatic events under future climate, however our general understanding of impacts of such events to plant communities in mountain ecosystems is limited. Among such extreme events, untimely/unseasonal snowfall at the time of growing season when it is unusual, can damage plant reproductive and vegetative structures and result in population dieback in the high-altitude ecosystems. Here, we report the findings from observation following an untimely snowfall event during the growing season (13 September 2021) in alpine region (Rohtang: ~ 4450 m amsl) of western Himalaya. In this study, we report on major damage to11 snowbed-restricted plant species and show that a short extreme event could have potential detrimental effects that can have long-term ecological impacts. An assessment of plant damage for vegetative (leaves and stem) and reproductive (flower and fruits) structures was made by visual inspection on 30 individuals per species. Additionally, the mortality and adaptation pattern (recovery after event) of an individual was also estimated in terms of "sub-lethal" (reversible) or "lethal" (irreversible) damage to the tissues by re-surveying the site and reviewing the status of individuals after a period of one week. We observed thatvarious species, suffered damagein the range of 20-100% of individuals/specieswith injuries to leaves, 10-100% to stems, 23.33- 100% to flowers and 53.33-76.67% to fruit-set. Further, recovery from damages after injuries indicate that, among various species, plant stem (46.67-100%) has a high revival potential, followed by flowers (10-90.91%), leaves (13.33-86.67%), and fruits (18.18-82.60%). Further, the mortality percentage of individuals ranged between 3.33-16.67% for various species, while some species (*Sibbaldia cuneata, Sibbaldia purpurea, Saussurea gossypiphora* and *Saxifraga jacquemontiana*) exhibited almost nil mortality. Our results suggest that the reproductive structure of alpine plant species are more vulnerable to short-term extreme climatic events than the vegetative structures. Consequently, such extreme events have the potential towards catastrophic reproductive failure and population die-back in the alpine plants communities in the long-term, if the extent, intensity and harshness of these events increased in the future.

Keywords: Climate change, Extreme climatic events, Alpine plants, Unseasonal snowfall, Vegetative and reproductive structure, Himalaya, Rohtang

INTRODUCTION

Themountainecosystems across the globe are relatively more affected by climate change, with warming rates for the alpine regions reported to be more than double the global average (Shen *et al.,* 2022). Potential consequences of these changes in the atmospheric phenomenahas increased the probability of climate extremes which are predicted to continue to further increase in future (Kotlarski *et al.,* 2023). These exceptional and sudden climate extreme eventscould have a fundamentally different impact on plant growth, community composition, and ecosystem functioning than normal climatic variability (change in average temperature and precipitation in atmosphere)(Felton and Smith 2017). Usually, extreme events result in exceeding the physiological thresholds of adaptation of a species, which may lead to local population reduction or even extirpation of the species (Guha *et al.,* 2018). Previous empiricalstudies have reported destructive effects on plant phenology, physiology and morphology due to abrupt changes in temperature,precipitation or other events (Augspurger 2011; Caradonna and Bain 2016). Despite the fact that there are many experimental studies-investigating the influence of expected changes in climate (warming, drought, nutrient enrichment, etc.) on natural plant communities (Dolezal *et al.,* 2020; Prendin *et al.,* 2022; Kotlarski *et al.,* 2023), a general understandingof the consequences of climatic extreme events on these communities is still limited and it remains to be seen how individual plant species respond to the extreme events.

In alpine regions, the extreme climatic events, especially the low-temperature events (frost damage due to unseasonal snowfall) during the active growth period are very common (Ball *et al.,* 2011). Usually, frost events typically happen frequently in the alpine region and plants are generally frost-resistant (Neuner 2014), but, an extreme frost event which is due to anunseasonal snowfall might impose high risksto species during the growing season. Recently, a study of long-term climatic record (124-year) indicated that the frequency of harmful spring frost episodes have risen and the extent of damage in plants are reported to be species-specific, phenological stage-specific and tissue-specific (Augspurger, 2013). Specifically, reproductive structures (flowers and seed)of species are at higher risk of frost damage than the vegetative structures (leaves and stem)(Caradonna and Bain, 2016). In this study it was also found that thelate-flowering species were found to be more sensitive to frost damage than early-flowering species.Thus, it can be ascertained that although some efforts have been undertaken to study the effect of frost,yet the response of many alpine species to unusual frost events, such as in the Himalaya,are still poorly understood.

In the alpine region of Himalaya, plants have a short growing season, which graduallygets even more shorterwith increase in elevation. The seasonal changes in abiotic environmental conditions prevalent at higher elevations (low temperature, high UV, high wind speed etc.) play a crucial role in growth and development of plant species. Since, plantgrowth is sensitive to abiotic stresses,any extreme climatic event (such as frost) happening during or in between the short growing season can put additional stress on the life cycle of plants, which may have cascading effects on persistence of plant populations.Recent evidences from the Himalayan region suggest that the frequency of extreme climatic events are increasing and affecting the vegetation dynamics of high-altitude plant communities (Dolezal *et al.,* 2016, 2021). For instance, the extreme snowfall events during the growing season have roughly decreased one third of vegetation cover, for both alpine and sub-nival plant communities in the Ladakh region of western Himalayas (Dolezal *et al.,* 2021). However, most of the evidences of impacts of extreme climatic events on plants have been reportedfrom the variousagro-ecosystems (particularly the commercially cultivated crops). For example, in Kashmir valley, extreme snowfall events (frosty conditions) are reported to affect the flower parts, pollination process and fruit setof many horticulture crops such as apple, almond, peach, plum, apricot and cherry (Rashid *et al.,* 2020; Kumawat *et al.,* 2022). Besides this, no other efforts have been initiated to knowabout theconsequences of extreme events on natural plant communities in the region.Hence,in this study an attempt has been made to examine the response of Himalayan 'alpine-restricted'plant species to an extreme 'untimely snowfall' event that took place at Rohtang (13 September 2021) in the uppermost elevation zone (~ 4450m amsl) of occurrence of species communities during the growing season. During this event, we estimated the mortality rate and magnitude of damage in 11 plant species. The primary objective of this study was to know about the effect of extreme climatic events and its potential consequences as long-term impacts on the Himalayan alpine plant communities.

METHODOLOGY

Study site and selection of individuals

Our ongoing research project to evaluate the eco-physiological and phenological responses of alpine species to climate change covered this observational survey.In 2019,we had setup a snow manipulation experiment with 10 experimental plots (5x5 sq.m; 05 each for Early Snow-melt and control respectively) at 04 elevations,at Rohtang (32°22' N; 77°16' E) in the Western Himalayan region (Fig.1).

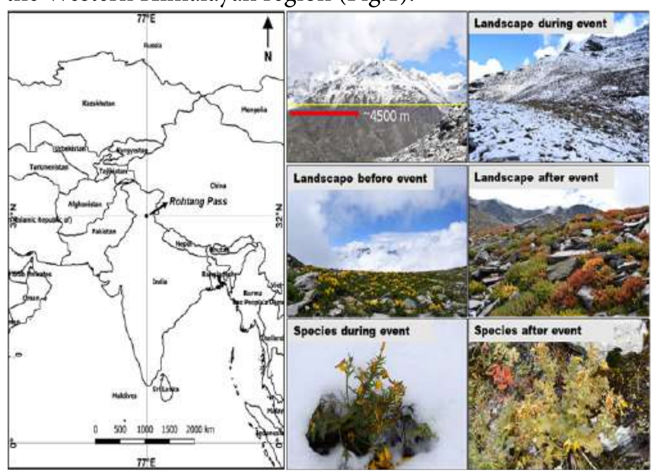

Fig.1. Study site and pictures depicting landscape-scale damage to alpine plants due to extreme event.

Within each of these plots, six individuals of various species were tagged for studying the changes in phenological responses.Thus, overall we were monitoring 30 individuals of various species.As the extreme event occurred on 13 September 2021with snowfall in the mountain peaks, our study elevation of 4450 m amsl became snow-covered (accumulating ~15 to 20 cm depth of snow), and individuals of different species (Table 1) were subjected tovarying levels of injuries on vegetative and reproductive tissues due to sudden frost. Thus, the already tagged individuals were carefully inspected in every plot to evaluate the aftermath of damage. Each individual was visually inspected for the visual deterioration in vegetative (leaves and stem (including branches)) and reproductive (flower and fruits) structures. Thus, in this study,all the individuals of species, which were tagged prior to the extreme event,were investigated twice:first, a day after the period of snowfall (i.e. on 14-15 September 2021),and second, after the snow pack melted (i.e. on 19-20 September 2021).

Table 1. Brief description of the plant species included in the study.

Species	Family	Life history	Growth form	Life form	Elevation Range (m amsl)*	Niche width*
Sibbaldia cuneata Schouw ex Kunze	Rosaceae	Perennial	Semi basal	Hemicryptophyte	3300-4700	19.37
Sibbaldia purpurea Royle	Rosaceae	Perennial	Dwarf shrub	Chaemaephyte	4100-4300	1.38
Saussurea gossypiphora D.Don	Asteraceae	Perennial	Semi basal	Hemicryptophyte	4500	1
Pleurospermum candollei (DC.) Benth. ex C.B.Clarke	Apiaceae	Perennial	Semi basal	Hemicryptophyte	4100-4500	2.59
Ranunculus brotherusii Freyn	Ranunculaceae	Perennial	Erect leafy	Hemicryptophyte	3100-4500	8.19
Anaphalis nepalensis (Spreng.) Hand.-Mazz.	Asteraceae	Perennial	Erect leafy	Hemicryptophyte	3300-4700	21.91
Corydalis meifolia Wall.	Papaveraceae	Perennial	Semi basal	Hemicryptophyte	3900-4700	1.57
Saxifraga jacquemontiana Decne.	Saxifragaceae	Perennial	Cushion	Hemicryptophyte	4500-4700	1.81
Rhodiola imbricata Edgew.	Crassulaceae	Perennial	Erect leafy	Hemicryptophyte	3300-4700	16.09
Primula elliptica Royle	Primulaceae	Perennial	Short basal	Hemicryptophyte	3300-4300	4.37
Cremanthodium arnicoides (DC. ex Royle) R.D.Good	Asteraceae	Perennial	Short basal	Hemicryptophyte	4300-4700	2.61

***Source:** Thakur and Chawla (2019) Functional diversity along elevational gradients in the high altitude vegetation of the western Himalaya. Biodiversity and Conservation, 28:1977–1996.

The observations after the snow-melt were made in order to assessfor how much species or individuals of species revived from the injuries due tosnowfall event. Every individual was again visually inspected for recovery in leaves, stem, flower and fruit-set. In addition, phenological stages of each specieswas recorded during the time of the event. For uniformity in results, assessment of injury to the species vegetative and reproductive structures was constantly performed by the same investigator.

ASSESSMENT OF PLANT DAMAGE

Species individuals were examined for injury in three approaches. In the first approach, an individual was assessed for the injury in nominal data format (Yes and No), that was further changed to binary scale (0 and 1) for data analysis. In this format, 'yes or 1' is specified to the individual that is injured due to event and 'No or 0' is specified to the individual who have no impact of the event. Injury to vegetative and reproductive structure was assessed when discoloration and wilting appears or structure became brown/black and brittle, linked to oxidation of polyphenols. In the second approach, visual inspection was made to assess the mortality of an individual. For this, we simply recorded that the individual is live or deceased. An individualmortality was considered dead when all vegetative and reproductive structureswere dead. In the third approach, the adaptation signals of the individual were estimated. For this, the recovery of individual was estimated in terms of "sub-lethal" (reversible) or "lethal" (irreversible) as two response classes. For this, we re-surveyed the plots and assessed the individuals after a week (when snow had fully melted) to study the responses. The fact that reversible injury could have cellular adaptation and the structure survives; vegetative or reproductive structure which revives (damage stops after the event and tissue revive) from the injuries after the event ends, was treated as "sub-lethal" and revival percentage estimated for various species. Similarly, irreversible injury with no cellular adaptation leads to loss of structure; with no visible signs of recovery after the event ends (damage continues after the event) was treated as "lethal"(Wheeler *et al.,* 2014; Min *et al.*, 2020).

RESULTS

Effects of extreme event on vegetative structure

All the plants of various species had fully matured in terms of their vegetative growthprior to the extreme event.However, due to the extreme event, all the species showedsome extent of damage to their vegetative structure. Leaves of the species started wilting and ultimately became brown or black in appearance, other symptoms observed were discoloration and shedding. Among the vegetative structure of plants, the leaves exhibited damage from 20% to 100%of all theindividualsof various species,whilethe stem damage (wilting, broken, bent, tiltingetc.) occurred between 10 to 100%. *Saxifraga jacquemontiana* depicted the least damage to leaf (20% of the tagged individuals) and stems (0%).

Effects of extreme event on reproductive structure

Among all the species, just prior to the extreme event, 06 were observed to be in their flowering stage, while, 03 species were in their fruit maturation stage (Table 1). Among the reproductive structures of plants, species in flowering stage exhibited 23.33% to 100% damage (in % of tagged individuals) in flowers (wilting and shedding), and species in the fruit maturation stage (wilting, partially broken and shedding) exhibited 53.33% to 76.67% damage in the seed-set. *Saxifraga jacquemontiana* showed the least damage to their flowers (23.33%), whereas, *Sibbaldia purpurea* depicteda comparatively less damage to the fruiting body (53.33%) and thereby less affecting theseed-set (Fig. 2; Table 2).

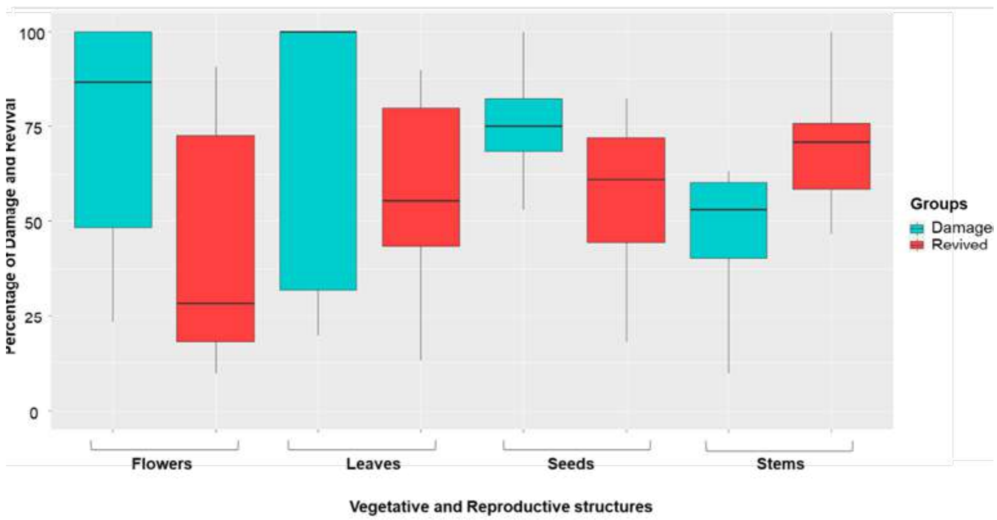

Fig.2. Damage and Recovery percentages of individuals of various species with respect to their vegetative and reproductive structures due to extreme climatic event.

Table 2. Damage%, revival % of vegetative and reproductive structures,and mortality rate (%) of individual of various species.

Species	Phenological stage of species during the extreme event	Damage Pattern				Revival Pattern				Mortality (%)
		Leaf damage (%)	Stem damage (%)	Flowers damage (%)	Seed damage (%)	Leaf revival (%)	Stem revival (%)	Flow-ers revival (%)	Seed revival (%)	
Sibbaldia cuneata	Fruit maturation	100.00	50.00	-	76.67	86.67	80.00	-	82.61	0.00
Sibbaldia purpurea	Fruit maturation	100.00	40.00	-	53.33	56.67	58.33	-	68.75	0.00
Saussurea gossypiphora	Peak Flowering	33.33	10.00	73.33	-	90.00	100.00	90.91	NA	0.00
Pleurospermum candollei	Peak Flowering	100.00	100.00	100	-	13.33	73.33	16.67	-	13.33
Ranunculus brotherusii	Fruit maturation	90.00	53.33	-	73.33	55.56	68.75	-	18.18	6.67
Anaphalis nepalensis	Peak Flowering	30.00	63.33	100	-	44.44	73.68	23.33	-	6.67
Corydalis meifolia	Peak Flowering	100.00	100.00	100	-	20.00	46.67	10	-	16.67
Saxifraga jacquemon-tiana	Peak Flowering	20.00	-	23.33	-	83.33	-	85.71	-	0.00
Rhodiola imbricata	Peak Flowering	23.33	56.67	40	-	42.86	58.82	33.33	-	3.33
Primula elliptica	End of Flow-ering	100.00	40.00	-	-	46.67	58.33	-	-	13.33
Cremanthodium arnicoides	End of Flow-ering	100	56.67	-	-	76.67	76.47	-	-	6.67

Adaptation pattern of individuals to extreme event

Focusing on the revival of vegetative structures after the end of snowfallreveals that leaf and stem revival rate of individuals ranged from 13.33% to 90.00% and from 46.66 to 100% for the various species (Table 2). Among the reproductive structures, flower revival rate ranged from 10% to 90.91%, whereas, revival ratefor fruiting bodies (achenes)ranged from 18.18% to 82.61% for various species. Individuals of Saussurea gossypiphora showed the maximum revival rate for leaves, stem and flowers. Further, the rate of revival of fruitwas maximum among the individuals of *Sibbaldia cuneata*.

Mortality rate of individual due to extreme event. After the end of the extreme event, mortality rate of the individuals of different species ranged from 3.33% to 16.67%. Highest rate of mortality was found for the individuals of Corydalis meifolia. Further, individuals of few species (*Sibbaldia cuneata, Sibbaldia purpurea, Saussurea gossypiphora and Saxifraga jacquemontiana*) depicted nil mortality due to the extreme event.

DISCUSSION

During our studies at Rohtang, in the growing season of 2021, we observed that an extreme climatic event, in the form of untimely snowfall, happened in the mid of September. The alpine-restricted species in the western Himalayan region inhabiting the uppermost habitats, suffered from the damageto both the vegetative and reproductive structures.In the current scenario, as several studiesbased on modelling of distribution of species,have found that species are moving upward to high-elevations to find refuge(Mamantov *et al.,* 2021), but the ecological possibility of surviving at higher altitudes is limited to some degree if the extreme weather events such as untimely snowfall continue to occur or the frequency of such events increases in near future. To the best of our knowledge, this is a first account of such an extensive plant damage from Himalayan alpine region. In general, this study showed that the rate of damage due to untimely snowfall is higher for reproductive structures of species than their vegetative structure. Similar responses of plant species are also reported from other regions to different climatic events. Caradonna and Bain (2016), investigating the effect of experimental frost on 08 sub-alpine species reported that, flowers were more sensitive to frost than leaves. Besides, we also observed varying level of mortality in individuals of species irrespective of adaptive potential to recover from the extreme event. However, the mortality of the individuals could be species–specific and indicate vulnerability to extreme events. Although, we didn't assess the effect of current year damage on growth and mortality of individuals of species in subsequent growing season in the following year, but, negative responses (individual dieback or reduced growth) could be expected due to trans-generational effects of extreme events (Mamantov *et al.,* 2021). Previous studies accounting for the effects of untimely frost damage on Aesculus glabra have reported that some of saplings of this species, which experienced frost damage in 2007, did not survive in 2009 (Augspurger, 2011). Thus, the short-term extreme events could have a long-term effect on community dynamics in alpine regions. The study also indicated that damage to individual's vegetative or reproductive structures is highly related to the phenological stage. The species which flowers later in the growing season (e.g.*Pleurospermum candollei*) were experiencing more damage to reproductive structures, while species flowering earlier in the growing season (e.g. *Ranunculus brotherusii*) experience more damage to vegetative structures. Thus, species flowering earlier can get a 'fitness benefit' in terms of reproductive output and survive more competently under extreme events conditions. Further, assuming that the reproductive damage in perennial plants to extreme events is widespread (Caradonna and Bain, 2016), it can affect the reproduction output by reducing the resources available to the pollinators (Walters *et al.,* 2022). Indeed, sudden extreme events have a potential to reduce the population of plant species by large mortality rates and consequently also reduce the population of pollinators due to diminished floral resources. Overall, this study advances the idea that extreme events such as 'untimely snowfall' can function as a selective force on Himalayan alpine restricted species and can completely reshape the community taxonomic composition in future.

CONCLUSION

Extreme weather events are among one of the important abiotic stresses restricting the distribution and abundance of alpine plants. As the intensity and frequency of such events continue to increase under future climatic conditions, understanding their ecological consequences on plant communities become essential. This observational study contribute to enhancing our general understanding of how untimely snowfall (extreme event; frost event) amid active growing season affects alpine plant species at their upper limit of distribution. Our results demonstrate that species reproductive structures (flowers and sees set) are more sensitive to extreme event than vegetative structures. Although, mortality due to extreme event is essentially species- specificand indicate the extent of vulnerability, but there might be chances that for some species, negative consequences could only be seen in the subsequent years. This study contributes to the general understanding of alpine plant sensitivity to extreme events and recommend that more studies (e.g. coordinated networks of manipulative field experiments) are required to improve the understanding of the responses of high-altitude or latitude plant communities to the unseasonal climatic extreme events.Further, the alpine species population community composition could be transformed or greatly affected if the extreme events happened multiple times in future years. Future experiments should be undertaken to understand:

(i) how such extreme events affect alpine species physiologically? and; (ii) what are its consequences on species reproductive output? and; (iii) How it affects the recruitment process of the alpine species?

ACKNOWLEDGEMENT

We are thankful to Director, CSIR-IHBT, for providing necessary facilities and encouragement to carry out research work. The study was supported with a financial grant from the Council ofScientific and Industrial Research (CSIR), India [MLP 0205, CeHAB] and later from Science & Engineering Research Board, DST (File no. CRG/2022/007618).

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CLIMATE CHANGE IMPACT ON CENTRAL HIMALAYAN FOREST AND RURAL ECOSYSTEM: AND ADAPTATION, MITIGATION AND COPING STRATEGIES

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INTRODUCTION

The central Himalaya is endowed in natural resources with a geographical area of about 55,845 sq.km (of which 90% hilly and 10% plain/ Tarai) representing 1.63% area of the country and is important part of the global system. It is rich in forest and has 35, 394 sq. km. of forest area which constitutes 64.8% of its total geographical area. The area represents typical Himalayan conditions with diverse geological and ecological zones; Sub-Himalaya, Lesser Himalaya, Higher Himalaya and Trans-Himalaya (Maikhuri *et al.,* 2001). The central Himalayan region considered most impacted regions around the world by recent changes in climate. In recent decades, this region has experienced rapidly increasing temperatureand decreasing monsoon precipitation (Yamada *et al.,*1992; Shrestha *et al.,* 1999; Hasnain, 2002; Duan *et al.,* 2006). The mountain region of the central Himalaya is highly sensitive and vulnerable to climate change. Although there is a lack of certainty in predicting and quantifying climate change impacts on mountain landscape and socio-economic systems. The rural landscape of the central Himalaya has close proximity to the forest ecosystems which provide livelihood to the local people inhabited there and it likely to be impacted by climate change in various ways as stated in the fourth assessment report of Intergovernmental Panel on Climate Change (IPPC, 2007). In addition, the National Action Plan for Climate Change (NAPCC) under National Mission for Sustaining the Himalayan Ecosystem (NMSHE) strongly highlighted this issue. Climate change directly affect agricultural systems, for example by influencing the types of crops that can be grown, or indirectly impact agriculture by affecting the forest resources and ecosystem services on which agriculture depends. Western Himalaya has a long heritage of subsistence economy, with agriculture being the core component involving over 70% of its population (Maikhuri *et al.,* 1997, 2001; Semwal *et al.,* 2004; Nautiyal *et al.,* 2002-2003; Maikhuri and Rawat, 2013) along animal husbandry supported by forest ecosystem. The diversity in the crops in this region is very high and about 40 different species of crops comprising cereals, pseudo cereals, millets, pulses, oilseeds etc. and their number of varieties are cultivated throughout the altitudinal gradient (Maikhuri *et al.,* 1996, 1997; Sen *et al.,* 2002; Rao and Saxena 1994; Nautiyal *et al.,* 2002-2003; Negi and Maikhuri, 2012). During recent past, as a result of rapid changes in land use caused by socio-cultural, climatic variation and various environmental perturbations, the agrodiversity, livestock population, natural resources in the region has decline and changed steadily. Changes in the climate may greatly affect the migration of some plant species to higher elevations and lead to the extinction of others (Maikhuri *et al.,* 2009; Rao *et al.,* 2012). Species composition, diversity, occurrence of rare and endangered species also affected, thus jeopardising the conservation value of Himalayan ecosystem (Maikhuri *et al.,* 2003). Accepting that climate change is occurring at rates unprecedented in recorded human history, people inhabiting the Mountainous region of Uttarakhand need to develop adaptation mechanisms and coping strategies to conserve the biodiversity and secure the related livelihood (Maikhuri et *al.,* 2003; Saxena *et al.,* 2005). Moreover, we need to develop the possible way of adaptation and coping strategies to overcome the climate change impact and formulate the better policies for the conservation and sustainable development of the natural resources particularly in the mountainous region of the Himalaya. Therefore, the present study is an attempt to analyze people perception on climate change impact and documentation of community response to vulnerability and adaptation in Central Himalaya.

STUDY AREA

Uttarakhand (28° 43' and 31° 28' N latitude and 77° 49' and 81° 03' E longitude) is a hill state located in the central part of the India and shares an international boundary with China in the north and with Nepal in the east. The state encompasses an area of 53,483 sq. km, which accounts for nearly 15.5 % of the total geographical area of western Himalaya and 1.63% of the total land area of India. The total population of the state is over 10 million (census-2011). Vegetation varies according to altitude and climatic conditions, from tropical deciduous forest in the foothills to Himalayan temperate forest at middle altitudes, coniferous, sub-alpine and alpine forest at higher altitudes, giving way to alpine grasslands and high altitude meadows. The region can be divided into three markedly different agro-climatic zones along the elevational gradient (vertical zonation) viz., lower altitude, 500 to 1000m, middle altitude between 1000 to 1800m and higher altitude, above1800 m (Fig.1). According to traditionally accepted criteria agricultural land in the region is identified either as the rainfed (locally known as Ukhar) or the irrigated (known as sera). Of the two, the former is predominant form of land use since only about 15% land falls in the latter category.

MATERIALS AND METHODS

Information on climate change assessment

Qualitative and quantitative methodologies were used to understand the people' perceptions and traditional ecological knowledge on climate change. Information on various sectors i.e. agriculture, animal husbandry and availability of forest resources was collected through a review of academic literature, governmental and local level data sources, household surveys, focus group discussions and other PRA tools i.e. community mapping, seasonal calendars, group discussion, historic information etc. Traditional ecological knowledge is a set of practices, beliefs and understanding about local ecology that has been shared across generations (Rao *et al.,* 2012).

Fig. 1. Location of Alaknanda Catchment

Sampling strategy

A sample of 54 villages from 9 mountainous districts involving 20 households of each village, with a total of 1080 households were selected randomly for understanding people perceptions and their adaptation to climate change. Both knowledgeable men and women were involved in group discussions and personal interview in order to collect information on climate variability and its impact on natural resources, agroecosystem, livestock husbandry and their adaptation strategies. Further the information was also confirmed by the field visits and vegetation analysis in high altitude villages and alpine meadows during working on different project of the Institute. Data on perception, responses and local knowledge of climatic variability were collected through individual interviews with local elderly and knowledgeable persons based on questionnaire and a checklist of issues adopted according to interview situations. The respondents were asked 'whether they have experienced, observed or witnessed the given climate change-related indicator' and allowed to choose one of the following three options: 'Yes, have experienced', 'No, haven't experienced' and 'Don't know about it'.

Qualitative assessments

An in-depth rural appraisal survey was undertaken in selected villages of Uttarakhand from 2014 to 2016 to collect information regarding the perception of local people about climate change impact over three decades in the region. Group discussions and meetings conducted at village level to establish the general perceptions regarding climate change impact and were verified through field surveys and personal interviews. Information were collected on different aspects such as climate variability, natural resources and water availability, status of plant diversity, agricultural and livestock productivity, problems related to pests and diseases to livestock and human health and its overall impact on socio-ecological system and its short and long term implications on the sustainability of mountain agro- ecosystem.

Quantitative assessments

People's perceptions and knowledge derived not from any direct measurements of climate but from the way climate affects their immediate surroundings and livelihood (Saxena *et al.,* 2004). For example people explained moisture stress as the condition arising from delayed on set or lower amounts of monsoon rainfall, nutrient stress as the conditions arising from lower rates of farmyard manure input due to decline in natural resources availability as a result of frequent forest fire over last several years as well as impact of climate variability in traditional agriculture system. The questionnaire survey was used to collect household data (i.e. farmer's perception and awareness towards climate variability and its impacts, types and preparedness and adaptation strategies adopted by people, etc.) which was further updated and verified with the data generated through the qualitative methods. The data collected following questionnaire-based approach includes natural resource availability, animal husbandry practices and changes in crops grown over a period of last three decades and reasons for these changes, indicators for climate change and adaptation measures adopted to cope up with from these changes. The survey was conducted in local language for better understanding so as to confirm the authenticity and validity of the questions.

RESULT AND DISCUSSION

Climate change impact on Mountain agrobiodiversity

A vast majority of the population (70 %) of the Central Himalayan communities, largely depend upon agricultural base activities for their livelihood. Inaccessibility, environmental heterogeneity, biological, sociocultural, and economic variations in the region have led to the evolution of diverse and unique traditional agroecosystems, crop species, and livestock which help traditional farming societies to sustain themselves (Maikhuri *et al*., 2000). During the recent past, as a result of rapid changes in land use caused by sociocultural and economic changes and various environmental perturbations the agrobiodiversity of the region has changed steadily. In the mountains particularly high-altitude areas of the Central Himalaya , the global shift in the environment is leading to a rise in temperature but in the very near future, however, it will bring more opportunities particularly for cash crops like tomatoes, cabbage, chillies, peas and medicinal plants (Maikhuri *et al.,* 2003). Notwithstanding, reduction in winter snowfall and spring rainfall and melt-water flows will produce a deficit of soil moisture that could limit any increase in yields resulting from temperature increases. It is believed that even minor changes in temperature could have a major impact on the severity of diseases. Amaranthus crops are most vulnerable to climate change as observed during the recent past when this crop was severely attacked by a disease called Hymenia rickervalis between 1,000 – 1,800masl, whereas between 2,200 – 2,800masl it grew well. It is assumed that poor rainfall during July and high temperatures and humidity, particularly in first and second weeks of September provided favourable conditions for the moth that damaged the crop and reduced the yield. Besides crops such as traditional legumes (Vigna unguiculata, Vigna angularis) important summer legumes growing between 1,000-2,000masl are facing problems of fruit setting because of the shift in peak rainfall time and other climatic factors. Diseases such as rust and blight were common in cereals and potato crops, and legumes such as Phaseolus spp were infected through soil- borne insects such as Coleoptera species (Maikhuri *et al.,* 1997). These insects damage the crops in the early stages of seed germination. One of the reasons for the occurrence of disease in these crops could be that the climatic conditions are favourable for the life cycles of the insects, i.e., an increase in moisture or humidity or milder winters (between 500-1,500 masl) in the lower regions. High-altitude agriculture in this region is definitely in transition and a rise in temperature in future may enhance agricultural productivity.

Impact of climate change on transhumant pastoralism

Animal husbandry constitutes an important component of the rural economy and well-being of the mountainous people and provides a wide range of services and products such as draught power, manure, wool, and supplementary nutrition (Maikhuri *et al.,* 2000). Transhumant pastoralism practices in the region have undergone rapid changes such as loss of alpine grazing land due to the constitution of new notified reserve areas etc. resulted loss of transhuman pastoralist. The reductions in the grazing areas were not sufficient to keep grazing intensity at the required optimum of about 0.3 hectare per animal unit (Nautiyal *et al.,* 2002).

As mentioned above, the livestock management also involves important relationships with lower altitudes (the Terai-Bhabhar tract). Currently, the lowland Terai region has dry spells from December to May (with the exception of winter storms). Although changes to the seasonal distribution of rainfall are highly uncertain, the light rainfall or no rainfall during winter would seriously jeopardise the long-standing regime in which some families send their livestock to the lowlands during the winter to early spring. This practice is also important for maintaining soil fertility in the lower areas. In the event of an increase in temperature in the Terai belts and at low altitude and also lighter than average rainfall, these areas would be under increasing pressure to provide adequate winter grazing. Climate change at high altitude would seriously affect the quantity and quality of forage available for the transhumant pastoral production system, increase disease and pests that spread disease (transmission of wind-borne, foot and mouth disease viruses), reduce water supplies and thus make it difficult to survive in extreme environments.

Impact of climate change on forest, timberline vegetations and alpine meadows

Central Himalayan region is predominated with *Pinus wallichiana, Quercus species*, mixed (pine-oak), *Cedrus deodara, Pinus wallichiana mixed conifer, Betula* and *Abies* and *Cupressus torulosa* along with scrubland and alpine and low-altitude grasslands. The timberline ecotone is the most prominent and significant ecological boundary where the sub-alpine forest terminates into the alpine medowsand could be effectively modelled and monitored for future climate change processes. In some locations, timberline vegetation represents evergreen conifers exclusively, while in some areas it is covered totally by deciduous broad-leaved trees (Purohit, 2003). The native species of the timberline are *Betula utilis, Abies* *pindrow*, and *Rhododendron companulatum*, constitutes a complex, unique habitats of medicinal and aromatic plants and wild edibles. These species with narrow ecological niche or amplitudes may disappear if they fail to compete with new arrivals under a warmer regime and to expand their ranges. Mid altitude species (1,600-2,000 masl), such as *Pinus roxburghii, Cedrus deodara, Cupress torulosa, Quercus dialtata, Q. semicarpifolia, Q. leucotricophor* and *Rhododendron* arboretum have a wider altitudinal range than alpine and sub alpine species and hence disappearance of the former is less likely than of the latter (Maikhuri *et al.,* 2003). A change in climate in the study area in future is likely to affect both species negatively and could lead to a decline in the area or a shift in their ranges towards higher altitudes. In addition, many important tree species in the timberline zone of the catchment have already been listed in the rare and endangered categories., *Taxus baccata, Juniperus* spp, and *Betula utilis*. These species are overexploited, legally or illegally, to a great extent and increased rates of destruction and the influence of a changing climate have made the situation worse. The communities residing the high altitude regions have observed that during recent past, the stem and leaves of Betula utilis growing in association with *Abies pindrow, Rhododendron campanulatum*, and *Taxus baccata* between 3,300 to 3,600masl were damaged severely by defoliator moths and this may be due to a decrease in snowfall in the past eight to 10 years and a gradual increase in temperature. Alpine meadows or grasslands are used extensively for grazing during summer (May-October) by various groups of local and transhumant pastoralists and are also an important reservoir of high-altitude medicinal and aromatic plants (MAPs). The growth period for plants is very short and the plants are very sensitive to changes in temperature. The distribution of alpine grasses and MAPs and their composition and association are determined by topography, shade and soil moisture, slope, light intensity, snowfall, and intensity of grazing and other biotic pressures. In the high-altitude areas (>3000 masl) current CO_2 levels are close to pre-industrial levels and in valleys at lower elevations they are close to the present global average (Maikhuri *et al.,* 2003). Thus, impact of CO_2 enrichment will vary spatially. Decline in biomass accumulation with decline in elevation in alpine species of Himalayas like *Aconitum balfourii, Aconitum heterophyllum* (Nautiyal, 1996) suggest that their growth is not limited by low CO_2 and low temperature conditions. Warming enhanced growth of *Allium strecheyi, Arnebia benthamaii, Pleurospermum anglicoides*, and *Dactylorhiza hatagirea* and reduced growth of *Angelica glauca* and *Rheum emodi*, although these species are similar in their ecological distribution (Kandari, 2005). When considering the likely impact of future climate change on alpine grasslands various factors should be considered which include changes in temperature, precipitation, and soil moisture as well as a direct response of grasses to enhanced atmospheric CO_2. The effects of increased CO_2 on grasses and plants also depend on the C_3 and C_4 photosynthetic pathways of plant species in a community. The alpine grasslands of the catchment could also be impacted by rising temperatures that would promote the upward migration of woody plants from lower elevations.

People's and farmers' perceptions on climate change impact

Indicators of climate-change impacts were analyzed through interactive discussions using qualitative and quantitative methods with 350 respondents in two different age groups (i.e., AG1,20-50 years and AG2, 50-80 years or above). The majority of respondents in both age groups agreed that during recent decades there had been many changes observed in the climate and they cited various examples (Table 1). It was observed that respondents who were 50-80 years or above had a deeper understanding, keener observation, more in-depth knowledge, and more experience of the changes in local climate changes than the respondents who were 20-50 years of age (Fig. 2). In-depth field observations of a team of scientists from G. B. Pant National Institute of Himalayan Environment (GBNIHE) and local people's perceptions revealed that advancement of flowering, leafing, and fruiting time (15-20 days) of medicinal and aromatic plants (i.e., *Rhododendron arboretum, Allium stracheyi, A. humile, Betula utilis, Meconopsisaculeate,and Saussurea obvallata*) and some prominent wild edible species (i.e., *Rebis orientale , Rosa webbiana,and R. sericea*) has been noticed and they considered it the most striking evidence of climate change.

Table 1. Kind of changes perceived by local communities due to climate change in central Himalaya

Climate change drivers	Kind of changes perceived by local people
Higher temperature with low rainfall and less precipitation	Vertical species migration of and extinction of some crops varieties increased Land use change that increases soil degradation/soil fertility and high species mortality Less infiltration affecting groundwater recharge and drying-up of natural springs and streams Decline in the moisture retention and water holding capacity of the soils has been linked to deforestation, abandonment of land resulting in loss of agrobiodiversity. Replacement of some crops with vegetable like cauliflower, tomato and cabbage in middle altitudes. Using higher amount of seeds during sowing resulted into increasing density of plants to cope drought condition. Cultivation of vegetables like pea, cauliflower and cabbage under kitchen gardens at higher altitudes. Fruits like papaya, banana, mango, litchi shifted to higher altitude areas. Decline in yield of apple and citrus spp. as it needs proper chilling during winters for proper fruit yield.

	Low land legumes shifted to higher altitude with decreased yield. Decline in the yield (80%) of Perilla frutescens, one of the important oil yielding crops. Increased grain yield of Elusine coracana, Amaranthus spp. and Sesamum indicum between 1500 -2200 masl. Decline in the grain yield of cash crops like Phaeolus vulgaries and Solanum tuberosum. Promoting low water requiring crops to prevent crop failure due to drought. Encouraging crop-livestock integration to increase soil organic matter, thereby increasing water retention capacity of soil for longer times. Farmers in these districts also indicated that inadequate draught power also inhibits their capacity to maximize the crop yields and their ability to prepare larger pieces of land. Adoption of alternate crop cultivation viz. Zingiber officinale and Curcuma longa floriculture (Gladiolus spp.and Lilium spp.) and fodder crops (Pennisetumpurpureum, Thysanolaena maxima etc) as option of livelihood. Conversion of irrigated to rain fed farming due to reduced flow of water in the streams as a result of warming and conversion of rainfed land at middle altitude to irrigated if water is available.
Higher temperature and more precipitation	High species mortality, loss of species and reduced agrobiodiversity at lower and middle altitude. Human migration due to extreme hydrological events and seasonal displacement. Unpredictable rain with impacts on harvesting and cropping patterns. Increase in population of invasive, exotic weeds (e.g. Lantana camara, Eupatorium spp. and Parthenium spp.). Cultivation of some medicinal plants at high altitude villages.
Decline in rainfall during March-May	Adverse impact in terms of decline in yield of Kharif crops due to large scale mortality and/or poor growth in the initial stage of crop growth. Abandonment of crop, e.g. Panicum miliaceum which used to be sown in March. This crop matures over a period of 3 months. The crop is badly affected if rainfall is delayed. Replacement of Amaranthus paniculatus by cauliflower, cabbage and potato.
High rainfall during August and September instead of the normal peak in July/August	Damage to rainy season crop when they are close to maturity. Shattering of crops before harvesting. Decline in the grain yield due to premature and post harvesting. Decline in the biomass of crop by-products negatively impact livestock production system.

Winter precipitation in January & February instead of December & January and decline in intensity of snowfall	Delayed ploughing/sowing of wheat, barley and mustard resulted into decreased yield as earlier it used to be done in November but now in December. Decline in grain yield of barley, brassica and wheat. Replacement of traditional cultivars of wheat by high yielding varieties. High yielding varieties of wheat and green pea which did not perform well earlier can perform well at higher altitude. Replacement of barely by green pea in middle and high altitudes. Declining fodder availability which indirectly affects Farmyard Manure (FYM) production, one of the essential components of hill agriculture. Farmers growing higher fodder yielding varieties with low grain production. Legumes like Macrotyloma uniflorum and Vigna angularis shifted to higher altitude. Climatic changes alter the pattern of blossoming, bearing and, therefore, fruit yield. The lack of be early cold in December and January is understood to adversely affect the chilling requirements. Shifted towards protected cultivation (polyhouse, shadenet, polypit etc)
Climate change (major changes felt by farmers)	Innovation of new agricultural practices characterized by high labour productivity and stress tolerant cash crops. Replacement of traditional staple crops by cash crops like potato, kidney bean, pea. Shifted towards vegetables cultivation in high altitude villages like cabbage, cauliflower, tomato, hybrid variety of other vegetables which never gown earlier. Adjusted agricultural production systems to production environment (short, early-maturing crops, short-duration, resistant and tolerant varieties, appropriate sowing). Early flowering, fruiting and maturity of winter crops, particularly wheat and mustard. Application of chemical fertilizers in farm particularly for cash crops increased as compared last twenty year A significant proportion of traditional agricultural land has been brought under off-season vegetables. This has adverse implications on the traditional agroecosystems; the agrobiodiversity of the region has shrunk over time. Change in phenological characters and decline in yield of many horticulture fruits (Prunus persica, P. cerasifera,P. armeniaca, Pyrus pyrifolia, Citrus spp. etc) Fruits like papaya, banana, mango, litchi shifted to higher altitude areas, disappearance/low availability of apricot, one of the very common fruit of middle altitude. Increased in frequency of human and animal disease.

	Early flowering, leafing and fruiting (20-45 days before the timing of their phenol-phases before 20-30 years) of medicinal and aromatic plants (i.e. Rhododendron arboreum, Prunus cerasoides, Allium stracheyi, Verginia ligulata, Betula utilis). Decline in forage and fodder production resulted into decrease in livestock population, which negatively impact livelihood and agriculture production system. Decline in average quantity of water in streams and drying up of water springs. Hiring of agriculture land increased by Nepali family for cultivation of seasonal and off-seasonal vegetables by applying high quantity of fertilizers Rural-to-urban and male out-migration and labour shortages for agriculture.

Table 2. Harvest (mean±SE) of important wild products in ten (10) villages of upper Himalayan regions

Botanical name	**Local name**	**Estimated price (Rs/kg)**	**Change in status/ availability**
*Aconitum heterophyllum**	Atis	175	Decline
Allium spp.	Sedum/Faran	200	Stable
*Angelica glauca**	Chippi	150	Decline
Bergenia ligulata	Shilphori	60	Decline
*Betula utilis**	Bhojpatra	150	Stable
Cedrus deodara	Deodar	50	Stable
*Dactylorhiza hatagirea**	Hathazari	550	Decline
Fagopyrum dbotys	Ban-oggal	10	Stable
Hippophae rhamnoides	Amesh	110	Decline
Juglans regia	Jungli Akhrot	50	Stable
Megacarpaca polyandra	Barmao	30	Stable
Morchella esculenta	Guchhi	6000	Decline
*Nardostachys grandiflora**	Mashi	250	Decline
Poeonia emodi	Chandra	30	Stable
*Picrorhiza kurrooa**	Katuki	150	Decline
Pleurospermum angelicoides	Choru	150	Decline
Prinsepia utilis	Bhinkal	125	Stable
Prunus persica	Kirol	200	Stable
*Rheum australe**	Dholu	80	Stable
Ribes himalayense	Darbag		Stable
Rumex hastatus	Chalmore	15	Stable
Rumex hastatus	Chalmore	15	Stable
*Saussurea costus**	Kut	150	Decline
Smilacina purpurea	Puyanu	25	Stable
*Thamnocalamus spathiflorus**	Ringal	5	Decline
*Taxus baccata**	Thuner	250	Decline
Viburnum cotonifolium	Ghenu	40	Stable

Source: *Medicinal and aromatic plants and wild edibles exploited to the verge of extinction (Maikhuri *et al.,* 2000).**Market value of 2016.

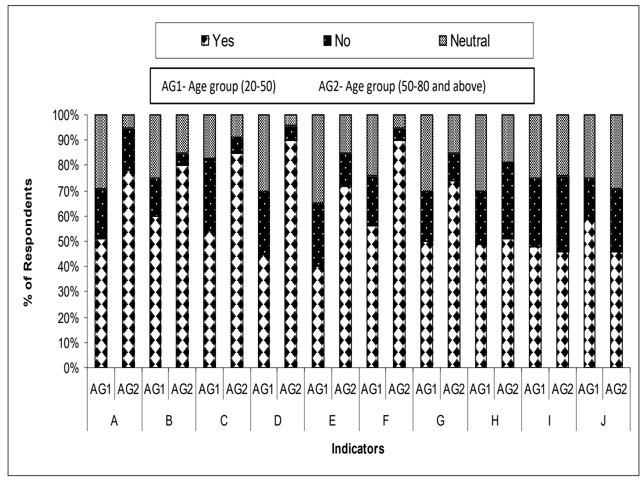

Fig. 2. Local people's (farmers/pastoralists) perceptions about the various indicators of climate change in Himalayan regions (% of people in different age groups responding in each indicator category)
(A) *Has the climate changed?* (B) *Increase in temperature,* (C) *Decrease in snowfall,* (D) *Decrease in rainfall /shift in rainfall,* (E) *Shift in flowering/fruiting (phenophases),* (F) *Low deposition/accumulation of snow,* (G) *Incidence of crop and livestock diseases,* (H) *Increased frequency and intensity of landslides/cloud burst,* (I) *Reduced water availability in rangelands for livestock,* (J) *Retreat of glaciers.*

*Total no. of respondents 350 (200 respondents of 50-80 years or above and 150 respondents of 20-50 years) belonging to 10 villages of Himalayan region.

More than 50% of respondents in the 20-50 age group and 90% of the respondents in the 50-80 or above age group noticed the significant decrease in snowfall and snow cover in the villages located between 3,000-3,600 masl. The recession of the Satopanth, Dunagiri, and other glaciers located in the region are also witness of the climate change and confirmed to the local people involved in trekking and mountaineering activities. In addition, transhumant pastoralist communities exhibit the most significant evidences related to sink of water resources over the decades used by livestock, particularly in several alpine pastures, low-altitude forests, and grazing areas. They attributed this change to a gradual increase in temperature and the consequent drying up of water bodies. Nonetheless, the possibility of modification of changes caused by the altered climate by non-climatic factors cannot be ruled out (Maikhuri *et al.,* 2003). 4.5. Socio-economic impact of forest and alpine meadows due to climate change. The traditional communities and local people of the high altitude region depends on the different forest types and alpine meadows for various bio-resources and medicinal and aromatic plants (MAPs) and NTFPs for household as well as commercial purposes (Maikhuri *et al.,* 2000, 2001; Nautiyal *et al.,* 2001). The bioresource diversity viz., MAPs and NTFPs collected/processed and their economic value were evaluated using the current market price method (Table 2). Presently, alpine meadows/temperate forest and sub alpine areas serve as a treasure of MAPs of high value-low volume. Table 2 provide summary of the MAPs and NTFPs species used, their purpose and any change in their availability and status over the past 15-20 years (Maikhuri *et al.,* 2001). The present trends in climate change may affect negatively the species existence, composition and advancement towards higher elevation ranges where their survival will be in danger.

COPING AND MITIGATION STRATEGIES

Conservation of wild biodiversity: strengthening of protected area network

Conservation of biodiversity is, perhaps, the most desirable need for adaptation and mitigation. Though we have a long history of planned conservation (9% area of the Himalayas is legally protected), our knowledge on people–biodiversity vulnerability linkages is very limited. Unsustainability of traditional grazing is more an assumption that a scientific conclusion (Maikhuri *et al.,* 2000a). Rarity of medicinal plant species is largely attributed to over- exploitation (Samant *et al.,* 1996), though this could also be due to inherent biological constraints delimiting their populations or to climate change. Ecological capital of protected areas derives from the ethos of sustainable resource use ingrained in traditional practices. Coping with climate risks is an important factor in shaping indigenous biodiversity may succumb to new global forces. Participatory research/ management could turn people's callous/ negative attributes to positive attitudes towards protected areas,(Maikhuri *et al.,* 2000a) together with improvement in scientific knowledge related to potential uses of biodiversity for coping and mitigation.

Rehabilitation of degraded forest lands:

The failure of afforestation and reforestation efforts to reclamation of degraded lands in the Himalayan regions could be attributed largely to the ignorance of people's essential needs and hence their non-cooperation. People's participation is now considered as a prerequisite to success of any land rehabilitation effort in the Himalaya. The practice and framework developed (Maikhuri *et al.,* 1997) for degraded land rehabilitation is now widely accepted, particularly by the locals. It has also been accepted as a major source of inspiration for the Forest Department, policy makers, NGOs, environmentalists, village institutions and other government departments involved in communities' development. More scientific research is required to establish the suitable plant species that are ecologically and socio-economically appropriate for landscape development of the communities thriving for the bioresources and land use problems particularly in the Himalayan regions.(Maikhuri *et al.,* 1997). Considering the diversity of ecosystems, indigenous knowledge, and socio-economic conditions in the mountains, a rehabilitation strategy has to be location-specific. There is a need for developing rehabilitation models suited to diverse sets of ecological and socio-economic conditions in the Himalayan Mountains (Maikhuri *et al.,* 1997; Rao *et al.,* 1999).

Promotion of traditional crops cultivation

In spite of the many virtues of traditional crops, and precious genetic diversity, the rivet of ecosystem stability, is gradually being lost (Maikhuri *et al.,* 2001). In addition, the region would lose the traditional knowledge of cultivation and the uses of these crops forever and would also lose the chance of being a diverse and nutritive food-producing region. In situ conservation of traditional crops and cultivars could succeed when these crops are strongly linked with the economic development of hill farmers. Institutional collaborative multidisciplinary research efforts are needed to evolve farming systems with appropriate selection of crops in view of future climate change which can provide enough quality food and economic security for the people of the region together with conservation of the traditional crop wealth, sustainability of the production systems and environmental conservation.

Cultivation and conservation of medicinal and aromatic plants

High altitude regions of the Himalaya are facing huge problems in cultivation and trade of medicinal and aromatic plants species due to variousfactors viz., Forest policies, PAs regulations etc . Besides, less interest of the farmers for advance scientific technologiesand cultivation techniques particularly in the agriculture sectorwhich can provide the diverse multi-cropping plant species based on the farmers preferences as a contingency measure to ensure substantialreturns during climatic stress spells. GBPNIHE, is one among several organizations which are involved in testing, developing and demonstrating action research to create a conducive atmosphere and relation between farmers, extension officers and research and development institutions. These relationships are thought to be a fundamental tool, allowing scientists to collect appropriate data/ information and to transform them into developed technologies/ products adapted to farmers needs. Though the government of Uttarakhand has introduced policies to promote and strengthen cultivation and conservation together, these policies have not shown desired results. In developing a policy regarding the medicinal plant sector, inputs from all the stakeholders not only provide important insight, but are also necessary for developing effective sustainable management plans

Adaptation and risk management

Current changes in the climatic system tend to increase the vulnerability of livelihoods in two main ways first, due to the fact that many of such livelihoods are exposed to more frequent and intense extreme events causing increasingly negative impacts. The second reason is related to the long-term impacts of changes in temperature and rain patterns. Understanding climate-development interplay is crucial for local adaptation and challenging for scientists as for policy makers and those engaged in development and extension activities. However, in order to plan and carry out more cost-effective adaptation measures, anticipatory measures and actions aimed reducing vulnerability and increasing resilience are becoming in the sense that it is based on some assessment of conditions in the future. Due to the changes in environmental patterns existing problems are exacerbated and new ones can appear. As a consequence, many vital factors for sustainable development such as water, food security, bioresources, health, infrastructure and natural ecosystems are seriously endangered. Although the impacts of climate change were not part of the original programme, design, GBPNIHE Garhwal Regional Centre have addressed extreme climate problems by showing evidence of reducing local vulnerability and improving livelihoods through resource harnessing and sustainable practices like organic farming, soil improvement, technology adoption, and land rehabilitation.

Capacity building and skill development

Scientific technologies adoption can play a major role to enhance the socio-economic condition of the region. However, the lack of appropriate site specific technologies and poor access caused the poverty and degradation of natural resources in the most of Himalayan regions.. Therefore, establishment of rural technology demonstration and training centre is required in the Himalayan region which can help to search the viable options for improving the yield of farm produce, enhance income generation from off- farm activities and resulted the existing pressure on forests and alpine meadows and other bioresources can be reduced. The appropriate technologies can also be encouraged for high altitude region including protected cultivation, organic compost and bio-fertilizers, off-farm technologies and other supporting technologies (Maikhuri *et al.,* 2007a).

Bioprospecting, conservation and management

Communities interest towards wild bioresources collection has grown significantly with the increasing awareness in linking forest conservation with rural development and poverty alleviation. Thus,sustainable harvest and appropriate management practices is essential for the availability of the bioresources to meet the demand of dependent communities for market supply and their own consumption. . It is realized that in order to influence policy planners and forest management practices one must understand the broader context such as sustainability, extraction rates, growth, yield, and biological possibilities for increasing production and the local variations in the value of wild edible species (Maikhuri *et al.,* 2007b).

Conservation and management of alpine meadows

The State management authorities should come forward to develop the stringent policies to restrict the livestock population in the region designated for the conservations purposes with regulate the grazing in some limited areas to fullfill the demand of fodder from the resided communities. Although villages with transhumant pastoralist populations are reducing livestock holdings because of non- availability of grazing resource in winter , the villages that are now settled continued to show increases in the numbers of cattle, which are required for draught power and manure. In addition, the keeping of small ruminants by these people for economic benefits is adding to the growing pressure on village commons and surrounding forest. For effective management of available resource in the region, continuance of transhumance by villages within the limits of carrying capacity is required, as is reduction of cattle populations and replacement of small ruminants with alternative options such as medicinal plant cultivation and organic food production in settled villages. This strategy could provide the required economic benefits to both settled and transhumant populations and also support conservation goals by reducing the overall pressure.

Institutional cooperation, coordination, collaboration and capacity building to address climate change

In central Himalayan region particularly Uttarakhand state has many Research and Development institutions with significant infrastructure and scientific/ technical capacity. However, so far these institutions have not much focused on climate change research, which includes modeling, field ecological and biodiversity related studies etc. There is inadequate capacity in several research and developmental institutions working on environmental and conservation issues in relation to climate change. There is a utmost need to create awareness and enhance capacities at individual and institutional level and also include other stakeholders i.e. NGOs etc. Local communities depending on natural resources for their livelihood have poor financial, technical and institutional capacity to adapt to adverse impacts of climate change. Therefore, it is important to enhance capacities of local people's (whose survival entirely depends on their surrounding forest vegetation) who are likely to be vulnerable to projected climate impacts.

Priority research areas for the future

(i) Documentation of communities knowledge and experiences about the variability in climatic pattern of high altitude regions and its impact on forest, agriculture, livestock and humans alonwith the possible indicator of change.

(ii) Effect of climate on seasonal variability and reliability on agriculture production, forestry and water resources.

(iii) Interface with policy issues, administration, local com-

munities and research and academic institutions regarding the broad aspects of adaptation options and livelihood.
(iv) In- depth studies on the variation in phenological changes on various forest types along an elevational gradient by establishment of permanent experimental plots for long term monitoring and assessment in response to the climate change.
(v) Capacity building of the local people, researchers/scientists in the field of climate change and encourage them for advance studies.
(vi) Conduct in-depth studies on genetic diversity of traditional mountain crops which may provides a platform to identify suitable thermal and drought tolerant cultivars for meeting climate change.
(vii) Installation of appropriate whether and meteorological stations on important and ecological sensitive areas with regional projections of climate parameters for developing regional climate models.
(viii) Conduct the studies on the natural resource utilization pattern and vulnerability assessment responsible for degradation of forest bioresources particularly in the in the Himalayan regions.

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INDICATIONS OF ELEVATION DEPENDENT WARMING ALONG TREELINE ECOSYSTEM IN WESTERN HIMALAYA- POSSIBLE IMPACTS ON ALPINE VEGETATION

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ABSTRACT

Alpine treeline ecotone, occurring between a subalpine forest and alpine meadow, is an extremely temperature-sensitive transition zone. Sensitivity of plant species to change in temperature and other abiotic factors (e.g. radiation, moisture, wind, slope exposure, topography) is high across this ecotone. Over past few decades accelerated rates of warming are noticeable in most of the Himalayan regions. There are increasing evidences that the Elevation-dependent warming (EDW) in mountain regions across the globe is more rapid than in the low altitude areas. This can accelerate the rate of change in mountain ecosystems. This study presents Temperature Lapse Rates (TLRs) using observed data for treeline transects in western Himalayan region and showed that annual mean TLR was shallow than the commonly used values. It increases with decreasing moisture, being high for dry and low for moist region in Himalaya. The One-Way ANOVA confirms that the TLR varies significantly among seasons (F=3.2175; P = 0.03). The lowest monthly for all three sites was found in December varying between -0.24 to -0.320C/ 100m. Relative humidity, snow elbedo and reflectance possibly play a key role as controlling factors on TLR in treeline environments. The observed shallow TLRs signify that EDW is amplified with elevation in Himalayan region under the influence of climate change. The EDW, indicated by lower values of TLR, may have several impacts on the dynamics of treeline ecotone in Himalaya, including change in snow and moisture regime, increased evapotranspiration and water stress, change in albedo and surface energy balance resulting to modify distribution patterns, range shift and growing season of alpine vegetation in the Himalaya.

Keywords: Elevation dependent warming, Temperature lapse rate, Climate, Treeline, Himalaya

INTRODUCTION

The Himalayan region, with the largest snow and ice cover mountainous region in the world, regulates climate of a large landscape in the South Asia. It is home to major river systems, largest cryosphere area outside the polar region, one of the global biodiversity hot spots, and support life of nearly 300 million people. This region also holds significant importance in terms of biological and socio-cultural diversity, and encompasses wealth of a large number of endangered endemic species. This region is experiencing swift changes driven by climate change and other anthropogenic factors such as urbanization, infrastructure development, migration, tourism and globalization, which may lead to enormous consequences; both at regional and global level. During past 102 years (1901-2003) the rate of warming in Himalayas during the last century has been higher (0.9-1.60C) than average global warming rate with relatively higher warming during the recent decades (Joshi and Kumar, 2013). Further, rise in both minimum and maximum temperatures has resulted into relatively warmer winters in the region with greater increases in minimum temperatures than maximum temperatures.

There are growing evidences that the rate of warming is amplified with elevation; high elevation areas including alpine region experience higher increase in temperature than lower elevation areas (Pepin *et al.,* 2015). This elevation-dependent warming (EDW) can modify various ecosystems in the mountain, including cryosphere, hydrological regimes and biodiversity. EDW is governed by important feedback mechanisms of snow, albedo, land surface, water vapour, latent heat release, and radiative flux changes, temperature change, and aerosols. Combinations of these factors may account for contrasting regional patterns of EDW across various mountain regions which would lead to rapid warming in high elevation regions including alpines than lowland areas across the world (Rolland, 2003; Joshi *et al.,* 2018).

The high altitude environments are influenced by the free atmosphere temperature gradients or temperature variation with altitude known as "temperature lapse rate (TLR)"; it varies on daily and seasonal scales in mountains (Joshi *et al.,* 2018) due to local surface energy balance and different weather types or synoptic conditions (Pepin *et al.,* 2015). It is useful for determining the elevational distribution of temperature along a transect in absence of the in-situ temperature measurements. In such cases, average temperature gradients of -0.60 0C or -0.65 0C/100 m (Barry and Chorley, 1987) are often used to simulate and model various ecological processes when high precision is not required. However, temperature and precipitation gradients vary considerably with space and time and controlled by topographical features in mountains. Hence, assuming a constant value of TLR may lead to inaccurate results while examining elevation dependent warming and its impacts on different high altitude ecosystems.

Studies on temperature gradients for treeline environments in Himalayan region are scarce. Initial estimates of annual TLR (-0.53 0C/100 m) treeline environment in Western Himalaya were given by Joshi *et al*., (2018), wherein it was found that TLR vary according to different seasons and along the aspect. In recent decades, air temperature at higher elevations has increased more rapidly thereby decreasing near-surface air temperature lapse rates in warmer climate (Pepin, 2001). Hence, the lower value of mean TLR for treeline region in Western Himalaya may be due to the enhanced EDW in high altitude areas and a consequence of global warming. The existing knowledge gap in this domain calls for further studies to analyse seasonal and synoptic variations in TLRs for climatically sensitive treeline environments in Himalaya.

Hence, this study aims to quantify the temperature lapse rate and examine its seasonal variations a treeline transect in western Himalayan region and explains the influencing factors on lapse rate. Considering that treeline environments are extremely temperature-sensitive transition zone for many plant species endemic to the region, possible implications of shallow TLR on alpine vegetation and impacts on critical ecosystems have been discussed in this manuscript.

MATERIAL AND METHODS

Study area

The present study was carried out along the Chopta-Tungnath transect- a treeline transect located in western part of the Indian Himalayan region (Fig. 1). The study transect is located in Rudraprayag district of Uttarakhand state in India is a typical monsoon rainfall (June-September) dominated site (State annual average 1549 mm). Climatic characteristics of the study transect is presented in Table 1. The treeline in this transect is found ranging between 3200-3400m asl. The forests at the higher elevations of the study area fall under the subalpine zone, which gives way to alpine meadows beyond the timberline ecotone (Rai *et al.,* 2012). In higher altitudes, above treeline (3400–3700 m) region of the study transect, grasslands are dominated by herb species of *Anemone, Potentilla, Aster, Geranium, Meconopsis, Primula* and dotted pockets of shrubs of *Rhododendron anthopogon* and *Juniperus* species. Whereas, in sub-alpine region (i.e. between 2900–3400 m) mixed forests dominated by species like *Rhododendron arboreum, R. campanulatum* scattered with a few *Abies pindrow* and *Taxus baccata* trees are present. Below 2800 m, broad leaved forests are dominated by *Quercus semecarpifolia, Betula utilis, Abies spectabilis, Acer caesium, Rhododendron arboreum* and *Sorbus foliolosa*.

Meteorological Setup and Data

Ten (10) portable ONSET HOBO Pro-V2 temperature loggers were installed along the transect at different elevation ranging between 1500 and 3680 m along South Eastern (SE) and North Western (NW) aspect (Table 2). These sites were selected based on the availability of suitable and safe sites. Further, an advanced AWS consisting of ARG100 tipping bucket with HOBO event data logger, and temperature probe

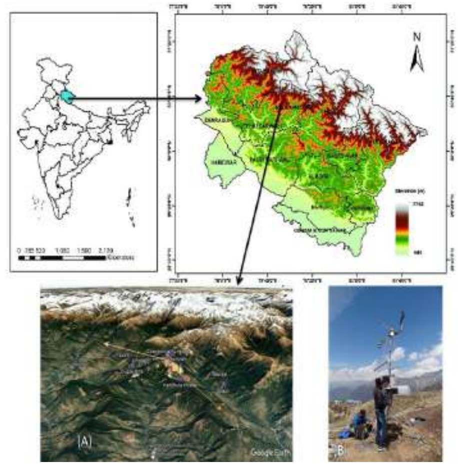

Fig. 1. (a) Map of the study transect (b) An AWS installed at 3360m (30029.57' N, 79012.95'E) within the transect.

Table 1. Climatic characteristics of three study transect in Indian Himalayan region

Parameter	Climatic characteristics
Annual Mean Temeprature	10.5±4.2°C
Mean growing season temperature	10.3±1.4°C
Precipitation pattern (State annual average precipitation)	Moderate annual precipitation (approx. 1549 mm)

(109-L) was also installed at 3300 m asl within the transect. Data from the temperature sensors and raingauge was recorded at 30-minute interval for 2017 and 2018 and were analysed for different months and seasons. Within the study transect, the winter season at high altitude is chareterized by the lowest temperatures and precipitation mostly in the form of snow. The pre-monsoon season is chareterized by relatively high temperatures gradually increasing till the onset of monsoon. During this period, high variability in diurnal temperature and scanty rainfall are observed. During the monsoon season heighest amount of rainfall is received along with relatively high temperature, and charecteristically low diurnal varibility. A steady decrease in temperature alongwith less amount of rainfall is the key feature of winter season.

Table 2. Details of the meteorological stations alongwith altitude (in masl)

NW aspect		SE aspect	
Station	Altitude	Station	Altitude
Ukhimath	1500	Siroli	1600
Taala	1820	Mandal	2100
Dugalbhitta	2500	Kanchulakharak	2675
Chopta	2870	Saukharak	3100
Tungnath	3360	Chandrashila	3680
Chandrashila	3680		

Method

The Pearson's correlation coefficient was calculated between observed monthly mean temperature and elevation of the station and the significant correlation coefficients were considered for further analysis to estimate the measure of the strength of the linear relationship between the two variables. Under well-mixed atmospheric boundary conditions air temperature often decreases/increases linearly with elevation. Hence, following Joshi *et al.,* (2018), TLRs (0C/100m) were estimated by developing a regression equation using all point level observations of mean temperature and elevation. TLRs were calculated separately for all the months well as for each of four seasons; viz. pre-monsson (MAM)), monsoon (JJAS), post-monsoon (ON), and winter (DJF). Statistical analyses were performed to test the significance of results alpha level $\alpha=0.05$ (or CI=95%) and p values ($p<0.05$). Further, relationship of TLR with two other climatic parameters (i.e. rainfall and relative humidity) was studied to analyze the factors for seasonal variation in TLRs. Based on the observed temperature data, saturation vapour pressure (es) lapse rate was calculated for CH transect using the equation based on the Clausius–Claperon relationship (Joshi *et al.,* (2018)).

RESULTS AND DISCUSSION

Analysis of correlation coefficient show that the correlation between monthly mean temperature and elevation was significantly negative at $p<0.01$ and $p<0.05$ for all the months (Table 3). The lowest correlations were for the winter months, where r varied between 0.83 and 0.94, when temperature was lowest across the transects. The heighest values of correlation coefficient (ranging between 0.83-0.94) was estimated for May to September.

Monthly, seasonal and annual varaitions in TLR

The lowest monthly TLR was found in the month of December (Table 3). However, TLR differed in terms of the seasonal pattern. The monthly TLR varied to an extent from -0.240C (in December) to -0.680C (in May), and the values were constantly high during pre-monsoon months (-0.630C to -0.680C), whereafter it decreased to an intermediate level during monsoon months (-0.490C to -0.630C) subsequently it decreased sharply from October (-0.510C) to December

Table 3. Monthly TLR values and Correlation coefficient (r) between mean temperature and elevation

Month	TLR (°C/100m)	R
January	-0.42	-0.94*
February	-0.52	-0.96*
March	-0.63	-0.95*
April	-0.67	-0.98*
May	-0.68	-0.99*
June	-0.63	-0.99*
July	-0.52	-0.99*
August	-0.49	-0.98*
September	-0.54	-0.98*
October	-0.51	-0.97*
November	-0.38	-0.94*
December	-0.24	-0.83**

*Correlation values significant at P < 0.01; *correlation values significant at P < 0.05.

(-0.240C) (Fig. 2).

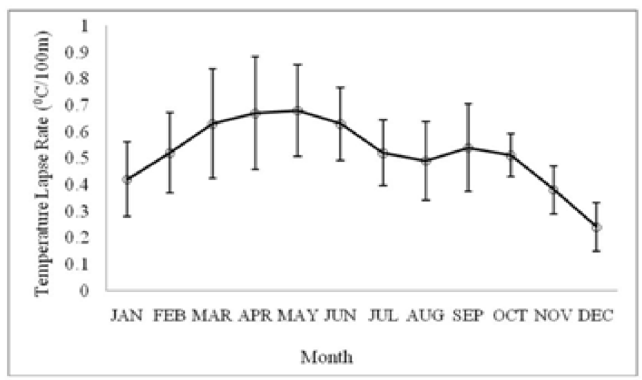

Fig. 2. Monthly variation in Temperature Lapse Rates (the bars represent statndard deviation)

The highest mean TLR values were observed during pre-monsoon months (-0.520C/ 100m) (Table 4).

Table 4. Seasonal values of mean TLR for the study transect

Season	TLR (in 0C/100 m)
DJF	-0.41
MAM	-0.66
JJAS	-0.53
ON	-0.47

Because of the absence of clouds and higher temperature during pre-monsoon, land surfaces receive more incoming solar radiation compared with outgoing radiation. This results in a rise in day time surface temperatures and large sensible heat flux which presumably enhanced strong dry convection in the daytime. The pre-monsoon season also had the highest daytime saturation varour pressour (es) lapse rate; as a result the TLR reached a maximum value in that season. The lowest mean TLR value (-0.410C/100m) was observed during winter season (DJF) which implies that snow elbedo or reflectance play a more important role as controlling factors on TLR. The One-Way ANOVA confirms that the TLR differ among seasons (F=3.2175; P= 0.03) indicating that the seasons have strong influence on TLR. These results are also in agreement with shallow TLRs estimated by Kattel *et al.,* (2013), Immerzeel *et al.,* (2014) and Joshi *et al.,* (2018) for other parts of Himalaya. The annual TLR was found to be -0.520C/100 m for the study transect. The annual mean TLR estimated for the study transect was also compared with tree line transects in Jammu & Kashmir and Sikkim. It was relatively lower to the value estimated for tree line transect in Kashmir Himalaya (-0.660C/100m) but higher than the value of annual TLR estimated for similar environment in Sikkim (-0.500C/100) (Joshi *et al.,* 2023). This implies that the TLR increases with decreasing moisture and is dissimilar for different precipitation regimes across the Himalaya. The annual mean TLR was found distinctly lower than the commonly used value of -0.65 0C/100 m (Barry and Chorley, 1987).

Relationship of TLR with controlling factors

Relationship of TLR with rainfall and saturation vapour pressure (es) lapse rate was analyzed to examine role of controlling factors in seasonal variation in TLR. An inverse relationship between daily rainfall and mean TLR (r=−0.27; R2=0.073) and a statistically significant positive relationship (r=0.63, $p<0.05$) between mean TLR and mean saturation vapor pressure (es) lapse rate suggests that variations in TLR for is associated with variations in es lapse rate but not consistently associated with rainfall. It shows that TLR gets enhanced during dry and moist conditions (in pre-monsoon season) and reduced during wet conditions (monsoon season). Hence, the atmosphere water vapour content and its seasonal variations play an important role in forcing the variations in lapse rate. The temperature inversion together with elevation dependent warming and change in snow cover could have resulted in the lowest TLR in December. Because of more warming of cool high elevation areas and less warming of low elevation areas, TLR is expected to have decreased due to global warming (Fig.3).

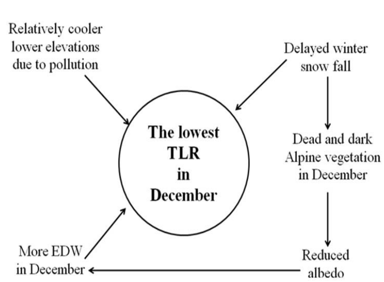

Fig. 3. Factors causing decrease in December TLR in Himalaya

Relationship between TLR and Treeline in Himalaya

For 2017 and 2018, the mean growing season temperature at treeline of study site was observed as 10.3±1.4°C, which is much higher than globally defined mean growing season temperature (6.5±0.8 ^{0}C) (Körner and Paulsen, 2004). This further confirms pronounced warming at high elevation areas and hence EDW signified by shallow TLRs across Himalayan region. Since the Himalayan treelines are unable move up, they are getting warmer for last several decades of global warming with several consequences for ecosystem services.

Possible impacts of elevation-dependent warming

Low TLRs particularly during winter and monsoon months are likely to contribute to high treeline in Himalayas. Heat deficiency is considered the main cause of treeline formation (Körner 1999). Winters in Himalayas are mild partly because day lengths remain long (> 10 hr), and days are sunny. As for growth of trees in high elevations, monsoon (warm and moist) is the key period, when much of the net primary productivity occurs (Joshi *et al.,* 2023). Hence, elevation dependent warming is likely to be now a major contribution to elevational rise of treeline. The treeline vegetation is largely constrained by the environmental factors including temperature, radiation, wind and water stress (Joshi *et al.,* 2023). Plants in treeline ecotone are influenced by climatic changes by way of morphological and physiological adaptations (such as stunted growth forms and small leaves, low thermal requirements for basic life functions, etc.). Location of the Himalayan treeline is usually governed by growing season air temperature combined with topographic factors. Therefore, the shallow TLRs may have several impacts on treeline ecotone vegetation such as change in treeline dynamics, loss of potential habitats of many threatened plant species, tree species distribution and upward migration of species to suitable habitat in response to EDW. As a consequence, the upwards migration of the treeline would significantly alter the surface characteristics and local climate of alpine regions by modifying the albedo and surface energy balance resulting into possibility of new assemblages of plant and animal species in these regions. Further more, EDW may also modify the frequency of fire outbreaks as forests tend to transpire most of the available soil moisture under dry and warm conditions. Hence, prolonged dry and warm season would exceed environmental and biological thresholds for fire outbreaks and consequently convert fire sensitive areas into regions of sustained fire hazard. Therefore, it is presumed that the response of ecosystems to EDW in treeline zones will be extremely vital at ecotones in terms of the loss of the coolest climatic zones and upslope shift of ecotone vegetation.

Under warm and dry conditions, treeline vegetation is likely to affected by increased evapotranspiration and water stress. Because of EDW, vegetation communities that grow in snow beds could be subjected to summer desiccation. An upward shifting of the tree lines by nearly 200m has been reported in the European Alps due to EDW and increase in the plant cover of dwarf shrubs in alpine areas (Cannone *et al.,* 2007) since the early twentieth century. Therefore, because of more warming at higher elevation, the endemic species occupying the highest elevation zones and having narrow range of extents are likely to face extinction. Thus loss of potential habitats of many threatened plant species and increase in the plant cover of dwarf shrubs in alpine regions, and increase in species richness at summits could be the likely effect of EDW.

CONCLUSION

Relatively shallow mean annual TLR exists for treeline in western Himalayas than the frequently used average value which suggests that the warming in high elevation regions is more pronounced than in lowland areas and also indicates elevation dependent warming (EDW) in Himalaya. TLR is expected to further decrease due to the effect of EDW and global warming. This study showed that the variation in TLR is controlled by moisture. The lower values of TLR may have several possible impacts on the dynamics of treeline in Himalaya, such as change in snow and moisture regime, increased evapotranspiration and water stress, change in albedo and surface energy balance resulting to modify distribution patterns, range shift and growing season of alpine vegetation. The decrease in TLR would reduce the temperature range in a given elevation range, enhance the upper elevation limit of vegetation and species, leaving increasingly narrower belts for survival of plants and snow covered systems. The restricted space may limit a species to form viable population to achieve upward shift. Information about changing climate and vegetation patterns of climatically sensitive alpine ecosystems is very crucial for a comprehensive understanding of their current and future states. Therefore, long-term climate observations are essential to study the change in TLRs vis-a-vis change in climate and its impacts on treeline environments by generating illustrative facts on climate change and its potential impacts on ecotone vegetation in Himalayan region.

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CHANGING ECOSYSTEM OF A HIMALAYAN RIVER: A CASE OF MICROBIAL DYNAMICS AND THEIR BEHAVIOUR ALONG ALTITUDINAL GRADIENT OF TEESTA RIVER IN EASTERN HIMALAYAN RANGE

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ABSTRACT

The rapid expansion of the human population, industrialization, and unsustainable agricultural practices has significantly increased pollution in rivers due to unregulated effluents and anthropogenic activities. Urban rivers face contamination from various sources, including wastewater from communities, hospitals, pharmaceutical industries, and animal husbandry. Point and non-point discharges contribute to increased nutrient loads, faecal bacteria, and a wide range of pollutants. Heavy metals and antibiotics have emerged as emerging environmental contaminants, posing a severe threat to aquatic life forms. To assess the health of rivers along their course, researchers analyse the dynamics of microbial communities present. It has been found that anthropogenic inputs can cause both short-term and long-term alterations to natural microbial communities. To understand the microbial dynamics in the once pristine Teesta River, located in the Eastern Himalayan range, researchers analysed water and sediment samples for bacterial composition and metals contamination. The study revealed that heavy metal concentrations were higher in the lower stretches of the river compared to the upper stretches. Isolated heavy metal-resistant bacteria from polluted sites demonstrated significant tolerance to various heavy metals, including lead, zinc, copper, and cadmium. Moreover, a high incidence of multiple antibiotic-resistant (MAR) bacteria was observed in the lower stretches of the river, indicating resistance to multiple antibiotics. Genetic adaptation played a crucial role in conferring resistance against specific heavy metals and antibiotics. The phenomenon of co-selection in bacteria from anthropogenically influenced sites was identified as a rich source of antibiotic-resistant phenotypes.

Keywords: Eastern Himalayan river, Anthropogenic activities, Microbial dynamics, Heavy metals, Antibiotics

INTRODUCTION

Rivers serve as vital arteries for human communities, playing an indispensable role in supporting domestic, agricultural, and industrial activities.The Himalayan rivers are vulnerable to climate change and anthropogenic pollution thereby changing the water quality and affecting the fragile river ecosystem. The unregulated flow wastewater from various point and non-point polluting sources has rendered rivers vulnerable to pollution. Heavy metals are an emerging environmental contaminant and heavy metal contamination of the riverine ecosystem hasbeen attributed to anthropogenic activities (Singh *et al.,* 2017; Patel *et al.,* 2018). Indian rivers are found to be polluted by elevated levels of toxic heavy metals like Hg, As, Pb, Cd, Cr, Zn and Cu have been reported from various Indian rivers (Mahato *et al.,* 2017; Singh *et al.* , 2017; Patel *et al.,* 2018). The concentrations of heavy metals in certain Indian rivers have been discovered to surpass the permissible limits set by both the Bureau of Indian Standards (BIS) and the World Health Organization (WHO) for drinking water (Prasad *et al.,* 2020). The microbial ecosystems play a vital role in aquatic ecosystems, contributing significantly to biogeochemical cycling, microbial food webs, pollutant degradation, and energy flow (Guan *et al.,* 2018). However, the discharge of wastewater from urban areas and industrial effluents has had a considerable impact on bacterial community composition, species abundance, and their functions, leading to disruptions in nutrient cycling processes, adverse effects on energy flow, and causing biodiversity loss. When exposed to toxic levels of heavy metals, numerous aquatic organisms, including microorganisms, develop a certain degree of tolerance. As a result, there is a notable shift in bacterial communities, with the resistant and tolerant species gaining dominance over the native species.(Klerks and Weiss, 1987). Rivers are subject to contamination from various sources, including pesticides, heavy metals, pharmaceuticals, and potentially allochthonous microorganisms. Hence, it becomes imperative to investigate how microbes respond at both the structural and community levels and establish their sensitivity to pollutants.

MATERIAL AND METHODS

Study area and sample collection

The current investigation was conducted on the Teesta River, which originates from the glacial lake, Khangchung Chho. This lake is situated at an elevation of 5280 meters and is nourished by the Teesta Khangse glaciers, located above 7068 meters in the northwest region of the state of Sikkim, in the Eastern Indian Himalaya. The Teesta River stretches across 414kilometers, covering a catchment area of 12,159 square kilometers, which is spread across mountains, hills, and plains (ENVIS, 2019). The unchecked industrial growth in the state of Sikkim has frequently led to the uncontrolled exploitation of rivers, resulting in the deterioration of their overall health. Water and sediment samples were collected during the monsoon and winter seasons of 2018–2019 from six sampling sites (S1-S6) along theriver. These sites varied in altitude, ranging from 5183 to 293 meters above mean sea level. The samples were collected in pre-sterilized containers and were carefully transported to the laboratory under ice-cooled conditions.

Determination of heavy metals in water and sediment samples

Heavy metal concentrations in the water and sediment samples were estimatedby using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES-Model No. iCap 7600 Duo) following thestandard protocols of APHA, (1998) with slight modifications. 500 mL of water samples from each site were pre-concentrated to 50 ml by heating on a hot plate at the optimal temperature. Onegram dry weight of sediment sample was acid digested in digestion vessel. The concentration of 7 heavy metals namely, cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), lead (Pb), nickel (Ni), and zinc (Zn) in the samples were analysed in the present study.

Isolation, enumeration and identification of bacteria

Isolation of bacteria from both the sediment and water samples was done by the standard spread plate technique. The sediment samples were serially diluted up to 10-5 and 0.1 ml of inoculum from each 10-3, 10-4 and 10-5 dilutions were spread on Luria Bertani (LB) agar. Water samples were diluted in 0.85% saline up to 10-4 fold dilution and subsequently 0.1 ml each from 10-2, 10-3 and 10-4 dilutions were plated on Reasoner's 2A or R2A agar plates. The inoculated plates were incubated at 28 °C for 24-48 hours. Bacterial colonies were selected based on their morphological characteristics. Bacterial count was enumerated as colony forming units (CFU) and expressed as CFU/ml in case of water samples and CFU/grams for sediment samples.

The number of bacteria in the samples was calculated as follows

CFU/ml or CFU/grams= CFU x Dilution factor/ Volume of sample plated.

Genomic DNA of the selected isolates was extracted using the Bacterial genomic DNA kit (HiMedia, India). The isolated DNA was amplified targeting the 16S rRNA gene using the universal bacterial universal primers 27F and 1492R.The amplified PCR products were sequenced from Macrogen, South Korea.

Heavy metal and antibiotic tolerance studies of the isolates

Bacterial tolerance to heavy metals (lead, zinc, copper, and cadmium) was conducted by determining Minimum Inhibitory Concentration (MIC) of the bacterial isolates. Likewise, antibiotic susceptibility of the bacterial isolates was conducted using agar diffusion technique on Mueller-Hinton agar (MHA) plates following the guidelines of the Clinical Laboratory standard Institute (CLSI) 2013. A total of 15 antibiotics were used in the study which are as follows Meropenem (10mcg), Imipenem (10mcg), Erythromycin (10mcg), Ciprofloxacin (5mcg), Nalidixic acid (30mcg), Netillin (30mcg), Novobiocin (30mcg), Neomycin (30mcg), Tigecycline (15mcg), Lincomycin (15mcg), Rifampicin (5mcg), Polymyxin B (300U), Co- Trimoxazole (25mcg), Ceftazidime (30mcg), Trimethoprim (5mcg). After incubation, the plates were observed for clear inhibition and the diameter of the zone was measured with a zone scale (Tao *et al.*,2010). The multiple antibiotic resistance (MAR) index for the test isolates was determined by calculating a/b, where 'a' represents the number of antibiotics to which the isolates showed resistance, and 'b' represents the total number of antibiotics used against the isolate, as described by Krumperman in 1983. MAR index value greater than 0.2 indicates that the isolate exhibits multiple antibiotic resistance, while an MAR index value of 0.2 or lower suggests very low or negligible antibiotic resistance.

RESULTS

Heavy metal concentration

The summary of the mean concentrations of heavy metals detected in the water and sediment samples are presented in Table 1.The estimated concentrations of all the heavy metals detected in the water samples were found to be under the threshold values as prescribed by BIS. In the sediment samples, highest concentration of Pb (15.9 mg/Kg) was detected at site S6 and lowest concentration (2.35 mg/Kg) was detected at site S1. Similarly, highest concentration Zn (61.7 mg/Kg) and Cu (25.05 mg/Kg) was estimated at site S3, and highest concentration of Cd (0.85 mg/Kg) was detected at site S6.

Total Bacterial Count

The mean bacterial count in water sample was highest at site S3 (monsoon-28.8±3.4; winter-11±1.78) and lowest at S1, (monsoon-3.1±0.35; winter-2.8±0.9). In the sediment sample, highest bacterial count was found at S5 (monsoon-165±9.5; winter-175±22.0) and lowest at S1 (monsoon-4.6±0.9; winter-4.4±1.3). The graphical representation of the total viable count expressed as colony forming unit (CFU) in both water and sediment samples are presented in Fig. 1.

Table 1. Summary of mean concentrations of heavy metals in water and sediment samples

Sample		Water				Sediment			
Sites	Season	Pb	Zn	Cu	Cd	Pb	Zn	Cu	Cd
S1	Mn	0.00355	0.00835	0.00285	0.00015	2.35	7.55	4.45	0.07
	Wn	0.00155	0.00115	0.00035	0.00035	4.65	3.49	5.3	0.15
S2	Mn	0.0057	0.01415	0.0034	0.0005	5.2	50.2	6.35	0.165
	Wn	0.0044	0.0051	0.0009	0.0004	17.4	24.85	3.6	0.35
S3	Mn	0.00885	0.0063	0.01445	0.0005	15.05	35.9	7.5	0.22
	Wn	0.00765	0.04375	0.0132	0.00055	15.03	61.7	25.05	0.45
S4	Mn	0.00625	0.02725	0.00285	0.00025	7.29	27.35	8.6	0.215
	Wn	0.00195	0.0051	0.0018	0.00035	11.45	24.9	7.7	0.45
S5	Mn	0.01855	0.03125	0.00375	0.0004	12.35	34.75	5.4	0.69
	Wn	0.00275	0.0043	0.00185	0.0004	9.65	21.8	12.2	0.65
S6	Mn	0.01155	0.03615	0.0061	0.00035	2.6	36.75	5.75	0.14
	Wn	0.0057	0.0385	0.00995	0.00055	15.9	32.3	20.15	0.85
BISa		0.01	15	0.009	0.003	-	-	-	
WHOb		0.01	3	0.09	0.003	-	-	-	
IRSc		-	-	-	-	11	16	28	

*Mn-monsoon, Wn-winter; [a]Bureau of Indian standards; [b]World Health Organization (2006), [c]Subramanian *et al.,* (1987).

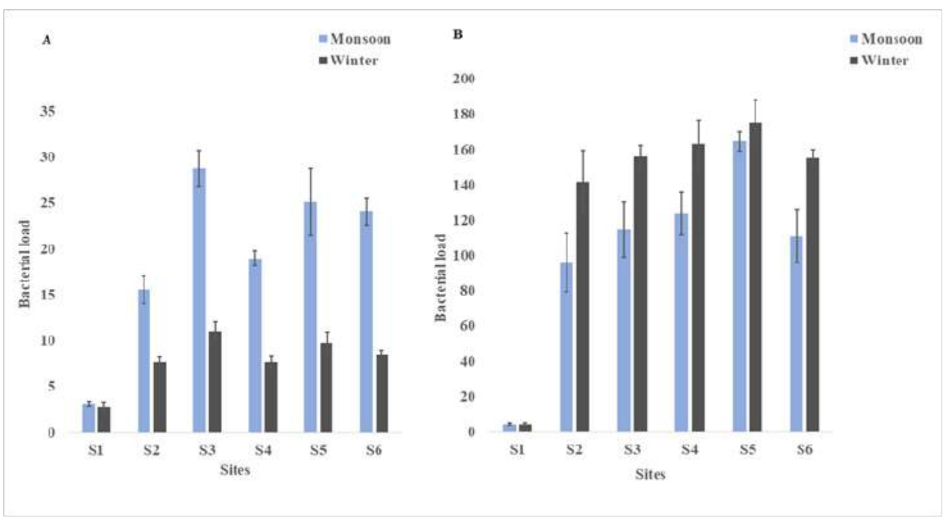

Fig.1. Mean bacterial load at each site for (a) water, and (b) sediment samples. Error bar represents the standard error of mean.

Bacterial diversity at Phylum and Genus level

A total of 245 bacterial isolates were obtained and characterized based on their colony characteristics and 16S rRNA gene sequencing. Among these isolates, 147 were retrieved from water samples, and 98 were obtained from sediment samples. The analysis revealed that the most dominant phylum in all samples was Proteobacteria, followed by Actinobacteria, Firmicutes, Bacteroidetes, and Deinococcus-Thermus. These 245 isolates belonged to 69 different genera. Fig. 2 shows the prevalent genera detected in both water and sediment samples, indicating a high bacterial diversity in both types of samples. However, the water samples displayed a higher number of bacterial genera compared to the sediment samples. In the water samples, *Pseudomonas* (13%) was the most abundant genus, accounting for 43% of the isolates, followed by *Microbacterium* (5.79%), *Bacillus* (5%), *Acinetobacter* (5%), *Brevundimonas* (4.34%), *Serratia*(3.6%), *Arthrobacter* (2.8%), and *Janibacter* (2.8%). These genera were found prevalent in more than two sampling sites, while the remaining 57% of isolates were unique to fewer sites. Similarly, in the sediment samples, *Pseudomonas* (11.57%) was the most abundant genus, comprising 40% of the total isolates, followed by *Microbacterium* (10.5%), *Acinetobacter*(8.4%), and *Arthrobacter* (6.3%). The rest of the isolates (60.1%) belonged to less prevalent genera. The study also revealed that downstream sites impacted by human intervention showed a higher frequency of opportunistic pathogens, such as *Serratia, Acinetobacter, Pseudomonas, Staphylococcus, Microbacterium, Gordonia* and *Brucella*. These findings highlight the potential implications of human activities on the bacterial composition in the river's downstream areas.

Heavy metal and antibiotic tolerance of the isolates

The tolerance of 107 bacterial isolates towards four heavy metals (Pb, Zn, Cu, and Cd) was investigated by determining their Minimum Inhibitory Concentration (MIC) values. The isolates were collected from different sites along the river, with the downstream isolates generally showing higher MIC values compared to those from the pristine uppermost site (S1).Regarding Pb, most isolates (82%) displayed a MIC of 2000 ppm, while 13% and 42% of isolates showed MIC values of 1000 ppm and 500 ppm, respectively, towards Zn. As for Cu, 8% and 67% of isolates displayed MIC values of 1000 ppm and 500 ppm, respectively. In the case of Cd, a significant proportion (82%) of isolates exhibited a MIC above 50 ppm.The antibiotic resistance profile of the bacterial isolates is presented in Table 2. Site S1 had the lowest percentage of MAR bacteria, while the percentage of MAR bacteria increased in the downstream sites of the river. The highest percentage of MAR bacteria was observed at site S6.

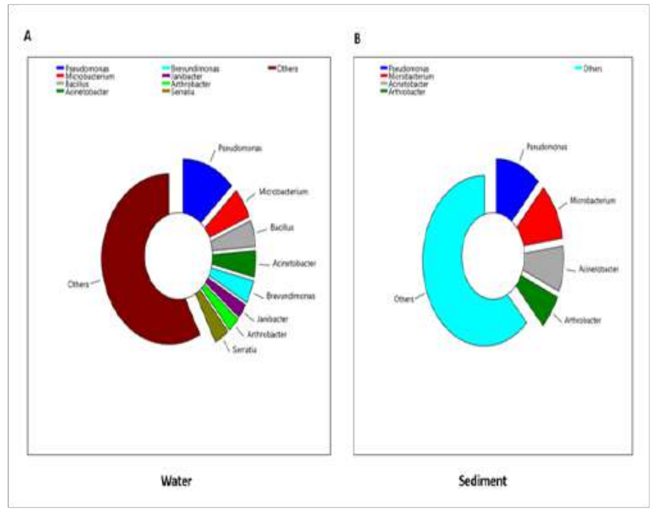

Fig. 2. Representation of major genera detected in water and sediment samples.

CONCLUSION

The Teesta River is vulnerable to pollution from the various small-scale industries and large-scale pharmaceuticals and has put the health of the river at stake. The measured concentrations of heavy metals in the river water were found to be below the threshold limit of BIS whereas the concentrations of Pb and Zn in the sediment samples at some sites exceeded the threshold limit provided by IRS. The lowest concentration of all the studied heavy metals were detected at site S1 which is least influenced by human activities. The increased concentration of heavy metals in the downstream sites can be attributed to the anthropogenic influence on the river. Detection of opportunistic pathogens in the downstream sites of the river can pose tremendous health risk to humans exposed to the water and moreover water is an easy means of dissemination of these pathogens.Co-resistance of the to various heavy metals and antibiotics was observed amongst the isolates studied. The occurrence of co-resistance of bacteria to different metals and antibiotics could be explained by the mechanism of co-resistance where genetically linked factors are expressed simultaneously; or cross-resistance, where the same factor or gene is responsible for resistance to several antimicrobials. These findings indicate that Teesta River may serve as a reservoir of heavy metal and antibiotic resistance genes and indicated major concerns regarding such genes associated with human pathogens. The changing ecosystem of the once pristine Eastern Himalayan River, mainly caused by human and developmental activities, has resulted in de-

Table 2. Antibiotic resistance profile of the isolates. The values in bold are the number of antibiotics to which the isolates at each site showing resistance

Sites	No. of isolates	Number of isolates showing resistance to corresponding number of antibiotics										Percentage of MAR bacteria at each site
		1	2	3	4	5	6	7	8	9	MAR index range	
S1	5	5	3	2	-	-	-	-	-	-	0.13-0.2	Nil
S2	18	17	17	14	11	6	5	4	1	1	0-0.46	61
T1	21	21	20	15	13	6	3	3	3	-	0.13-0.53	62
S3	14	14	14	13	9	9	9	9	1	-	0.2-0.53	64
S4	26	26	26	24	24	17	8	8	6	-	0.13-0.53	92
S5	23	23	23	23	22	18	3	2	-	-	0.2-0.46	95

graded water quality, and has become a potential pool for the dissemination of pathogenic and antibiotic-resistant bacteria.

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UNDERSTANDING THE MICROBIAL DIVERSITY OF ANTARCTICA; FROM THE ERA OF FUNDAMENTAL CULTIVATION TECHNIQUES TO MASSIVE SEQUENCING DATA

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ABSTRACT

Antarctica is situated in the Southern Ocean and boasts a climate that is famously cold, dry, and windy, constituting the majority of freshwater on the planet. This distinctive ecosystem provides a valuable opportunity for the examination of psychrophilic microorganisms experiencing polyextremophilic conditions. These polyextremophilic microorganisms in Antarctica play a vital role in biogeochemical processes, carbon sequestration, and greenhouse gas regulation. They are involved in various essential processes, including biogeochemical recycling and bioremediation. Moreover, their extraordinary adaptability holds promise for numerous biotechnological and industrial applications in this remote and challenging region. Unfortunately, due to the rising global temperatures in Antarctica, the native microbial diversity is getting drastically affected. Thus, it is crucial to bring these concerns to light and target the conservation of the microbial diversity in such a pristine environment. However, safeguarding these microorganisms proves to be a challenging task, given that cultivating them in laboratory settings often encounters difficulties in replicating their native environment effectively. Despite attempts to culture microorganisms in the laboratory, only a small fraction (less than 1%) has been successfully characterized, leaving the majority still unknown and unexplored. To mitigate these challenges high throughput sequencing has emerged as a robust method for assessing the total microbial diversity and identification, bolstered by improved databases. This technology facilitates the comprehensive analysis of various microorganisms, aiding in their accurate classification. Additionally, the next generation sequencing data offers insights that contribute to cultivating previously uncultured microorganisms. Moreover, these advanced techniques are instrumental in revealing the impact of climate change on microbial diversity by monitoring changes in microbial communities over time, yielding crucial insights into the influence of environmental shifts on these vital ecosystems, thus aiding conservation and mitigation endeavors.

Keywords: Antarctica, polyextremophiles, next generation sequencing, climate change

INTRODUCTION

Antarctica, the fifth largest continent in the world, is considered inhospitable for life due to its extreme climatic conditions (Smith *et al.,* 2009). Despite its harsh environment, this remote and frigid continent has revealed itself to be a thriving habitat for a unique group of microorganisms known as polyextremophiles (Cary *et al.,* 2010). These polyextremophilic microorganisms have evolved to thrive in a multitude of extreme environmental conditions including very cold temperatures, low availability of nutrients and high ultraviolet (UV) radiations.These polyextremophiles inhabit the glaciers, ice sheets, permafrost, and subglacial ecosystems of Antarctica (Bowman *et al.,* 1997; De Maaye *et al.,* 2014). The traditional methods, used for cultivating these Antarctic microorganisms in the laboratory settings, face significant hurdles due to the complexities of replicating their natural polyextremophilic conditions ex-situ (Cowan and Tow, 2004). Until now, only a small portion of microorganisms have been isolated from this unique ecosystem leaving the majority uncultivated and unexplored. However, recent advancements in sequencing and analysis techniques have provided a revolutionary approach for studying these resilient organisms. The integration of high-throughput sequencing and cultivation-dependent strategies has allowed researchers to delve into the genetic makeup and functional capabilities of these microorganisms, shedding light on their unique adaptations and potential biotechnological applications (Yergeau *et al.,* 2007). Further, the application of "omics" approaches, coupled with in-depth physicochemical analysis, have unveiled a deeper understanding of the ecological roles and metabolic potentials of Antarctic microbes (Teixeira *et al.,* 2010). In particular, high-throughput sequencing technologies have emerged as a powerful tool to comprehensively assess microbial diversity and identify these polyextremophiles (Cary *et al.,* 2010). This advancement has not only expanded our knowledge of the hidden microbial world within Antarctica but has also enabled us to explore how these microorganisms might respond to the impacts of climate changeby monitoring shifts in microbial communities over time. (De Maayer *et al.,* 2014). This deeper understanding of the microbial response to environmental shifts contributes to ongoing conservation efforts and mitigation strategies, ultimately safeguarding the unique microbial diversity of Antarctica and its potential contributions to biotechnological applications before we lose them.

Navigating cultivation challenges through the potential of Next Generation Sequencing

Microorganisms, the invisible architects of life, hold the key for understanding the world's ecosystems, human health, and industrial processes. However, unraveling the secrets of these microscopic entities has been a long-standing challenge, primarily due to the limitations of traditional cultivation methods. Using conventional methods, numerous microorganisms, particularly those dwelling in extreme environments or engaging in intricate symbiotic interactions, defy the attempts of cultivation under artificial laboratory conditions. Their unique requirements for growth, slow proliferation rates, and unidentified growth factors have stymied traditional methods, preventing the scientists from fully comprehending their complexity and importance.

Specifically talking about the cultivation of microorganisms from Antarctica, it has presented challenges due to constrained resources, inadequate equipment, and research facilities, required for the transportation of samples back to the research laboratory for cultivation. This process of sample transportation results in delay that ultimately impacts the cell viability and may lead to the loss of microbial diversity (Kajale *et al.,* 2020). Furthermore, another significant challenge lies in developing an appropriate laboratory environment that can effectively support the growth of these organisms, within controlled settings. As far as culturing of microorganisms in artificial or laboratory environment is concerned, it is of utmost importanceto provide the conditions as close as possible to their native environment. The shorter incubation periods pave a way to other fast growing life forms which colonize within a couple of days, rendering the organism of interest unable to flourish due to depletion of the nutrients. Hence, the cultivation of Antarctic microorganisms is difficult and demands for more rapid and reliable means of identification. Given these challenges, comprehensively capturing all the microorganisms through cultivation becomes a complex endeavor, underscoring the intricacies of investigating microorganisms from Antarctica (Jani *et al.,* 2022). With the progression of next-generation sequencing (NGS) technologies, it has become feasible to delve into the complete microbial diversity linked to a specific sample. Illustrated in Fig. 1 is a visual depiction of both cultivation and next-generation sequencing approaches.

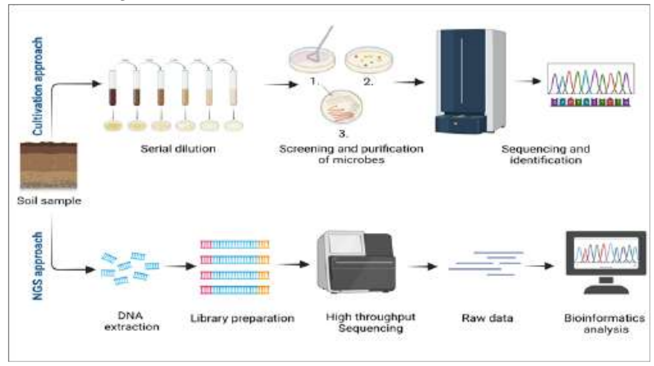

Fig. 1. Cultivation and identification approaches for the assessment of microbial diversity (Created with BioRender.com).

The advent of NGS technologies has cast aside these barriers, empowering the researchers with an unparalleled tool to unlock the enigmatic world of microorganisms. Platforms such as Illumina, Ion Torrent, and Oxford Nanopore Technologies epitomize the NGS revolution, enabling rapid and high-throughput sequencing of genetic material. Crucially, NGS has significantly bolstered our understanding of uncultured microorganisms. Metagenomic sequencing, a cornerstone of NGS applications, enables the comprehensive analysis of genetic material extracted directly from theenvironmental samples. The NGS approach helps in detecting the rare, uncultivable, low abundance taxa, playing significant roles present in Antarctic ecosystem, which might remain uncaptured using the traditional cultivation approaches (Jia *et al*., 2022). This technique not only identifies microbial diversity but also sheds light on functional capabilities encoded within their genomes. Metatranscriptomics takes this a step further, revealing gene expression patterns and allowing researchers to decipher the roles these microorganisms play within the intricate ecosystems.NGS effectively bridges the gap between uncultured microorganisms and their potential contributions to various fields. It provides a holistic view of microbial diversity, unshackling us from the constraints of cultivation bias. This newfound ability has profound implications for diverse domains, from environmental studies and biotechnology to medical research and beyond. It is also important to note that next generation sequencing has revolutionized genomics research not only by enabling faster and more comprehensive analysis of genetic material but also by significantly lowering the costs, thereby expanding its availability to laboratories worldwide. Advances in technology, increased competition among sequencing providers, and economies of scale have all played a pivotal role in driving down the expenses associated with NGS. This cost reduction has democratized genomic research especially the microbial genomics related studies, empowering more laboratories to engage in sequencing, generating data and further accelerating our understanding of microbial diversity and their possible functionalities (Dijk *et al.,* 2014). This technology has not only eclipsed the limitations of traditional cultivation but also paved the way for groundbreaking discoveries that promise to reshape our understanding of thecrucial roles played by these microorganisms in biotechnological advancements and the well-being of humans, animals, plants, and the overall health of our planet. As NGS technologies continue to evolve from the first generation to the third generation, it brings in the promise of even greater and advanced platforms with cost effectiveness. The first generation of sequencing technologies consisted of chain termination and chemical sequencing methods (Sanger *et al.,* 1977; Maxam and Gilbert, 1977). The automated Sanger's chain termination DNA sequencingwas used to sequence the first human genome under the Human Genome Project (Collins *et al.,* 2003), but its high cost (~300 million USD) and low throughput data demanded the development of newer and cost-effective sequencing technologiesknown as the next generation sequencing (NGS) technologies. The second-generation sequencing platformscomprised of the short-readsequencers such as the Roche 454 genome sequencer (2005) which implied the principle of pyrosequencing, Illumina which used bridge amplification for sequencing by synthesis, Sequencing by Oligo Ligation Detection (SOLiD) which incorporated sequencing by ligation andthe Ion Torrent Personal Genome Machineused semiconductor sequencing. Theprice of sequencing a genome reduced to almost 10, 000 times (~600 to 1000 USD per genome), as compared to the cost in 2004, with the second-generation sequencers (van Dijk et al., 2014) while the data output increased (Fig. 3). The third-generation sequencing platforms include the long-read sequencers of the Pacific Biosciences which utilizes single molecule real time (SMRT) sequencing and Oxford Nanopore Technologies in which ionic current passes through nanopores. The evolution of sequencing platforms over the yearsis illustrated in the Fig. 2.

Fig. 2. The advancement of sequencing platforms along the years (*Source: https://www.thermofisher.com, https://dnatech.genomecenter.ucdavis.edu*)

Impact of climate change on the native microbial diversity of Antarctica

Antarctica, despite being a pristine continent, remains susceptible to the effects of human induced activities and industrialization, undermining its otherwise undisturbed state. The high UV radiation and rising temperatures across the continent is ultimately causing the glaciersto melt thereby leading to climate changes in Antarctica (Jani *et al*., 2022). Global warming and climate change, whether originating naturally or through anthropogenic activities, have emerged

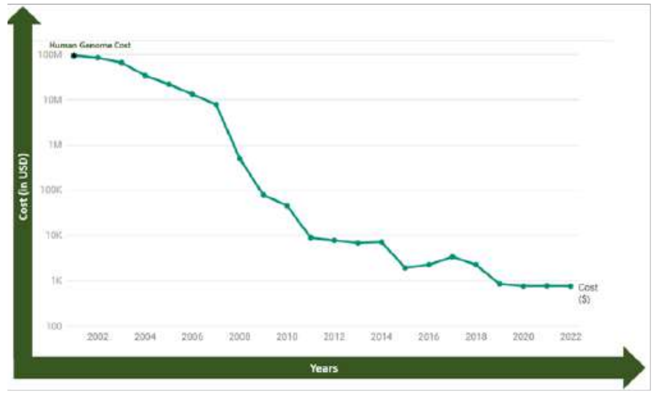

Fig. 3. The trend of cost reduction of sequencing a genome with the devel- opment of sequencing technologies along the years *(Source: www.genome. gov/sequencingcostsdata)*

as the most significant threats to both humanity and the biodiversity of the planet. Antarctica, hosting a vast array of life, faces peril from the gradual melting of ice caps due to rising temperatures. The rapid expansion of industrialization and urbanization has profoundly altered atmospheric chemistry, leading to the ozone layer depletion through the emission of chlorofluorocarbons (CFCs), resulting in increased penetration of UV radiations at the continent's surface. This rise in penetration of UV radiation has lead to a considerable impact on native microbial life. Previous studies have revealed that UV radiation induces the development of defense mechanisms,causes DNA damage, changes in cellular ultra structures, reduces photosynthetic pigment production, and disrupts metabolism (Midt *et al.,* 2010; Pichrtová *et al.,* 2013; Jani *et al.,* 2022).Variations in the temperature and increased UV radiation lead to changes in bacterial diversity, suggesting that bacteria could serve as indicators of climate change.The other major abiotic factors affecting terrestrial life include soil composition, temperature, and water availability (Bissett *et al*., 2014). Fluctuations in these factors allow certain microorganisms to adapt well, and it's important to continually monitor these changes to understand the shifts in microbial communities (Verde *et al.,* 2016). Understanding the impact of climate change on microbes is crucial for mitigating serious consequences and creating a sustainable environment (Cavicchioli *et al.,* 2019). The microorganisms, once thriving in their optimal habitats, are now at risk due to the melting of ice caps caused by rising temperatures and shifting climate conditions (Doytchinov and Dimov, 2022; Ortiz, 2011; Jani *et al.,* 2022). Deglaciation due to global warming causes shift in both rare and abundant microbial communities in Antarctica (Yan *et al.,* 2017). Thus the retreating glaciers caused by global warming have provided opportunities for the colonization of various algae and bacterial species (Świątecki *et al.,* 2011). These microbial communities are highly responsive to environmental changes, making them valuable for studying the effects of alterations in their surroundings. It is widely recognized that the primary reason of climate change in Antarctica is the warming of the continent. This warming initiates the melting of ice and disturbs the marine microbial ecosystem due to higher amounts of freshwater entering the system. Consequently, this disruption has an impact on soil microbial communities, especially within the ponds formed by the melting ice (Santos *et al.,* 2023). Persistent increases in UV radiation might render certain organisms unable to adapt to their changing habitat, leading to extinction and exacerbating imbalances in the ecosystem (Blumthaler *et al.,* 2007). Consequently, the polar ecosystem, encompassing glaciers and coastal areas, serves as a valuable model for investigating how ecosystems respond to the changing environmental conditions. Specifically, an upsurge in the Firmicutes phylum was noted in response to the harsh conditions in Antarctica (Doytchinov and Dimov, 2022). Notably, few studies have highlighted shifts in the growth of blue-green and eukaryotic algae due to rising temperatures, further emphasizing their potential as indicators of climate change (Ilicic *et al.,* 2022; Survey *et al.,* 1996). The melting of permafrost and ice glaciers can unleash microorganisms harmful to humans, driven by alterations in soil composition that stimulate growth. Inert permafrost thawing initiates biotic processes and releases soil carbon as greenhouse gases (methane and CO_2) into the environment. Consequently, the Antarctic continent has been losing around 1.3x 1014 kg of ice per year (Dutta and Dutta, 2016). The presence of the microalga Vicicitus globosus denotes elevated CO_2 levels in oceans, disrupting trophic level food chains.Frequent UV exposure has hindered the establishment of zonal fungi in terrestrial Antarctic environments, limiting their growth (Hughes *et al*., 2003). This underscores the prospect of utilizing microbial communities to unravel the consequences of climate change in Antarctica. Diatoms offer another avenue for tracking climate change in Antarctica. The presence of diatoms, traditionally hindered by ice covers, signifies ice melting due to temperature elevation. Warming conditions induce deglaciation, enriching the environment with nutrients and influencing diatom species compositions (Spaulding *et al.,* 2010). Further, the climate change elevates the risk of emergence of novel diseases, influences host and parasite acclimatization and facilitates horizontal gene transfer, ultimately leading to increased antibiotic resistance in human infections. Climate change-induced geographic shifts have repositioned once non-pathogenic microbes into a context of highly invasive, antibiotic-resistant infections (Tiedje *et al.,* 2022). Collectively, alterations in microbial composition or metabolites can be positively correlated with climatic shifts in Antarctica, establishing microbes as credible indicators of climate change. The combination of both culture-dependent and culture-independent methodologies holds the potential to offer more profound understanding regarding the changing climate in Antarctica and the potential risks posed by the release of long-frozen pathogens within the permafrost.Taking this into account, we collected samples from various sites near Bharati and Maitri stations in India (Fig. 4 A and 4 B) during the 38th Indian Scientific Expedition to Antarctica supported by the National Centre for Polar and Ocean Research, Goa, an institute of Ministry of Earth Science, Government of India. Our aim was to evaluate microbial diversity through conventional cultivation and targeted amplicon sequencing methods. Employing traditional cultivation, resulted in the description of a novel species named Marisediminicolasenii (Jani *et al.,* 2021). Simultaneously, our amplicon sequencing analysis enabled us to examine how subtle environmental changes influence bacterial and fungal communities. Our findings revealed that Cyanobacteria constituted the predominant bacterial group, while Ascomycota was dominant in case of fungi (Jani *et al.,* 2021; Jiya *et al.,* 2023).

Fig. 4. Indian research stations at Antarctica (A) Bharati Station and (B) Maitri Station

CONCLUSION

In conclusion, the progression of sequencing technologies has been instrumental in advancing our understanding of microbial diversity. From the labor-intensive Sanger sequencing to the high-throughput capabilities of NGS platforms and the long-read capabilities of third-generation sequencers, each generation has contributed to uncovering the genetic makeup, functional potential, and ecological roles of microbial communities. The continued advancements in sequencing technologies, coupled with bioinformatics advancements, hold great promise for further unraveling the intricate world of microbial diversity, enabling us to explore the hidden realms of microorganisms and their impact on our planet. In recent years, the integration of sequencing data with bioinformatics tools and databases has further enhanced our understanding of microbial diversity. Comparative genomics, metagenomics, and phylogenetic analyses have become routine approaches to investigate microbial communities and their interactions. The availability of reference databases, such as GenBank, SILVA and NCBI,etc, has facilitated the taxonomic and functional annotation of microbial sequences, enabling comprehensive exploration of microbial diversity across different habitats and ecosystems.

ACKNOWLEDGEMENT

Dr. Avinash Sharma is DBT Wellcome Trust Fellow and Principal Investigator of the "Centre for Excellence, National Centre for Microbial Resource". Our studies on Antarctic microbiology were supported by the Science and Research Engineering Board (SERB), Department of Science and Technology (DST), Government of India, New Delhi, under the project grant (SRG/2022/000126). The authors are grateful to NCPOR, Goa for providing the logistics support during the 38th Indian Scientific expedition to Antarctica. We are extremely thankful to Dr. Shailendra Saini for his remarkable suggestions on sampling strategies.

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EXPLORING THE EPIPHYTIC BACTERIA ASSOCIATED WITH MACROALGAE IN ANTARCTICA

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INTRODUCTION

Seaweeds - or marine macroalgae – are important components of coastal Antarctic ecosystems. Macroalgae are major primary producers in the Southern Ocean, playing a crucial role in the organic matter cycling, as well as providingsubstrate and shelter for diverse species of marine microbes and fauna. A remarkable feature of the Antarctic marine flora is the high level of endemism. A total of 151 species of macroalgae has been recorded in Antarctica (85 Rhodophyta, 34 Ochrophyta and 32 Chlorophyta) with an endemism of 27 %(Oliveira *et al.,* 2020). Probably the macroalgal diversity is underestimated due to limited sampling in remote parts of Antarctica and the scarcity of molecular taxonomic studies. Antarctic macroalgae are also important in terms of biomass, with levels comparable to temperate kelp forest (Quartino *et al.,* 2020) (Fig. 1).

Fig.1. The endemic brownmacroalgaeAscoseira mirabilis Skottsberg, one of the dominant species in the uppersubtidal of the Antarctic Peninsula. Photo credits: Ignacio Garrido.

Antarctic marine macroalgae are emerging as an important source of a unique microbial diversity. The epibiotic and endobiotic microbial communities are closely associated with the macroalgal host, playing crucial roles in their metabolism, morphogenesis and evolution. Much of our understanding of the microbial communities associated with marine macroalgae comes from studies in tropical and temperate latitudes, with very few studies analyzing themicrobiomes associated with macroalgae of the white continent. The diversity, ecological role and biotechnological relevance of Antarctic macroalgae-associated microorganisms are still quite unexplored.

BACTERIA ASSOCIATED WITH ANTARCTIC MACROALGAE

Studies in tropical and temperate environments have shown that marine macroalgae associate with bacterial populations that differ from the free-living bacteria in the surrounding seawater (Lachnit *et al.,* 2011).The surface of marine macroalgae is a fluctuating environment, where biological and abiotic factors, such as the age of the algal tissue and irradiance are important in shaping microbial community composition.For instance, in a study with intertidal seaweeds in South Africa, (Selvarajan *et al.,* 2019) reported that the diversity of epiphytic bacteria in macroalgae surfaces is greatly influenced by algal organic exudates as well as the profile of chemical elements, which triggers chemotaxis in bacterial epibionts. The epiphytic bacteria in turn produce secondary metabolites which defend the algal host from opportunistic pathogens and help to counteract bacterial dysbiosis (Li *et al.,* 2022b). In turn, macroalgaealso release secondary metabolites with antimicrobial activity, as has been recently demonstrated for Antarctic macroalgae (Martín-Martín *et al.,* 2022). Culture-based studies have shown that Antarctic macroalgae harbor a diverse and bioactive fungal community. A recent study by (Martorell *et al.,* 2021) reported a diverse community of Ascomycota fungi (*Antarctomyces, Cadophora, Cladosporium, Penicillium, Phialocephala* and *Pseudogymnoascus)* isolated from six species of Antarctic macroalgae. The authors also concluded that Antarctic macroalgae are a source of novel fungal taxa with promising bioprospecting and biotechnological potential. A recent compilation made by (Ogaky *et al.,* 2019) reported that the algicolous fungal communities obtained from 16 macroalgal species is composed of a few dominant species, as well as a balance among endemic(e.g. Metschnikowia australis), indigenous, and cold-adapted cosmopolitan species (e.g. *Penicillium* sp.). Recent culture-based studies have also shed light on the diversity and bioactive potential of the bacterial community living in association with the surface of Antarctic seaweed. The epiphytic culturable bacterial community is dominated by Gram-negative organisms and includemembers of Gammaproteo bacteria (e.g. *Paraglaciecola, Pseudoaltermonas, Psychrobacter*), Alphaproteo bacteria (*Sulftobacter*) and Flavobacteriia (e.g. *Algibacter, Flavobacterium,Olleya*). Previous work of our group reported the diversity of epiphytic culturable Gram-negative bacteria associated with Antarctic brown (*Adeno cyst is utricularis, Himantothallus grandifolius*), green (*Monostroma hariotti*) and red macroalgae (*Iridaeacordata, Pantoneura plocamioides, Plocamium cartilagineum*), especially those with the ability to degrade algal phycocolloids. 16S rRNA identification showed that the agar-degrading bacteria belonged to the genera *Algibacter, Cellulophaga, Colwellia, Lacinutrix, Olleya, Paraglaciecola, Pseudoalteromonas,Winogradskyella* and *Zobellia* (Alvarado and Leiva, 2017; Sánchez Hinojosa *et al.,* 2018). A cold-active agarase was also purified from the culture supernatant of a potential new species of the genus *Olleya* sp. (strain HG G5.3) isolated from the surface of the Antarctic subtidalmacroalgae *Himantothallus grandifolius*. The purified agarase exhibited activity at 4°C, retaining more than 50% of its maximum activity at this temperature (Sánchez Hinojosa *et al.,* 2018). The recent study of (Yuanyuan *et al.,* 2020) has expanded the list of Gram-negative, agar-degrading bacteria isolated from Antarctic macroalgae to representatives of *Flavobacterium, Halomonas, Shewanella, Photobacterium, Pseudomonas, Psychrobacter* and the family Thiotrichaceae. Recent metagenomic and culture-based studies have highlighted the potential of Antarctic macroalgae-associated bacteria as a novel source of new algal polysaccharide-degrading enzymes (agarases, carrageenases) with promising applications in various industries (Gui *et al.,* 2021; Li *et al.,* 2022a; Sánchez Hinojosa *et al.,* 2018). The scarce information available on the occurrence of Antarctic epiphytic Gram-positive bacteria shows a strong presence of the phylum *Actinobacteria*, with a small proportion of Firmicutes, which contrast with there ported dominance of the latter on tropical and temperate macroalgae (Gaitan-Espitia and Schmid, 2020). Our studies on three co-occurring Antarctic intertidal macroalgae of King George Island, *Adeno cyst is utricularis* (brown alga), *Iridaeacordata* (red alga) and *Monostroma hariotii* (green alga), reported 17 genera of Actinobacteria, with a dominance of members of Micrococcaceae and Micro bacteriaceae. In contrast, representatives of *Staphylococcus* and *Planomicrobium* were the only *Firmicutes* isolated from these macroalgae. The dominance of *Actinobacteria* can be attributed to their ability to colonize plant surfaces, persist in harsh environments, as well as the synthesis of a wide range of antimicrobialsand extracellular hydrolytic enzymes against plant pathogens (Desai and Amaresan 2022). Ultraviolet (UV) resistance can also be postulated as one of the reasons for the dominance of actino bacteria on the surface of intertidal Antarctic macroalgae. Pigmentation and a high G+C content have been shown to increase protection from UV radiation in bacteria (Newton *et al.,* 2011). Antarctic ozone depletion has enhanced the spring and summer UV exposure of terres trial and marine biota (Robinson 2023). Interestingly, (Leiva *et al.,* 2015) found a diverse community of pigmented *Actinobacteria* associated with intertidal marine Antarctic macroalgae. The pigmented *Actino bacteria* contained species of the genera *Agrococcus, Arthrobacter, Brachybacterium, Citricoccus, Kocuria, Labedella, Microbacterium, Micrococcus, Rhodococcus, Salinibacterium* and *Sanguibacter* (Fig 3). Pigmentation may help epiphytic Actinobacteria to successfully tolerate the harsh environment found in the Antarctic intertidal zone in spring and summer, characterized bydamaging levels of UV radiation and frequent freeze–thaw cycles. Both culture-dependent and culture-independent studies have shown that marine macroalgae harbor host-specific bacterial communi-

Fig.2. Adenocystisutricularis (Bory) Skottsberg (brown, small and sac- catemacroalgae) and Iridaeacordata (Turner) Bory de Saint-Vincent(red and leatherymacroalgae) in the intertidal zone of Punta Rodríguez, King George Island, Antarctica. Photo credits: Sergio Leiva.

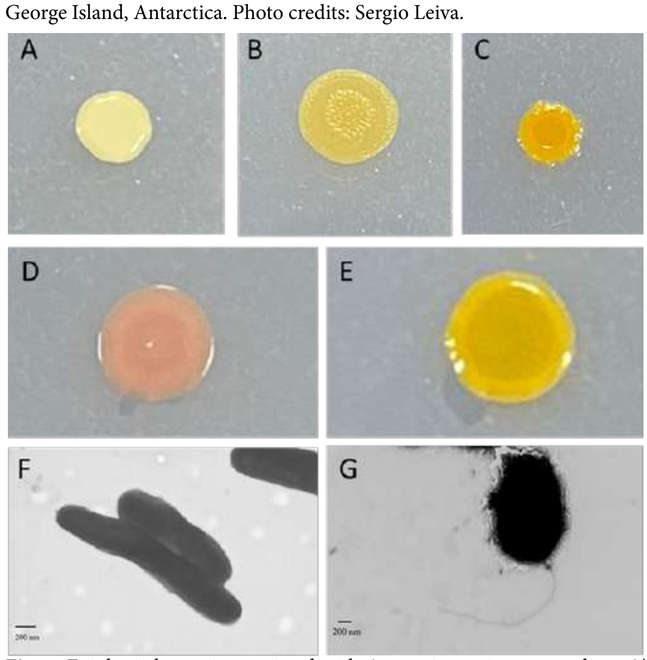

Fig. 3. Epiphytic bacteria associated with Antarctic marine macroalgae. A) Colony of *Kocuriapalustris* isolated from *Desmarestiaantarctica*. B) Colony of *Flavobacteriumfrigidarium* isolated from *Pantoneuraplocamioides.* C) Colony of *Cellulophagaalgicola* isolated from *Himantothallusgrandifolius,* D) Colony of *Shewanellapolaris* isolated from *Georgiellaconfluens.* E) Colony of *Maribacteraquivivus* isolated from *Plocamiumcartilagineum*. F) Transmission electron micrograph of a strain of Maribacteraquivivus isolated from Himantothallusgrandifolius. G) Transmission electron micrograph of a strain of *Pseudoalteromonas* sp. isolated from *Plocamiumcartilagineum*. Photo credits: Sergio Leiva.

ties on their surfaces (Lachnit *et al.,* 2011; Wang *et al.,* 2009). Interestingly, in a study aimed at assessing the diversity of the culturable Gram-positive bacteria associated with the surface of three co-occurring intertidal Antarctic macroalgae (the brown algae *Adeno cyst is utricularis*, the red algae *Iridaea-cordata* and the green algae *Monostromahariotii*) (Fig 2), we found that the Gram-positive epiphytic community exhibits a clear host-specificity, with no species common to all three macroalgae species (Alvarado *et al.,* 2018). The Gram-positive isolates were grouped into 29 phylotypes, with 16 phylotypes associated with *A. utricularis*, 9 phylotypes with *I. cordata* and 10 with *M. hariotii*. Most of the bacterial phylotypes were unique to one host macroalgae (23 out of 29 phylotypes), only 6 phylotypes were shared by two macroalgae species, and nophylotypes were common to all three macroalgae species.

ANTARCTIC MACROALGAE AS A SOURCE OF NOVEL ACTINOBACTERIAL SPECIES

Extreme, unusual and isolated environments such as the Antarctic are of great interest as a source of novel actinobacterialtaxa that can be of medical and biotechnological importance. New species of actinobacteria has been described in the last 5 years from differentAntarctic environments, such as marine macroalgae, glacier samples and various types of soils. The novel species belong to diverse actinobacterial genera, such as *Amycolatopsis, Arthrobacter, Marisediminicola, Nakamurella, Paeniglutamicibacter, Paraconexibacter, Pseudarthrobacter, Rhodococcus* and *Tessaracoccus* (Chen *et al.,* 2022; Da *et al.,* 2019; Jani *et al.,* 2021; Kim *et al.,* 2023; Sakdapetsiri *et al.,* 2021; Sun *et al.,* 2023; Vodickova *et al.,* 2022; Wang *et al.,* 2018; Zhou *et al.,* 2020). In this regard, marine macroalgae are an attractive source for the isolation of novel bacterial taxa, although so far, most of the new species have been Gram negative, isolated from a few wide spread macroalgal genera and from samples collected at higher latitudes. Examples of new species of Gram- negative bacteria isolated from marine macroalgae around the world in recent years can be found in the International Journal of Systematic and Evolutionary Microbiology (IJSEM), and include representatives of diverse taxa such as *Flavobacterium, Mangrovicoccus, Nitratireductor, Octadecabacter,Tenacibaculumand Zobellia*. With respect to Gram-positive bacteria, recentnovel actinobacterial descriptions from algae are *Cellulomonasalgicola* from a fresh water alga (*Chetophoraceae*) collected from a river in Japan (Yamamura *et al.,* 2019) and *Ornithinimicrobiumlaminariae* from the kelp Laminaria japonica in China(Cao *et al.,* 2022). Recently, (Wang *et al.,*2018) described a novel species of the actinobacterial genus Amycolatopsis (*A. antarctica*), isolated from the intertidal Antarctic macroalga *Adenocystisutricularis*. The genus Amycolatopsis (*Pseudonocardiaceae*) has approximately 70 species, and very few of marine origin, and *A. antarctica* was the first new species of *Amycolatopsis* described from a marine macroalgae. In turn, (Wang *et al.,* 2020) described *Putridiphyco bacterroseus*, a novel species of Bacteroidetes which was recovered from an unidentified rotten seaweed collected in King Geroge Island, Antarctica. This suggest that Antarctic macroalgae may host large numbers of undiscovered bacterial species.

CONCLUSIONS AND PERSPECTIVES

Our understanding of the multifaceted macroalgae-microbe interactions in Antarctica remains very limited. Future research should take advantage of the technical, practical and conceptual progress in microbiome research in recent years, which will revolutionize our understanding of the biology, diversity and evolution of Antarctic bacterial epiphytes, their mechanisms of adaptation to the extreme Antarctic marine environment, metabolic potentials, ecological roles, and the biotechnological applications of their biomolecules and enzymes. Metagenomics Next-Generation Sequencing (mNGS) and associated bioinformatics tools will allow a deeper analysis of microorganism diversity and will accelerate our understanding of the extremely complex microbial community associated with macroalgae in Antarctica.A greater attention should be put on gaining insights into how microbiomes influence various aspects of the macroalgal host, which may uncoverundescribedand critical microbe-host relationships under the harsh and challenging conditions of the Antarctic marine environment. Furthermore, microbial culturomics combined with genome-guided cultivation efforts will help retrieve the microbial dark matter (MDM) associated with Antarctic macroalgae, i.e. the currently undescribe dprokaryote diversity, still recalcitrant to conventional cultivation. There is an urgent need to gain a better understanding of the direct and indirect impacts of climate change on macroalgae-associated microbiomes in Antarctica. Future studies should examine how the interaction smacroalgae-microbiome will respond to increased temperatures in Antarctica at molecular, biochemical and ecological level. Antarctica is an extreme and remote environment, a challenging region to carry out research due to the inaccessibility of the coastline and the inherent logistical, financial and safety concerns. But Antarctica is a marine biodiversity hotspotand significant efforts must be devoted to improve our knowledge in both the marine biota diversity as well as the host-associated microbiomes.

CONFLICT OF INTERESTS

The author declares no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by a grant of Instituto Antartico Chileno (INACH, Grant RT_06-13).

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COLD-ADAPTED FRIENDS AND FOES: MICROBES IN ANDEAN GLACIAL ECOSYSTEMS

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ABSTRACT

Glaciers are colonized by abundant and diverse microbial communities. Many of these microbes are alive and metabolically active, despite being confined in ice for centuries or millennia. Almost no information is available concerning the microbiology of tropical glaciers, and even less is known about tropical Andean glaciers. The main results obtained by the Venezuelan group that leaded the microbiological research in the last two Venezuelan glaciers are summarized below. Altogether, the results indicate that, before their disappearance, these glaciers harboredviable bacteria which could be considered either as our potential "friends" or as our potential "foes".The information was originally presentedin the form of a conference, originally included in the program of the International Conference on "Mountain ecosystems: Biodiversity and adaptations under Climate Change scenario" hosted by Graphic Era Deemed University, Dehradun, India and organized in collaboration with the International Centre for Integrated Mountain Development, ICIMOD, Nepal (22-24 March 2023).

INTRODUCTION

Cryosphere is the term we use to refer to the regions of the Earth where water is permanently frozen. The term was introduced by Antoni Dobrowolski in 1923, and it includes glaciers (which cover almost 10% of the Earth' surface), snow, ice sheets, sea ice, and permafrost (which represents more than 20% of terrestrial soils in the northern Hemisphere), among others. If we only consider glaciers, we are referring to almost 33 million cubic kilometers of ice worldwide (Ohmura 2015). For many years it was thought that glaciers were devoid of life, that they were almost completely sterile. But this vision completely changed when Soviet scientists discovered that glacier ice is, in fact, colonized by an unexpected amount of microbes. This happened almost 45 years ago, in the mid-eighties, when Sabit Abyzov and its colleagues were studying ice cores collected in Antarctica, several kilometers down, when drillinginto the glacier located above Lake Vostok, the biggest sub-glacial lake. When analyzing the ice cores, of several thousand years of age, they discovered that they were plenty of microbes and, more astonishing, that many of these microbes were viable (Abyzov *et al.,*1982). Almost 20 years later, Brent Christner and his colleagues found and reactivated bacteria in ancient ice samples, collected in many glaciers worldwide (Christner *et al.,* 2000, 2002). Later on, many more scientists confirmed these findings, when studying ice samples ranging from 500 to 157,000 years old, collected in Greenland and Antarctica (Knowlton *et al.,* 2013). Today, the presence of (viable and culturable) microbes in glacial ice is acknowledged worldwide. In fact, glaciers are considered true ecosystems, home to all kinds of living beings: there are lots of microorganisms on their surface (a supraglacial ecosystem), in their interior (an englacial ecosystem), and at their base (a sub-glacial ecosystem) (Boetius *et al.,* 2015; Anesio *et al.,* 2017).Even the meltwaterthat emerges from the glacier' snouts, as a result of their melting, is loaded with high amounts of microorganisms that reach aquatic ecosystems downslope. How many microorganisms colonize glacier ecosystems ? It has been estimated that frozen environments can harbor between 1025 and 1028 microbial cells (Boetius *et al.,* 2015). Many of them remain confined within glacier ice,in theenglacial ecosystem; some have been preserved there for thousands of years. That is why glaciers are considered as paleoarchives of ancient life forms (Zhong *et al.,* 2021). Deep within these glaciers, many of these microorganisms are metabolically active, and many of them are multiplying actively, although at very low rates inside very small interstitial venules located in the margin of ice crystals (Price and Sowers 2004; Price 2007; Nikrad *et al.,* 2016). Many glaciers have been studied through out the years, around the globe. In one of the most recent of these studies,which was performed on Tibetan glaciers by a team of Chinese scientists, they published the largest catalog of glacier genes and genomes to date (Liu *et al.,* 2022). The microbial communities in glaciers are diverse, with members of the Proteobacteria, Bacteroidota, Actinobacteriota and Firmicutes phyla as the most abundant. Most of the taxa identified are endemic, and many of the sequenced genomes have a very low percentage of identity with others previously known, which suggest they belong to unknown species.

GLACIER ICE MICROBES

Surviving in frozen environments poses significant challenges for microorganisms. They must endure a number of harsh conditions like nutrient scarcity, the presence of oxygen radicals, periodic cycles of freezing and thawing, low water activity (in fact, glaciers are considered frozen deserts), high levels of ultraviolet radiation, and high hydrostatic pressure. However, the most challenging of all are the prevailing low temperatures, which drastically affects cell structures and functions. Cold environments limit the diffusion of solutes because of the water viscosity increases and the membrane fluidity decreases; for the same reason, uptake of nutrients and release of metabolites are also restrained (D'Amico *et al.,* 2006; Rodríguez and Tiedje 2008). Furthermore, the formation of ice crystals is particularly dangerous to live cells, as they can damage fragile cytoplasmic membranes. Finally, low temperatures severely affect enzymes' functionality, as their catalytic activity decreases exponentially with temperature drops (Feller 2013). Despite all these constraints, metabolically-active glacier-ice microbes occur with in liquid water brines found inside microscopic veins, located between ice-crystals, and at temperatures below a freezing point (Price 2007). Psychrophilyis the ability to grow at low temperatures (Cavicchioli 2015). To achieve this, psychrophiles evolved diverse strategies, including regulating membrane fluidity, producing cryoprotectants and antifreeze proteins, adjusting ion channel permeability, synthesizing cold-shock chaperones, and entering seasonal dormancy (Georlette *et al.,* 2004; D'Amico *et al.,* 2006). Also, certain bacterial species form spores or develop thick cell walls and polysaccharide capsules to endure extreme conditions (Priscu *et al.,* 2007).

Among these adaptations to cold- environments, the most crucial feature that enables psychrophiles to grow and multiply is the extreme flexibility of their enzymes' structures (Feller 2013). This flexibility compensates for reduced molecular movements, ensuring thus a high catalytic efficiency even at sub-zero temperatures. Unfortunately, glaciers also store pathogenic microbes (Houwenhuyse *et al.,* 2018; Rogers and Castello 2020; Sajjad *et al.,* 2020). Their reactivation and release, due to ice melting, can pose an enormous threat to other living beings, including humans. In fact, some years ago an anthrax epidemic impacted the villagers of a remote region of Siberia (Selyaninov *et al.,* 2016). The epidemic was related to the thawing of permafrost and the release of spores contained inthe carcasses of reindeers that died almost a century before, due to an epidemic caused by Bacillus anthracis (Timofeev *et al.,* 2019; Liskova *et al.,* 2021). Glaciers also contain many viruses, and many ancient viruses, a subject that has been poorly studied. For instance, human pathogenic viruses, like caliciviruses, influenza viruses, and enteroviruses, have been detected in glacier ice samples (Smith *et al.,* 2004). Many other viruses, which are unknown to science, have been stored and conserved inside these glaciers for millennia and, although the information concerning their diversity is still limited, a few studies are beginning to shed light on the subject (Rassner 2017). Other microbes, such as certain yeasts and bacteria, related to human pathogens, have been also detected in glacier ice and streams (Goodwin *et al.,* 2012, Turchetti *et al.,* 2015; Brad e*t al.,* 2018; Perini *et al.,* 2019).

GLACIER ICE AS RESERVOIRS OF MICROBIAL GENES AND GENOMES

Cryospheric environments, particularly glacier ice, are considered to be a vast reservoir for microorganisms, many of which can remain viable for thousands of years when trapped in ice crystals or concentrated in microscopic liquid veins (Mader *et al.,* 2006). When ice melts, the confined microbes are released, being able to spread throughout the environment. Some studies estimated that approximately 2.9 x 1022 microbes will be discharged every year, into downstream ecosystems,solely from northern Hemisphere glaciers, due to their melting (Stevens *et al.,* 2022).Since some of the released microbes are either ancient pathogens or closely related to contemporary pathogens, some warned about the potential threats they pose to natural ecosystems, and even to humans, animals and plants (Rogers *et al.,* 2004, 2020; Edwards 2015; IAP 2020). Apart from the release of these microbes, there is also significant concern about the potential spreading of their genes and genomes into aquatic ecosystems, and their mixture with contemporary genomes. This phenomenon has been called 'genome recycling' (Rogers *et al.,* 2004) and can introduce important changes in the metagenomes of microbial communities, by drastically modifying allele proportions. In fact, it is well-known that horizontal gene transfer (HGT) between microorganisms, either closely or distantly related, occurs at high rates and influences the evolution of many microbial species, including eukaryotes (Soucyet al. 2015). HGT is of particular when involving antibiotic resistance genes (ARGs), found frequently in pristine glacial ecosystems (Ushida *et al.,* 2010, Segawa *et al.,* 2013). This abundant and diverse group of ARGs include determinants of resistance against antibiotics used to control human, animal, and agricultural diseases, which emphasizes their dangerousness.

MICROBIOLOGY OF ANDEAN TROPICAL GLACIERS

Tropical glaciers are located on top of high mountains in regions of the where three geographical zones coincide: (1) the astronomical tropics (radiative delimitation), (2) any area in which the daily air temperature amplitude exceeds the annual temperature amplitude (thermal delimitation), and (3) the area of oscillation of the Intertropical Convergence Zone (ITCZ) (hygric delimitation) (Kaser 1999). Even though a few (small) tropical glaciers exists in East Africa (Mt. Kenya, Mt. Kilimanjaro, and Rwenzori Mountains) and in New Guinea (Irian Jaya), approximately 99% of them are found in the Tropical Andes region of South America, a vast region that spans from southern Venezuela to Northern Argentina. Contrary to what happens with glaciers located at higher latitudes, and despite their significance and biotechnological value (Margesin and Collins 2019), studies concerning microbes immured in Tropical Glaciers are rather scarce (Yarzábal 2021). This lack of information is worrying; even more when we consider these frozen environments are disappearing faster than ever (Jackson *et al.,* 2019; Ramírez *et al.,* 2020), leaving scientists with very little time to study them. For instance, the last two Venezuelan glaciers, located in Pico Bolívar and Pico Humboldt, the highest in the Venezuelan Andes, almost vanished just four years after samples were collected by the author and their team (Rondón *et al.* , 2017). Consequently, crucial information about the microbial communities trapped within these glaciers (as well as other important aspects and resources) has been lost. Forever. Since 2012, my research team at the University of Los Andes in Mérida (Venezuela), dedicated its efforts to study the microbes entrapped in the last two remaining Venezuelan glaciers. The initial report, published in 2014, confirmed the presence of an abundant community of bacterial heterotrophs (>106 CFU/ml) in ice samples collected from La Corona glacier (Humboldt's Peak) at an altitude of approximately 4950 m.a.s.l. (Ball *et al.,* 2014). Many of the isolates were reactivated readily when cultivated invitro on agarized media, with several identified as members of the Proteobacteria (α, β, and γ), Actinobacteria, and Flavobacteria. Pseudomonas strains were frequently detected in the collected samples, with many isolates closely related to previously identified psychrophilic or psychrotolerant strains.Notably, a significant proportion of these isolates produced cold-active proteases and amylases and many were resistant to various antibiotics and heavy metals. Similar results were obtained in our second expedition when prospecting glacier ice from another Venezuelan glacier located atop Bolivar Peak, at around 4,850 m.a.s.l. (Rondón *et al.,* 2016).Antibiotic resistance in microorganisms occurring in Tropical Glaciers is particularly important, but has received little attention. As said, antibiotic-resistant bacteria are abundant in glacier ice from two Venezuelan glaciers. For instance, almost two thirds of the strains isolated in La Corona glacier (located at Mount Humboldt's Peak) were resistant to high doses of ampicillin (>100 μg/ml), andmore than half of the strains were resistant either to nalidixic acid, penicillin, or chloramphenicol (Ball et al. 2014).We showed in this study the presence of multiresistant isolates, since almost 60% of the isolates were simultaneously resistant to at least three different antibiotics, and more than 20% resisted five antibiotics. The same was true in the case of isolates reactivated from glacier ice collected at Pico Bolívar Glacier (Rondónet al. 2016). Furthermore,a high proportion of the isolates were also able to grow in the presence of high doses (i.e. 100 ppm) of several toxic metals, including Ni++, Zn++, and Cu++. It is a well-known fact that ARGs are often located within mobilizable plasmids (Pal *et al.,* 2015). In line with this fact, we were able to detect low- and high-molecular-weight plasmids in approximately one half of the strains tested. To date, almost no further information has been published concerning this topic, even though it is of primary importance considering the impact that the dissemination of these resistance genes in natural ecosystems might have. On the other side, in 2015 we discovered that some of the Pseudomonas spp. isolates behaved, in vitro and at low temperatures, as plant growth promoting bacteria (PGPB) (Balcazar *et al.,* 2015). The traits exhibited by the isolates included the ability to dissolve mineral phosphates, to synthesize plant hormone-like compounds(like indol-acetic acid), and the ability to produce highly microbicide volatile metabolites, such as hydrogen cyanide. Confirmation of these biocontrol traits came from co-cultivation experiments performed in the presence of three phytopathogens (*Pythiumultimum, Fusariumoxysporum,* and *Phytophthorainfestans*). As a result, we hypothesized that some of these strains could be valuable for developing cold-active biofertilizers. This hypothesis received experimental confirmation in 2019, when Rondónet al. reported that wheat (*Triticumaestivum*) seedlings, previously inoculated with some of the aforementioned Pseudomonas spp. isolates, developed longer roots and shoots. Further more, bacterized wheat plantlets, grown in sterile sand or soil at 15°C, were protected against P. ultimum infection. This report represents the first evidence, not only of the PGP abilities of ice-trapped bacteria, but also of their potential use for developing cold-active biofertilizers and/or biocontrol agents.

SUMMARY AND CONCLUSIONS

In the last ten years, relevant information has been added to the literature, concerning the microbiology of Tropical Andean glaciers. The most important results confirm that these glaciers are, indeed, colonized by an abundant and diverse community of bacteria and fungi, and that psychrophilic heterotrophs can be readily isolatedin vitro and at low temperatures. Some of these isolates exhibit important traits, such as resistance (and multiresistance) to antibiotics, which can be considered relevant from a public health perspective. On the other side, some isolates were also shown to act as PGPB, both in vitro and in plantae experiments. These results indicate that these glaciers harbored bacteria which can be considered either as our potential "friends" or as our potential "foes". Obviously, more work is needed to confirm such assumptions, and to properly describe the main characteristics of these microbial communities. Unfortunately, most of these projects had to be interrupted due to the socio-political and economic crisis that has affected my country for almost two decades. Even though some enthusiastic and courageous young Venezuelan researchers continue to make their best to generate as much information as possible, they face serious limitations. It is surprising, however, that –with a few exceptions- almost no information has been published to date on this subject, since our group did so a few years ago. This is even more surprising if we take into account that, as I said a moment ago, we are dealing with glaciers on the verge of extinction. Mérida, were I lived and worked for more than 25 years, has traditionally been known as “the city of eternal snows”. When we began our work in 2012, the “eternal snows” could still be seen on top of the highest Andean peaks. Today, the glaciers vanished, and Venezuela has the dubious honor of being the first modern country to have lost all its glaciers. To many this is, sadly, the end of eternity.

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MICROBIAL BIODIVERSITY IN THE COPAHUEGEOTHERMAL REGION-AN EXTREME ENVIRONMENT DOMINATED BY AN ACTIVE VOLCANO IN CORDILLERA DE LOS ANDES

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ABSTRACT

The Copahue geothermal area is located on the Northwest of Neuquén province in Patagonia, Argentina, on the Cordillera de los Andes, one of the world's most important mountain systems. This naturally acidic extreme environment is dominated by the still active Copahue volcano, a stratovolcano of approx. 2965 m.a.s.l., whose cyclic eruptive periodshave shaped the landscape,thegeo-physicochemical characteristics of the place and the microbial diversity that inhabits it. The Copahue geothermal area, of approx. 250 Km^2, has two differentparts, both extreme environments: the Copahue volcano-Río Agriosystem and the geothermal ponds. Río Agrio is a natural acidic riverthat originates at two geothermal ponds a few meters below the volcano crater and runs down its hill maintaining the low pH values for almost its entire path, despite receiving many tributary neutral water courses.On the other hand, the geothermal manifestations,pools, ponds and hot springs with a wide range of temperature and pH, are a constantly changing environment, highly dependent on the volcanic activity. In this work wediscuss the rich biodiversity and complex community structure of the microbial species, bacteria and archaea, that inhabit the Copahue geothermal area, and their corelation with the geology and physicochemical characteristics. The Copahue-Caviahue geothermal system is a clear example of how the environmental and geochemical condition defined by a mountain, in this particular case, the Copahue volcano, determines the shape and dynamics of the native microbial communities.

INFLUENCE OF THE COPAHUE VOLCANO IN ITS SURROUNDINGS AND DESCRIPTION OF THE COPAHUE CAVIAHUE GEOTHERMAL SYSTEM

The Copahue Geothermal region is in the Northwest corner of the Neuquén province, in Cordillera de los Andes. Cordillera de los Andes occupies the western part of South America, bordering its entire coastline on the Pacific Ocean. It is 8500 kilometres long, making it the longest continental mountain system on Earth. It has the highest mountains outside Himalayas and presents many active volcanoes, among them, the Copahue volcano, which dominates the Copahue geothermal system. The Copahue Volcano is an imposing stratovolcano, characterized by its towering height of approximately 2,997 meters above sea level. Its distinct geological composition consists of alternating layers of volcanic ash, lava flows, and pyroclastic deposits, reflecting its complex history of past eruptions. Geographically, the volcano's coordinates are approximately 37.85°S latitude and 71.17°W longitude. The particular geology associated with the volcanic activity and the condensation of gases such as HCl (hydrogen chloride), H_2S (hydrogen sulfide), SO_2 (sulfur dioxide), make the Copahue geothermal region an environment rich in sulfur and iron compounds. Over the last few decades, Copahue has been a subject of heightened scientific interest due to a series of significant eruptions that have generated ash plumes that blanketed nearby areas, temporarily disrupting air travel and also generating ejection of pyroclastic material and volcanic gases, contributing to temporary shifts in atmospheric composition. These eruptions have led to alterations in local physicochemical conditions, including fluctuations in temperature, with hydrothermal springs reaching temperatures of up to 90°C during eruptive periods, a temperature that is close to boiling point of water at the area's height. Additionally, the release of volcanic gases during these eruptions has influenced pH levels, causing acidification of the water bodies of all the system. These environmental and landscape characteristics as well as theperturbationscaused by the volcanic activity havedirect impact in the diversity and dynamic of the microbial communities that inhabit the different geothermal water bodies and the waters of Río Agrio. For studying purposes, the Copahue-Caviahue geothermal region is divided in two areas: the Copahue volcano - Río Agrio system and the geothermal field. Fig 1 shows the location of the region in the map and images of both areas. In the crater of the Copahue volcano there is a very acidic lake with pH varying between 1.1 and 2.0 and temperatures ranging from 4°C to near the boiling point of water during eruptive periods. In fact, the lake disappeared after the eruption of the year 2000 and it has only been possible to sample from it in 2018.Río Agrio originates approximately 100 metres below the Copahue volcano crater as a very narrow course at two highly acidic geothermal affluents. The physicochemical conditions of these hot springs vary greatly with the volcanic activity; with temperatures fluctuating from 70 to 30°C and pH values from 0.5 to 2. After emerging from its geothermal source,the Upper Río Agrio (URA) rapidly cools down to approx. 15°C, a temperature that maintains all through its path. Regarding pH, waters remain acidic all through the course of the river, in spite of receiving many tributary courses mostly of snowmelt origin. The URA then flows down through a series of very beautiful water falls until it discharges in the Caviahuelake. The lake is from glacier origin, the waters are acidic (measured pH around 3), however due to the discharge of fluids from Caviahue village, emplaced in its west side, it is

Fig.1. Location of Copahue Geothermal field in Coordillera de los Andes (Argentina,South America).Left side: images of the Copahue volcano and the Río Agrio. Right side: images of the different geothermal manifestations

contaminated. After emerging from Caviahuelake, Lower Río Agrio (LRA) is wider and the borders are covered by red-orange rocks and sediments due to the precipitation of iron minerals produced by the rise in pH above 3 caused by the massive input of tributary courses of waters. Approximately 5 Km down the lake LRA has a spectacular waterfall, named Salto delAgrio. Then, the river continues its course slowly raising its pH for more than 40 Km when it finally reaches neutrality and merges with another mayor river. The other study area, the geothermal field, is even more influenced by the volcanic/geothermal activity. They are a series of pools, ponds and hot springs, some of them used for recreational or therapeutic purposes. Geological studies have determined that the waters in contact with the muds are acidic, with high concentration of ions, especially chloride and sulphate, and rich in sulfur. The geothermal manifestations are a much less stable environment that the river, the size, temperature, pH, shape and even presence of the ponds and hot springs vary significantly with the volcanic activity.

MICROBIAL BIODIVERSITY IN THE COPAHUE GEOTHERMAL SYSTEM

Our research group has studied the microbial diversity of this area for many years, using a variety of approaches, including enrichment cultures, high throughput molecular ecology techniques, and other culture-independent methods. This has allowed us to gain a comprehensive understanding of the microbial communities and their interactions with the environment.

Copahue volcano - Río Agrio system

The natural acidic waters of Río Agrio are inhabited by a quite diverse community of acidophilic, sulfur and/or iron oxidising prokaryotes that is richer near the origin of the river, where the environmental conditions are more extreme. As the river flows down the Copahue volcano hill and the environment gets more stable, the microbial community also stabilises and the better adapted species endure. According to microscopic cells recount done using fluorescence in situ hybridisation (FISH) with specific probes for bacteria and archaea and high throughput ampliconsequencing of the 16S rRNA gene, Archaea represent between a 20 and 40% of the prokaryotes of Río Agrio, nevertheless they have not been detected in the highly acidic crater lake of the Copahue volcano. At the Phylum levelthe archaeal diversity was represented by four phyla with a clear dominance of the genus Ferroplasma, comprised of extremely acidophilic, moderately thermophilic, iron oxidising, pleomorphic species, from the domainEuryarchaeota. Thanks to high throughput sequencing it was also possible to detect all over the URAAcidianuscopahuensis, a thermoacidophiliccrenarchaeota autochthonous of the Copahue geothermal system.Regarding Bacteria, the diversity was described by twenty-four phyla, with Proteobacteria being the most abundant phylum along the Copahue volcano-Río Agrio system, followed by Firmicutes. In order to gain deeper insights into the relationships among microbial species, their metabolic activities, and the interplay of physicochemical and geochemical factors, we conducted various biostatistical analyses to establish correlations within these variables.For instance, a canonical correspondence analysis between the points in the system, their physicochemical variables and microbial species showed in Fig. 2 B.clearly separated the Copahue volcano-Río Agrio system in three different habitats.One group is the URA, which positively correlates with temperature, organic matter, conductivity, iron, and sulphate concentrations and negatively correlate with pH.The points of the URA as well as the microbial species that inhabit there (represented by the blue dots) are very close together meaning that all the upper river is very similar in both environmental characteristics and biodiversity. On the other hand, the crater lake is clearly separated from the points of the URA, especially by its negative correlation with temperature, organic matter, conductivity, iron, and sulphate concentrations; it also shows its own biodiversity with only a few species shared with the other groups. Salto delAgrio, in LRA, is also separated from the URA, it correlates positively with pH and negatively with the other variables and, like in the other groups, the microbial species are very close together, sharing almost no taxa with the crater lake or the URA. These three environments are inhabited by different microbial communities, the relative abundances with the most representative species highlighted can be seen in Fig. 2 A. The crater lake is dominated by species related to Sulfuriferula, a neutrophilic and mesophilic genus able to oxidise a variety of sulfur compounds. The remaining speciespresented lower abundances and were mostly acidophiles, mesophiles, or moderately thermophiles related to sulphur or iron metabolism and found in other acidic environments including Río Agrio. Among them, Ferrithrix, extremely acidophilic, moderate thermophilic and obligate heterotrophic iron oxidisers, and Thiomonas, moderate acidophilic, mesophilic, quimioheterotrophicsulfur oxidisers, were only detected at the crater lake. The occurrence of species at pH and temperature conditionsthat are apparently distant from their optimum for growth can be explained byconsidering the changing nature of the lake. It could also be expected that the relative abundances of species vary greatly with pH and temperature modifications favouring the better adapted ones. In the particular case of Sulfuriferula and Ferrithrix, they have been detected by high-throughput sequencing in other environments with conditions similar to the crater lake and distant from their optimum. On the other hand, in the URA the microbial community is more stable. It is dominated by acidophilic, mesophilic or moderately thermophilic, sulphur and/or iron oxidising species, includingAcidithiobacillus, Sulfobacillus, Leptospirillum,Acidibacillus and the archaeonFerroplasma. Among these genera, some of the sequencesretrieved have the peculiarity of being distantly related to cultivated species (97% or less) but being 99–100% similar to different uncultured sequences found in other acidic environments such as Rio Tinto, hot springs, and mine tailings. Particularly, all the OTUs affiliated with the genus Acidibacillus were 99% similar to uncultured clones retrieved from Río Agrio but distantly related to cultivated species. In the same way, the archaealsequences affiliated with the family Thermoplasmataceae were 99–100% similar to sequences retrieved from the Copahue-Caviahue system and from an acid mine drainage in China; however, they were around 90% similar to cultivated species of the genus Thermoplasma. These findingssuggest that there may bepotential indigenous novel species or even new generayet uncultured butubiquitous and characteristic of acidic environments. Finally, the surroundings of the big waterfall Salto delAgrio had a completely different, much more diverse microbial community with no clear dominant members. There was a higher abundance of anaerobic species and no significant occurrence of the acidophiles found in the URA or the crater lake. Such differences could be explained considering the higher pH, lower Eh and iron concentration at this site. Despite the low abundances of the most common acidophiles in thehigh throughput sequencing data analysed, species related to iron and/or sulfur oxidising species like *Acidithiobacillus, Acidiphilium*, and *Leptospirillum* have been cultured from samples collected from Salto delAgrio.

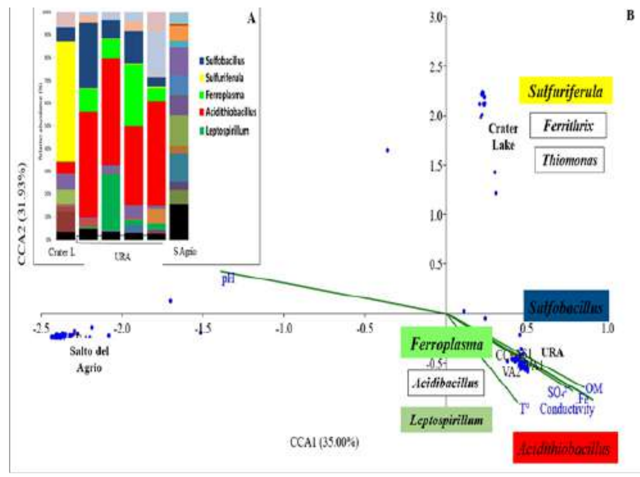

Fig 2. (A)**.** Relative abundance of bacteria and archaea in the Co- pahue volcano-Río Agrio system. (B)**.** Canonical correspondence analysis of microbial diversity and environmental parameters in the sampling points of the Copahue volcano-Río Agrio system.

Agrio system. B. Canonical correspondence analysis of microbial diversity and environmental parameters in the sampling points of the Copahue volcano-Río Agrio system.

Connecting the environmental and biodiversity results we were able to outline a geomicrobiological model of the URA, schematised and summarised in Fig 3A: the geothermal origin of the river and its closeness to the Copahue volcano provide a variety of sulfur and iron compounds, and work also as the reservoir of the bacterial and archaeal species that inhabit all the URA. The oxidation of these iron and sulfur compounds catalysed by the acidophilic bacteria and archaea helps to maintain the low pH all through the URA, in spite of the constant input of snowmelt and other neutral waters. Also, the metabolically catalysedsulfur oxidation justifies the grate amount of sulfate measured in the waters of Río Agrio. Among cations, Fe(II) is the most relevant, its concentration is quite high at the origin of the river, where the diversity of iron oxidisers is higher and then the concentration decreases, in part for the dilution but mostly because the slight increase in pH produces the hydrolysis and precipitation of Fe(III) compounds, that can be seen in the margins of the river. Such chemical process also releases H+ contributing to the maintenance of the acidic pH.

GEOTHERMAL FIELD

The geothermal manifestations are a much more complex environment, more dependent on the volcanic activity and with a wider range of ecological niches. Theunrooted phylogenetic tree of Fig.4Ashows the main species found in the water, sediments and biofilms of the geothermal ponds. The species marked in green, most of them photosynthetic, were detected exclusively on the biofilms. On the other hand, archaea were dominant in the higher temperature niches, represented by species of thermoacidophilic genera such as Sulfolobus and Vulcanisaeta and the autochthonous Acidianuscopahuensis. This distribution was confirmed by comparing the universal DAPI stain, in blue, with the images of the FISH assay using the archaea specific probe in the ponds of different temperatures. Figure 4 A and B, respectively, shows the charts with the cell's recounts using probes specific for Bacteria and Archaea domains and the hybridisations with the fluorescent probe specific for archaeaand DAPI images for different ponds and hot springs of high temperature. The moderate temperature pools were inhabited by sulfur and/or iron oxidising bacterial species, similar to those found in Río Agrio, such as Acidithiobacillus, Acidiphilium and Thiomonas and other species very common in geothermal environments like Hydrogenobaculum.

Based on the geological and volcanic characteristics of the area that led to the accumulation of different sulfur compounds and our findings that all ponds, pools and hot springs were inhabited by acidophilic, sulfurand/or iron oxidising species, either strict or facultative autotrophs, we proposed two geomicrobiological models.One for the moderate temperature niches (Fig. 3B) and other for the ones with high temperatures (Fig. 3C), bothspecially focused on the carbon and sulphur cycles.Basically, in both niches the metabolic processes and reactions are the same, the aerobic oxidation of sulfur compounds, autotrophically or at the expenses of organic matter, releases H+ contributing to the maintenance of the acidic conditions of ponds and pools and also releasing sulphate, which explains the high concentrations measured. In the sediments the sulphate reducing microorganisms (SRM) take care of the anaerobic reduction of such sulphate. Various SRM have been found in anaerobic sediments collected from different ponds and poolsfrom the Copahue geothermal field, including potential novel species, like Desulfotomaculumcopahuenis,Desulfosporosinussp. and-Desulfotomaculum sp. At certain pools and ponds with less extreme conditions, where the waters are quieter, biofilms and mats develop, and they are inhabited by photosynthetic species that contribute to carbon fixation.

CONCLUSION

In conclusion, our comprehensive study of the Copahue geothermal system over more than twenty years, with a particular focus on the influence of the Copahue volcano's activity, has shed light on the remarkable microbial diversity within this unique environment.The frequent eruptions of the volcano have not only disrupted local atmospheric conditions but have also significantly altered the properties of geothermal water bodies, including temperature, pH, and chemical composition.

Within the Copahue volcano - Río Agrio system, we observed distinct microbial communities inhabiting different habitats. The crater lake, characterized by extreme acidity and fluctuating temperatures, harbours sulfur-loving species and a minority of iron-oxidisers. In contrast, the Upper Río Agrio maintains a stable microbial community dominated

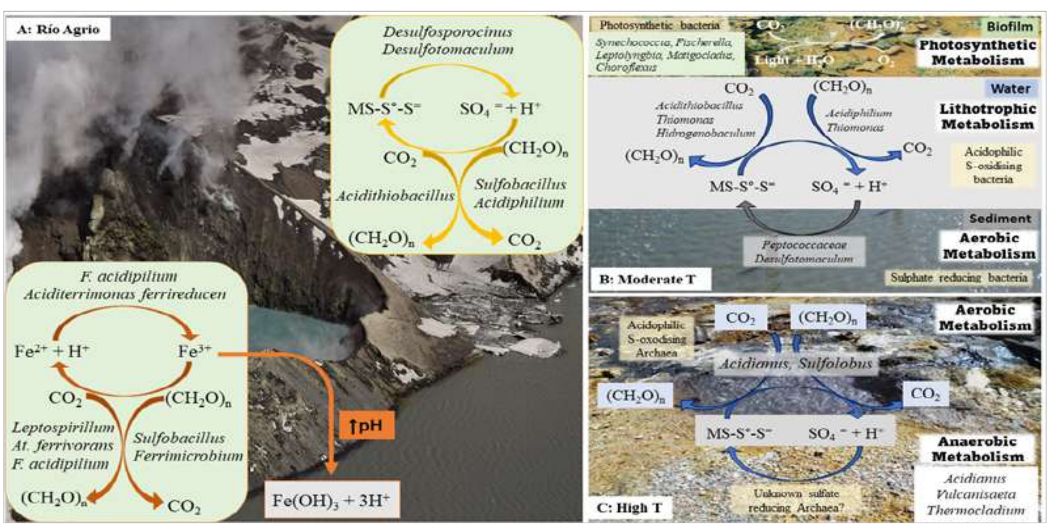

Fig. 3. Geomicrobiological models of the Río Agrio **(A)**. Geothermal ponds of moderate temperature **(B)**.geothermal ponds and hotsprings of high temperature **(C)**.

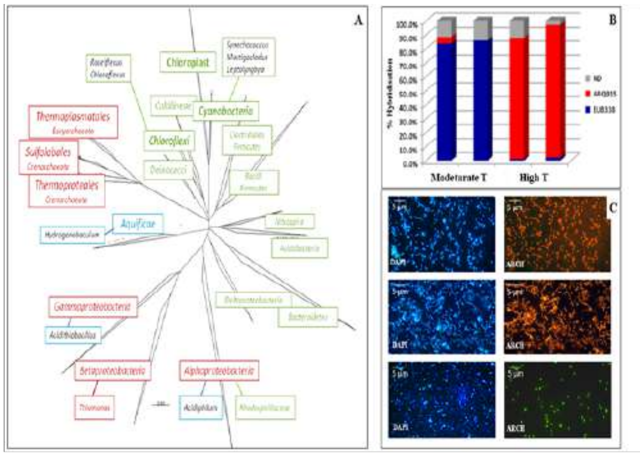

Fig.4. **(A).**Unrooted phylogenetic tree of the microbial species found in water, sediments and biofilms of different geothermal manifestations in the Copahue geothermal region. **(B).** Chart of the percentage of hybridisation with bacteria (EUB338) and archaea (ARQ915) specific probes in relation to the total number of cells stained with the universal DNA colorant DAPI in pools with moderate temperature (20 to 30°C) and high temperature (50 to 85°C). **(C).** Images of DAPI stain and hybridisations with the probe ARQ915 in different high temperature pools and hotsprings.

by acidophilic and thermophilic species such as Acidithiobacillus and Ferroplasma. Salto delAgrio, with its unique environmental conditions, hosts a diverse microbial community, including anaerobic species and a variety of iron and sulfur oxidizers.The geothermal field,highly influenced by the volcanic activity, exhibited even greater microbial diversity across various niches. Autochthonous archaea thrived in the higher temperature zones, while moderate temperature niches were inhabited by sulfur and iron oxidizing bacteria, similar to those found in the Río Agrio. Our findings suggest that the Copahue geothermal system is a dynamic and highly adaptable ecosystem. The influence of the Copahue volcano makes the whole area a captivating natural laboratory for understanding microbial ecology in extreme environments. Our research contributes to a deeper comprehension of the intricate relationships between microorganisms, their environments, and the biogeochemical processes that drive this unique extreme ecosystem.

ACKNOWLEDGEMENT

We specially thank the Organising Committee of the International Conference “Mountain ecosystems: Biodiversity and adaptations under Climate Change scenario” and the Guest Editors of this Special Issue for the invitation to participate and the opportunity to share our work.

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DISTRIBUTION AND ANTI-INFECTIVE PROPERTIES OF CULTURABLE ACTINOBACTERIA FROM THE INDIAN HIMALAYAN REGION(IHR)

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ABSTRACT

The aim of this study is investigating the distribution of culturable actinobacteria in the selected Indian Himalayan region (IHR) and its evaluation for anti-infective properties. The anti-infective potential activity of HS2C1 which was isolated from three different altitude of Himalayan region was confirmed by agar plug method. Based on the results, the strain HS2C1 showed broad spectrum of activity except against Candida sp. Active metabolite from Streptomyces sp. HS2C1 exhibited 77% inhibition at 25 ug/mL concentration against Escherichia coli and 56% against Acinetobacter baumanniiwith low IC50 values. Also, it exhibited anti-biofilm property against carbapenem resistant E. coli for about 84% of biofilm inhibition at 200 ug/mL concentration. Based on their phenotypic and molecular traits, taxonomic position of the potential strain HS2C1 was confirmed as Streptomyces rochei HS2C1. To conclude, S. rochei HS2C1 isolated from KMVN base camp of Himalayan region is a promising source for anti-infective molecules with anti-bacterial and anti-biofilm properties.

Keywords: Anti-bacterial activity, Anti-biofilm activity, Carbapenem resistance, Darma valley, Himalayan region, Streptomyces rochei

INTRODUCTION

Opportunistic infections by the pathogens of Enterobacteriaceae family can easily infect both healthy and compromised hosts, causing Urinary Tract Infection (UTI), wound infections, pneumonia, gastroenteritis, and meningitis, and contributing to mortality and morbidity worldwide (D'Agostino and Cook, 2016). The family Enterobacteriaceae are gram-negative facultative anaerobic bacteria and are members of the ESKAPE pathogenswhich includes Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter baumanniithat are life-threat to human beings (Mancuso *et al.,* 2021). Bacterial infections are treated with conventional antibiotics. However, the emergence of carbapenem-resistant infections, poses a potential threat to the patient's life as the majority of the current antibiotics fail to work on those multidrug resistance microorganisms (MDR) as a result of the overuse of antibiotics (Murray *et al.,* 2022). Exopolysaccharide (EPS) production and the formation of biofilm by A. baumannii, P. aeruginosa, K. pneumoniae, and E. coli worsen this situation. According to Sankhwaret al. (2023) and Zhou *et al.,* (2015), a biofilm is a group of microorganisms that is encased in an extracellular polymeric matrix. EPS has a complex structure made up of multiple layers of cells and extracellular material, which can easily hinder the penetration of antimicrobial substance to access the biofilm's cells. They can therefore be resistant to antibiotic treatment and are a leading global cause of persistent and recurrent infections brought on by clinically important pathogens. (Qian *et al.,* 2021). The development of new antibacterial drugs is urgently required to combat the biofilm organisms due to the severity of biofilm infections and the lack of effective antibiotics against biofilm associated infections. According to Sankhwar *et al.,* (2023) and Maiti *et al.,* (2020), microorganisms that produce natural bioactive compounds thrive in Himalayan soil, which has less or negligible anthropogenic activity. The genus Actinomycetes contain the majority of the antimicrobial compounds that have been studied so far. Actinobacteria are characterized primarily by their production of secondary metabolites, which is influenced by diverse environmental conditions. Actinomadura, Actinoplanes, Amycolatopsis, Marinispora, Micromonospora, Nocardiopsis, Saccharopolyspora, Salinispora, Streptomyces, and Verrucosispora are the main genera of actinobacteria with the greatest potential to produce commercially significant bioactive natural products (Selim *et al.,* 2021). In the genome of Streptomyces, the biosynthetic gene clusters are the major factors which are responsible for the identification of the majority of the antibiotics. Recent research suggests that variations and diversity in BGCs were responsible for the various secondary metabolite productions in closely related strains. This recombination may have occurred as a result of differing environmental conditions causing inter- and intra-species variation. According to Belknap *et al.,* (2020), polyketide synthase (PKS-I), non-ribosomal peptide synthase (NRPS), and lanthipeptides are the BGCs in Streptomyces that are widely studied. Since terrestrial soil has been exhausted because of low metabolite content and the repetitive isolation of the same strains, we have explored the natural soil of the Himalayan region with negligible anthropogenic activities for the isolation of novel and broad-spectrum antibiofilm agents.

MATERIALS AND METHODS

Collection and Pre-treatment of soil samples

Soil samples were collected from three different altitudes of the Indian Himalayan region of Drama Valley, Uttarakhand state, India in clean plastic bags and shifted to the laboratory and preserved at 4 ± 1 °C. About 15 g of the soil sample was weighed and dried at room temperature for 3 days. (Radhakrishnan *et al.,* 2016).

Isolation of Actinobacteria

Actinobacteria was isolated by adopting standard spread plate method using different media such as nutrient agar (NA), actinomycete isolation agar (AIA), starch casein agar (SCA) which was incorporated with Nalidixic acid and Nystatin. About 1 g of soil sample was added into 9 mL of sterile distilled water in 100 mL conical flask. It was kept in shaker for 30 min and serially diluted upto 105 level of dilutions in sterile distilled water. Hundred μL aliquot from 103, 104 and 105 dilutions were taken and evenly spread on all the isolation agar plates and kept incubated at 28 °C for one month. After incubation, the colonies with suspected actinobacterial morphology were selected and inoculated in International Streptomyces Project (ISP) 2 agar plates. After incubation at 28°C for 7 days, growth, consistency, presence of aerial mycelium, colour, reverse side pigment and soluble pigment production of all the cultures were recorded (Shirling and Gottlieb, 1966). The pure cultures were preserved in ISP 2 agar slants as well as in 20% glycerol broth at 4oC and -80oC, respectively.

Primary screening of actinobacteria for antimicrobial activity bioactive metabolites from isolated actinobacterial cultures were produced by agar surface fermentation. All the isolates were inoculated into ISP 2 agar plates and incubated at 28°C for 10 days for the production of secondary metabolites. The fresh colonies of test pathogens were inoculated into Muller Hinton Broth (MHB) and incubated at 37°C for 24 h. Standardized inoculum was prepared by adjusting the overnight culture with 0.5 MacFarland standard (1*108 CFU/mL; OD600 = 0.08 to 0.1). Antimicrobial activity of Actinobacterial cultures were tested by adopting agar plug method (Soundarya *et al.,* 2020). In brief, the test pathogens were swabbed on Muller Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) plates using sterile cotton swab. Agar plugs with 6 mm in diameter were cut from ISP2 agar plates grown with actinobacterial culture and were placed over agar plates seeded with test pathogens. All the plates were incubated at 37 °C for 24 h and observed for zone of inhibition.

Effect of fermentation method on secondary metabolite production

Based on the results of primary screening, strain HS_2C_1, which showed broad spectrum antimicrobial activity, was prioritized as potential culture for further studies. Effect of solid state and submerged fermentation on bioactive metabolite production by the strain HS_2C_1 was investigated (Soundarya *et al.,* 2020). Spores of the strain HS_2C_1 was inoculated into ISP_2 agar plates as well as in 100 mL of ISP_2 broth. The plates were incubated at 28 °C for 10 days whereas the broth culture was incubated in rotatory shaker with 95 rpm at 28°C for 10 days. For every 24 h, agar plugs from the plates were tested against the target pathogens whereas 2 mL of broth culture was collected from shake flask and centrifuged at 10,000 rpm for 10 min. The resultant cell free supernatant (CFS) was also tested against the target pathogens by adopting well diffusion method.

Extraction of bioactive metabolites

Secondary metabolite production from the potential strain HS_2C_1 was performed using submerged fermentation. The strain was pre cultivated by inoculating their spores into 100 mL of ISP_2 broth and incubated for 4 days at 25 °C in rotary shaker at 95 rpm. About 10% of seed culture was used to inoculate 500 mL of ISP_2 media in 1L Erlenmeyer flask, followed by incubation in a rotary shaker at 160 rpm for 6 days. After the production, CFS was collected by centrifuging at 7500 rpm for 15 min at 4 °C. Crude extracellular metabolites from the CFS were extracted using ethyl acetate at 1:1 ratio. The solvent phase was concentrated using rotary evaporator to get the crude extract (Hussain *et al.,* 2018).

In vitro Anti-infective study

Determination of Minimum Inhibitory concentration (MIC)
The MIC of the crude extract from the potential strain HS_2C_1 against the selected pathogens was determined by microbroth dilution method using resazurin dye as per CLSI pro-

tocols (Qian *et al.,* 2020; Elshikh *et al.,* 2016; CLSI 2012). The optical density (OD) reading at 600 nm was measured after incubation using UV spectrophotometer (Bioteck – EPOCH 2) and compared with negative control (NC). The percentage of antimicrobial activity was calculated by using the formula:

$$\text{Percentage of inhibition} = \frac{OD_{600}\text{ of NC} - OD_{600}\text{ of Test}}{OD_{600}\text{ of NC}} \times 100$$

Then, 20 µL of 0.015% resazurin dye was added to all the wells and incubated at room temperature for 30 min and observed for colour change from blue to pink. The MIC was defined as the lowest concentration at which the microbial growth was inhibited. The IC50 value of active extract of HS_2C_1 was defined as the smallest proportion of ethyl acetate extract at which the growth of microorganism was inhibited by 50%.

ANTI-BIOFILM ACTIVITY OF HS_2C_1 EXTRACT

Anti-biofilm activity of the extract HS_2C_1 against biofilm pathogens (E. coli, K. pneumoniae, P. aeruginosa and A. baumannii) was determined by performing crystal violet microtitre plate (CV-MtP) assay (Thenmozhi *et al.,* 2009). To perform the experiment, bacterial suspension was prepared in Trypsin soy broth (TSB) incorporated with 1% glucose and adjusted to 0.5 McFarland standard. Hundred µL of sterile TSB was loaded in all the wells. Hundred µL of 100 µg/mL concentration of extract was added to the first well and two-fold dilution was done until the desired concentration was achieved. Then, 50 µL of standardized inoculum was added to all the wells except blank. The well with broth + gentamicin (30 µg/mL) + pathogen was served as positive control (PC) while broth + pathogen was growth control (GC) and broth + 10% DMSO + pathogen well serve as carrier solvent control. All the plates were incubated at 37 °C for 24 h. After incubation, the spent media was discarded by gently tapping the plate and rinsed with 1X PBS for twice and dried effectively. Later, 200 µL of 0.1% crystal violet dye was added to all the wells and incubated at room temperature for 30 min to stain the biofilm in the wells. The dye was discarded and washed with distilled water to remove excess dye and dried. To resolubilize the stained wells, 200 µL of 95% ethanol was added in each well and the intensity of the colour was estimated by measuring the absorbance at 570 nm. The percentage of biofilm inhibition was calculated using the below formula:

CHARACTERIZATION AND IDENTIFICATION OF POTENTIAL STRAIN

$$\text{Percentage of biofilm inhibition} = \frac{OD_{570}\text{ of GC} - OD_{570}\text{ of Test}}{OD_{570}\text{ of GC}} \times 100$$

Micromorphology

Micromorphological characteristics of potential strain HS_2C_1 was studied by adopting slide culture method. The recorded microscopic characteristics include the presence of aerial and substrate mycelium, mycelial fragmentation and the details of spore chain morphology. Spore structure and spore surface details were recorded using light microscope (Radhakrishnan *et al.,* 2014). Cultural characteristics were studied by inoculating the growth of actinobacteria strain HS_2C_1 into different ISP media such as tryptone agar (ISP_1), yeast extract-malt extract agar (ISP_2), oatmeal agar (ISP_3), glycerol-asparagine agar (ISP_5), tyrosine agar (ISP_7), AIA and SCA. All the media were prepared by following the guidelines described by Shirling and Gottlieb (1966). All the plates were incubated for 10-14 days at 28°C. Cultural characteristics recorded include the nature of growth, consistency, aerial mass colour, presence of reverse side pigment and the details of soluble pigment production, if any.

Physiological Characteristics

Basal agar medium recommended by Shirling and Gottlieb (1966) was used for carbon utilization studies. After sterilization of basal agar medium by autoclaving at 15 lb for 15 minutes, about 1% of various filter sterilized sugars were added and poured into petri plates. Well grown culture of actinobacterial strain HS_2C_1 was inoculated in different sugar containing basal agar plates. Growth was recorded after 10 days of incubation at 28 °C. The same procedure was used to study the utilization of nitrogen sources by the potential actinobacteria strains. Sugars used in this study include glucose, lactose, xylose, mannitol starch, inositol, raffinose (Hi media). Nitrogen sources used include soyabean, malt extract, peptone, yeast extract, KNO_3. Effect of different mineral source (NaCl, $MgSO_4$, $FeSO_4$, $MnCl_2$, $CaCl_2$) and pH (5 to 9) and temperature (20°C, 25°C, 35°C and 40°C) on growth was determined by inoculating the actinobacterial strain HS_2C_1 onto ISP_2 agar medium with respective physiological condition. All the plates were observed for growth after 10 days of incubation.

Molecular characterization

The molecular characterization of the strain HS_2C_1 was done by performing 16s rRNA gene sequence analysis at Bioserve Biotechnologies Ltd, Hyderabad. The genomic DNA of potential strain HS_2C_1 was extracted using Solute ready genomic DNA kit (Gen EluteTM). The polymerase chain reaction (PCR) amplification of the 16s rRNA gene was carried out in Eppendorf Master Cycler Gradient by using primers 27F (5-AGA GTT TGATCC TGG CTC AG-3) and 1492R (5-GGT TAC CTTGTT ACG ACT T-3) (Lane, 1991). The final volume of reaction mixture of 50 µL contained 100 ng of genomic DNA (2 µL), 10x buffer (5 µL), 1 mg ml_1 of BSA (4 µL), 2.5 mM of dNTP mixture (1 µL), 3.5 U of Taq DNA polymerase and 10 μM of each primer (1 μL). Conditions of PCR is as follows: 95 °C for 5 min followed by 35 cycles at 95 °C for 30 sec, at 58 °C for 45 sec and at 72 °C for 1 min, finishing with extension at 72 °C for 7 min. The 16s rRNA gene sequencing of purified PCR product was done by Sanger sequencing method. To edit the sequences and assemble the contigs, DNA baser software version 3 was used. The edited sequences were then compared with previously deposited sequences in the GenBank database by BLAST analysis. With MEGA 11 software, the phylogenetic tree was constructed using neighbour joining algorithm (Saitou and Nei, 1987). Bootstrap analyses with 1000 replications were used to determine the confidence values for the phylogenetic tree branches (Felsenstein, 1985). The partial 16s rRNA nucleotide sequence of the strain HS_2C_1 was deposited at GenBank database by using Bankit tool (http://www.ncbi.nlm.nih.gov/WebSub/?tool=genbank).

RESULTS AND DISCUSSION

Actinobacteriaisolated from a variety of natural habitats, remain the most promising candidates for the development of new antibiotics. However, research on actinobacteria from Himalayan ecosystems is less explored with special reference to anti-infective drug discovery (Prudence *et al.,* 2020). To explore bioactive actinobacteria, a total of 38 actinobacterial cultures were isolated from soil samples collected from three different locations in the Indian Himalayan region (IHR) (Fig.1 & Table-1). In general, unicellular bacterial and fungal culturesoutgrow the number of actinobacterial culture. However, the heat treatment method and antibiotics supplemented in this study substantiated the growth of unwanted bacterial and fungal colonies and supported the recovery of actinobacterial culture in more numbers. Majority of the recovered actinobacterial cultures looks like Streptomyces in cultural and micromorphology. Most of the isolated actinobacterial cultures showed powdery growth with aerial and substrate mycelium (Table-2). Streptomyces speciesare reported to produce wide range of pigments with different

Fig. 1. Soil samples from snow covered Darma valley, Indian Himalayan region (IHR)

Table 1: Details of samples collected from Indian Himalayan region (IHR)

S. No	Sampling site	Latitude	Longitude	Altitude	No. of Isolates
1.	Zero point	30°13’25” N	80°29’41” E	3757 m	9
2.	KMVN base camp	30°14’31” N	80°31’30” E	3417 m	10
3.	Dugtu Village	30°14’55” N	80°32’35” E	3218 m	19

biological activities (Meng *et al.,* 2021). Similarly, in the present study also five cultures were observed to produce extracellular pigments. According to Berdy (2012), only 1.5% of antibiotic compounds are active against gram negative bacteria while a large number of antibiotic compounds show exclusive activity against gram positive bacteria. Biological and metabolic activities as well as diversity of actinobacteria are significantly influenced by the ecophysiological parameters of a given ecosystem (Muralidharan *et al.,* 2021). In the present study, out of 38,23 actinobacterial strains showed activity against at least any one of the tested pathogens (Table-3). The antimicrobial metabolites producing actinobacterial strains were pre-dominantly recovered from Dugtu and Camp region. About 16 isolates (42%) produced antimicrobial activity against *M. smegmatis*, 14 isolates (37%) were against gram positive; 19 (50%) showed activity against gram negative and one showed antagonistic activity against Candida sp. Among other isolates, HS_2C_1, HS_2C_5, HS_2D_1, HS_2D_3 and HS_2D_{30} showed maximum antimicrobial activity. Moreover, the isolate HS_2C_1 showed the highest level of inhibition against carbapenem resistant *A. baumannii* and *E. coli* with a zone of inhibition of 12 mm in diameter. Fermentation is a cost-effective way of mass production of bioactive molecules. There are multitude antibiotics that are produced by fermentation process (Subramaniyam and Vimala, 2012). Therefore, fermentation technology must be decided based on the strain that is being used for production. In the present study, cell free supernatant (CFS) of HS_2C_1 by submerged fermentation showed maximum zone of inhibition of around 17-19 mm of test pathogens when compared to surface fermentation. Thus, the crude bioactive metabolites were obtained by submerged fermentation which results in good extraction of bioactive metabolites around 60 mg in 100 mL of ISP_2 medium at 6 days of incubation when extracted with ethyl acetate. The medium polar solvent ethyl acetate is used to extract the majority of actinobacterial metabolites, which are extracellular in nature (Gangotry *et al.,* 2022) (Fig- 2&3). In order to highlight the antimicrobial potency of the active strain HS_2C_1, the interaction of antimicrobial metabolite with test pathogens were studied by MIC. The MIC values of ethyl acetate extract of HS_2C_1, were 25 μg/mL (77%) against E. coli CRH. It was also effective against A. baumannii CUM with an MIC of 200 μg/mL. Here, standard strains (i.e.,) E. coli ATCC and A. baumannii MTCC were employed for subsequent experiments.

The inhibitory effects of natural products against E. coli and A. baumannii pathogens have previously been reported in several studies. The IC50 value was found to be around 12.5 μg/mL (58%) against E. coli. The IC50 value of gentamicin was found to be 30.00 μg/mL against E. coli respectively. Ac-

Table 2. Characteristics of isolated actinobacteria

Strain no	Sampling site	Media	Growth	Consistency	AMC	Reverse pigment	Soluble pigment
HS_2D_1	Dugtu	NA	Good	Powdery	white	white	-
HS_2D_2	Dugtu	NA	Good	Powdery	white	yellow	-
HS_2D_3	Dugtu	NA	Good	Powdery	light grey	yellow	-
HS_2D_4	Dugtu	NA	Good	Powdery	grey	light brown	-
HS_2D_9	Dugtu	NA	Good	Leathery	dark grey	light grey	-
HS_2D_{14}	Dugtu	AIA	Good	Leathery	brown	brown	+
HS_2D_{15}	Dugtu	AIA	Good	Leathery	orange	yellow	+
HS_2D_{16}	Dugtu	AIA	Good	Powdery	White	White	-
HS_2D_{18}	Dugtu	AIA	Good	Powdery	white	yellow	-
HS_2D_{20}	Dugtu	AIA	Good	Leathery	white	yellow	-
HS_2D_{25}	Dugtu	SCA	Good	Powdery	white	yellow	-
HS_2D_{26}	Dugtu	SCA	Good	Powdery	white	orange	-
HS_2D_{28}	Dugtu	SCA	Good	Powdery	Grey	Pale brown	-
HS_2D_{29}	Dugtu	SCA	Good	Powdery	light brown	light brown	-
HS_2D_{30}	Dugtu	SCA	Good	Leathery	white	brown	-
HS_2D_{31}	Dugtu	SCA	Good	Powdery	grey	white	-
HS_2D_{33}	Dugtu	AIA	Good	Powdery	white	yellow	-
HS_2D_{34}	Dugtu	AIA	Moderate	Leathery	Pale brown	Pale brown	-
HS_2D_{35}	Dugtu	SCA	Good	Leathery	dark grey	white	-
HS_2D_{36}	Dugtu	AIA	Good	Leathery	yellow	white	-
HS_2C_1	Base camp	NA	Good	Powdery	Grey	Pale yellow	-
HS_2C_5	Base camp	NA	Good	Powdery	brown	yellow	-
HS_2C_6	Base camp	NA	Good	Powdery	grey	light brown	-
HS_2C_7	Base camp	NA	Good	Leathery	white	white	-
HS_2C_8	Base camp	NA	Good	Powdery	dark brown	brown	-
HS_2C_9	Base camp	SCA	Good	Leathery	brown	dark brown	-
HS_2C_{12}	Base camp	SCA	Good	Leathery	grey	grey	-
HS_2C_{15}	Base camp	AIA	Good	Leathery	yellow	orange	-
HS_2C_{19}	Base camp	AIA	Good	Leathery	light grey	brown	-
HS_2C_{20}	Base camp	AIA	Good	Leathery	yellow	yellow	-
HS_2Z_9	Zero point	AIA	Good	Leathery	Orange	Yellow	+
HS_2Z_{11}	Zero point	AIA	Good	Leathery	yellow	Pale orange	+
HS_2Z_{12}	Zero point	SCA	Good	Powdery	Grey	dark brown	-
HS_2Z_{14}	Zero point	AIA	Good	Powdery	white	Yellow	+
HS_2Z_{16}	Zero point	AIA	Good	Powdery	Pale grey	Brown	-
HS_2Z_{19}	Zero point	SCA	Good	Powdery	Pale grey	white	-
HS_2Z_{20}	Zero point	SCA	Poor	Rough	Fade white	Pale brown	-
HS_2Z_{21}	Zero point	AIA	Poor	Rough	Fade white	Pale brown	-

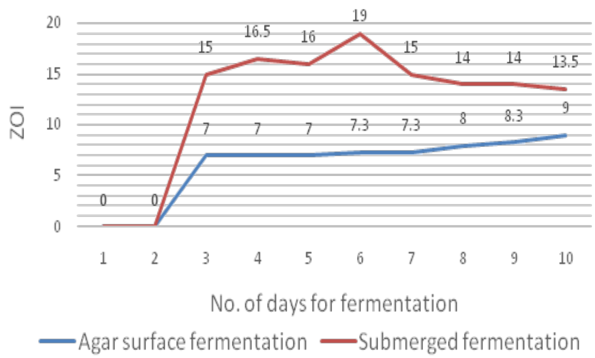

Fig.2. Effect of fermentation media against *E. coli*

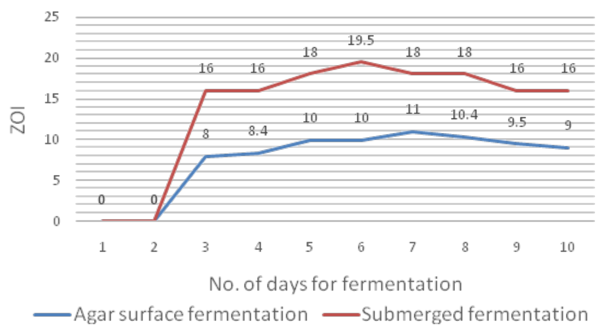

Fig.3. Effect of fermentation media against *A. baumannii*

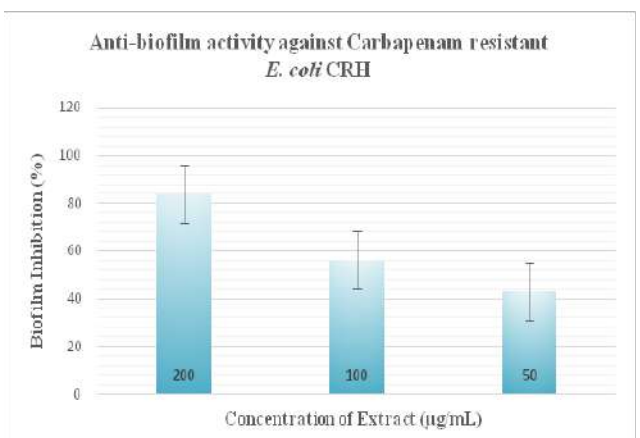

Fig. 4. Percentage of biofilm inhibition using CV-MtP assay

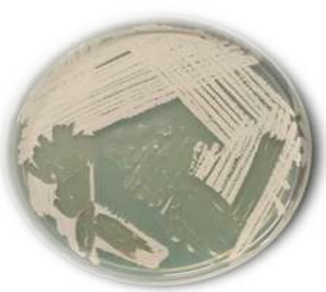

Fig.5. Culture morphology of the potential actinobacteria HS_2C_1

cording to earlier literature, bioactive ansamycins produced by a Streptomyces sp. from the hyper-arid desert (Salar de Atacama) exhibit promising antibacterial activity against methicillin-resistant S. aureus (Rateb *et al.,* 2011). Two novel antibiotic compounds from Saccharothrix SA198, isolated from Saharan desert soil, have also been reported by Boubetra *et al.* (2013). These antibiotic compounds exhibit strong activity against Mucor ramannianus and Aspergillus carbonarius and moderate activity against both gram-positive and gram-negative bacteria.Biofilm mediated resistance and chronic infections caused by drug resistant biofilm forming bacteria create chaos on human beings, animals and other organisms (Rather *et al.,* 2021). They also contribute to environmental threats by creating biofouling related issues. Previous research by Mi *et al.,* (2018) revealed that bacteria in biofilms are up to 1,000 times resistant antibiotics than their planktonic counterparts. Due to the ability of these compounds to inhibit initial adherence and prevent subsequent attachment of pathogens that form biofilms, which is a necessary condition for the growth of a persistent biofilm, antibiofilm agents hold promise for a better future (Padmavathi & Pandian, 2014). Some of the antibiofilm compounds identified so far even have the ability to disrupt pre-formed mature biofilms by disposing the extracellular polymeric substances (Panlilio and Rice, 2021). Our findings demonstrated that the metabolites from HS_2C_1 exhibit active biofilm property that could assist in combating the biofilm resistance. At 200 µg/mL concentration, 84% of E. coli biofilm was inhibited when compared with untreated biofilm (Fig. 4). The treatment of HS_2C_1 extract at IC50 resulted in a considerable reduction (56-84%) in the biofilm formation. Overall, the extract inhibited the formation of biofilm by E. coli in a dose dependent manner. Many studies proved that Actinobacteria are amongst the potential sources that could be utilized for the development of anti-biofilm compounds (Amin *et al.,* 2020). Slide culture method revealed that the substrate mycelium was filamentous and the reproductive aerial mycelium had a spiral appearance. The growth patterns and appearance of actinobacteria in various kinds of culture medium are referred to as their culture characteristics. International Streptomyces Project (ISP) 2 - 5 are the standard culture media for morphological study for all Streptomyces (Shirling and Gottlieb, 1966). However, actinobacteria can also be identified and preserved using Starch Casein Agar (SCA) medium (Syiemiong and Jha, 2019).The cultural characteristic of HS_2C_1 revealed that the isolate grew well on several media include ISP 1, 2, 3, 5, 7, SCA and AIA (Fig. 5). The aerial mycelium colour was varied from grey to greyish brown in most of the tested media. The consistency of the isolate was almost powdery. Reverse side of colony was greyish yellow to yellowish brown in colour. No soluble pigment was found in any of the medium. This morphological growth and change were observed after 10 days of incubation at 28 ± 2 °C strictly according to the procedures done in International Streptomycesproject. The same outcomes were reported by Saravanan *et al.,* (2022).The taxonomic characterization and identification of distinct Streptomyces sp. have been based on the morphological and biochemical parameters and were acquired from the physiological test suggested by Siddique *et al.,* (2014). The strain isolated at mesophilic temperature (25–37 °C) was consistent with the results of the earlier research, proving that the majority of Streptomycesstrains are amenable for isolation under these conditions. A variety of physiological characteristics were carried out to have a comprehensive view of the phenotypic characteristic of potential strains. Our study was in concurrence with Saravanan *et al.,*(2022), the isolated strain HS_2C_1 has utilized all the sugars as a sole carbon source. At the same time, all the nitrogen sources supported the growth of the isolate HS_2C_1. The strain grew well under mesophilic conditions (25 – 40 °C) at pH 5 to 9. The morphological and physiological characteristics revealed that the potential strain HS_2C_1 belongs to Streptomyces sp. which was further confirmed with 16s rRNA gene amplification and the resulting sequences of 908 bp in length was recognized as Streptomyces sp. Based on the Tamura – Nei model, the phylogenetic tree for the potential strain HS_2C_1 along with its closest relatives of genus Streptomyces is shown in Fig. 6. It is accurately depicted that the strain HS_2C_1 was closely clade with Streptomyces rochei MN795133.1 (99.89%). Based on the results, the strain HS_2C_1 was identified as Streptomyces rochei. The gene sequence of the potential strain Streptomyces rochei HS_2C_1 was submitted to NCBI GenBank with the accession number OQ975947.1.

CONCLUSION

The present findings showed that the Himalayan ecosystem is a promising source for bioactive actinobacteria and the Streptomyces rochei HS_2C_1 from this ecosystem is a promising strain for the isolation of bioactive compound for clinical important carbapenem resistant strains.

ACKNOWLEDGEMENT

The authors acknowledge the management of Sathyabama Institute of Science and Technology, Tamil Nadu, India for the research facilities provided. Thenmozhli Geetha Saravanan and Radhakrishnan Manikkam thank the Department of Biotechnology (DBT), New Delhi for their support in the form of research grant (BT/PR41474/NDB/39/760/2020 dated 28.09.2021).

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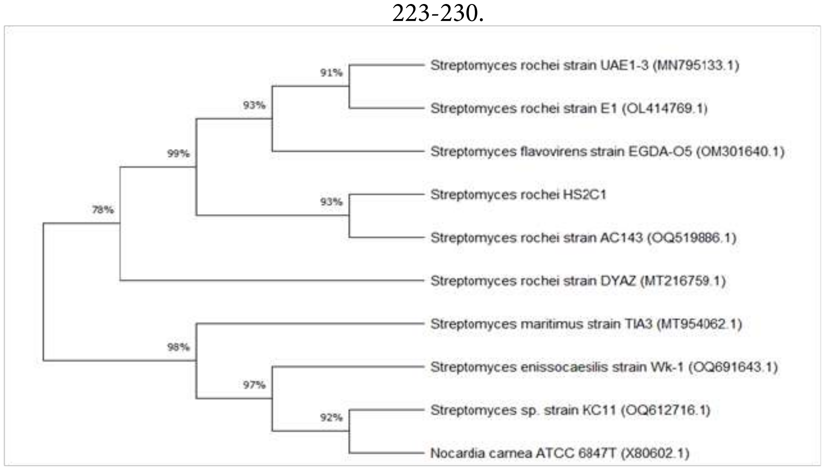

Fig.6. Neighbour joining (NJ) phylogenetic tree based on 16S rRNA gene sequences comparing potential Streptomyces strain HS2C1 to other Streptomyces sps collected from NCBI BLAST.

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DEVELOPMENT PERSPECTIVES AND MOUNTAIN DEVELOPMENT APPROACH

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ABSTRACT

Development is an easy word to say, but a difficult and complicated one to define. Although, the term is used abundantly in common parlance, as well as in academic discourse, yet its understanding and interpretations has always remained as a debated issue among the people, planners, decision makers and researchers. But, in the context of mountains development has been not in conformity with the mountain environment and society. Mountains are among the most disadvantaged regions in the world, with the highest poverty rates and some of the greatest vulnerability to global climatic, environmental, and socioeconomic change and related risks. The existing challenges of and increasing pressure on mountain people and resources enforce unsustainable land management practices and land abandonment, which in turn might imperil the provision of key mountain services. As the world's second-most-populous country and one of its fastest-growing economies, India faces both unique challenges and unprecedented opportunities in the sphere of development in general and mountain in particular. In this paper an attempt is being made to understand global and national development perspectives and then what approach should be adopted for mountainous regions in general and Himalayan in particular.

INTRODUCTION

Development Perspectives

Development is an easy word to say, but a difficult and complicated one to define. Although, the term is used abundantly in common parlance, as well as in academic discourse, yet its understanding and interpretations has always remained as a debated issue among the people, planners, decision makers and researchers. However, development and its related terms, remain fundamental to contemporary thinking. There is no agreement among developmental thinkers regarding a common definition of development. Some say it is an increase in income and productivity while others lay emphasis on the quality of life, provision of basic needs, happiness and wellbeing. The Millennium Development Goals as declared by the United Nations General Assembly on September 8, 2000 has also delineated the paradigm of development for the nation state, which includes, eradication of extreme poverty and hunger; achieve universal primary education; promote gender equity and empower women; reduce child mortality; improve maternal health; combat HIV/AIDS, malaria and other diseases; ensure environmental sustainability; and develop a global partnership for development. Now, it is clearly observed that development is not merely raising economic growth, but that it goes much beyond it, and economic growth is one of the components. As rightly described by Tadaro (1977) "development is both a physical reality and a state of mind in which society has, through some combination of social, economic, and institutional processes, secured the means for obtaining a better life". Thus, in order to achieve development, all societies must try to increase the availability and widen the distribution of basic life sustaining goods such as food, shelter, health, and protection to all members of society. Further, to raise levels of living, higher incomes, provide more jobs, better education, and more attention to cultural and humanistic values. These all are required not only to enhance material wellbeing but to generate greater individual and regional and national self-esteem.Now development scientists including V.R. Panchamukhi (1990) give more importance to New Paradigms of Development, which has the following observations about development: (i) development can no longer be identified as a process of shifting surplus labour from agriculture to the industrial sectors (ii) growth is no longer the guarantor for trickle down effects (iii) market as an instrument for ensuring optimum allocation of resources has been discredited (iv) concepts such as take off, big push, great support, backward and forward linkages, have also lost their essence. Some of facets of the development paradigm are that development not only means the increase of GNP, but includes aspects such as social, human, cultural, and political dimensions; the focus has shifted from the one sector model to dual sector models, for example, rural-urban; agriculture and industry linkages. The shift from a centralized to a decentralized model for faster development; inclusive development, where every individual participates in development processes, and exclusion is minimal; development with a humane face removing poverty, hunger, and human misery from society. Some of the most widely accepted components of development by the development economists and development scientists are increase in income and productivity; increase in social welfare through the provision of better healthcare and education; increased access to basic needs, i.e., food, clothing, and shelter; enhanced choices and opportunities; increase in income and employment opportunities; reduction of disparities; enhance quality of life of the people by way of increased and improved social services; reduction of poverty, malnutrition and diseases; increased access to potable drinking water and sanitation; provision of infrastructure facilities for better living conditions such as road, electricity, and information gadgets. Development planners and researchers have emphasized that development should also be people-centered; democratically organized; responsive to the whole environment, not only the ecological and the economic, but also political, social and cultural, and also be balanced between centre and periphery, between public and private, between the roles of men and women. Development must be human-centered, coming from within, rather than imposed from outside. With this brief development perspective background, the understanding of mountain specificities and their interrelationshipsmake it very difficult to correlate and proceed on development issues and integrated approach to mountain development. It is a well known fact that mountains are highly fragile ecosystems and important repositories of rich biological and cultural diversity. They are also established tourist destination and target areas for recreation of diversified cultural heritage. Occupying about one-fifth of the world's land surface area, mountains provide a direct and indirect life-support base for about one-tenth of humankind as well as goods and services to more than half the world's population. Mountain ecosystems are essential to the survival of the global ecosystem, and important regulator of climatic conditions and important source of world's major river systems. However they are now rapidly changing and are susceptible to accelerated soil erosion, landslides and rapid loss of habitat and genetic diversity. There is widespread poverty among mountain inhabitants and loss of indigenous knowledge systems. As a result, inhabitants are migrating out, and most global mountain areas are experiencing environmental degradation. The conditions in the Himalayan Mountain are no different from the mountainous regions of the world. Himalayan region is also experiencing irreversible unsustainable human action. Therefore, the fragility of Himalayan ecosystems represent a considerable challenge to sustainable development due to the fact that the impacts of unsustainable development are more rapid, heavier and more difficult to correct than in other ecosystems. In addition, the recent impact of globalization has already begun to erode the social and cultural integrity of Himalayan societies due to increased and accelerated contact with the market forces and outside cultures and lifestyles. Unsustainable land use practices, agricultural encroachment in village common lands and pasture lands have been observed in most of the Himalayan states. The adoption of unsustainable human activities ignoring the traditional management practices relating to their forest, rangeland management and water management. The bypassing of traditional institutions of decision making, ignoring the concept of collective survival and collective decision making has added momentum to the land degradation.

Himalayan States Specificities

The Indian Himalaya, which covers a vast area along the northern frontiers of the country and is, spread over five Indian States Jammu and Kashmir, Himachal Pradesh, Uttarakhand, Sikkim and Arunachal Pradesh from west to east. For the mountain people living in these states, the Himalayan mountaincontinues to be the predominant factor in their lives. Having acted as a natural and political barrier for centuries, the Himalaya has isolated a number of communities, cultures and customs. The Indian Himalaya marks the crossroads of Asia's three main religions. Kashmir -- formerly a paradise on earth -- is largely influenced by Islam. The foothills of Jammu, Himachal Pradesh and Uttar Pradesh form the northern boundary of Hinduism. The entire Trans Himalayan region, from Ladakh (Jammu and Kashmir) through Tibet and onto the eastern state of Sikkim, has seen a dominating influence of Buddhism. It is a well known fact that the Indian Himalaya has nurtured a number of ethnic groups of human race spread over in different valleys isolated and cut off from each other due to natural barriers. The true divisions of the Indian Himalayas are based on the mountain ranges rather than the state boundaries. From west to east, the Indian Himalayas can be divided into a number of such valleys: Kashmir, Ladakh, Zanskar, Lahul and Spiti, Chamba, Kinnaur, Garhwal, Kumaun, Sikkim and Arunachal Pradesh. A great variety of tribes and cultures have developed in these regions of the Indian Himalaya. This has been aided by the fact that the mountain people have mostly lived in valleys isolated from the rest of the world, with entry and exit points lying over high mountain passes. As a result, they have been able to develop their own distinct cultures and traditions. Offering some of the finest trekking and mountaineering challenges, the Indian Himalayas are a hot spot among climbers and trekkers throughout the world. Since most of India's northern boundary lies in these mountains, many areas close to the international borders have been declared off-limits for tourists, especially for foreigners.

Demography and Development Issues

It is a well known fact that development also shapes the population trends, for example, rising incomes and education level usually leads to falling birthrates as we find in Metropolitan cities and developed countries. But the reverse is also true: population trends can impede or hasten development. In isolated Himalayan areas it is easier to establish the macro and microlevel connections between demographic trends, poverty, and economic growth. However, precise links between population and poverty has remained unclear, and similarly, the economic growth alone does not pull individuals out of poverty and reduced fertility rates do not necessarily yield higher economic growth.Changes in population size, structure and spatial distribution affect the economic, social and environmental situation of a society which is not connected directly with the larger mainstream societies, and the Himalayan states are good examples to establish this link between population and development.The population size of most of the Indian Himalayan States districts was less than 4 lakhs (0.4 million) with only 10 districts having a larger population.During the past four decades the populations of these states haverecorded an increase of 2.7 times. While during the last census block (1991-2001), the population growth rate (25.43%) showed a significant decline as per the 2001 Census, but, it was, still higher than the national growth rate (21.35%). Each Himalayan state's society is at a different stage of the demographic transition, due to education and employment levels, out migration and availability of basic infrastructure to settle down after retirement. In the majority of the developed societies, population ageing is rapid. In the few states where fertility and population growth rates remain high, the number and share of young people continues to grow. However, there is common population and development related challenges and opportunities in the Himalayan region. Inequity in access to services, including reproductive health services; unmet need for family planning; high maternal mortality rates; gender inequality; large numbers of youth with limited access to health, education and employment opportunities; population ageing; internal and cross-border migration; environmental degradation; and vulnerability to natural disasters are some of the main challenges in this regard.The biggest threat of population growth in the Himalayan societies is that the already slow development process in these Himalayan states is getting even slower when the population increase is not connected with economic growth. The greatest threat occurs, when the already slow development process in those countries gets even slower when population explosion is not connected with economic explosion. Currently this phenomenon is occurring in the Himalayas.The population density and total population of mountain areas is less than the people living in plains and lowlands Even though only10% of the world's population live in mountain areas, the mountains areeven more important for people who live in the lowlands (Messerli and Ives, 1997). Thus, the actions of people living in the mountains, which aredirectly linked to population size, are so important. It is worth noting thatin some parts of the world, the mountains are the only source of freshwater (80–100% in some tropical areas), so they are extremely importantfor world food security. This clearly shows that mountain areas and theirresources are important for billions of people, not only those living withintheir immediate vicinity, but also downstream and further afield (Price and Messerli, 2002; Price, 2004). Thus, the relationship that existsbetween the populations settled in the higher regions of Himalayan states and the populations settled in the lowlands are totally dependent on each other for production food crops, animal husbandry, water for irrigation, drinking and supply in urban settlements. The Himalayan population is more than mere numbers––it is an extremely diverse collection of people of varied backgrounds and has maintained distinct cultural diversity. The solution to both thepopulation and development problems of the Himalayan state lies in employment and intensive growth of rural economy. Himalayan rural economy cannot be improved without addressing the persisting issues relating to water, forests and agriculture. The improvement of infrastructure and opportunities in health, education, and social equality can address the population problem to some extent. But, how to connect this and bring improvement in the fields of rural economy is an all time issue, and has not been addressed so far in almost entire rural Himalayan areas, and as a result, the rural to urban out migration is still increasing continuously. Migration often contributes to economic and social development in countries of origin, destination and transit. Greater access to education and lifelong learning improves health, economic prospects in the labour market and the opportunities for women to contribute to society. With adequate policies and programmes, these challenges can be addressed, and opportunities harnessed.

Vulnerability and Environmental Issues

The various kind of natural disasters in different Himalayan states, and recent events of flash floods and landslide in Himachal Pradesh and Uttarakhand have shown, that we need a development strategy for the Himalayan region, which can takes into account the vulnerability of the region and the need for environmental protection from natural and man-made disasters. The need for economic growth and development should not be at the cost of the environment. As we have already witnessed how the development initiatives in various parts of the Himalaya have already made the risk-prone and ecologically fragile region more vulnerable to environmental disasters and degradation.A number of studies carried out in the high Himalayan villages have reflected that how development programmes and projects have ignored forest resources, which is the most essential for fodder and water sources for sustenance of agro-farming practices along with source of water supply to down below low lands and plains. This resource has to be explored both in requisites of its opportunity along with risk to its ecology and economy.

Forest Based Economy

The Himalayan region has seen a very good regime of good forest resources availability, and a period of ecological stability and availability of plenty of water resources, rich flora and faunal biodiversity. This phase was also the extraction of forests for commercial and other money making activities to support all kinds of development. Human population in the Himalaya has been demolishing and over exploiting forest resources for several decades ever since agriculture came into existence and finally leading to deforestation and degradation of forest lands (Mahat *et al.,* 1986; William, 1997). The degradation of forest resources, which finally leads to diverse problems like soil erosion, slope failures, depletion of soil fertility, paucity of fuelwood and fodder, amplified overland flows, abridged ground water recharge hammering biological diversity and accelerated siltation of river beds in lowlands areas (Mahapatra *et al.,* 2018).This phase led to widespread deforestation in the region, which increasedthe vulnerability to landslides as well as deprivation among people dependent on forests for their basic survival. Later, a new phase of activities started, and it was the diversion of large tracts of forests for the construction of hydropower and road projects. It is a well known fact that all categories and types of forestsare an important reservoir of biodiversity, they also provide protection of Himalayan land against soil erosion and increased flooding in the low lands and plains, and also work as sinks for carbon (carbon sequestration). The importance of ecosystem services has been well researched and discussed, the methodology has also been prepared for a strategy to "pay" for these ecosystem services of the standing forests of the region and accordingly agreed to share the money collected in the name of ecosystem services with the local communities. The 12th and 13th Finance Commissions have also included the concept of compensating the states for maintain their forests. Unfortunately, the funds provided for these services are very less for initiating any developmental work. Some governments of Himalayan states like Himachal Pradesh have initiated work on assessing the ecosystem and carbon sequestration services for its standing forests. This issue requires more importance and discussions to evolve a common format for valuation of the forests. Many studies carried out in the high Himalayan villages have reflected forest resources as most essential for fodder and water sources for sustenance of agro-farming practices along with source of water supply to plains flowing high glaciers and mountains (Zobel and Singh, 1997; Anonymous, 2009; Raina, 2009). There is a social and public need to devise mechanisms to strengthen the existing forest resources, so that the local economies are benefitted, and local people get more income for maintain their forests.

Non Destructive Tourism

Sustainable tourism is a concept that covers the complete tourism experience, including concern for economic, social and environmental issues as well as attention to improving tourists' experiences and addressing the needs of host communities. On similar lines, a tourism that does not cause any destruction to the natural environment, social and cultural fabric of the region, and the local economic structure is regarded as non destructive tourism. Sustainable tourism should embrace concerns for environmental protection, social equity, and the quality of life, cultural diversity, and a dynamic, viable economy delivering jobs and prosperity for all. It has its roots in sustainable development and there can be some confusion as to what "sustainable tourism" means. Sustainable tourism as defined by the UN Environment Program "tourism that takes full account of its current and future economic, social and environmental impacts, addressing the needs of visitors, the industry, the environment and host communities". There is now broad consensus that tourism should be non-destructive and sustainable.In fact, all forms of tourism have the potential to be sustainable if planned, developed and managed properly.Tourist developments organizations are promoting sustainable tourism practices in order to mitigate negative effects caused by the growing impact of tourism, for example its environmental impacts. There is enormous potential for non destructive and sustainable tourism in the Himalayan region. Based on the existing forms of prevalent tourism, like the religious tourism, adventure tourism, educational tourism, sightseeing and recreational tourism, film making and photography, health recovery etc., local people and private business agencies like the hoteliers, local people, NGOs, transport business, tourist guides and service providers need to interact more frequently for achieving the goals of sustainable tourism. There is also a need to create similar to sanctuaries and national parks, create a provision of buffer areas, surrounding the pilgrimage sites, where development is restricted. In order to include the local people to participate in providing the supply of local products for the pilgrimage activities and to share the benefits from the sale of such locally produced items as approved by the temple committees. Further, the local communities should also be included in the management and upkeep of such sites.

Sustainable Urbanization

Urbanization is a worldwide phenomenon after the industrial revolution and a product of the development of modern industry and infrastructural progress. The benefits and comforts of urban facilities and urban living have always attracted the rural population, but the unaffordable cost of urban living has always dissuaded the majority. However, the development of roads and the impact of market forces have created urban like facilities in a number of new locations in the Himalayan regions and have been driving social and infrastructural factors for rural to urban out migration in these new urban centers are better education and better health facilities, opportunities for small business and employment opportunities as compared to their rural settlements. Now most of the road head settlements located in remote and far flung areas have urbanized because of the growth of domestic tourism, marketing, and the search for new tourist destinations. All this has led to a tremendous increase in the size, area, number, and complexity of urban settlements in The Himalayan region including Uttarakhand. The Himalayan states are considered the most densely populated and most rapidly urbanising mountain ecosystem in the world and Uttarakhand is the most rapidly urbanising state, both in terms of urban population and number of sprouting new towns. According to the 2011 Census, more than 30 per cent of Uttarakhand's population lives in urban areas as against the 25 per cent average urban population of all Himalayan States. In the absence of an urban land-use policy has also pushed urbanization from the geologically more stable mid-slopes and ridges to the environmentally sensitive higher elevations and right down to the floodplains. Unfortunately, this whole process of urbanization is unplanned, unregulated, and unsystematic. The absence of any urban development planning policies is glaring. This has increased the vulnerability of most such areas to a variety of geohydrological hazards. Urban encroachment into forests has not been regulated, disruption of natural drainage has not been stopped, cutting and deep excavations on hill slopes to erect buildings and infrastructure have been allowed has catapulted the frequency of landslides to dramatic proportions in Himachal Pradesh and Uttarakhand. The encroachment, obstruction, and obliteration of natural drainage channels have increased the vulnerability of the slopes to geohydrological risks, putting most towns under the threat of repeated landslides and mass movements. It is important to monitor and restrict the expansion and growth of urban areas in the ecological fragile zones. The building of public awareness amongst the local people to devise strategies for consolidation of urban settlements, which are governed through land-use planning incorporated in the municipal master plan should be discussed publicly before allowing for any land use change. In other words, unmanaged and unchecked urban growth should not be permitted at any period of time. The absence of an urban land-use policy has pushed urbanisation from the geologically more stable mid-slopes and ridges to the environmentally sensitive higher elevations and right down to the floodplains. Unfortunately, this whole process of urbanisation is unplanned, unregulated, and unsystematic. The absence of any urban development planning policies is glaring. All this will require the creation of strong regulatory institutions in the towns. The municipal byelaws must provide for construction activity to be banned in areas, which fall in hazard zones or areas close to rivers, springs and watersheds of the towns. In many cases these provisions exist in the byelaws, but have not been strictly enforced. Hence, there is a need for a zero-tolerance policy on the matters of construction work in the newly extension of urban settlements issues.

CONCLUSIONS

The Indian Himalaya holds key to India's ecological and social security by virtue of being centre of biological and cultural diversity and store house for water and other resources. Apart from harboring rich cultural and biological diversity, the Indian Himalayan region (IHR) is the major supplier of timber, medicines, fiber, oils, spices and condiments, firewood, organic manure, fodder and hydropower. It is reckoned as 'gene bank' and continues to remain an important centre for the origin of the crop diversity and numerous under-utilized and potential future crops. The north eastern region of the IHR has been recognized as a world heritage of biodiversity hot spot. The IHR is complex, unique in geomorphology, vulnerable, fragile and reacts sensitively to population pressure and global climate change. It is invariable to note that urban expansion in the Himalayan mountainregion are becoming a major concern for the local people and planners in view ofincreased incidences of natural disasters.

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CITIZEN CENTRIC INNOVATIONS IN E-GOVERNANCE: GOVERNMENT OF INDIA INITIATIVES IN THE PUBLIC SECTOR

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ABSTRACT

As per the United Nations, bolstering democracies requires trustworthy State institutions and high-quality public services that are available to everybody. The lack of State capability has been linked to numerous issues, such as poverty and social disintegration, which nations face today. The COVID-19 pandemic has compounded the difficulties of governmental management. To find low-cost answers to complex challenges, businesses must encourage a culture of innovation. Innovation can be crucial in providing citizens with valuable services. Innovations in the Public sector have been a contemporary subject that has attracted government and scholars alike (Osborne and Brown 2011; Walker 2014). The Innovation in National Education Policy in India, Direct Benefit Transfer Welfare Scheme, Mahatma Gandhi National Rural Employment Guarantee Act (MGNREGA) 2005, and Ayushman Bharat Yojana are some examples of government initiatives aimed at improving public service delivery. Complex interactions between internal antecedents, resources, and players in the public sector and external environmental antecedents lead to shifts in e-governance. Studies of public invention investigate not just the innovations themselves but also the organizational and environmental settings in which they emerge, as well as the causes and conditions that make them possible. The pioneers in the innovation process and the means of public service delivery are the focus of our research review. We will share what we learned about how novel approaches to delivering e-governance services to citizens fared during the pandemic.

Keyword: e-Governance, Innovations, State capability, Environmental antecedents, Democracy

INTRODUCTION

On 17th Feb 2022 Honerable PM Mr Narendra Modi while speaking in the seventh edition of the Economic Times Global Business Summit in New Delhi said that once former PM Mr R Gandhi had said that "only 15 paise of every rupee sanctioned by the government reached the poor", and "now due to Direct Benefit Transfer (DBT) 100% delivery of the amount to the intended beneficiary. ". INR 28 lakh crore from various schemes were transferred successfully to the beneficiary using a digital platform DBT . This profound example tells volumes on how digitalization (Mobile, bank connectivity, Aadhar) has helped reduction in poverty , corruption and increase in inclusive growth. According to the United Nations, one of the requirements for bolstering democracy is to have trustworthy and efficient state institutions as well as public services that are open to everyone and of a high quality. It is now generally accepted that a lack of state capacity is the root cause of a significant portion of the challenges that nations worldwide are confronted with today, including poverty and social disintegration. Any business that wants to solve complicated challenges at a reasonable cost needs to embrace innovation. Public administration innovation

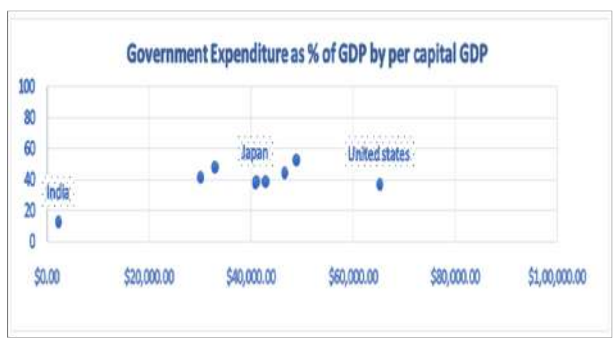

Fig. 1. Country government expenditures (as % of GDP) by per-capita GDP

provides citizens with valuable services. New research shows that there has been some innovation in India's public administration, but more work has to be done to boost citizen satisfaction.The development has been uneven among the Indian states (Ahluwalia, 2000), partly because some states have developed their governance structures and processes more than others (Khandwalla, 2010). Citizens' expectations are increasing globally, which puts pressure on the government to deliver efficient and high-quality public services. Public service providers must become cost-effective, accountable, and capable of providing benchmarked services to their citizens, thus becoming citizen-centric. Innovation is one of the core elements of public management, resulting in economic and political messages signaling good governance. (Gupta *et al.,* 2019).

NOTEWORTHY CONTRIBUTION TO THE FIELD

There is a lack of theoretical work that especially addresses innovation in the public sector. In addition, there is a lack of empirical research in three key areas that are critical to comprehending the innovative processes of public sector organizations:

•The nature of public services themselves,

•The environment in which public-sector organizations must function, and

•Interactions with parties inside and outside the government.

Complex interactions between internal antecedents, resources, and actors and external, environmental antecedents, actors, and resources produce innovation in the public sector. (See Fig. 2. Types of Public Sector innovation types applied) Studying open innovation entails investigating not just the innovation themselves, but also the organizational and environmental settings in which they emerge and the conditions that make them possible.

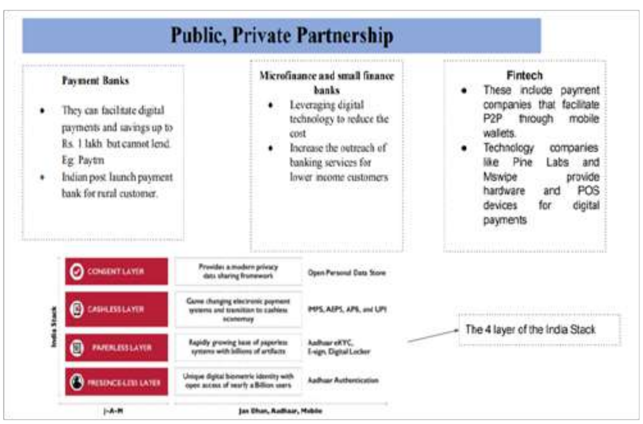

Fig. 2. Public sector innovation types applied

Made evident by a survey of the existing literature on the subject of innovation, research (Hanna *et al.,* 2015) has classified four innovation types, process, products/services, and government conceptual innovation.

Indian experience:

•Innovation is the key to finding cost-effective solutions to complex problems for any organization.

•India faces a lot of complexity and challenges in governance due to its diverse culture, geographies, and prevailing inequities.India is a hopeless case for studying innovations in the Public sector. (Mckinsey, 2019).

Our research-related contribution concerns the antecedents in the innovation process as well as the method of delivery of public service. "Moreover, there is a need to look deeper into the goals and effects of the innovation process since, whileinnovation and improvement have often been assumed synonymous, this is by no means always the case "(Osborne and Brown 2013,).

Table 1. Prime Minister's Awards for Excellence in Public Administration

Innovation type	Focus	References	Examples
Process innovation	Optimizing both internal and external procedures to increase quality and productivity	Walker (2014)	
Administrative process innovation	Creation of novel organizational structures, managerial strategies, and operational practices.	Meeus and Edquist (2006)	Creation of a 'one-stop shop' by a municipality where citizens can access various services at a single location
Technological process innovation	The creation and implementation of cutting-edge technologies within an organization to benefit its customers and the general public.	Edquist et al., 2001	Digital assessment of taxes
Product or service innovation	Creation of new public services or products	Damanpour and Schneider (2009)	Creation of youth work disability benefits
Governance innovation	Creation of novel structures and procedures for resolving pressing social issues	Moore and Hartley (2008)	Governance practice that attempts to enhance the automated and self-organizing capacities of policy networks
Conceptual Innovation	Reframing the nature of individual problems and the potential answers to those problems by introducing novel concepts, frames of reference, or paradigms.	Bekkers *et al.,* 2011	The introduction of the paradigm that, when assessing a person's work disability, insurance physicians no longer analyze what people cannot do, but instead analyze what they can still do it, hence

Ref: De Vries, H.A., Bekkers, V.J.J.M., L.G. Tummers ,Innovation in the Public Sector: A Systematic Review and Future Research Agenda. Public Administration

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DATA AND METHODOLOGY

'As public sector service provision often revolves around providing services cost-effectively and creating societal well-being, the value in the public sector is more complicated than in the private sector. It can, therefore, be harder to measure' (Mulgan and Albury, 2003). Management innovations make up the bulk of awardees at the Prime Minister's Awards for Excellence in Public Administration, with technology breakthroughs coming in a distant second. They provide clues as to the general path taken in terms of good governance and reproducibility. To improve the economy as a whole, innovation must be fostered across all sectors, especially in the public sector."However, innovation policies and strategies relating to the public sector are far less developed than those targeting the business sector." "There are important differences between the public and the private sector in terms of incentives and motivation, resource allocation, and attitudes towards risk, which are inherent in the different roles played by the two sectors in the economy, and which have a profound impact on how innovation is carried out and how policy can support it. "United Nations Economic Commission for Europe UNECE 2017. By establishing links with existing theories, it could be possible to develop better explanations of the actual impacts of innovations, thereby answering the question: did these innovations matter and make a difference in India? Most of the empirical studies on Innovation examined failed to address this issue. (Hanna *et al.,* 2015). In conclusion, a new and promising innovation in the battle against corruption is the widespread implementation of information and communication technology to increase government openness and transparency. For the government to be more effective, efficient, and accountable to its citizens, technological advances in the public sector may prove to be a key catalyst.

Innovation Type	Focus	Case Study
Process innovation	Improvement of quality and efficiency of internal and external processes.Ex: Ease of doing business	•GST- one nation, one tax, one market (2018) • Ease of doing business in India Innovation(AS/JS&Dir./DS category) (2018) • Swatch Bharat Mission (Gramin) (2016) •Village health and nutrition day in complete convergence Mode, Tripura(2010-11) • Sickle cell Anemia Control program, Government of Gujrat(2009-10) • Sustainable Plastic Waste Mgmt in Himachal Pradesh: From concept to policy(2009-10)
Administrative process innovation	The development of novel organisational structures, the adoption of cutting-edge administrative practises, and labor-saving innovations are all examples of reimagining of the workplace Ex:A municipality sets up a "one-stop shop" where residents may get many different types of help in one convenient place.	• Swatch Bharat mission (Gramin) (2016) •"Surguja Fulwari Initiative" Chhattisgarh (2013-14) • Achieving 'total financial Inclusion' in the west Tripura district through E ROR(2013-14) • Education initiatives in Dantewada (2011-12) • Sugarcane Information System, Uttar Pradesh (2011-12) • Making Medicines affordable, Chittorgarh Rajasthan (2008-09) • Computerization of Paddy procurement and public distribution system, Chhattisgarh (2008-09)
Technological process innovation	The development and implementation of cutting-edge technology within an organization to improve the quality of care provided to customers and constituents. Example Automatic tax evaluation in the cloud	• Unnaya Banka-Reinvesting Education using Technology (2018) • Cashless village palnar (2017) •GST- one nation, one tax, one market (2018) •"Saving the Womb" An initiative to address and redress malpractices in the implementation of RSBY in Samastipur, Bihar (2013-14) • Achieving 'total financial Inclusion' in the West Tripura district through E ROR(2013-14) • Sugarcane Information System, Uttar Pradesh (2011-12) • Computerization of Paddy procurement and public distribution system, Chhattisgarh (2008-09) • Integrated Taxpayer Data Management System (2008-09)

Product or service innovation	Creation of new public services or products. Example;Creation of youth work disability benefits	• Swatch Bharat Mission (Gramin) (2016) •"Surguja Fulwari Initiative" Chhattisgarh (2013-14) • Education initiatives in Dantewada (2011-12) • Sugarcane Information System, Uttar Pradesh (2011-12) • Sickle cell Anemia Control programme, Government of Gujrat(2009-10) •Cervical Cancer Screening, Chennai, Tamil Nadu (2008-09)
Governance innovation	Creating novel structures and methods to solve pressing social issues. By way of example, a governance strategy that seeks to improve policy networks' self-regulatory and self-organizing abilities	• Improved policy network self-regulation and self-organization is an objective of this governance strategy. •GST- one nation, one tax, one market (2018) • Sugarcane Information System, Uttar Pradesh (2011-12) • Bridging the gap Bihar • Medicines (2008-09) • Recognition of forest rights, Madhya Pradesh (2008-09)
Conceptual Innovation	Reframing the nature of individual problems and the potential answers to those problems by introducing novel concepts, frames of reference, or paradigms.	• Information, Education and communication of Swatch Bharat Mission (Gramin) Innovation (AS/JS & Dir. / DS Category) (2018) • Soil health Card scheme (2016) • Sustainable Plastic Waste Mgmt in Himachal Pradesh: From concept to policy(2009-10) • Removal of Encroachments of Structures- maintaining Communal Harmony. Jabalpur,MP (2008-09)

Our chapter will contribute as follows:

•Opening the black box of why and how of public sector innovation.

•Assist in shaping the method, environment, and policy decision-making for fostering innovation in the public sector

•Strengthening study in the realms of innovation and the public sector

We conducted indpeth case studies of the following seven public policy innovations

Case study 1: Integrated Taxpayer Data Management System

Case study 2: Promoting Digital Payments

Case study 3: Computerization of Paddy Procurement and Public Distribution System in Chhattisgarh

Case Study 4 : Innovation in National Education Policy in India

Case study 5: Direct Benefit Transfer Welfare Scheme

Case study 6: Mahatma Gandhi National Rural Employment Guarantee Act (MGNREGA) 2005

Case study 7: Ayushman Bharat Yojana

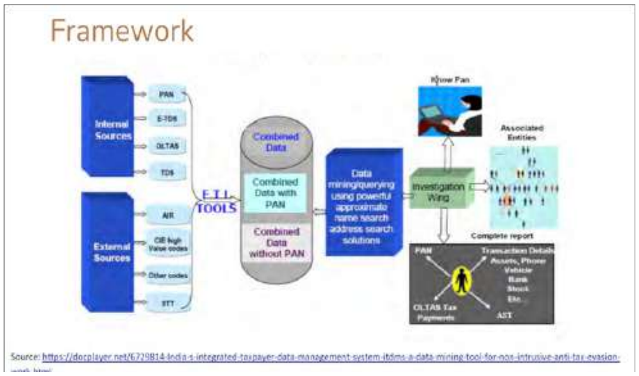
Learnings from case studies:

Case study 1: Integrated Taxpayer Data Management System

Integrated Taxpayer Data Management System ITDMS (2008-09): This was one of the central government's first E-governance projects.ITDMS was created to help the IT department's investigation division follow potential violators in tax payments. It is a data mining solution implemented by the tax department. It generated a 360-degree transaction profile of an individual by combining the data on the entity from all the possible data sources for a full-fledged solution. The system gathers data from various sources and connects them with a unique identifier to predict cases of significant tax evasion. ITDMS also was expected to promote voluntary compliance.

Databases used before ITDMS had the following details:

PAN: Name, DoB, address, type of business,OLTAS: Online tax accounting system from banks give info about payments of tax from bank,TDS: Tax deduction particular from employers, Annual Information Returns (AIR) (2005) requiring third parties like- banks, stock exchanges, RBI Bonds Mutual Funds, IPOs credit card companies, Registrars of Immovable Properties, Assessed incomes and other external databases like high networth individual, telephone directory, high-value transactions. These databases could not be married together, resulting in unstructured and non-standardized data from various sources meant errors in records if merged (different spellings of the same word). There was no technology available to manage such amount of enormous data sets. Due to these issues, the data was underutilized, and no actionable insights could be inferred. The government had to develop an integrated profile through which the data could be leveraged for tax evasion checks. A unique identifier with PAN can be created forefficient profile data generation- Internal and External Databases, including Annual Information, Returns. The working model of ITDMS had an ETL tool - like Microsoft SQL. Added Identity search – Primematch: which could handle variations. A Family tree - name and address correlated through a unique identifier.The technology platform and server depth made the response time in milliseconds. In 2008 it was probably the most significant data mining in government worldwide. First, a Prototype Anin-house team in Delhi Investigation Unit was developed. Then, aFull-fledged solution -1 was foundbycreatinga search engine suitable for Indian names. Full-fledged solution -2 (POC on over 4 million records). Final Solution (Identity search tool with high recall and precision). Even after developing a robust and customized system, many issues werefaced, namely i) Difficulty in obtaining data from different taxation bodies, ii) the need for more than a search engine (analysis required) – iii) Difficulty in dealing with cross-border transactions. The government developed INTRAC - Data analytics in tax admin-



istration and developed CMCPC - approach for voluntary compliance. For legal compliance MoU between CBDT and CBIC was commissioned for the exchange of information. DGARM - National Customs Targeting Centre for Cargo & Passenger data was created and integrated.

KEY STAKEHOLDERS:

Income Tax Department:When establishing the Integrated Taxpayer Data Management System, the tax department is the most crucial stakeholder. The tax agency was accountable for: - Rollout of the software to several departments - Development of tax agency-wide ICT system policy; Development of execution strategy and vision - Approval of tax administration business process reengineering Management of System Planning, Development, and Deployment - Enhancing the organization's internal IT, digitizing and migrating data, and training and educating employees.

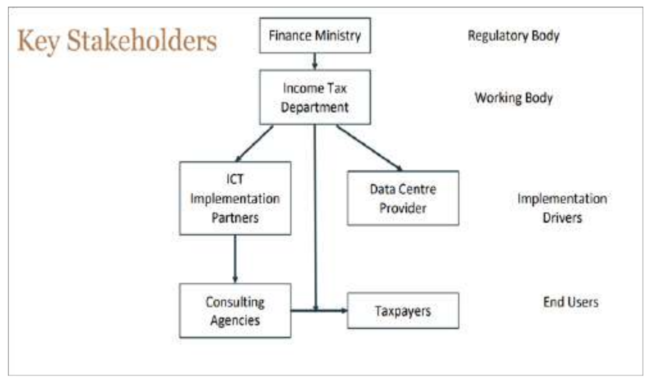
Finance Ministry: The Finance Ministry was responsible for national tax policymaking as the supreme revenue and taxing authority. Once the new system is in place, their duties will expand to include providing financing assistance to all tax agency offices and overseeing the system's deployment throughout all tax agency offices.

Data Center Service Provider: As governments worldwide move toward exclusively online service delivery, the function of the data center service provider (cloud computing) becomes increasingly important. The data center service provider was in charge of hosting the new system application in both the test and live environments and performing routine data backups and archiving.

Consulting Agencies: Consultants played multiple roles during the system implementation lifecycle, including support to the tax agencies in implementing the system. This included supporting business process reengineering, defining a target operating model, and conducting an independent audit and quality assurance.

ICT system implementation partners(third-party vendors):Integrated Taxpayer Data Management relies heavily on designing, developing, and implementing its information and communication technology systems. Data digitization, user acceptability testing of the application software, and support for the ICT system's ongoing operation and maintenance are all part of the process.

Taxpayers (General Population): These end users for the integrated system interface directly with the design and the software to submit their requests and queries. They are either assisted in this process by the consulting agencies or work independently with the system.



The Government of India beautifully managed the stakeholders' roles and responsibilities and made it customer centric (IT payers). The government has started the third step in enhancing the IT system through AI-ML Integration & Big Data Analytics. As technology improves, the government needs to take meaningful steps to keep up with technological changes. Incorporating next-gen AI-ML algorithms into the existing system, coupled with rigorous,extensive data analysis, would ensure that all key stakeholders can generate richer insights.

Scaling Up - Government can leverage the rich data available to further simplify taxpayers' lives by allowing them to consent to use this data for other uses, such as credit ratings, loan approvals, etc. Such integrated systems would also incentivize more people to start paying taxes. An Example: New York City has effectively used its data management system to catch underreporting taxes by businesses. Having studied patterns of income and tax payments of similar businesses, NYC searched for outliers (where taxes were underreported). It led to 12 times more efficient auditing of fraudulent companies and has reduced the time consumed to sniff a fraud by 60 percent.

Case study 2: Promoting Digital Payments

"Faceless, paperless, and cashless" is a declared goal of Digital India. The Reserve Bank has taken several steps to promote payment digitization in the country. Significant progress has been made in making several digital payment solutions available, and their use has grown over time. The government plans to increase the use of digital payment methods such as the Unified Payments Interface (UPI), Unstructured Supplementary Service Data (USSD), Aadhar Pay, Immediate Payment Service (IMPS), and debit cards by setting a goal of 2.5 billion transactions in the fiscal year 2017–18.

The Government of India's primary goals in encouraging the use of digital payment methods was:

•Promoting and ensuring the formation, expansion, and sustainability of a strong, secure, and inclusive national digital payment ecosystem.
•Promotional campaigns, training, and teaching are used to raise awareness about the advantages of digital payments.
•Developing and facilitating acceptable standards for digital payment services that are efficient, economical, and secure;
•Ensure that the digital payments ecosystem is secure.

Factors that lead to success in the digital payment ecosystem:

•Increase in the dedicated payment systems
•Increased mobile users
•The proliferation of the internet

Jan Dhan-Aadhaar-Mobile (JAM) trinity: Government of India initiative to integrate Jan Dhan accounts, mobile numbers, and Aadhaar cards of Indians. This has made it possible for people to check their account balances, apply for and earn scholarships and fellowships, and receive subsidies for things like fertilizer, LPG, disability pensions and farm income assistance in their bank accounts. NPCI's efforts, including the Rupay payment system, Immediate Payment System (IMPS), Aadhaar enabled Payment system (AePS), and Bharat Bill Payment System, are helping to lead India into the Digital Revolution.Thanks to their reliance on the Aadhaar and JAM digital infrastructure. With UPI, users can instantaneously transfer money from one bank account to another by pushing (making a payment) or pulling (collecting) the money using a mobile device. Digital costs on the UPI platform rose from 0.1 million in 2016 to 1.3 billion in 2020.With multiple bank accounts, users can link all their bank accounts on a single app, making it very convenient and more like a super wallet. The entry of big tech players has caused increased competition & enhanced services; recently, payment services have been launched through WhatsApp. Under Digi Dhan Abhiyan, startups have been asked to expand digital payment avenues in unserved sections. The government still faces challenges like poverty, lack of financial literacy, inadequate physical infrastructure, transaction charges, small &medium enterprises/ small retailers,privacy,and cyber security perception of consumers. Government has plans to overcome these challenges through legal interventions, communication with stakeholders, and citizen partnerships.The Government of India needs to also take a leaf from global experiences like tax incentives provided for digital payments (Argentina, Columbia, Uruguay, and South Korea) helped increase in usage of digital payments. France, Portugal, Spain, Bulgaria, and Greece implemented disincentives for cash transactions. Hong Kong's Octopus Card Payment System, South Korea T- the money are single prepaid card. Single prepaid cards are also a good source of increasing digital payments. GOI needs to develop physical infrastructure in rural areas too. Most of the digital infrastructure is restricted to urban areas and needs to build the same for rural areas too. Co-Branded Cards- Rupay Cards can be launched in collaboration with other brands such as IRCTC, e-commerce sites, etc. Incentivize digital payments by cashback - Cashbacks and tax rebates can be offered for digital transactions, as in petrol payments. UPI and QR-based payments are promoted in governments through government offices and collection centers –For example, electricity boards, municipal corporations, etc. Enabling digital payment facilities at Revenue (Tehsil) offices, ration shops, fertilizer shops, etc.
Case 3: Computerization of Paddy Procurement and Public Distribution System in Chhattisgarh.
India's Food grainsupply chain landscape is bogged down with multiple challenges. The entire supply chain can be covered under the twogovernment schemes below:
a)Procurement at MSP: The majority of farmers are poor. They need to sell off the produce quickly due to the need for funds and lack of storage. This has led to exploitation by many intermediaries. Chattisgarhgovernment implemented fair prices with MSP for its main crop: Paddy. The Government did three million tonnes procurement on MSP at 2400 Crores and benefited 1 million farmer families. Paddy gets converted into rice in mills, goes ahead for PDS.
b)Targeted PDS: The Government of Chhattisgarh has implemented a programme to provide 35 kilograms of rice to BPL families at Rs.6.25 per kilogram. The number of low-income families that benefited is 2.4 million. About 3.7 million low-income families each received 35 kilograms of rice at Rs. 3 rupees per kilogram.

Key Motivations for Technology Innovation
The massive discrepancy in the funds spent was seen atthe ground level. There was massive corruption in the supply chain. Extensive complaints about fair-price shops, leakages, and diversions were received. Due to staff shortages and complexities, it wasn't easyto monitor the system. Ration cards, food stamps, and other barcoded food vouchers failed to provide adequate nutrition.
Public Distribution Systems are widely criticized for delivery leakages and diversion. Among the five basic necessities of life - education, water, PDS, electricity, and healthcare—it

ranks third in terms of corruption. According to estimates, almost 25% of the ration is stolen before it reaches the recipients.

There are four key regions where people are diverted:

•Diversion in the procurement itself.

•Diversion in the movement of commodities between CGSCSC (CGSCSC: Chhattisgarh State Civil Supplies Corporation Limited)warehouses.

•Diversion while transporting to FPS from CGSCSC warehouses.

•Diversion at the FPS level.

Use of ICT-Computerization to Check Corruption

Adaption of three stage strategy in the delivery mechanism of the Public Distribution System:

Step 1: Computerizing PDS operations, making data publicly available online, and updating users with critical information via short message service (SMS) all contribute to greater operational transparency.

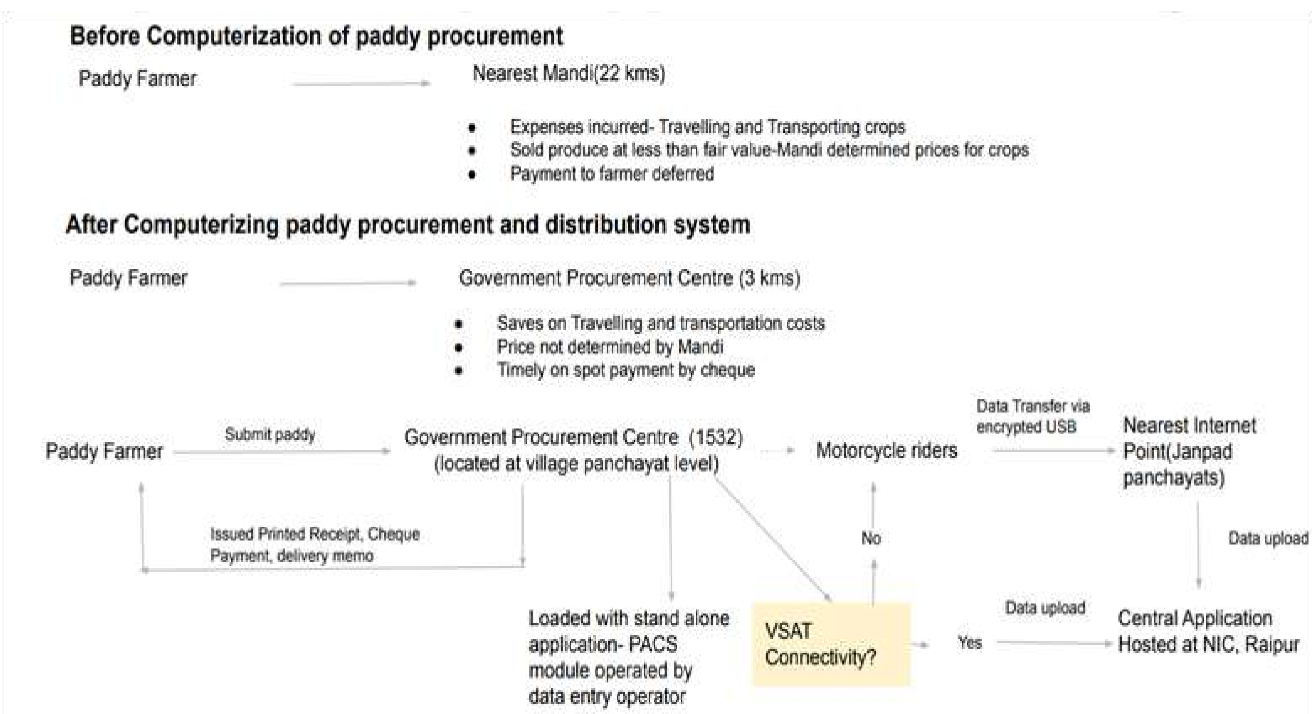
Step 2: A quick and painless way to voice opinions and concerns - Chattisgarh now has access to a toll-free call center. Calling or going online to file a complaint is standard procedure.

Step 3: Build public confidence- Put Complaint Monitoring System in place to check the effectiveness of the complaint redressal mechanism by monitoring the complaints for speedy disposal.

The outcome of such a strategy was that increased transparency aided the Central Cooperative Bank of Chattisgarhin recovering Rs. 400 cr. of loans taken by farmers to grow paddy.

Computerization of Total Food Grain Supply Chain

From paddy purchase from farmers to its storage, grinding, and distribution to 3.7 million ration card holders through 10,416 Fair Price Shops, the Chhattisgarh government has fully computerized the food grain supply chain (FPS). 1532 paddy procurement facilities, 50 storage centers, all relevant district offices, 99 civil supplies corporation distribution locations, and 35 FCI rice reception centers have all been computerized as part of this initiative. Cheque generation, Miller registration, Agreement with millers, Delivery Order expiration dates, etc., are all automated at paddy procurement centers, along with the rest of the purchasing and issuing process. There is now a database of 3.7 million people who have ration cards. A computer program now determines how much money the FPS will give each month. From 8:00 AM to 10:00 PM (local time), residents could call the toll-free number 1800-233-3663 to voice any concerns they had or receive any more information they sought regarding paddy procurement and public distribution. A website with a citizen interface has been set up to encourage more people to use their voice in regulating the theft of PDS goods. Computerization of Paddy procurement & Milling. The project can be categorized into four sections, each contributing to the overall goal of computerizing the entire food grain supply chain and monitoring it.



Paddy Procurement and Milling:
About one million Chhattisgarh farmers' paddy is acquired at the minimum support price (MSP) by 1532 procurement centers across the state. Since most of these centers are located at the village panchayat level and lack internet access, a separate form-based stand-alone module was developed to facilitate the online purchase and distribution of paddy to mills, storage facilities, and food processing industries. A million farmers have benefited greatly from the ability to generate their own cheques instantly on computers. There were 1532 local data entry operators hired and instructed how to use the PACS Module. Excitingly, data transmission from distribution centers to the central server and back again is being handled by motorcyclists (250 of whom were engaged). These motorcyclists also obtain the latest software updates and delivery order details from the block-level server and deliver them to the distribution hubs.

Unified Ration Card Database and issue of PDS commodities to FPS
Ration cards are now printed from a central database that has been compiled for this purpose. In Chhattisgarh, only ration cards with a barcode and serial number generated by that system can be used. Shop-specific distributions are now handled mechanically, thanks to the computerization of the whole ration cards database. All Fair Price Shops must report their prior month's inventory and sales before issuing PDS commodities. The software determines the precise quantity of PDS commodities to be delivered to the FPS based on allocation, stock, and sales data from the FPSand then issues a delivery order via the web interface. The central server now has allocations, stocks, issues, and sales data for each FPS. Every month, at least 10% of this data is physically validated by the food department employees, and any FPS submitting incorrect claims is subject to disciplinary action.

Citizen Participation website
The citizen's knowledge and engagement in the public delivery system is a crucial check on diversion and leakage. As a result, a public-facing website has been developed. There is a channel for feedback and concerns. To monitor whether or not vehicles are being diverted from PDS to FPS from the warehouse, this website also gives way for citizens to get involved. Interested citizens can register their phone numbers on the site and choose from among a number of FPSs to participate in the PDS monitoring. When the warehouse sends PDS goods to the FPS, a text message is issued to the subscribed phones. Listed in this transmission is the truck number, the quantities of PDS items loaded into that truck, the time of departure, and the date of departure. The citizen can file a complaint online or over the phone if the required number of entities does not show up at the FPS within a reasonable time frame.

Call center and Complaint Monitoring System
A call center with a toll-free number of 1-800-233-3663 is operational. Immediately upon receipt, complaints are put into the system, and a complaint number is sent to the caller. All complaints received by the call centre or online can be viewed in the inbox of the relevant officer. The officer must document the findings of the investigation and the subsequent actions taken if the complaints were justified. On request, the complainant is updated on the status of their case. Fast resolution of complaints is ensured by constant monitoring at the directorate and secretariat levels.

INNOVATIVE IDEAS ADAPTED

Adopting Data transmission via motorcyclists: Most PACS can be found in extremely remote areas with no connectivity to the internet. Internet access via V-Sat is provided at Chhattisgarh's administrative centers, including the Janpad Panchayat and block headquarters. Motorcycle couriers are used regularly to move information from computers in the procurement center to the block headquarters, where it is then uploaded to a central server over the internet. Thanks to this development, near-real-time information may be gathered independently of the internet.

SMS Delivery of Truck Dispatch Information to Citizens: Using this website, citizens can register their phone numbers and select one or more FPSs to assist in the monitoring of PDS. When a shipment of PDS items arrives at an FPS, an SMS is sent to everyone of the FPS's registered mobile phones to notify them of the arrival. This notification includes the truck number, the quantities of PDS items being supplied by that truck, the date and time of dispatch, and the truck's location. This led to greater openness and citizen engagement.

Rice Festival (Chaval Utsav): Distributing PDS goods takes place in a hat bazar in the village on a specific, publicized day each month in the presence of the general public and designated government officials. On this day, BPL households receive PDS items and other benefits, like old-age pensions. This concept enhanced civic participation and transparency.

Truck photograph to the server with coordinates of truck position: In order to use a GPS-enabled mobile phone with a camera in the warehouse, a J2ME application was developed and installed on the device. The programme snaps a picture of the delivering truck and uploads it to the server whenever a truck containing rice or other items arrives at the warehouse. The server-side computer checks the truck's coordinates against the warehouse's coordinates to guarantee that the truck will arrive at the warehouse at the appointed time. This innovation helps to a limited extent in confirming claims made by reception centers without actually receiving the truck sent.

OUTCOMES OF THE PROJECT
In order to monitor the implementation of Agreements with fraudulent millers, this season saw the introduction of com-

puterized miller registration. The statewide standardization of procedures is primarily due to the advent of computerization. When using a manual approach, it could be challenging to guarantee that every district follows the same steps. Automating processes using computers has led to statewide consistency. Micromanagement of stock with a web app increased milling throughput, decreased rice and paddy damage, and yielded significant cost savings. Automatic inconsistencies and abuses in allocating funds to FPS were eradicated after calculating monthly allocations. It's computed mechanically depending on the stock levels of cards. PDS supplies were automatically received and issued at distribution facilities, allowing for better lift monitoring and greater transparency. Citizens are encouraged to participate in PDS monitoring through an SMS alert system and a citizen interface website.The lessons of Chattisgarh show how adversity can be turned into success. The government had difficulties due to poor connection at paddy procurement hubs. So the government hired motorcyclists to carry information. This year, the government installed generators and uninterruptible power supplies at procurement centers because of unreliable power. When entering a large amount of beneficiary information into the Ration card database, the government must use decentralized data entry and ensure sufficient validation checks are in place to catch any mistakes. A readable font was available for Hindi text. The government employed Unicode to facilitate simple interoperability. It was a problem for the government to find enough qualified workers. With a total of 2500 person-days of training delivered, the government accomplished a Herculean feat by developing trained human resources. Considering the three aforementioned examples, it is apparent that the government can do anything provided it sets forward well-defined goals and is motivated to improve the current system. If used correctly, technology has the potential to curb wrongdoing. On the other hand, technology is not the real safeguard. Technological advancements aim to replace labor-intensive manual processes with ones that are more open and accountable to the average citizen. For any e-governance project to be successful, buy-in from higher authorities, capability building, and connectivity are necessary. Data collection at the moment of generation is preferable to an MIS programme that allows data entry after manual processing.

Case Study 4: Innovation in National Education Policy in India

Introduction: The National Education Policy (NEP) 2020 in India highlights the importance of innovation in transforming the education system. This case study aims to explore the various aspects of innovation in the NEP and highlight some statistics that illustrate the current state of education in India. Background: India has one of the largest education systems in the world, with more than 1.5 million schools and over 250 million students. However, despite this, the quality of education in India remains a concern. The NEP 2020 is a comprehensive document that aims to address the challenges facing the education system in India. Technology-driven Education: The NEP proposes the use of technology in education, such as digital resources, online tools, and artificial intelligence, to make learning more engaging and interactive. According to the Annual Status of Education Report (ASER) 2020, only 11% of rural households have access to a computer for online learning, highlighting the need for the NEP's emphasis on technology-driven education. However, the report also notes that 63% of children have access to a smartphone, which can be used for digital learning.

Flexible Curriculum: The NEP proposes a flexible curriculum that allows students to choose from a range of subjects and pursue their interests. According to the National Sample Survey (NSS) 75th Round (2017-18), only 7% of students in India pursue vocational education, highlighting the need for the NEP's proposal to introduce vocational and skill-based courses. The policy also encourages interdisciplinary learning, which integrates different subject areas, and multiple entry and exit points, which allows students to switch between academic and vocational streams.

Experiential Learning: The NEP promotes experiential learning, which involves hands-on activities and real-world projects. According to the ASER 2020 report, 43% of students in rural areas do not have access to libraries, highlighting the need for experiential learning opportunities that do not depend on access to traditional resources. The policy encourages field trips, internships, and community service projects that allow students to apply their learning in practical settings.

Collaborative Learning: The NEP emphasizes the importance of collaborative learning, which involves working in groups and learning from peers. According to the NSS 75th Round (2017-18), 74% of students in India study in government schools, which may have limited resources and opportunities for collaborative learning. The policy encourages the creation of learning communities where students can interact with each other and share their ideas, as well as the use of peer-to-peer learning and mentoring.

Teacher Training: The NEP recognizes the importance of teacher training in promoting innovation in education. According to the District Information System for Education (DISE) 2019-20, there are approximately 11.7 lakh schools in India, but only 66% of them have internet connectivity, highlighting the need for training in the use of technology. The policy encourages schools to provide professional development opportunities for teachers to help them develop the skills and knowledge needed to incorporate innovative teaching methods into their classrooms. One example of innovation in the NEP is the Delhi government's Happiness Curriculum. The curriculum, which is currently being taught to students from Nursery to Class VIII in Delhi government schools, is designed to promote values such as empathy, mindfulness, gratitude, and critical thinking, among others. The curriculum consists of 45-minute sessions conducted every day, where students participate in activities such as meditation, storytelling, and group discussions. The Happiness Curriculum has been well-received by students, teachers, and parents alike. According to a survey conducted by the Delhi government, over 90% of students reported feeling happier and more positive after participating in the curriculum. Additionally, teachers have reported an improvement in students' concentration levels and behaviour. The success of the Happiness Curriculum has garnered international attention, with several countries expressing interest in adopting similar programs. The curriculum's approach to promoting mental health and happiness has also been praised by organizations such as the World Health Organization. Overall, the Happiness Curriculum is an excellent example of how education can be used as a tool to promote holistic development and well-being among students. By incorporating values such as empathy and mindfulness into the curriculum, students can develop the skills and attitudes necessary to lead fulfilling lives and contribute positively to society.

Conclusion: Innovation is a key aspect of the National Education Policy (NEP) 2020 in India. It aims to transform the education system by promoting creativity, critical thinking, and problem-solving skills among students. While there are challenges to the adoption of the NEP's proposals, such as limited access to resources and training, the statistics highlighted in this report indicate that there is a need for change in the education system. The NEP's emphasis on technology-driven education, a flexible curriculum, experiential learning, collaborative learning, and teacher training has the potential to lead to positive changes in the education system in India.

Case 5: Direct Benefit Transfer Welfare Scheme

Introduction: The Direct Benefit Transfer (DBT) scheme is an initiative by the Indian government to transfer subsidies and other welfare benefits directly to the bank accounts of beneficiaries. The scheme aims to reduce leakages and ensure that the benefits reach the intended recipients.

Background: India has several welfare schemes for various sections of society, such as the Public Distribution System (PDS), Mahatma Gandhi National Rural Employment Guarantee Act (MGNREGA), and the National Social Assistance Program (NSAP). However, the implementation of these schemes has been plagued by leakages, corruption, and delays. The DBT scheme was launched in 2013 to address these issues and improve the efficiency and transparency of welfare schemes.

Implementation: The DBT scheme is implemented through the Aadhaar card, which is a unique identification number issued by the government to residents of India. Beneficiaries are required to link their Aadhaar card to their bank account to receive the benefits directly. The scheme also uses the National Payments Corporation of India's (NPCI) platform to transfer the funds. The NPCI is a not-for-profit company that operates the Unified Payments Interface (UPI) platform, which enables real-time interbank transactions.

Benefits:

The DBT scheme has several benefits, including:

a.Reduced leakages: The scheme aims to eliminate intermediaries and ensure that the benefits reach the intended beneficiaries. According to the Ministry of Finance, the DBT scheme has resulted in savings of over INR 1.7 lakh crore ($23 billion) since its inception.

b.Improved transparency: The scheme promotes transparency by providing a clear record of the transactions and the beneficiaries.

c.Reduced corruption: The scheme reduces the opportunities for corruption by eliminating intermediaries and ensuring that the benefits reach the intended recipients.

d.Improved efficiency: The scheme reduces the time taken to transfer the benefits and eliminates the need for physical documentation, resulting in improved efficiency. The implementation of the DBT scheme has had a significant impact on various welfare schemes in India. One such scheme is the PDS, which provides food subsidies to the poor. The PDS has been plagued by leakages and corruption, leading to the benefits not reaching the intended beneficiaries. After the implementation of the DBT scheme in the PDS, the Ministry of Consumer Affairs, Food and Public Distribution reported significant savings. The Ministry stated that the DBT scheme resulted in savings of INR 17,500 crore ($2.4 billion) in the financial year 2019-20. Another scheme that has seen the benefits of the DBT scheme is the MGNREGA, which provides employment to rural households. Implementing the DBT scheme in the MGNREGA has resulted in timely payment of wages to beneficiaries and improved transparency. According to the Ministry of Rural Development, the DBT scheme has resulted in savings of INR 10,140 crore ($1.4 billion) since its implementation in the MGNREGA. As of December 2022, the DBT scheme has covered 310 schemes across 53 ministries and departments of the Indian government. According to Ministry of Electronics, more than six trillion (6.3 lakh crore) were transferred to the beneficiaries under the DBT scheme in the financial year 2021-22.

Challenges:

a.Aadhaar seeding: The scheme requires beneficiaries to link their Aadhaar card to their bank account, which has been a challenge in some cases, especially for elderly and marginalized sections of society.

b.Connectivity: The scheme relies on internet connectivity, which can be a challenge in rural and remote areas.

c.Bank accounts: The scheme requires beneficiaries to have bank accounts, which can be a challenge for those who do not have access to banking services.

d.Exclusion errors: The Aadhaar-based authentication can sometimes result in exclusion errors, where eligible beneficiaries are not able to access the benefits due to technical or other issues.

Conclusion: In conclusion, the DBT scheme has been a significant step towards transforming India's welfare delivery system. It has helped to eliminate corruption, reduce leakages, and ensure that the benefits reach the intended beneficiaries directly. The scheme has resulted in significant savings for the government and improved the lives of millions of Indians. However, the scheme faces several challenges, and the government must address them to ensure that the benefits reach all eligible beneficiaries, especially those from marginalized and remote areas. With continued implementation and improvement, the DBT scheme has the potential to transform India's welfare delivery system and improve the lives of millions of Indians.

Case 6: Mahatma Gandhi National Rural Employment Guarantee Act (MGNREGA)

Introduction: The Mahatma Gandhi National Rural Employment Guarantee Act (MGNREGA) is a flagship program of the Indian government aimed at providing employment opportunities and ensuring livelihood security to rural households. This is accomplished by guaranteeing 100 days of wage-employment in a given fiscal year to rural households in exchange for the adult members' willingness to perform unskilled manual labor as volunteers. It has grown to become one of the most extensive public works programmes in the entire world. It has grown to become one of the most extensive public works programmes in the entire world. The programme hopes to achieve its dual objectives of alleviating poverty in rural regions and enhancing the region's physical infrastructure.

Objectives: As a key goal of India's development policy, the MGNREGA was created to end poverty, providing a legal guarantee for wage employment. The MGNREGA has the following objectives:

a.Providing not less than one hundred days of unskilled manual work as a guaranteed employment in a financial year to every household in rural areas as per demand, resulting in creation of productive assets of prescribed quality and durability.

b.To empower women by providing them with equal employment opportunities and wages.

c.To promote sustainable development and economic growth in rural areas.

d.Strengthening Panchayati Raj Institutions.

Impact of the MGNREGA:

The implementation of the MGNREGA had a significant impact on the village. The following are some of the key findings:

a. Employment generation: The MGNREGA generated employment opportunities for rural households in the village. It provided them with a steady source of income, which enhanced their livelihood security. In the financial year 2020-21, 3.9 crore households were provided employment under the scheme.

b. Wage payment: The timely payment of wages was one of the key features of the MGNREGA. The government disbursed a total of Rs. 1,00,000 as wages to the households in the village. In the financial year 2020-21, the government disbursed a total of Rs. 66,714 crore as wages under the scheme.

c. Asset creation: The MGNREGA contributed to the creation of durable assets and infrastructure in the village. In the financial year 2020-21, a total of 4.4 lakh works were completed under the scheme, including the construction of roads, water harvesting structures, and irrigation facilities.

d. Women empowerment: The MGNREGA provided equal employment opportunities and wages to women, thereby empowering them economically and socially. In the financial year 2020-21, 57% of the total person-days generated under the scheme were by women.

e. Poverty reduction: The MGNREGA contributed to reducing poverty in the village by providing employment opportunities and enhancing the rural economy. It improved the standard of living of the households and reduced their dependency on agriculture. In the financial year 2020-21, the scheme provided employment to 9.2 crore households, thereby providing them with a steady source of income.

One suitable case study example for the impact of the Mahatma Gandhi National Rural Employment Guarantee Act (MGNREGA) is the village of Piplantri in the Rajsamand district of Rajasthan. In 2013, the village started implementing the MGNREGA scheme with the help of the local government and NGOs. Under the scheme, the village created several assets, including check dams, ponds, and community buildings. The scheme generated employment opportunities for rural households and ensured the timely payment of wages. Women played a significant role in the implementation of the scheme, and their participation led to their economic empowerment and social inclusion. The implementation of the MGNREGA in Piplantri had a significant impact on the village. It helped in improving the standard of living of the households, reduced their dependency on agriculture, and enhanced their livelihood security. The scheme also contributed to the development of the local economy by providing a steady source of income to the households and creating durable assets. The success of the MGNREGA implementation in Piplantri is a significant example of how the scheme can be a vital tool in promoting sustainable development and inclusive growth in rural areas of India.

CHALLENGES:

Despite its achievements, the MGNREGA has faced several challenges, such as

a. Delayed wage payment: Delayed payment of wages to the registered households is one of the major challenges faced by the MGNREGA.
b. Corruption: Corruption in the implementation of the MGNREGA is another significant challenge. It leads to the misappropriation of funds and benefits that should have gone to the rural households.
c. Limited coverage: The coverage of the MGNREGA is limited to rural areas only, which leaves out the urban poor.
d. Seasonal nature of work: The nature of work provided under the MGNREGA is seasonal, which limits its effectiveness in providing year-round employment opportunities.
5.Conclusion: In conclusion, the MGNREGA has been a significant initiative in providing employment opportunities and enhancing the livelihood security of rural households in India. The case study of a remote village in Bihar has highlighted the positive impact of the scheme, including the generation of employment, timely wage payment, asset creation, women empowerment, and poverty reduction. However, challenges such as delayed wage payment and corruption need to be addressed to ensure the effective implementation of the MGNREGA and achieve its objectives of promoting sustainable development, reducing poverty, and strengthening the rural economy. Despite these challenges, the MGNREGA has been a vital tool in promoting inclusive growth and improving the standard of living of rural communities.

Case 7: Ayushman Bharat Yojana
1.Introduction: Ayushman Bharat Yojana is a health insurance scheme launched by the Indian government in 2018 to provide health coverage to vulnerable sections of society. The scheme covers over 50 crore people across the country, making it the world's largest government-funded health care program. The programme is part of the Indian government's National Health Policy. It is a centrally sponsored scheme and is jointly funded by both the union government and the states.
2.Implementation of Ayushman Bharat Yojana: Under the scheme, eligible beneficiaries are entitled to receive health coverage of up to Rs. 5 lakh per family per year for secondary and tertiary care hospitalization. The scheme is being implemented in partnership with public and private hospitals, and it covers more than 1,500 medical procedures, including surgeries, diagnostics, and treatments.
3.Impact of Ayushman Bharat Yojana: Since the launch of the Ayushman Bharat Yojana, there has been a significant impact on the healthcare sector. The following are some of the key findings-
Increase in Health Coverage: The Ayushman Bharat Yojana has significantly increased the number of people covered under health insurance in India. As of February 2022, the scheme has covered more than 12.5 crore beneficiaries, including 6.5 crore rural and 6 crore urban families.
Reduction in Out-of-Pocket Expenditure: The Ayushman Bharat Yojana has led to a reduction in out-of-pocket expenditure for healthcare services. The scheme covers medical expenses up to Rs. 5 lakh per family per year, which has reduced the financial burden on households.
Reduction in Hospitalization Expenses: The scheme has reduced the hospitalization expenses for beneficiaries. The government has provided an estimated Rs. 25,000 crores to private and public hospitals under the scheme, which has reduced the financial burden on healthcare institutions.
Increase in Hospitalization Rates: The Ayushman Bharat Yojana has led to an increase in the hospitalization rates in the country. The scheme has provided access to affordable healthcare services to vulnerable sections of society, leading to an increase in hospitalization rates.
Improvement in Health Outcomes: The Ayushman Bharat Yojana has led to an improvement in health outcomes for beneficiaries. The scheme has provided access to affordable healthcare services to vulnerable sections of society, leading to a reduction in morbidity and mortality rates.

CASE STUDY OF HARYANA:
Haryana is a state in northern India with a population of approximately 2.6 crore people. Since the launch of the Ayushman Bharat Yojana, the state has made significant progress in providing health coverage to its residents.
a. Increase in Health Coverage: As of February 2022, the Ayushman Bharat Yojana has covered more than 46 lakh beneficiaries in Haryana. The scheme has significantly increased the number of people covered under health insurance in the state.
b. Reduction in Out-of-Pocket Expenditure: The Ayushman Bharat Yojana has led to a reduction in out-of-pocket expenditure for healthcare services in Haryana. The scheme covers medical expenses up to Rs. 5 lakh per family per year, which has reduced the financial burden on households.
c. Increase in Hospitalization Rates: The Ayushman Bharat Yojana has led to an increase in the hospitalization rates in Haryana. The scheme has provided access to affordable healthcare services to vulnerable sections of society, leading to an increase in hospitalization rates.
d.Improvement in Health Outcomes: The Ayushman Bharat Yojana has led to an improvement in health outcomes for beneficiaries in Haryana. The scheme has provided access to affordable healthcare services to vulnerable sections of society, leading to a reduction in morbidity and mortality rates.
e. Reduction in Infant Mortality Rate:
The Ayushman Bharat Yojana has also contributed to a reduction in the infant mortality rate in Haryana. The state has recorded a decline in the infant mortality rate from 33 per 1,000 live births in 2016 to 26 per 1,000 live births in 2021, which is attributed to the scheme's implementation.

Challenges:
(i) With an aim to provide healthcare to 10 crore families, the magnitude of the scheme is a challenge due to the underdeveloped infrastructure, like the lack of availability of hospital beds in rural areas as well as Tier II and III cities.
(ii) With a national budget with a relatively low central government investment in health care - about one percent of the GDP, India was still facing fundamental healthcare obstacles such as a lack of physicians and an increase in infectious disease cases before the launch of the scheme. Hence, the scheme could fall flat if it did not ensure a minimum level of quality.
(iii) System inadequacies exist in the healthcare system; hence lack of uniformity of hospital procedures and protocols for doctors is another challenge. With primary care in disarray, most hospitals that are already overburdened with secondary and tertiary care are then forced to assume additional responsibilities. Additionally, some private corporate hospitals have not joined the program. Since profits primarily guide them, they report that they would not be able to offer their services at the government low price, even with a government subsidy.
(iv) Lack of participation of all states during the implementation of the scheme till certain concerns of those states would be addressed since they had existing health assurance schemes.

Conclusion: The Ayushman Bharat Yojana has been a significant initiative in providing health coverage to vulnerable sections of society in India. The scheme has significantly increased health coverage, reduced out-of-pocket expenditure, hospitalization expenses, and improved health outcomes for beneficiaries. However, challenges such as a limited hospital network and awareness and information need to be addressed to ensure the scheme's effective implementation and achieve its objectives of promoting universal health coverage and strengthening the healthcare system in the country. Despite these challenges, the Ayushman Bharat Yojana has been a vital tool in promoting inclusive growth and improving the health outcomes of vulnerable sections of society in India.

Outcomings:

This paper explores a common thread among various case studies in India's recent policy landscape for example- the Innovation in National Education Policy, the Direct Benefit Transfer Welfare Scheme, the Mahatma Gandhi National Rural Employment Guarantee Act of 2005, and the Ayushman Bharat Yojana. These policy interventions demonstrate India's innovative and proactive approach to tackling key social and economic challenges. The Innovation in National Education Policy, for example, aims to transform the education system by providing a more practical and holistic learning experience. As of 2021, the policy has been implemented in 33 states and Union Territories, covering over 27 crore (270 million) students. The policy emphasizes early childhood education, vocational training, and the use of technology to improve learning outcomes. The policy also aims to increase the Gross Enrollment Ratio (GER) to 50% by 2035, up from the current GER of 27.1%. The Direct Benefit Transfer Welfare Scheme, launched in 2013, seeks to ensure that government welfare benefits reach the intended beneficiaries in a timely and efficient manner through the use of technology. As of August 2021, over 5.5 crore (55 million) beneficiaries have received cash transfers through the scheme, amounting to a total of over Rs. 17.5 lakh crore (USD 236 billion). The scheme has also helped to weed out duplicate beneficiaries, leading to savings of over Rs. 1.78 lakh crore (USD 24 billion) in the past 7 years. The Mahatma Gandhi National Rural Employment Guarantee Act of 2005 (MGNREGA) provides employment opportunities to rural households, with a focus on women and marginalized communities. As of 2021, over 13 crore (130 million) households have availed of employment opportunities under the scheme, with a total of 37.7 crore (377 million) person-days of work generated. The scheme has also played a crucial role in reducing poverty and improving rural livelihoods. The Ayushman Bharat Yojana, launched in 2018, aims to provide universal health coverage to all Indian citizens, especially those who are vulnerable and marginalized. As of January 2022, over 2.5 crore (25 million) beneficiaries have availed of the scheme's benefits, which include cashless hospitalization and access to free medical treatment. The scheme has also played a crucial role in reducing out-of-pocket healthcare expenses for families, which can be a major cause of financial distress. In conclusion, the convergence of these policy interventions highlights the Indian government's commitment to social welfare and innovative solutions to address key challenges. By leveraging technology, promoting employment, improving education, and providing access to healthcare, India is taking significant steps towards a more inclusive and equitable society.

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SAFFRON (CROCUS SATIVUS L.) CULTIVATION IN THE NORTH-WESTERN HIMALAYAS: A CASE STUDY

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ABSTRACT
Saffron isa low volume high value crop but there is a huge gap between its production and demand. One way to fill this gap is the extension of its cultivation to non-traditional areas. Three-year trial from 2018-2021s were conducted at seven different non-traditional locations, of north western Himalayas. These areas were selected as they had similar pedo-climatic conditions for saffron cultivation, as in traditional areas. The overall yield and the stigma quality were evaluated along with soil and climatic properties of traditional as well as non-traditional sites of saffron cultivation. It was observed that yield attributes, viz., flower number, stigma length, fresh and dry weight were significantly higher at Mandi during the three year trials. Mandi is located in Poonch district at an altitude of 1580 m amsl with >1000 mm annual rainfall, sandy soil texture, and high Nitrogen Phosphorus and Potassium content in the soil. Principal component analysis (PCA) concluded that there is a positive relation between environmental factors such as temperature, rainfall and altitude with soil health and with the overall yield of the saffron. Based on yield of spice Mandi and Assar were found to besuitable for saffron cultivations. Extension of saffron cultivation to these areas will not only fill in the gap between demand and supply of saffron but will also increase the income of farmers living in NW Himalays.

Keywords: Saffronnorth west Himalayas, non-traditional areas, agricultural extension, alternate farmi

INTRODUCTION
The dried stigmas of Crocus sativus L. are used to make saffron, which plays a vital role in the agricultural economy. It has been utilized as a human disease treatment since ancient times and was used to dye for clothing and in culinary condiments (Magotra *et al.,* 2021).Due to its application in the food sector, cosmetic and health industries, it's demand is increasing and so is its cost (Husaini and Wani, 2020). Saffron has tremendous medicinal properties such as; neuroprotection,cytotoxicity, anti-depression, anti-inflammatory,anti-oxidant, anti-carcinogenic and visual improvement effect (Abu-Izneid *et al.,* 2022). Iran produces the majority of the world's saffron, accounting for roughly 90% of it with India coming in second with about 4.0 kg/ha net production (Agriculture Department, Jammu and Kashmir, UT, India) but ranking 12th in the world for saffron exporters (Husaini and Wani, 2020). In India, saffron is mostly grown in Jammu and Kashmir's four districts of Pulwama, Budgam, Srinagar, and Kishtwar (Gupta *et al.,* 2020). Being sterile, it vegetatively propagates through corms (Bhagat *et al.,* 2021). It is reported that factors such as; size and health of mother corm, plantation time, depth and density during plantation, post-harvesting management, drying, and storage conditions influence the yield and quality of saffron (Cardone *et al.,* 2019). Corm sprouting, initiation of flowering and flowering time are generally governed by temperature, altitude, photoperiod, topographical locations, and soil characteristics (Cardone *et al.,* 2020). The saffron crop can grow well in loose, well-irrigated, well-drained and friable clay calcareous soils with pH ranging between 6.8–7.8 and having electrical conductivity (E.C.) less than 2 dS m-1 (Kothari *et al.,* 2021).In the present report Keeping in view the growing demand of saffron and the requirements of saffron cultivation in various parts of the world, seven non-traditional areas in the western Himalayas were selected for evaluation as potential sites for commercial saffron cultivation.

MATERIALS AND METHODS
Experimental sites:
2018-2021, three-year saffron cultivation trial has been done on seven locations in north-western Himalayas (Table 1) which were selected on the basis of literature review for environmental conditions for saffron cultivation (Table 2). Physicochemical properties of the soil were done at Yara Fertilisers India Pvt. Ltd India (Table 1).

Agronomic field trials:
Saffron healthy corms of weighing 8-10 g each were purchased from traditional farmers of Pampore of Pulwama district in Kashmir valley (74.93° E Latitude, 34.02° N longitude),India. Land preparation was done by ploughing 2-3 times before plantation.300 saffron corms were planted per

location with 10 X 15 cm distance (Cardone *et al.,* 2019). Weeds wereremoved manually.In flowering phase, flowers were harvested manually early in the morning before sunrise. Soon after harvesting, stigmas were separated manually, dried at 40 °C for 24 h, and stored at 18-22°C in dark glass jars for qualitative analysis. Total numbers of flowers were recorded to find the actual yield / location/ year.

Table 1: Meteorological data and physiochemical properties of soil of selected locations.

Site	District	Altitude (amsl)	Latitude (°N)	Longitude (°E)	Rainfall (cm)	pH	Sand%	Silt%	Clay%	OC (%)	N (kg/ha)	P (kg/ha)	K (kg/ha)
Gursai	Poonch	1580m	33.6047118	74.254532	885	7.1±0.15b	36.6±1.1b	32.6±0.5e	30.8±0.51f	1.8±0.05c	217.5±2.0b	4.3±2.5a	118.5±1.6d
Mandi	Poonch	1580m	33.742084	74.267270	1001	7.4±0.15bc	50.3±0.5d	22±1.0b	27.7±0.57e	2.4±0.1d	290.1±4.0e	28.9±0.75g	153.9±1.8f
Dagwar	Poonch	1577m	33.783094	74.108690	964	7.4±0.11bc	47.6±0.5c	34±1.0e	18.4±0.51a	1.0±0.03a	255.5±1.5d	22.5±3.3f	121.5±2.8d
Kanthi	Ramban	662m	33.253473	75.239106	1031	7.3±0.05b	51.3±0.5d	24.3±0.5c	24.4±0.53d	1.5±0.05b	237.9±2.9c	16.3±1.5e	53.1±1.1a
Assar	Doda	1107m	33.168612	75.348206	1041	7.3±0.05bc	51.6±0.5d	19.6±0.5a	28.8±0.52e	2.0±0.2c	234.5±1.6c	11.8±1.8c	129.3±0.7e
RS Pura	Jammu	270m	32.603955	74.739167	790	7.8±0.1c	55±1.0e	22.6±0.5bc	22.4±0.5b	1.0±0.1a	181.6±1.4a	5.4±2.4b	97.5±1.2b
Mantalai	Udhampur	1225m	33.0020728	75.356851	764	6.4±0.3a	48±1.0c	28.3±0.5d	23.7±0.5c	1.3±0.1ab	249.5±1.8d	13.5±3.0d	109.1±1.6c
Pampore	Pulwama	1573m	33.9981412	74.9114286	743	7.1±0.1b	28.7±0.2a	38.1±0.3g	33.2±0.2g	3.7±0.1f	298.5±0.2f	35.7±0.3i	168.1±0.4h
Kishtwar	Kishtwar	1638m	33.3526488	75.6902432	960	7.4±0.2bc	27.6±0.1a	36.2±0.2f	36.2±0.4h	2.8±0.2e	289.5±0.4e	30.2±0.5h	159.3±0.5g

Table 2: Physio-climatic properties of saffron growing countries worldwide

Countries Variants →	**India**		**Iran (Kho-rasan)**	**Spain (La Man-cha)**	**Italy (Abruzzo)**	**Morocco (Taliou-ine)**	**Greece (Kozani)**	**Afghan-istan (Herat)**	**Australia (Tasma-nia)**
	Pampore	**Kishtwar**							
Altitude (m, asml*)	1573	1638	1540	610	714	1007	710	920	1617
Air Temperature °C	-1 to 29	-2 to 30	-2 to 31	1 to 30	-1 to 29	3 to 37	-1 to 28	-4.4 to 33	3 to 23
Photoperiod (h. min)	9. 52 to 14.25	9.56 to 14.21	9.53 to 14.25	9.25 to 14.55	9.05 to 15.16	9.47 to 14.31	9.17 to 15.02	9.51 to 14.27	9.06 to 15.14
Annual Rainfall (mm)	740	740	57.8	170	702	233	461	158.8	214
Rainfall one month prior to flowering (mm)	50	50	5	7.5	47	4.9	47	2.2	21.3
Humidity	69%	69%	26%	55%	68%	74%	62%	36%	74%
Humidity one month prior to flowering	69%	69%	14%	47%	69%	56%	57%	27%	65%
Soil type	Silty- clay loam	Silty-clay loam	Silty sandy-Calcareous texture	Calcareous texture	Calcareous texture	Sandy silty texture	Sandy clay loam	Sandy loam	Sandy loam
Soil pH	6.9 to 8.8	6.91 to 8.0	6.5 to 8.1	6.1 to 8.0	6.0 to 8.0	6.1 to 8.0	6.9 to 8.3	6.4 to 8.2	7.1 to 8.4

Meteorological data:
For each flowering phase every year and from each experimental location, meteorological data was collected in which temperature; relative humidity, and rainfall were recorded at the time of the start of flowering. (https://www.timeanddate.com/)

Spectrophotometric analysis of saffron components:
Saffron from all different locations were subjected to qualitative analysis as per ISO-3632, (Sereshti *et al.,* 2018). 500 mg of powdered saffron was taken which was filtered through a 0.5 mm sieve. The powder was transferred to a 1000 ml volumetric flask which contained 900 ml distilled water. The solution was stirred for 1 hour in dark and then the final volume was raised to 1000 ml with distilled water. The resultant extract was diluted to 1:10 with deionized water, and filtered through polytetrafluoroethylene filters (PTFE) of 15 mm diameter with 0.45µm pore size, the final saffron aqueous extract was analyzed for crocin, picrocrocin, and safranal by taking absorbance of 1% aqueous saffron extract at 440, 257 and 330 nm respectively by UV–visible spectrophotometer (Thermo Scientific GENESYS™ 10S UV-Visible Spectrophotometer) and as per equation 1.

$$A^{\wedge(1\%)}\,1cm=((Dx20000))/((100-H)) \qquad \text{Eq. 1}$$

Where: D is the absorbance at 440, 330, and 257 nm respectively; 20 000 is dilution factor for saffron extract sample; H is the volatile and moisture content, (as a mass fraction). H was determined as per ISO 3632, by placing 2.5±0.01g of saffron sample in the oven for 16 h at 103±2 °C for drying, and was calculated as the percentage of the initial weight of the sample as per equation 2.

$$H= (m_0-m_1)*(100/m_0)\%'\,' \qquad \text{Eq.2}$$

Where: m0is mass (g) of saffron before drying, and m1is mass (g) after drying.

Statistical analysis:
A mean of ten replicates was used to represent all the tests. Version 26 of IBM SPSS statistics was used for the investigation. The Tukey's test was then used in a post-hoc analysis with a 5% threshold of significance.

RESULTS AND DISCUSSION

Physiochemical properties of soil:
All the selected locations have sandy soil except Gursai where sand, silt, and clay were evenly distributed. The pH of all selected soils are alkaline except Mantali which has slightly acidic pH. Organic carbon, Nitrogen, Phosphorus, and Potassium in the various concentrations (Table 2). The traditional sites in India, Pulwama and Kishtwar have silty-clay loam texture, pH (6.3 to 8.3), (Ganaie and Singh, 2019). A variety of soil types have been reported for saffron cultivation around the world, including silty-sandy-calcareous soil in Iran, sandy-loamy soil in Azerbaijan, calcareous soil in Spain, and sandy-loamy soil in Afghanistan (Cardone *et al.,* 2020). Other reported soil types include clay loam in Greece and sandy clay in the Sardinia region and Morocco (Husaini and Wani, 2020).

Flowering and climatic condition:
In Kanthi, Mantalai and RS Pura flowering started in November when temperature ≤ 17 °C 3 whereas at other locations flowering started in October when temperature ranged between 16 °C to 17 °C. In the traditional saffron-growing region of Kashmir, the flowering phase, which lasts for approximately a month, has been observed between mid-October and mid-November (Husaini and Wani, 2020). These authors have reported that for flower emergence and proper flowering day temperature should be 17 °C which was observed to be true in the present study as well.

Saffron growth and yield at selected locations:
Out of 300 corms planted per selected location, the maximum number of flowers was produced in Mandi in Poonch and minimum in RS Pura in Jammu in the first year of cultivation i.e. 2018-19. During the second year, i.e. 2019-20 number of flowering increased at every location, again with maximum flowers were produced in Mandi and very few flowers in RS Pura. The number of flowering produced decreased in the third year i.e. 2020-21 at every selected location except for Mandi and no flowering was observed at RS Pura (Table 3).Numbers of flowers are directly linked to the yield of saffron, so the yield is as per flowering number with maximum in Mandi of Poonch district during all the three years. In all locations during first year the saffron is in grade I category (as per ISO-3632) but in third year only Mandi's saffron is in grade I category. Mandi has a sandy soil texture but it has the highest altitude, OC, and NPK content as compared to other selected locations, as reported by Kothari et al. 2021, sandy soil increases root growth, penetration, and development along with corm size, flower numbers, and yield. Mykhailenko *et al.,* (2020) reported that at high altitude, the better quality of crocin and picrocrocin might be due to air temperature, rainfall, solar radiations along with soil texture and soil properties (OC, N, P, K).

Principal component analysis (PCA):
Principal component analysis was done to find the relationship between soil properties, saffron yield and with different climatic conditions. The selected parameters were reduced to two principal components that have total variance of 69.42%. PC 1 have37.89% and PC 2 have21.84% of the total variability. Three soil parameters (N, P, and OC), two climatic pa-

rameters (rainfall RF and altitude ALT), and three plant parameters (flower number FN, Stigma fresh weight STIFW and overall yield YLD) have a positive relationship with both PC1 and PC2 (Figure 1). EC has a negative relationship with both PC1 and PC2. The score plot shows a clear separation between cultivation sites. Mandi, Dagwar, Mantalai, and Assar have a positive relation with PC1, and Gursai, Mandi, Dagwar, and Kanthi have a positive relationship with PC2. Only Mandi and Dagwar show positive relationship with both PC1 and PC2 whereas RS Pura shows negative relation for PC1 and PC2 and lies just opposite to Mandi. From PCA analysis it is concluded that altitude, rainfall, and soil nutrition are important for saffron cultivation. Cardone et al, 2020,reported that factors including temperature, height, and soil moisture content affect both the quality and quantity of saffron, and the majority of saffron growing locations are in the 600–1700 m amsl range in altitude.

Location Year	Gursai	Mandi	Dagwar	Kanthi	Assar	RS Pura	Mantalai
Number of flowers (out of 300 corms)							
2018-19	33	120	42	30	90	10	24
2019-20	175	280	245	245	214	12	205
2020-21	42	388	15	16	150	NF	11

Data represent total number of flowers produced per location during complete flowering phase every year.
NF- No Flowering

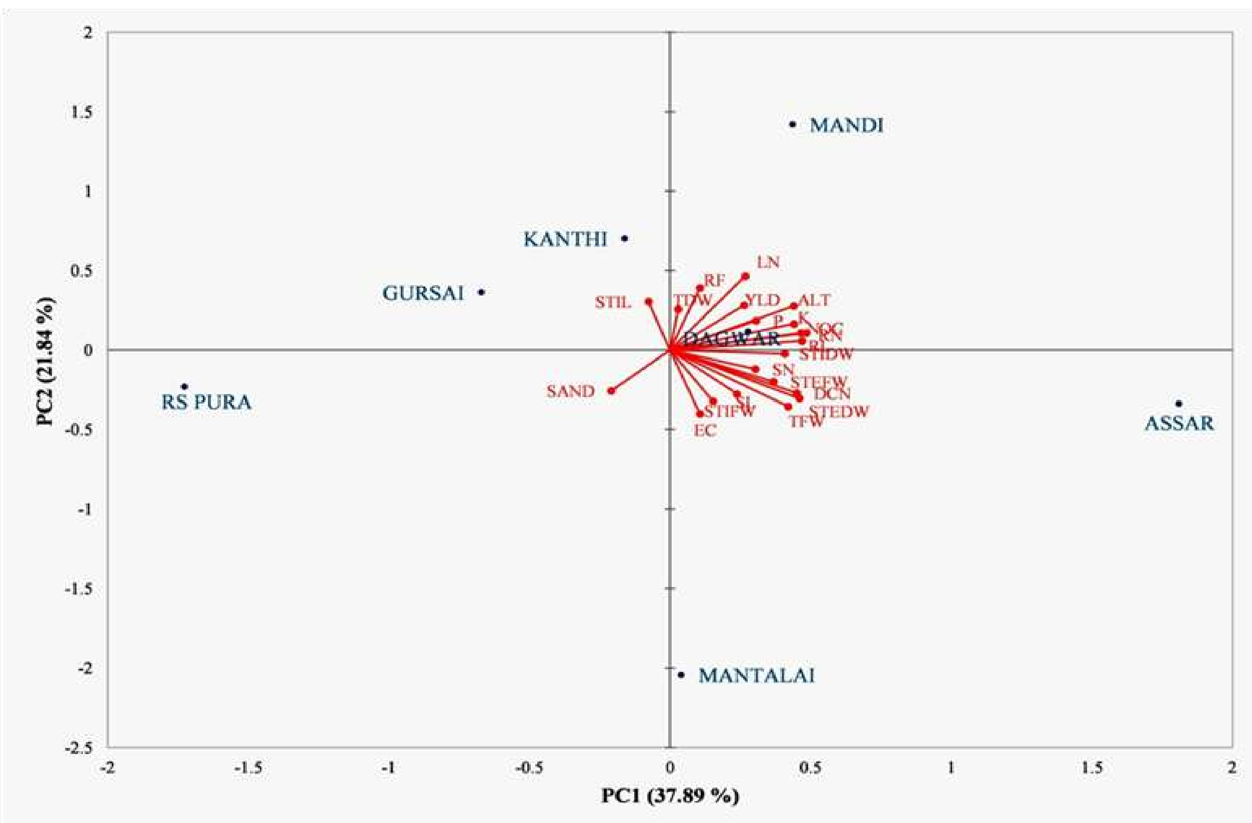

Fig .1. Biplot representing two-dimensional space projection of soil, climatic and plant parameters along with different geographic locations. SN (Shoot Number), SL (Shoot Length), RN (Root Number), RL (Root Length), LN (Leaves Number), DCN (Daughter Corm Number), STIL (Stigma Length), STIFW (Stigma Fresh Weight), STIDW (Stigma Dry Weight), STEFW (Stamen Fresh Weight), STEDW (Stamen Dry Weight), TFW (Tepal Fresh Weight), TDW (Tepal Dry Weight), EC (Electrical Conductivity), SAND (Sand), OC (Organic Carbon), N (Nitrogen), P (Phosphorus), K (Potassium), ALT (Altitude), RF (Rainfall), and YLD (Yield).

CONCLUSION

The introduction of its cultivation in non-traditional places can close the gap in saffron output. Due to its low volume and high value, the farmer's income will grow. For each of the features taken into consideration, the results of this study shown considerable variations between various regions. Therefore, choosing a precise geographic location is crucial for effective cultivation. The mixture of soil properties, including OC and NPK levels, as well as altitude, is the most important aspect in the growth of saffron. According to our research, saffron may be grown wherever that has a climate comparable to Mandi by modifying the soil's NPK concentration.

ACKNOWLEDGEMENTS

Authors also thank UGC-SAP, DST-PURSE, DST-FIST, and MHRD-RUSA. Authors also thank GBP-NIHE, Almora. Tahir Ali is thankful to UGC for the fellowship under NET-JRF Scheme.

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NANOCURCUMIN-BASED FORMULATION FOR COMBATING CARDIAC AND SKELETAL MUSCLE DAMAGE AT HIGH ALTITUDE

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ABSTRACT

High Altitude (HA) exposure leads to various adverse effects on cardiovascular system and skeletal muscles. Since general oxygen availability declines under hypoxia, the right ventricles pump more blood towards the pulmonary bed in order to improve oxygenation of blood and undergoes excessive volume overload. Under such conditions, cardiovascular system modulates its function by acute increase in stroke volume, blood pressure and cardiac output. These functional changes are realized in terms of modulation in the morphological and physiological attributes of the heart and development of compensatory right ventricular hypertrophy (RVH). Apart from that there is decreased aerobic work capacity following skeletal muscle atrophy at high altitudes. However, the effects of HA on myofiber remodeling and mitochondrial Ca2+ homeostasis are largely unexplored. A distinctive combination of nanocurcumin (NC) and pyrroloquinolinequinone (PQQ) has been formulated (named as NCF, Indian patent no. 302877) in our lab to address the high-altitude maladies. Current work presents experimental proofs for therapeutic evaluation of cardio-protective efficacy of NCF in vitro and in vivo under hypoxia-induced pathological RVH and associated pulmonary impairments. NCF supplementation effectively prevented the transition of RVH from state of acclimatization to de-compensation secondary to pulmonary damages. NCF also alleviate skeletal muscle atrophy and improve exercise exhaustion time following rescuing myofiber transition from slow to fast which leads to enhanced physical endurance under hypobaric hypoxia. Altered expression of mitochondrial calcium uptake 1 (MICU1) and impaired mitochondrial membrane potential ($\Delta\psi m$) results in apoptosis and mitochondrial dysfunctionality. NCF prevented skeletal muscle atrophy, resisted transition of myofibers and improved mitochondrial dysfunctionality. Thus, NCF could work as a therapeutic of choice for rescuing both cardiac as well as skeletal muscles from hypoxia-associated damages.

INTRODUCTION

Millions of visitors including pilgrims, adventure seekers, tourists, and army personnel go to high altitudes every year. Most of the visitors are unaware of the illness and adverse physiological effects associated with a rapid ascend to high altitudes. The high altitude induces acute mountain sickness which may further lead to potentially life-threatening pathologies like high-altitude pulmonary edema, high-altitude cerebral edema, right ventricular hypertrophy, thrombosis, and a severe decline in physical activity (Basnyat and R. Murdoch, 2003; Gallagher and Hackett, Selvamurthy and Basu, 1998; Taylor, 2011). The heart is a dynamic muscular organ that continuously remains in rhythmic contractile motion in order to pump blood to maintain constant supply oxygen and nutrients to the body. Although the cardiovascular system encompasses remarkable acclimatization potential against hypoxic stress, chronic deprivation of oxygen might cause significant changes in cardiovascular physiology and enhances the risk of disease progression (Poupa *et al.,* 1966). A decline in oxygen availability (hypobaric hypoxia) is a main physiological stress during altitude exposure leading to right ventricular hypertrophy, reduced muscle mass (atrophy) and oxidative work capacity (Boyer and Blume, 1984; Hoppeler and Vogt, 2001). Maintenance of homeostasis and normal functioning of body rely upon un-interrupted supply of oxygenated blood to conduct aerobic respiration. Since general oxygen availability declines under hypoxia, the right ventricles pump more blood towards the pulmonary bed in order to improve oxygenation of blood and undergoes excessive volume overload (Berger *et al.,* 2012). Under such conditions, cardiovascular system modulates its function by acute increase in stroke volume, blood pressure and cardiac output (Ge and Helun, 2001; Huez *et al.,* 2007). These functional changes are realized in terms of modulation in the morphological and physiological attributes of the heart and development of compensatory right ventricular hypertrophy (RVH) (Leon-Velarde *et al.,* 2010). However, the excessive volume overload on right heart is further intensified by trans-vascular leakage in the pulmonary vasculature, thus exerting pressure overload on the right heart. This sustained pressure and volume overload might eventually transform the compensatory hypertrophy into a state of de-compensation leading to failure in adaptation. Various pre-clinical and clinical reports have shown that de-compensation and dilatory cardiac hypertrophy remain critical initial symptoms of clinically important and potentially lethal conditions such as heart failure (Berger *et al.,* 2012; Di *et al.,* 2007; Bradley and Floras, 2009; Vercesi *et al.,* 2018). The cardiac muscles require an un-interrupted supply of energy for continuous rhythmic contractile function, the preservation of mitochondrial homeostasis is of prime importance (Di *et al.,* 2007). In addition, hypoxia is also an etiological factor in the global burden of chronic diseases, such as cardiovascular diseases (Bradley and Floras, 2009), type II diabetes mellitus (Aurora and Punjabi, 2013), sarcopenia (Di *et al.,* 2009), chronic obstructive pulmonary disease (De *et al.,* 2011), acute respiratory distress syndrome (Nitsure *et al.,* 2020), cancer cachexia, and disuse, restraining physical activity (Semenza, 2014). Hypobaric hypoxia may lead to various consequences in skeletal muscle by imposing severe oxidative stress, skeletal muscle atrophy, myofiber remodeling, mitochondrial dysfunction, and impaired myogenesis (Grocott *et al.,* 2007; Chaillou, 2018). Skeletal muscle atrophy is pronounced during acute and chronic exposure at high altitudes in humans and animals due to oxidative stress-induced proteostasis alterations (Agrawal *et al.,* 2017; Hoppeler and Vogt, 2001; MacDougall *et al.,* 1991). Oxidative stress is widely considered a major cause of muscle atrophy not only at high altitudes but also in most chronic diseases, triggering the oxidative modifications of proteins and activation of catabolic pathways involved in their degradation (Powers *et al.*, 2005; Chaudhary *et al.,* 2012). Strong evidence indicates that both calpain and the ubiquitin-proteasome system play important roles in proteolysis (Powers *et al.*, 2005). Ample studies suggested that muscle atrophy can be limited by inhibiting calpain activity and NF-κB signaling in various stress conditions (Salazar *et al.,* 2010; Jackman and Kandarian, 2004; Bartoli and Richard, 2005). Skeletal muscle is metabolically active and sensitive to hypoxia and largely dependent on oxidative metabolism for ATP supply because of its high energy demand during prolonged contraction. Basically, skeletal muscle has two types of fibers: 1) Slow/oxidative fibers and 2) Fast/glycolytic fibers. Slow fibers are fatigue-resistant and responsible for oxidative work capacity with high mitochondria numbers. In contrast, fast fibers are fatigue-susceptible with few mitochondria numbers. Importantly, fast fibers (Gastrocnemius muscle) are more susceptible to hypoxia-induced oxidative damage than slow fibers (Soleus muscle) (Chaudhary *et al.*, 2012; Rahar *et al.,* 2019). Hypoxia-induced oxidative stress triggers alterations in the phenotype expression of muscle fibers and modulating muscle contractile properties (Perrey and Rupp, 2009; Itoh *et al.*, 1990; Chaillou *et al.*, 2014), however, molecular insight into myofiber remodeling in skeletal muscle at high altitudes is remain to understand. Chronic exposure at extreme altitudes (beyond 6400 m) reduced mitochondrial densities by 21%, with a loss of 73% of subsarcolemmalmitochondria, this could be an adaptive change to reduce ROS leakage and oxidative stress (Levett *et al.*, 2012; Murray and Horscroft., 2016). Hypobaric hypoxia limits mitochondrial function with a concomitant decrease in oxidative phosphorylation (Hoppeler *et al.*, 1990; Magalhaes *et al.*, 2005), but the mechanism of mitochondrial dysfunction is largely unexplored. Indeed, calcium and ATP are crucial factors for excitation-contraction coupling in skeletal muscle (Brookes *et al.*, 2004). Mitochondrial plays an important role in calcium homeostasis during intracellular calcium fluctuations in the heart and skeletal muscle (Williams *et al.,* 2013). A recent study elucidated the mechanism of impaired intracellular calcium handling at a simulated high altitude and suggested that dysregulation of the ryanodine receptor following a redox modification allows excessive leakage of Ca_{2+} from the sarcoplasmic reticulum to cytoplasm (Agrawal *et al.*, 2020). Impaired intracellular handling linked to mitochondrial calcium overload and disease progression (Jadiya *et al.*, 2019; Santulli *et al.*, 2015; Vercesi *et al.,* 2018). However, the effect of impaired intracellular calcium handling on mitochondrial function is not well elucidated at high-altitude hypoxia. Considering that a large number of low landers ascend the high altitudes annually, including military personnel, pilgrimages, trekkers and tourists, it remains absolutely necessary to not only acknowledge these cardiovascular impairments and physical activity decline but also demand to develop potential therapeutic candidates to help in improving acclimatization. Thus, in the light of these evidence, we planned a cohort of study to not only established in vitro and in vivo models at high altitudes, but also developed a therapeutic strategy to help improve acclimatization. To better understand the mechanism of reduced oxidative work capacity at high altitude the present study investigated the alterations in oxidative fibers and mitochondria function. The present study also suggested a therapeutic intervention prepared in our lab by combining nanocurcumin and pyrroloquinolinequinone at a certain ratio (Fig. 1) named nanocurcumin formulation (NCF, Indian patent No. 30877). Nanocurcumin (size 200nm) is a nanotized form of curcumin (a plant extract from Curcuma Longa) with improved bioavailability and biostability. Further nanocurcumin was added with another potent molecule, pyrroloquinolinequinone, to address high altitude-induced multidimensional consequences in vital organs.

NCF imparts protection to cardiomyocytes under hypoxia

The whole study cohort was divided into multiple stages of initial screening, in vitro investigations and finally in vivo investigations and validation. The initial experimentations

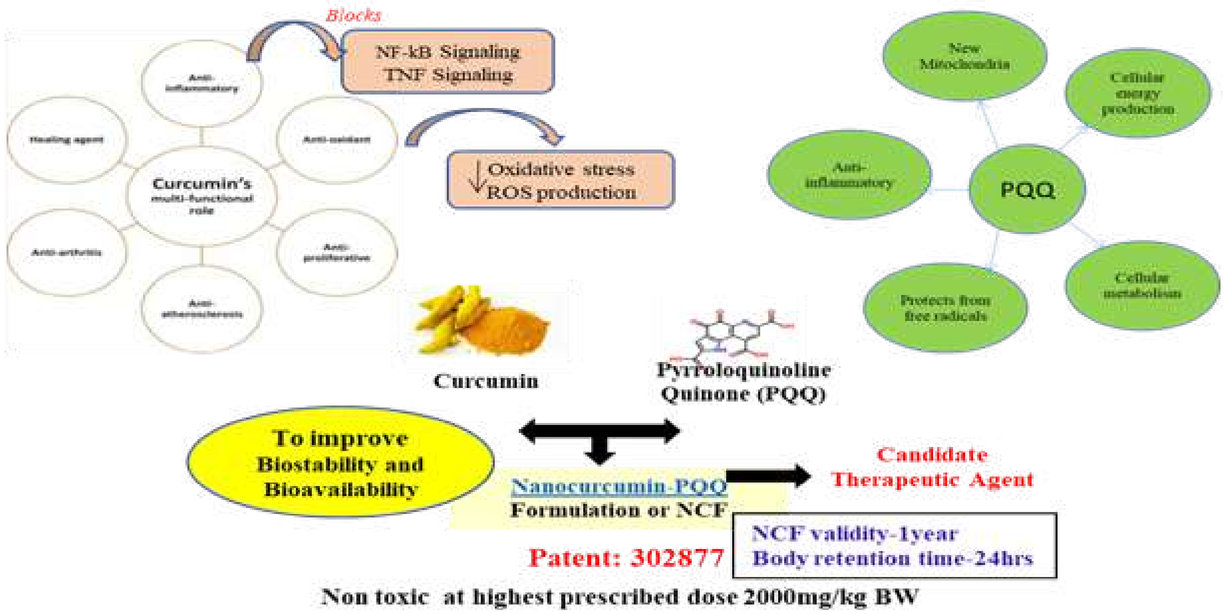

Fig. 1. Preparation of Nanocu rcumin based formulation

targeted to investigate the efficacy of NCF in improving adaptation were performed using multiple established cell lines (MRC5, A549, N2a and H9c2) representing variety of tissue representatives. Also, a set of experiments were performed to investigate the effect of NCF on modulating acute hypoxia induced pulmonary hyper-ventilatory response, changes in exercise capacity by treadmill-exercise test, investigations on changes in cardiac morphometry along with cerebral and pulmonary edema formation and trans-vascular leakage. These initial set of experiments provided sufficient data demonstrating enhanced cyto-protection under in vitro conditions as well as improvement in acclimatization at a relatively small dosage of supplementation. The observations and results obtained from these initial set of studies were claimed as intellectual property in the form of Indian patent (2749/DEL/2013A). The first set of pre-clinical investigations was performed using rodent ventricular cardiomyoblast H9c2 as experimental model. The cells were exposed to hypoxic stress (0.5% Oxygen) for 24, 48 and 72 hours to assess hypoxia induced cell-death. Results demonstrated that 24 hours of exposure was sufficient to bring about cell death in H9c2 cells. Further, in order to investigate appropriate dose of nanocurcumin (NC) against raw curcumin (C), the cells were supplemented with a 100-1000 ng/ml of NC just prior to exposure to hypoxia. It was found that 500 ng/ml of NC effectively improved cell survival under hypoxia. Further investigations demonstrated that NC supplementation improved cellular survival by regulating caspases of mitochondrial origin. In this regard, the activation of caspase-3,-7 was found to be suppressed by NC supplementation while simultaneously restoring cellular redox balance by maintenance of lipid peroxidation, GSH/GSSG content and reducing excessive ROS leakage. Finally, the molecular markers of survival (Akt/Erk pathway), hypoxia-responsive elements (HIF-1α) and hypertrophy (ANF and excessive accumulation of essential amino acid) were also effectively modulated by NC supplementation. Together, these set of evidences demonstrate that supplementation with NC was effective in maintaining cellular homeostasis under hypoxia (Nehra *et al.*, 2015). The leads obtained from the preliminary study on H9c2 cardiomyoblasts were used to perform further detailed investigation in primary human ventricular cardiomyocytes (HVCM) to demonstrate the efficacy of NC against C in an experimental cellular model close to humans. These studies were targeted primarily to investigate the molecular mechanisms behind NC-mediated modulation in cardiomyocyte hypertrophy as well as detailed mechanism of action in providing protection against apoptosis. The data obtained from this study suggested that NC modulated hypoxia induced hypertrophy by preventing histone-3 acetylation and down-regulating the activation of histone acetyl transferase due to transcriptional activity of p300 in the HVCM cells. It was also found that p53 also played crucial role in regulating mitochondria mediated cell death in HVCM cells along with cellular expression of c-Fos and c-Jun proteins in a time-dependent manner. Also, a time-dependent effect of hypoxia and NC supplementation was also delineated in HVCM cells (Nehra *et al.*, 2015). Since the initial in vitro investigations provided encouraging data suggesting effective cyto-protection by NC under hypoxia, the first set of in vivo experiments was conducted in adult male Sprague Dawley rats for investigating effects of acute hypobaric hypoxia (HH, altitude 25000', effective oxygen ~8%, 72 hours) on pulmonary damages and chronic hypoxia (3 weeks) on cardiovascular impairments. The data revealed that acute HH provoked pulmonary vasoconstriction response in the experimental animals by modulating systemic and general expression levels of endothelins. The systemic levels of endothelins increased under acute HH suggesting physiological tendency to constrict the pulmonary vasculature. This finding was validated by increase in tissue expression of endothelins in the lungs with modulation in the expression levels of is receptors A and B. Further, severe oxidative stress was also evident in the pulmonary vasculature with compromised fluid-clearance capacity and thus edema formation. Investigations revealed that supplementation of animals with NC not only maintained redox balance in Akt/Erk dependent manner, but also regulated the fluid-clearance capacity by modulating expression levels of Na+K+ATPase, but also maintained the pulmonary vasoconstriction by balancing the expression levels of endothelins and its receptors (Nehra *et al.*, 2015). Further, we intended to investigate the potential damaging effects of chronic HH upon the cardiovascular system in terms of dilatory RVH. The experimental animals were exposed to HH for different time periods (3, 7, 14 and 21 days) to investigate the onset of RVH and associated changes. It was found that molecular regulators of RVH started increasing by day 3 whereas morphological appearance of the heart altered by 14 days. The initial markers of cellular damage were evident by day 14 whereas extensive cell death was observed by day 21. These observations suggested that the cardiovascular system underwent highly dynamic changes starting from acute exposure to HH and underwent eventual irreversible damages under chronic exposures. Supplementation of the animals with NC not only reduced the tendency of the rodent hearts to undergo right ventricular dilation, but also protected them from undergoing apoptotic cell death. The molecular investigation also revealed time-dependent efficacy of NC supplementation in providing cardio-protection under chronic HH (Nehra *et al.*, 2016). The studies conducted so far established in vitro and in vivo models of hypoxia induced hypertrophy and associated damages in various cell lines and the animal model. The data so far evidently demonstrated enhanced cardio-protective efficacy of NC as compared to C and PQQ alone. Thus, further experiments were performed to investigate the cardio-protective efficacy of NCF vs NC in both in vitro (Fig. 2) and in vivo (Fig. 3) models. The pharmacokinetic assessment of NCF was performed in Sprague Dawley rats along with toxicological and safety assessment for 28 days. The results demonstrated physiologically acceptable pharmacokinetic properties of NCF while no evidence of toxicity was observed. Molecular investigations revealed dynamic changes in mitochondrial homeostasis inrodent right ventricles as evident by modulation in transcriptional and translational regulators forfatty acid metabolism (PPARα/β/γ), redox-function (Nox-2, Cox-2), bio-energetic (UCP-2, UCP-3), bio-genesis (mtTFA, Nrf1, Nrf2 and PGC1α) and apoptosis (Bcl2/Bax). Damage to mitochondrial homeostasis was further confirmed by analysis of activities of electron transport chain complexes I-V. NFκB mediated pathological effects on hypoxia induced hypertrophy was further analyzed in right ventricles and HVCM cells. The data demonstrated immense cardio-protective potential of NCF as compared to NC and PQQ alone by effective modulation in various markers of damage and delineating the molecular mechanisms for the same (Nehra *et al.*, 2017).

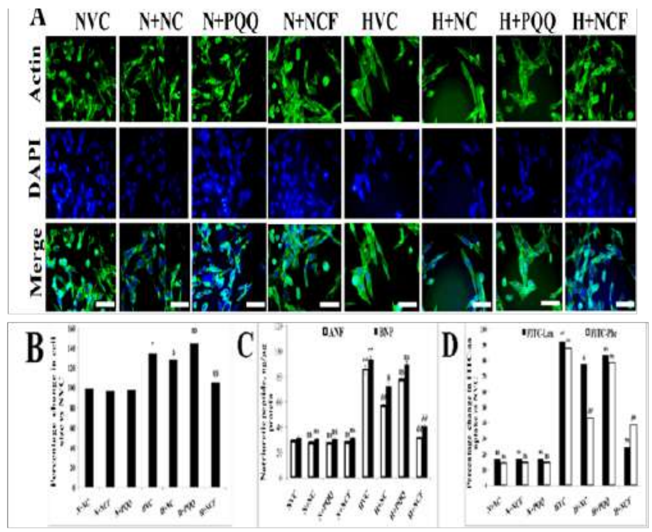

Fig. 2.(A-B) Hypertrophy in HVCM due to hypoxia and protective efficacy of NCF (Nehra et al., 2017, Experimental & Molecular medicine).

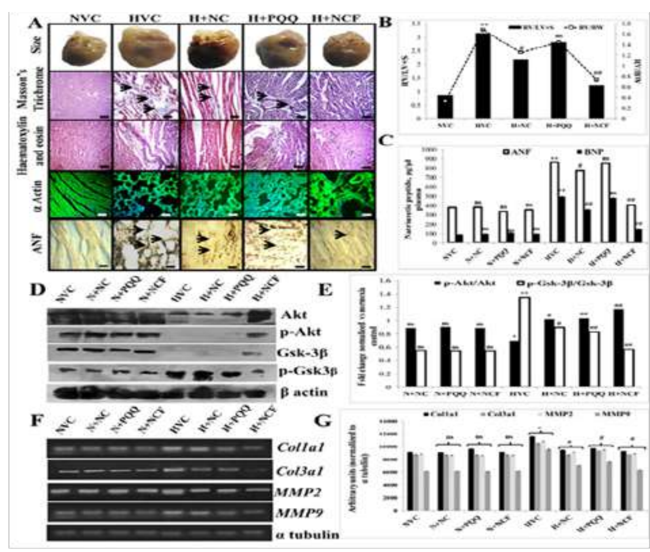

Fig.3. (A-G) Changes in heart size and physiology due to cHH and modulation by NCF (Nehra et. al 2017, Experimental & Molecular medicine)

Cumulatively, the study cohort provides experimental proofs which establish a pre-clinical in vitro model of cardiac hypertrophy under hypoxia in rodent embryonic cardiomyoblasts and validate the same in primary human cell line. The data further revealed the progression of pathological RVH in the rodent model under chronic HH. The study also outlines the time-course of various bio-chemical and molecular events which delineate the transformation of clinical state of hypertrophy from compensatory acclimatization to de-compensatory ventricular dilation under hypoxia. Hypoxia-induced oxidative stress, redox imbalance and calpain activation have a debilitating effect on skeletal muscle mass. In the first phase of the study, we examined the therapeutic potential of NCF to ameliorate skeletal muscle atrophy by known pathways including calpain activation and NF-kB signaling in Sprague Dawley rats exposed under simulated high altitude at 7620m for 1,3 or 7 days. Greater lengths of hypoxic exposure caused progressively increased NF-kBp65 and MuRF-1 expression along with significant increased calpain activity by day 7 in the gastrocnemius and soleus muscles. Moreover, intracellular Ca2+ and inflammation were also higher in hypoxia exposed animals than normoxia groups. Myofibers cross-sectional fiber area of gastrocnemius muscle was decreased more than soleus muscle after HH-exposure. Myosin heavy chain type-I (slow oxidative fibers) significantly decreased in gastrocnemius (> 50 %) and soleus (> 46 %) muscles by the seventh day of exposure. NCF supplementation showed improvement in skeletal muscle acclimatization through effective alleviation of oxidative damage, and changes in calpain activity and atrophic markers at HA when compared to hypoxia control or treatment alone with NC/PQQ. Thus, NCF-mediated anti-oxidative, anti-inflammatory effects lead to decreased proteolysis resulting in mitigated skeletal muscle atrophy under hypobaric hypoxia (Fig. 4)(Kushwaha and Saraswat, 2022).

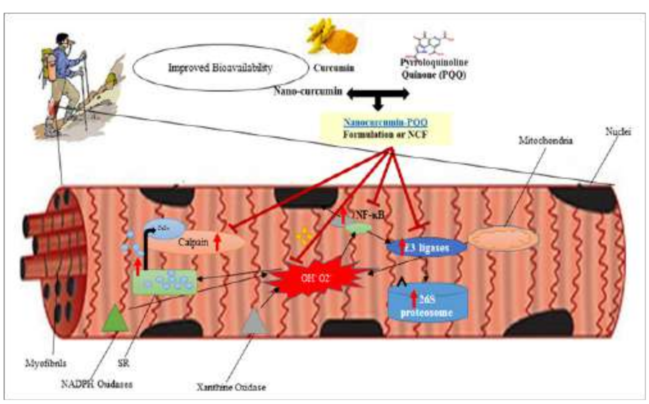

Fig. 4. Pictorial representation of hypobaric hypoxia induced myofiber damage and NCF therapeutic targets in skeletal muscle. Ca2+ : calcium ion, NF-κB: Nuclear factor- κB, SR: Sarcoplasmic reticulum, NADPH: Nicotinamide adenine dinucleotide phosphate (Kushwaha *et al.*, 2022; high altitude medicine and biology).

Having observed the more vulnerability of gastrocnemius muscle (predominant fast fibers) than soleus muscle (predominant slow fibers) till seventh day of exposure we further explored the intricate pathways of myofiber composition after hypobaric hypoxia. To accomplish this phase of the study animals were exercised with endurance training protocol prior hypobaric hypoxia exposure with or without NCF. This phase of study also evaluate the ameliorative potential of exercise-preconditioning and nanocurcumin formulation on muscle oxidative work capacity. The salient findings of the study revealed a significant reduction in slow-oxidative fibers under hypoxia. There was also a marked decrease in exhaustion time in hypoxia control rats, indicating a reduced work capacity. Mitochondria function was decreased following a significant decrease in electron transport complex activities after hypobaric hypoxia exposure. Exercise preconditioning along with NCF supplementation significantly increased slow-oxidative fiber proportion, and exhaustion time while maintaining mitochondrial homeostasis. These findings suggest that HH leads to an increased transition of slow oxidative fibers to fast glycolytic fibers and increased muscular fatigue. While administrating NCF in combination with exercise-preconditioning restored this myofiber remodeling and improved muscle oxidative work capacity (Fig. 5) (Kushwaha *et al.*, 2023).

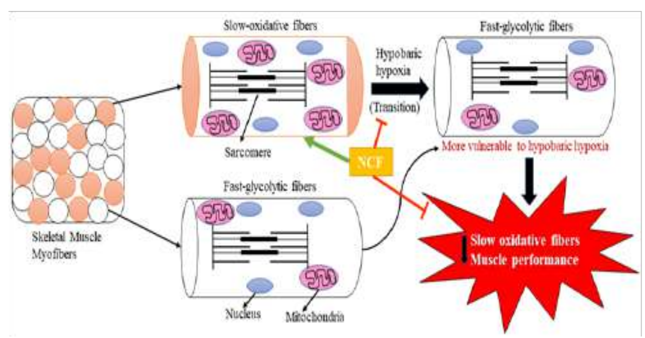

Fig. 5. The graphical abstract illustrates the therapeutic role of nanocurcumin formulation (NCF) in rescuing hypobaric hypoxia-induced transition of myofibers from slow-oxidative to fast-glycolytic in skeletal muscle to improve anti-fatigue ability (Kushwaha et. al 2023, Journal of physiology and biochemistry).

With mounting evidence for the role of impaired intracellular Ca2+ handling in mitochondrial dysfunction in various disease models including heart failure, Alzheimer, sarcopenia our next phase of the study significantly progresses in mitochondrial Ca2+ homeostasis in the skeletal muscles during hypobaric hypoxia. Hypoxia increases intracellular Ca2+ level by dysregulating the release of Ca2+ from ER ryanodine receptor. The most interesting outcome of the current phase of the study was the mitochondrial Ca2+ overload due to altered expression of MICU1 and MICU2, impaired mitochondrial membrane potential (ΔΨm) and allows opening of mitochondrial permeability transition pore. p53 stabilization and its translocation to the mitochondria were observed following disrupted mitochondrial membrane integrity in myoblasts under hypoxia. Furthermore, the downstream effects of p53 led to the upregulation of proapoptotic proteins (Bax, Caspase-3, and cytochrome C) in myoblasts under hypoxia. Nuclear translocation of p53 limits the myoblast differentiation attributed to decreased expression of Pax3/Pax7 and myogenic regulatory proteins (MyHC, Myf-6, myogenin and MyoD) under hypoxia. NCF administration was found to be beneficial in maintaining mitochondrial Ca2+ homeostasis and limiting p53 translocation into mitochondria and nucleus under hypoxia in muscle myoblasts. NCF treatment also modulates heat shock proteins and apoptosis-regulating protein expression in myoblasts. Conclusively, we proposed that mitochondrial Ca2+ overload due to altered MICU1 expression intensifies apoptosis and mitochondrial dysfunctionality (Fig. 6). However, NCF could improve mitochondrial Ca2+ homeostasis, anti-apoptotic ability and myogenesis in C2C12 myoblast under hypoxia (Kushwaha *et al.*, 2023).

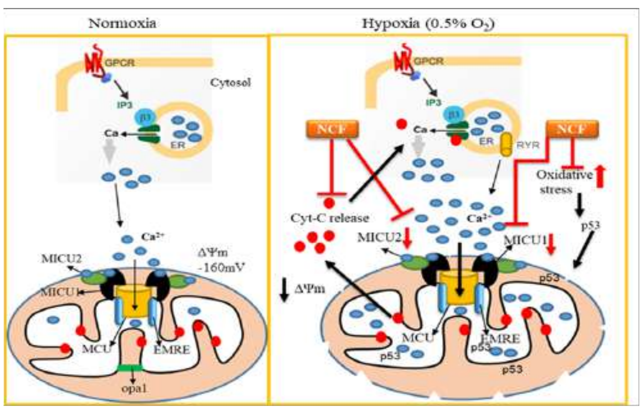

Fig. 6. A cellular mechanism approach to elucidate the mitochondrial dysfunction following Ca2+ overload and escalation of apoptosis under hypoxic insult. The symbol 'ΔΨm' indicates a change in mitochondrial membrane potential. NCF; nanocurcumin formulation, MCU; mitochondrial calcium uniporter, MICU1; mitochondrial calcium uptake protein 1, MICU2; mitochondrial calcium uptake protein 2, EMRE; essential MCU regulator, RYR; ryanodine receptor, ER; endoplasmic reticulum, IP3; inositol (Agrawal et al., 2020; Bartoli and Richard, 2005; Basnyat and Murdoch, 2003) triphosphate (Kushwahaet. al 2023, IUBMB Life).

CONCLUSION

To conclude cardiovascular findings, the study presents experimental proofs for therapeutic evaluation of improvement in cardio-protective efficacy of NCF in vitro and in vivo under hypoxia induced pathological RVH and associated pulmonary impairments. NCF supplementation effectively prevented the transition of RVH from state of acclimatization to de-compensation secondary to pulmonary damages. Collectively, the study establishes cardio-protective potential of NCF and represents the translational potential of NCF for ameliorating HH induced maladies in ascendants.

In skeletal muscle, NCF supplementation alleviates oxidative damage and resists changes in calpain activity and atrophic markers at HH. NCF-mediated anti-oxidative, anti-inflammatory effects lead to decreased proteolysis resulting in mitigated skeletal muscle atrophy under hypobaric hypoxia. Moreover, HH leads to an increased transition of slow oxidative fibers to fast glycolytic fibers, mitochondrial dysfunctionality and increased muscular fatigue. While administrating NCF in combination with exercise-preconditioning restored this myofiber. Conclusively, NCF would be a novel and promising therapeutic intervention to sustain cardiac adaptation and physical activity during high altitude exposure by limiting the hypoxia-induced debilitating impact on cardiovascular and skeletal muscle. The present study may also potentiate the use of NCF as a therapeutic candidate in oxidative–stress driven muscle atrophy and other muscle related pathologies which arises due to impaired mitochondrial homeostasis.

ACKNOWLEDGMENT

The authors are grateful to Defence Institute of Physiology and Allied Sciences, DRDO for providing funds and facilities to conduct the studies.

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MODERN METHODS OF POTATO (SOLANUM TUBEROSUM L.) SEED PRODUCTION FOR SUSTAINABLE HILL ECONOMY

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ABSTRACT

Seed potatoes constitute a significant share of the cost of potato incurred on crop, and the quality of seeds used significantly impacts the produce's overall yield and quality. Conventionally, potato seeds are produced in the hilly region because of the absence of vector populations. As the spread of viruses in this region is prevented, the harvested seeds have very low possibilities of viral infections.Virus infections are the primary concern in such vegetatively propagated crops; the continued production of virus-free seed material is essential for sustained and quality potato production, both for domestic and industrial use. Nowadays, based on micropropagation, many potato seed production technologies are in practice. In this article, an attempt has been made to discuss the possibilities of adopting such technologies for producingvirus-free potato seeds in the hilly states vis-à-vis economic development.

INTRODUCTION

Potatois a tuberous dicot plant (family Solanaceae) represented by about 98 genera worldwide (Olmstead and Bohs 2007). It is a starchy, tuberous food crop grown over 192,464,62 hectares and consumed by approximately 3 billion people (FAOSTAT 2016, Birch *et al.,* 2012). Potato is native to South Americaand was introduced in India by Portuguese sailors during the 17thcentury (Singh and Rana 2014). Since then, it has evolved as an essential food crop in the Indian consumption and production market.After China, India is the second largest producer of potatoes, with its cultivation on 21 lakh hectares spreading over 23 states (GOI 2018). Due to various minerals such as potassium, magnesium, calcium, iron, zinc, sodium, phosphorous and essentialvitamins in potatoes, it has been adopted as a staple food in European countries. It is used as a vegetable crop in India (Navarre *et al.,* 2009). Apart from its use as a food source, industrial applications of potatoes in starch, paper and alcoholic industry further add value to the crop (Gopal and Khurana 2006). Potato is usually propagated vegetatively using tubers from previous cropsdue to the non-availability of true seeds and the associated dormancy (Song *et al.,* 1986).The tubers of the prioryear's crop, which are used as propagules, are continuously exposed to various pathogens. The crop is constantly threatened by many seed-tuber-borne fungal, bacterial and viral pathogens causing many economically important diseases such as blight, scab, and bacterial wilt, and many viral diseases that significantly risk crop yield (Fig. 1 Khan *et al.,* 2008). Among these seed-borne diseases, the most important are viral diseases,as the tubers once infected with virus pathogens, it is challenging to get disease-free plants. During repeated cycles of cultivation using tubers, more and more propagules get infected,resulting in the cultivar's degradation, necessitating replacing existing seed stocks with new virus-free seeds and/or developing new cultivars. Thus the lifespan of a cultivar in the field is limited. If healthy material is not maintained, after a few years,developing a new cultivar through traditional breeding is necessary, which is laborious and time-consuming.Healthy tissues of a cultivar can be maintained in culture; this way, important cultivars can be preserved indefinitely. The healthy seed tubers can be propagated fromin vitro maintained pathogen-free (viruses) culture stocks and supplied to the producers as generation zero (G0) seed for further bulking as seed material for 2-3 cycles before commercial propagation.

Conventional potato seed productionand associated problems

Potato can be propagated both using true seed (sexual reproduction) as well using tubers (vegetative propagation) (Fig. 1). However, true seeds are not ordinarily available, and the commercial cultivars are highly heterogygous and can not be propagated through sexually produced seeds. Conventionally, these cultivars are propagated using tubers from the previous crop. During repeated cycles of vegetative propagation, tubers get infected/infested with many pathogens (Fig. 2), and the performance of plants raised from infected tubers gets affected (Shiwani *et al.,* 2021).From India, 6-7 viruses have been reported to infect potatoes, which is the primary cause of degradation of a cultivar in the field (Halabi *et al.,* 2019; Sangar *et al.,* 1988). Theseviruses are usually transmitted through specific vectors. Traditionally, the seed propagated in the hilly area is preferred as plants are less exposed to viruses because the population of vectors in hillyregions

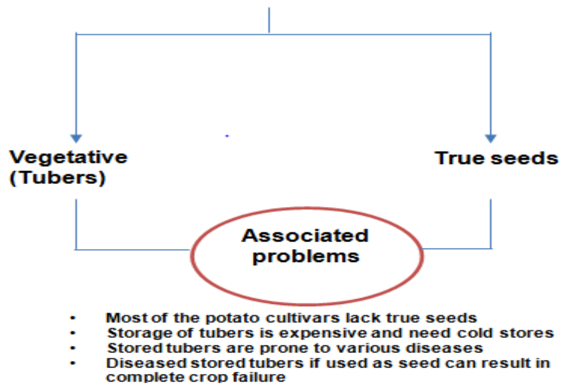

Fig.1. Conventional methods of potato propagation

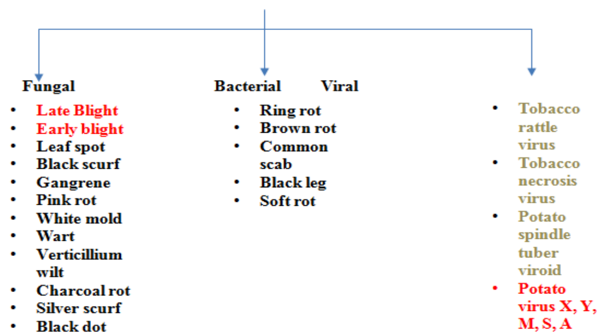

Fig.2. The diseases of potato caused by various fungi, bacteria and viruses

is either nonexistent or very low.However, propagation of potatoes for the seed tubers is also practised in some other areasunder protected cultivation (in greenhouses or tunnels covered with mesh 40). As some parts of Uttarakhand can be used for propagating quality seed tubers, the lower potato tuber multiplication in the field and the higher cost incurred for the seed crop make this activity less economical (Shiwani *et al.,* 2021). Thus other technologies need to be practised to increase the contribution of seed material from these regions.Therefore, there is a felt need to adopt alternative technologies to maintain and propagate disease-free seed stocks of a cultivar. Although such practices can be taken up anywhere, hill states havethe advantage of being a power surplus and easy and cost effect human resources availability.

Modern methods of producing healthy seed tubers:

Alternative approaches based on micropropagation for producing healthy seed tubers have been developed. Micropropagation is also used to maintain healthy stock cultures of important cultivars and propagate tubers from these healthy stocks, which are provided to farmers as seeds.Various technologies used for the production of tubers based on micropropagation is depicted in Fig 3. Micropropagation of potato for seed production includes the following important aspects

A. Virus indexing of potato tubers: Virus indexing ensures that the initial material used to establish the cultures is virus-free. The virus indexing can be done for all the possible viruses, whichin that geographical area might be present. For virus indexing, either Enzyme-linked Immunosorbent Assay (ELISA) or molecular techniques such as Real-Time-PCR (RT-PCR) are used.

B. Establishment of the culture: For the aseptic culture establishment, tissue (shoot buds) is taken from the virus-indexed tubers of the desired cultivar, and after surface disinfection, the shoot buds are cultured onto the Murashige and Skoog medium (1962) (MS medium).

C. Shoots were multiplied on either basal MS medium or MS medium supplemented with 0.01 mg/l 6-banzylaminopurine (BAP). These actively growing shoots have small hair-like roots, and these shoots, when transferred to the soil, started to grow with good post-soil transfer survival.

In vitro actively growing cultures of potato are maintained as virus-free stocks. Plants can be propagated from these stocks and used to produce tubers. The following are the main methods used to produce tubers (Fig 3), considered generation zero (G0) stocks.

1.Potato in vitro microtuber production

2.Potato minituber production

3.Aeroponic potato minituber producetion

4.Hydroponicminituber production

Potato microtuber production

Potato shoot cultures are induced to produce the tubers, called microtubers. Microtubers can be used as original seed material (G0), which can be grown in the field like their conventional counterparts.Microtuber production is a two-step process (Fig. 4). In the first step, cultures are grown in liquid MS medium containing BAP (0.1 mg/l), NAA (0.01 mg/l)and GA3 (0.4 mg/l)and incubated under fluorescent lights with 16-h photoperiod. In the second step liquid medium is replaced with a tuberization medium consisting of MS with 8% (w/v) sugar, BAP (10 mg/l), CCC (500 mg/l) and incubated in the dark at 16 ^{0}C for about 120 days. After about 30-40 days,the initiation of tubers can be seen, which further grew on this medium. After 120 days, about 20-25 microtubers can be harvested per culture bottle (Fig. 4E). The harvested tubers are then washed, treated with fungicide, and exposed to fluorescent light till they turn green. These freshly produced microtubers have dormancy levelssimilar to freshly harvested conventional potato tubers. Therefore, microtubers are stored at 2-4 ^{o}C for 40-50 days for the release of dormancy before these can be planted in the field. The complete protocol of microtuber production is dipcited in figure 5.

Potato minitubers production

Minitubers are produced from nicropropagated plants.In plant tissue culture, the plants can be propagated by shoot

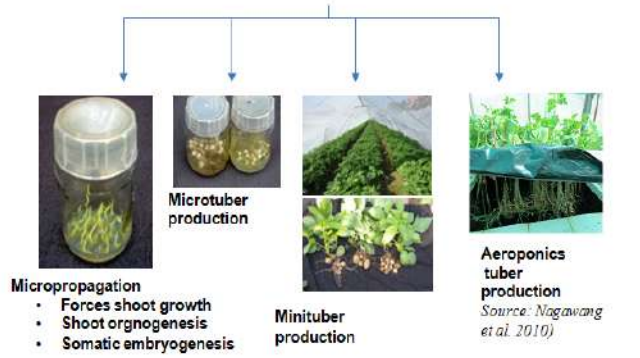

Fig. 3. Different technologies of producing healthy propagules of potato based on plant tissue culture

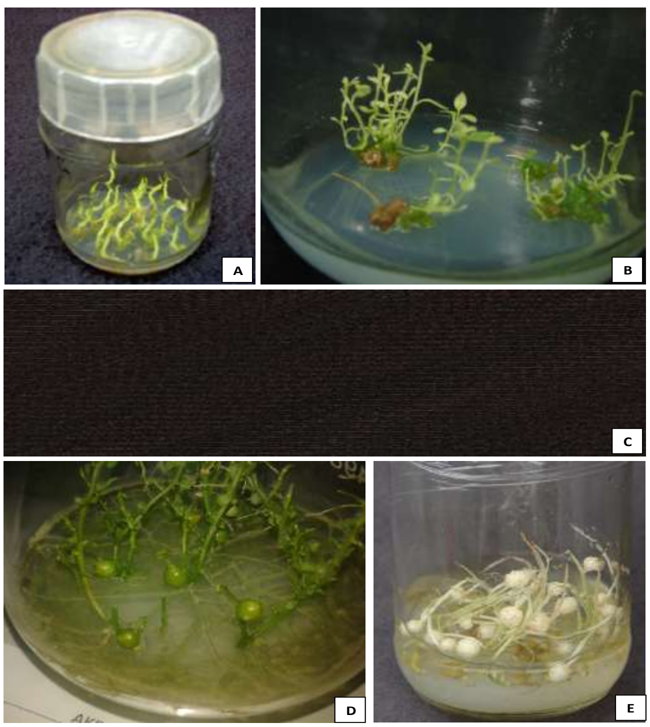

Fig. 4. Steps followed in potato minituber production. A: in vitro plant multiplication; B: shoot organogenesis; C Stages of somatic embryogenesis; D: in vitro microtuber development in old cultured of potato; E: in vitro microtuber formation on a tuber induction medium as mentioned in the text

multiplication, shoot organogenesis or somatic embryogenesis. However, for genetic stability, shoot multiplication is generally preferred. The total protocol of plant propagation through shoot multiplication is as follows.

a. Culture initiation and multiplication: Cultures are initiated from the virus-free tubers or virus-indexed cultures of the known cultivars. The same can be procured from Central Potato Research Institute (CPRI) Kufri, Shimla. The shoot multiplication can be achieved on basal MS medium containing 10 μM $AgNO_3$ with a multiplication rate of 3 per subculture cycle of 21 days. The multiplied potato shoots did not require additional rooting stepsas thesethese shoots have

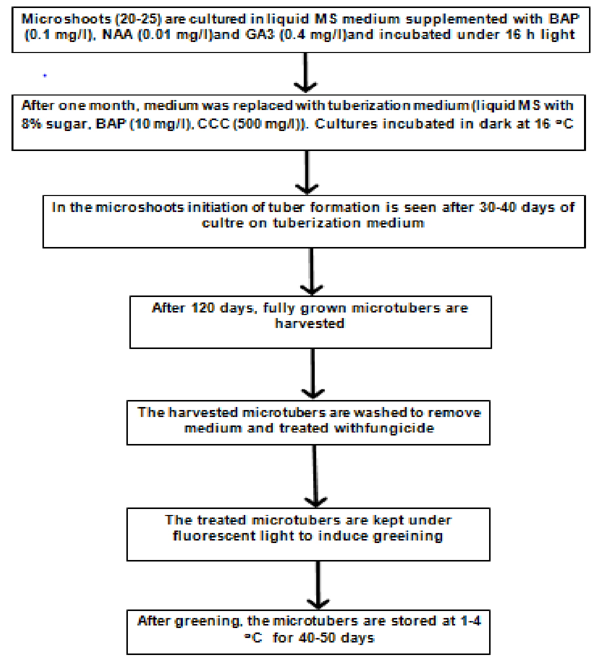

Fig. 5. Protocol followed for microtuber production in potato

fine roots. These shoots can be directly acclimatised in the polyhouse.

b. Acclimatisation: The successful acclimatisation can be carried out in a polyhouse with a fan pad cooling system from September under Patiala conditions.

Plants are taken out from the bottles, and cultures are washed softly to removethe agar medium and treated with water containing 1.0 % carbendazim (W/V). The plants are transferred to the hikko trays (209 spaces) containing potting mix consisting of soil and cocopeat (1:1). The plants are kept in the polyhouse with an average temperature of 25-27 °C. Plants start growing after 3-4 days showing the emergence of new leaves. After 10-12 days of growth in polyhouse,acclimatised plants can be transferred to the field (Fig. 6A). Generally, it is seen that at the beginning (September 25 – October 05),the survival rate of plants after ex vitro transfer is lowerand with the decrease in temperature; the survival rate increases during the second week of Octoberand from 21 -30 October the survival rate of about 90-95% can be attained.

c.Transfer to field and post transfer care

After 10-12 days of acclimatisation, the plants were transferred to the field in tunnels with 3 raised beds in each tunnel (one meter wide x 75 mt). The plant-to-plant space was kept at 4.5 inches, and the row-to-row space was 3.5 inches. After transfer, the plants were irrigated twice daily for 15 minutes each using micro-sprinklers and tunnels were kept covered with mosquito nets to protect the plants from vectors. The

Fig. 6. Stages of potato microtuber production A: acclimatization of plantlets; B: field preparation and erection of tunnels; C: Planted net tunnels in the field; D: 45 days old plants growing the net tunnel; E: plants with minitubers; F: harvested minitubers ready for dispatch

plants started to grow after ten days. The field was kept weed-free by manual removal of weeds. After 15-20 days, when plants have grown to a height of about 8-10 cm, the first soil support is applied to cover the root portion and emerging stolens. This way, about three earthing-up operations are carried out within 60-55 days. Plants are protected from viral vectors and other diseases by continuous sprays of pesticides. After about 90-100 days, the irrigation is stopped, and plants are sprayed with paraquat (weedicide) to dryout the aerial parts of the plants. After the plants have dried, in 10-15 days, the skin of the tubers is tested, and if mature, the crop is ready for harvest. The tubers are harvested manually and are washed and treated with fungicides. These are then graded according to size classes and packed for cold storage for >45-59 days to break dormancy. After dormancy breakage, these tubers can be used as generation zero (G0) seeds. For planting an acre, about 33000- 35000 seed tubers are required.

d. The effect of planting date in the field on tuber production

Asignificant effect of the planting date on tuber production was observed. The plants which were transferred to the field during 1st half of October produceda lesser number of tubers (5-6), but the average size of tubers is bigger (>15 g). However, with the season's progress, the number of tubers per plant increased, and the size of the tuber decreased (Table 1). Plants transferred during 2nd week of November can produce more than 12 tubers per plant, and the average size of tubers is significantly reduced (> 12 g).

Table: 1 The effect of date of planting in the field on average size and number of tubers per plant harvested

Size range (g)	Number of tubers per plant			
Planting dates	5-15 Oct	15-Oct 25	October 25 to November 05	5-Nov 15
>35	2.6	2.4	1	0.5
20 – 35	2.25	2.75	3.5	1.75
10-20	2.0	2.9	3.2	3.0
5-10	1.25	2.1	2.5	3.56
< 5	1.65	2.2	3.5	4.25
Average number of tubers per plant	8.75	10.60	12.50	12.80

Table. 2. Cost analysis of some of the propagules used for potato planting

Parameter	Microplantlets	Microtubers	Minitubers
Production cost per propagules (Rs)	3.5	3.5	2.5
Market price per propagule Rs.	10.0	8.0	5.0 (average of all sizes) *
Profit of production unit per propagule (Rs)	6.5	4.5	2.5
Number of propagules required for 1 acer	30000	40000	35000
Cost of propagules for one acer (Rs)	3,00,000	3,20,000	1,75,000

Aerophonic potato seed tuber production

This is emerging as a main seed production technology as it needs limited land resources and production is achieved in a much smaller nethouse with an installed facility. The seed multiplication rate of plants propagated in an aeroponic system is much higher (5-6 times) than minituber produced in the field. Further, as the exposure of the plants to the standard field conditions is very limited, there is also a lower risk of getting infected with viruses. The aeroponic plants are grown in nethuse on a support, the nutrient solution is provided as mist on a fixed interval, and plants are produced with the help of natural light (autotropic mode of nutrition). The in vitro propagated plants are acclimatised for 15-20 days and then planted on a support, and the nutrient mist is provided in the root zone, which is kept protected from the sunlight (Fig 7). The stolens start growing in the root zone, which later develops the tubers. The tubers are allowed to grow to get an optimum size and then harvested. The harvested tubers are hardened and then stored at 1-4 ºC to release dormancy before planting for the next crop.

Estimated cost of production of propagules

Cost of production of various propagules under Patiala condition is depicted in Table 2. The various propagules such as in vitro growing plantlets, acclimatised plantlets, minitubers, microtubers can be provided to the formars or any other stake holder. However the demand of minitubers is maximum as the performance of better than all other propagules and the handeling is very easy. As the maturation of minitubers is synchronised, thus the relase of dormancy is also synchronised and can be planted in a short time window.

Fig. 7. Stages of aeroponic production of potato minitubers (*Source: Nagawang et al., 2010)*

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Section II
Environment and **Ecology**

INSIGHT INTO APPLICATION OF WATER EVALUATION AND PLANNING MODEL TO STUDY THE IMPACTS OF CLIMATE CHANGE ONWATER DEMAND AND SUPPLY IN MOHALKHAD WATERSHED OF KULLU DISTRICT, HIMACHAL PRADESH

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ABSTRACT
Water demand and supply is one of the most important topic of discussion at present. Increasing population, change in land use, industrialization, and climate change has drawn heavily on water resource system in the Indian Himalayan Region (IHR). This challenges the water practitioners and planners to frame a sustainable water resource management plan that can cater the demand of water among the population, agriculture, livestock, industry as well as of the ecosystem. This needs the new versatile assessment tools which will evaluate the water resource system and help managers to make a comprehensive water management plan. Present study demonstrates the application of Water Evaluation and Planning (WEAP) model in Mohalkhad watershedlocated inKullu district. An attempt has been made to customize and demonstrate the capability of WEAP model using limited datasets to study the impacts of climate change on water supply and demands in the watershed. The model is applied to evaluate and analyze the existing and futurewater balance and possibleimpacts on the water demands and supply in the watershed.We generated the trends of water demand and supply as well as the scenarios for water resources management till year 2030.In thisfirst ever attempt of customization of WEAP model in Himachal Pradesh, it was found that WEAP model performs satisfactorily with few exceptions and able to simulate the climatic variation and corresponding water supply and demands of different sectors in context of climate change.

Keywords: WEAP, Climate Change, IHR, Water demand and Supply, Water resource development and management

INTRODUCTION
Many countries worldwide are facing water stress, and this is expected to become more critical in the future due to pressure of population growth and climate change. This situationchallenges water practitioners, planners and policy makers to frame a sustainable water resource management plan that can cater various demands. The consequences of the climate change are already witnessed in the several parts of the India including the Indian Himalayan Region (IHR) which is also known as Water Tower or Third Pole.There is increasing concern for allocation of limited water resources, maintaining environmental quality and policies for water use in sustainable manner (Mounir *et al*., 2011). To handle the impacts of climate change and to secure the current and future water supply, an integrated water management practices must be adopted (GWP, 2007).However, there is a challenge in processing the hydrological and water demand data as well as forecasting the effects of different management strategies under the changing climate (Hollermann *et al*., 2010). In recent years there has been considerable progress in simulation modeling of hydrological cycle by developing different physical as well as empirical climate and hydrological models at different spatial and temporal scale. Integrated study of these models helps planners in managing water resources. In the water resources planning and management system, assessment of water availability in the watershed is a basic requirement for watershed conservation. At present, there is inadequacy in developing framework for sustainable management of water resources using scientific solutions that supports the climate change adaptations strategies and development of policies in IHR.With growing attention on sustainable development of water resources, there is a need of assessment tool that can provide a comprehensive, flexible and user-friendly management framework for studying water balance, scenario generation and then by planning and policies making for water management.

Water evolution and planning (WEAP) developed by Stockholm environment Institute (SEI)is one such model having Integrated Water Resources Management (IWRM) framework to evaluate planning issues related to water resources.

By employing the transparent set of objectives and procedure, and user defined constraints, WEAP can be used in analyzing range of issues and uncertainties faced by water mangers, that includes climate change, watershed condition, anticipated water demand, need of ecosystem services, operational objectives and infrastructure (Suryawanshi and Shirke, 2014). The present study demonstrates the application of Water Evaluation and Planning (WEAP) model in Mohalkhad watershedlocated inKullu district of Himachal Pradesh. An attempt has been made to customize and demonstrate the capability of WEAP model acknowledging the limitation in required datasets to study the impacts of climate change on water supply and demands in the watershed. The model is applied to evaluate and analyze the existing and futurewater balance and possible impacts on the water demands and supply in the watershed.

LITERATURERE VIEW

The Water Evaluation and Planning (WEAP) model developed by the Stockholm Environment Institute (SEI), USA was selected for the present study. WEAP is a practical tool for water resources planning, which incorporates both the water supply and the water demand issues and guided by number of methodological considerations, integrated planning framework, scenario analysis to understand effect of different developmental activities (WEAP Use Guide). Being a semi-theoretical modeling framework, it needs calibration and validation(Abrishamchi *et al*., 2007).The model simulates water system operations with in a river system with the principles of water balancing on a user-defined time step, it computes water mass balance for every node and link in the system for the user defined simulation period (SEI, 2001, Yilmaz *et al*., 2010). Worldwide there are many research studies that uses WEAP model for water resource development and management, where WEAP has been applied for long term water demand evaluation and supply planning for future policy and water management decisions (Raskin *et al*., 1992, Yates *et al*., 2009) and in quantifying future state wide water demand under different urban growth and climate change scenarios applied in California Water Plan (Juricich *et al*., 2011). Purkey *et al*., (2008) deployed WEAP model to look at the impact of climate change on agricultural water management and the potential for adaptation in Sacramento Valley. Using WEAP model Saadon and Ali (2014), investigated the trend of supply and demand of water in Langat catchment. Theresearch on management of water resources of Alana valley (Iraq) by using the WEAP model carried out different future scenarios of water availability and was supported by GIS layers, climatic data and hydrologic data and other required data (Rasul *et al*., 2010). This model provides an advantage in analyzing concurrent scenarios related to hydrological change because it integrates hydrological modelling with a decision support system (Harma *et al*., 2012). Yates *et al*., (2005a, b) provided comprehensive details of the WEAP hydrologic module. Azadani (2012) projected the precipitation and temperature in smaller temporal and spatial scale by MAGICC/SCENGEN tool and simulated the impact of climate change on the water resource by WEAP software to provide result for the water managers and policy makers in Arkansas River basin in Colorado.In one of the study on effect of socio-economic development scenarios and climate change using WEAP model, Rochdane *et al*., (2012) analyzed Rheraya's future water situation until 2100. WEAP has been applied and used as a forecasting tool for future water balance in many similar studies. Ali *et al*., (2014) assessed water demand in Malaysia using WEAP system based on three scenarios: higher population growth, water year method and extended dry climate sequences. One of the significant uses of WEAP model in India was to evaluate the water availability against water demand in the link from Godavari River (at Polavaram) to Krishna River (at Vijayawada); where Bharati *et al*.,(2008) examine the effect of planned water transfers on the growing agricultural water demands in the Polavaram link command area. Malla *et al*., (2014) examine the impacts of climate change on the supply and demand of water and the resulting socio-economic implications for the Dachigam stream and Sindh stream of Srinagarcity (J&K). For hydrologically relevant assessment of potential adaptation options, Bhave *et al*., (2012) applied WEAP model in the Kangsabati river basin of West Bengal; where WEAP modeling provide useful scenario analysis in incorporating location specific current and future climatic conditions. Considering all above issues and variety of application, Water Evaluation and Planning (WEAP) model is used in present study. The goal of the study is to customize the WEAP model using limited datasets to get first hand information on demand and supply status of water in Mohalkhad watershed under the future climate scenario.

MATERIALS AND METHODOLOGY

Study Area

MohalKhad watershed (area of 54 km^2) is located between Latitudes 31050'7" and 31055'31"N and Longitudes 77001'30" and 77007'37" E in district Kullu of Himachal Pradesh in Indian Himalayan Region. The topography ranges from 1111 to 3126 m amsl. Average annual rainfall & temperature of the area is 733.4mm & 17.2^{0}C respectively. The main water source for the watershed area is the Mohalnala/stream which joins the Beas river at village Mohal. The stream was perennial in the past, but at present it is perennial only in upper reaches & lower reach stream dried out in summer season. Probable forcing factors for this change could be population growth, increased demand for agricultural, domestic and industrial use as well as climate change. The majority of the land use in the study area is a forest reserve, followed by agriculture which mostly comprised of vegetables and orchards (Fig. 1 and Table 1).

Input data

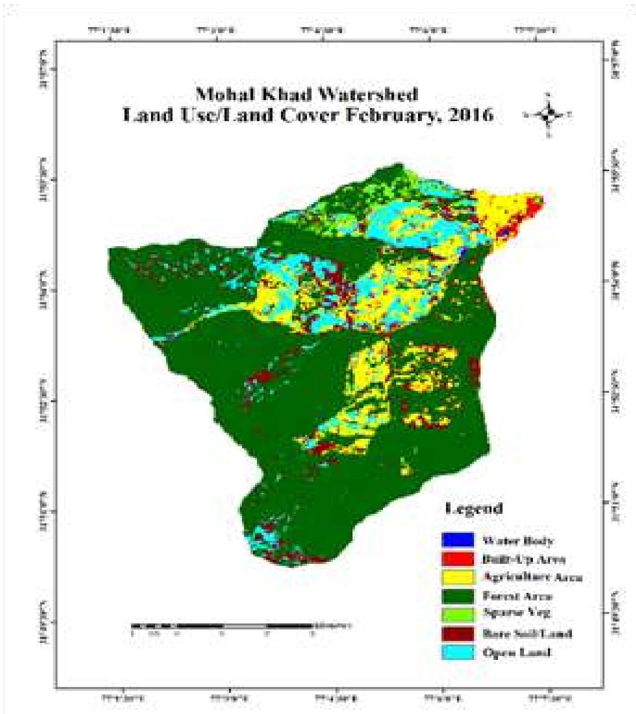

Fig. 1. Land use/land cover of MohalKhad watershed

Table 1. Land use/land cover of Mohalkhad watershed

Land use/cover categories	Area In Hectares (Feb., 2016)	
	Hectares	Percentage (%)
Water Body	9.54	0.175
Built-Up Area	47.97	0.883
Agriculture Area	745.38	13.734
Forest Area	3554.55	65.495
Sparse Vegetation	96.21	1.772
Bare Soil/Land	429.12	7.906
Open Land	544.41	10.031

Data set for the year 2015 were used for the present study, which denote the Current Year/Account (water system as it currently exists) in WEAP modelling. Socio-economic data of domestic population, livestock and agriculture were collected from Statistical Department, Patwari (Record keeping department), Agriculture Department of the Himachal Pradesh and through field survey. Watershed map, drainage network and land use/land cover map were prepared using Remote Sensing and GIS.

Daily rainfall data of the year 2015 was obtained from GBPNIHE, HRC, Kullu; and Mohalkhad discharge data were calculated by SCS-CN method using available rainfall data and land use/land cover map. All these data used as input in WEAP for current year 2015. Further, for building future climate change scenario, data (rainfall, temperature and humidity) of GCM model GFDL-ESM2M under RCP 4.5 were used for the years 2016 to 2030. During simulation modeling, in-built assumptions of WEAP model were used wherever necessary.

Overview of WEAP model

WEAP is developed by the Stockholm Environment Institute (SEI), USA. WEAP model works on the principle of a water balance. It can be applied to single watershed as well asto trans-boundary river basin system. It is considered as a policy oriented model due to its integrated approach to simulate water systems. The components of the natural system (e.g. watersheds, rivers and lakes) and the components of the technical system (e.g. reservoirs, diversions, canals, cities, wastewater treatment plants, hydropower and irrigated farms) are schematized (without geographical reference)using a network of inter-connected model elements: nodes and links. Nodes denote the water is demanded or made available for supply, and links denotes the transfer water between the nodes. The water management module in WEAP is driven by user-defined demand priorities, supply preferences and environmental requirements atvariednodes. The water allocation problem is solved using linear programming on a daily or monthly basis.

Methodology for customization and analysis of WEAP model

The major goal of the present study is to customize the WEAP model for the Mohalkhad watershed using available limited datasets and demonstrate the ability of WEAP model to simulate water demand and supply in context of future climate. Therefore, all the available input data as mentioned in above section was feed to the WEAP system. A schematicof WEAP for the Mohalkhad watershed has been prepared using different nodes and links between different demand and supply sites of the watershed. The demand sites in watershed are population, livestock and agriculture; and the supply sites are Mohal stream as a river water withdrawal and groundwater schemes. The highest demand priority was fixed to satisfy the requirement of population and livestock and subsequently for agriculture sector. Further to meet out demands of population and livestock, the river water withdrawal schemes were set as a first preference and groundwater scheme with second preference. For water balance simulation, we used Rainfall-Runoff simulation method of WEAP model. For WEAP simulation modelling we used year 2015 as a Current Year/ Account for which all the actual datasets were provided to the model; and future water demand and supply scenarioswere build from 2016 to 2030 (Reference Scenario) to see the possibleimpacts of climate change and other factors on water availability and demand management.The flowchart of methodology is given in Fig. 2.

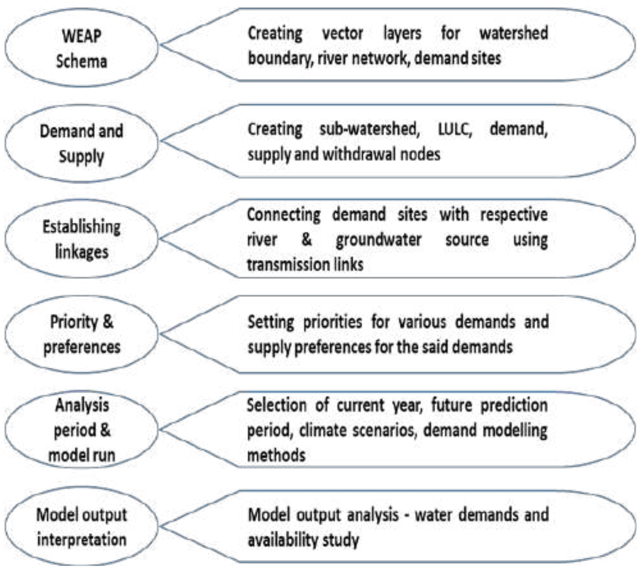

Fig. 2. Flowchart for customization of WEAP andanalysis of water demand and supply

RESULTS AND DISCUSSION

The computation of WEAP model was done by computing the entire model for the Reference Scenario that was generated using Current Account information. The results were divided into three section viz. Hydrologic analysis, Water allocation and Crop yield for the watershed.

Hydrologic Analysis

The hydrologic analysis of Mohal khad watershed includes the rainfall pattern during 2015 - 2030 over the watershed, groundwater flow/storages, runoff pattern and water demands for the domestic (population and livestock) and agricultural sectors.

Rainfall

The projected annual rainfall (mm) over the Mohalkhadwatershed, for GFDL-ESM2M with RCP4.5, during 2015-30 (15 years) is shown in Fig. 3. It has been predicted that the conditions to be very dry with an annual rainfall of 322 mm for the year 2020. Whereas, year 2024 would experience high rainfall (1889 mm) resulting in to wet conditions. Remaining years,rainfall would be above and below of the average value. The climate model predicted the future dry and wet conditions that may help in improving the already existing or newly planned water conservation strategies in the hilly watershed like Mohalkhad watershed where surface runoff is very high. The planning of future water resources project can include the harvesting of excessive projected rainfall of wet years to use it in dry year/period or to recharge ground water storage to improve water table level for satisfying demands of various sectors. Without the effective water resource management, the domestic and agriculture sectors would have experience the severe water problems in dry years to come.

Flow to the groundwater

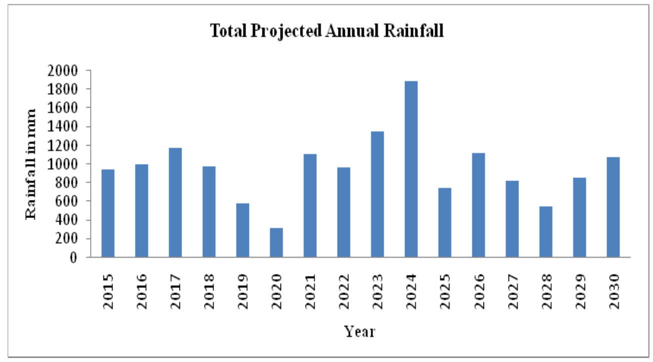

Fig. 3. Projected annual rainfall over Mohalkhad watershed

The Fig. 4 depicts average monthly flow to Groundwater from different land use/land covers of the watershed for 2015-2030.As obvious, the maximum flow to the groundwater has been found in the monsoon months as compare to the non-monsoon months. Due to hilly terrain, high slope and less ponding time for flowing water, only limited amount of rain water can able to flow to the groundwater. Usually northern region especially Himalayan region of the India receives late monsoon than rest of the India, the Fig. 4 depicts the same trade-off in flow to groundwater from the rainfall. Fig. 4 also shows the flow to groundwater in winter months because of the fact that the area also receives winter rainfall frequently due to its close proximity to the Himalayan Glaciers. It is also found that flow from the forest land to the groundwater is maximum because of the dual fact that about 65% of total watershed area is covered by forest and forest vegetation covers increases the ponding time of water to allow water to infiltrate into sub-surface zone of groundwater system. Contribution from build-up land to the groundwater flow is minimum among the all land use/land cover classes of the watershed because of two reasons: being imperious (infiltration process suppressed) area due to settlement activity and lowest percentage share (about 1%) of the total watershed area.

This flow to the groundwater helps in increasing the water table of the area which can further be used in dry years and with-in-year water scarcity period if any groundwater schemes developed in the area.

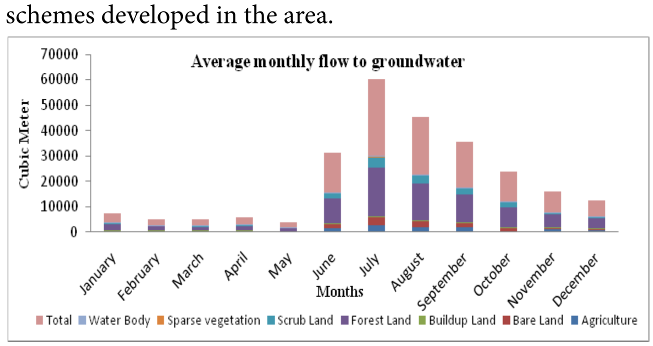

Fig. 4. Average monthly flow to groundwater from watershed

Further, Fig. 5 shows the total annual flow to the groundwater from the watershed. Year 2020 and 2024 contribution to the flow to groundwater is lowest and highest respectively among other years due to predicted respective driest and wet year. Rest of the years also shows the same trade-off of that respective predicted rainfall, which shows the good simulation modeling capability of the WEAP with respect to the natural system. This computation of groundwater flow could help in development of groundwater recharge structures in the watershed to replenish the water table for dealing water demands in dry period.

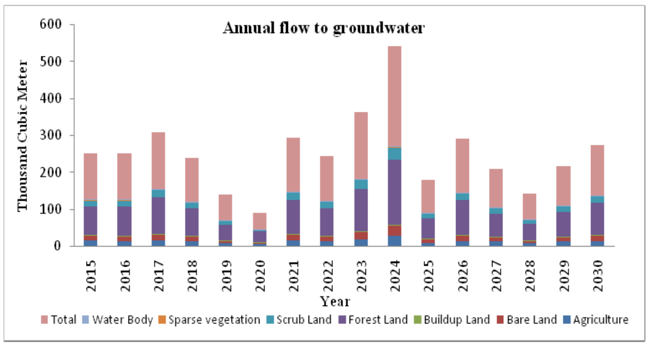

Fig. 5. Annual flow to groundwater from watershed

Surface Runoff

The scenario of average monthly surface runoff from the watershed is shown in Fig. 6. The WEAP model uses Natural Resources Conservation Service - Curve Number method (NRSC-CN) to calculate the surface runoff. Runoff gradually increases from June to August and is highest inthe month of July which follows the monsoon rainfall track.

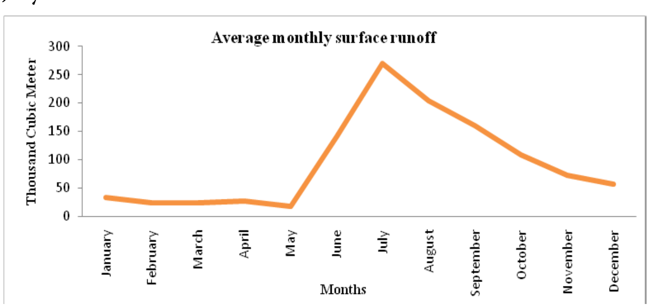

Fig. 6. Average monthly surface runoff from watershed

As the watershed also receive the winter rainfall, thereby showing runoff in winter months. Fig. 7 represents the annual total surface runoff from the Mohalkhad watershed and is showing the projected rainfall-runoff trade-off of 15 years, e.g. year 2020 shows lowest surface runoff being driest year among others and year 2024 shows highest surface runoff being wet year. The annual variation in surface runoff value is in correlation with the annual rainfall behaviour as expected. The years expected to encounter heavy rains also show high runoff value and vice versa. Fig. 6 & 7 shows the loss of water in the form of surface runoff from the watershed. Therefore, rain water harvesting measures are highly desirable to capture, conserve and use the projected amount of surface runoff for various demands.

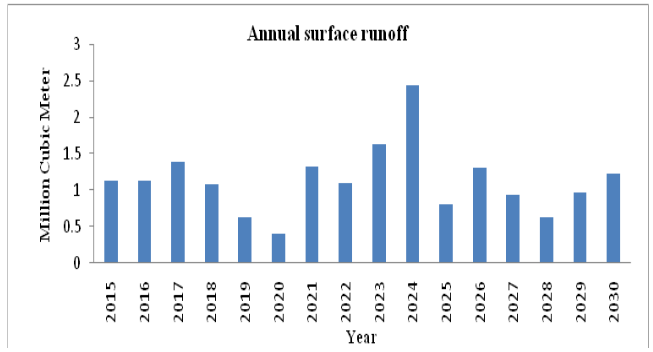

Fig. 7. Annual total surface runoff from watershed

Water demands

Population and Livestock water demands

Fig. 8 shows the average monthly water demands of the population and livestock computed by the WEAP model separately. The graph gives a clear picture of average monthly waterdemands of population and livestock in Mohalkhadwatershed.

For calculation of monthly water demands, the standard defined by Bureau of Indian Standards have been used (annual water use rate: For Domestic = 25.55 m3/person (70lpd) and for Livestock = 70m3/ livestock). The demands are calculated as per the number of days in the month.

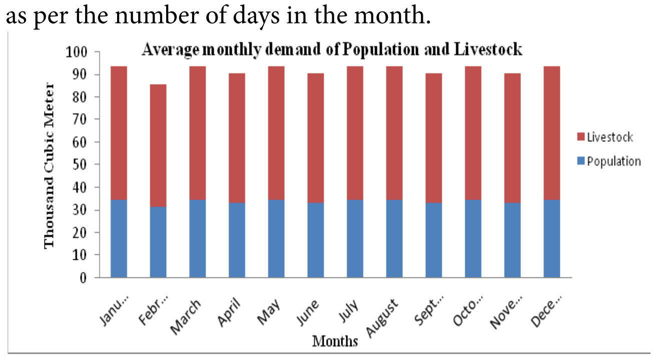

Fig. 8. Average monthly demand of Population and Livestock

Agriculture water demand

The WEAP model calculates the agricultural demand using Crop coefficient (Kc) approach of Rainfall-Runoff (Simplified Coefficient Method) method. It can be seen that rain water is available to meet out the crop water demand in monsoon months (Fig. 9).

The agriculture demand gradually increases from December end, towards nextMay. Average monthly demand of agriculture in March shows less demand than February and April because of the fact of winter rainfall. Further in summer, crops required more water to satisfy the consumptive use demand during high temperature, therefore showing water demand on higher side. However, for winter months, WEAP performance to predict the average monthly demand of agriculture is not showing promising results.

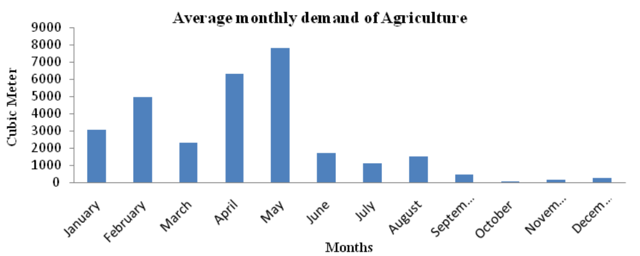

Fig. 9. Average monthly agricultural demand

The annual agriculture demand varies with the amount of rainfall to be received in the respective years (Fig. 10). Dry condition is predicted in the year 2020, therefore corresponding agricultural demands are higher side. In year 2024, annual demand of agriculture is expected to be lowest due to high rainfall prediction.

The annual agriculture demand varies with the amount of rainfall to be received in the respective years (Fig. 10). Dry condition is predicted in the year 2020, therefore corresponding agricultural demands are higher side. In year 2024, annual demand of agriculture is expected to be lowest due to high rainfall prediction.

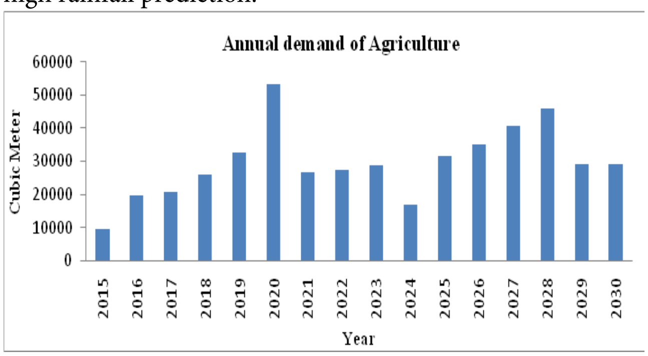

Fig. 10. Annual agricultural demand

Water Allocation

Average monthly and Annual Unmet Water demands of Population and Livestock

The demand which is not meet out is referred as an unmet demand here. Fig. 11 and 12 shows the projected average monthly and annual unmet water demands of population and livestock respectively. The demands were calculated using standard of Bureau of Indian Standard as explained in previous section. The calculated water demands are irrespective of age of population and livestock so are always on higher side than actual water use rates of population and livestock. The same trend has been reflected in WEAP modelingin unmet water demands. Demands are higher in non-monsoon months than in monsoon month as expected. The projected annual unmet water demands of population and livestock for 2015-2030 (Fig. 12) is showing no correlation with the amount of predicted rainfall in the respective years and showing constant values for all the years. This is due to fact that the population growth indexing was not considered during the study. WEAP performed poorly in simulating annual unmet water demand of population and livestock of Mohalkhad watershed.

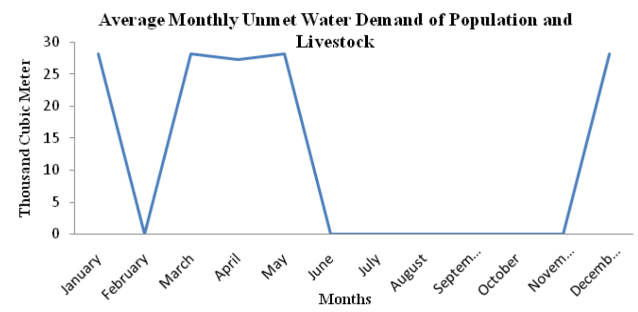

Fig. 11. Average monthly unmet water demands of population and livestock

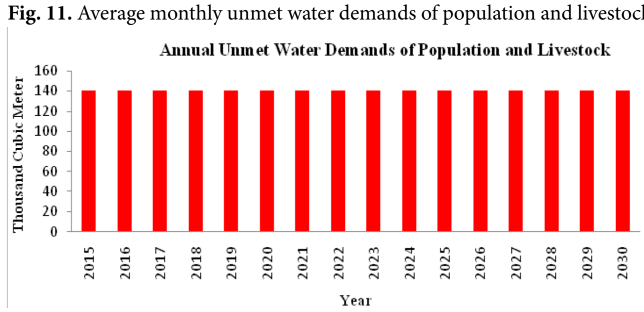

Fig. 12. Annual unmet water demands of population and livestock

Average monthly and Annual Water Supply to Population and Livestock

In present WEAP simulation, the highest demand priority was fixed to satisfy the requirement of the population and livestock demands first and second priority was fixed for the agriculture sector.

The WEAP simulation for average monthly supply delivered to population and livestock is shown in Fig. 13. While simulation, the standard water requirement of population and livestock was kept as per the Bureau of Indian Standard as mentioned in the previous sections. The water supply in monsoon months is significantly related to the projected rainfall over the watershed which increases the river water supply (the first preference) directly.

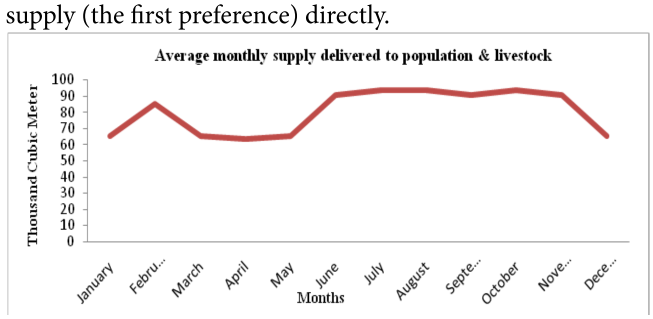

Fig. 13. Annual unmet water demands of population and livestock

The projected annual water supply for population and livestock (Fig. 14) for 2015-2030 showing no relation with the projected rainfall from the climate model. Annual supply delivered showing constant values for every year but when relates to unmet demands of population and livestock, it satisfying the need of the same. This shows that WEAP model outputs are able to satisfy the demands of population and livestock but fails to simulate the water availability trade-off with the projected rainfall for estimation of supply delivered to satisfy the unmet water demand in present case. This attributed to the supply preferences and priority fixation during customization of WEAP model which needs more prioritization ideas and corresponding calibration of WEAP model.

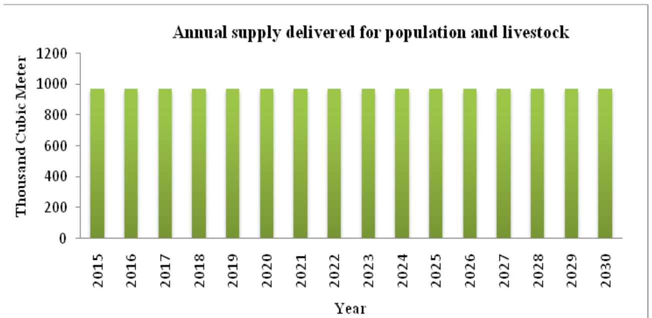

Fig. 14. Annual water supply delivered to population and livestock (2015 - 2030)

Average monthly and Annual unmet water demand of Agriculture

The WEAP model computes agriculture water demands usingCrop coefficient (Kc) for evapotranspiration component and effective precipitation. The average monthly unmet water demand of agriculture is shown in Fig. 15 which clearly shows that the unmet agriculture water demand is low in Kharif season than in Rabi season. This is because of the fact that in Kharif season, agriculture demands are mostly fulfilled by the rain water, whereas, in Rabi season, agriculture demands are mostly dependent on irrigation or groundwater or winter rainfall.

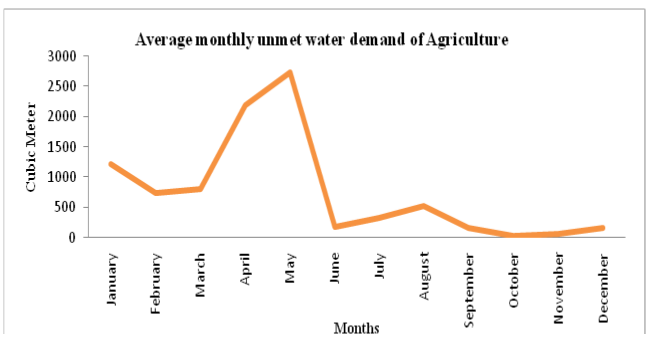

Fig. 15. Average monthly unmet water demand of agriculture

Average unmet demand of agriculture is highest in May due to fact of high temperature summer month without rainfall and absence of any other water source to satisfy the demand. WEAP model predicted the annual unmet water demands of agriculture (Fig. 16). Rainfall, being the major source ofwater supply to the watershed agriculture area; unmet demands of the agriculture follows the trade-off amount of projected annual rainfall. The unmet water demand of agriculture is highest in dry year 2020 and vice versa for year 2024.

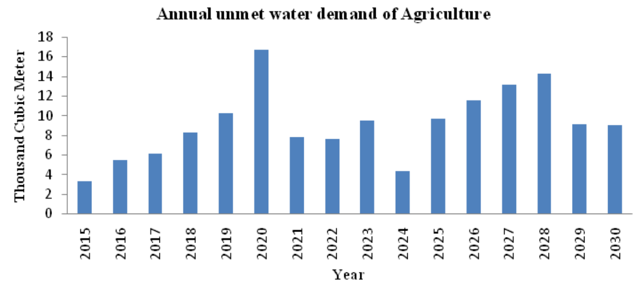

Fig. 16. Annual unmet water demand of agriculture (2015-2030)

Average monthly and Annual water supply for Agriculture

While customization of WEAP model for Mohalkhad watershed, the demand priority for agriculture was set as 2 i.e. after population and livestock sector and the supply preference to meet out the agriculture demands was kept the same (river and groundwater) as that of the population and livestock. The average monthly water supply delivered for agriculture shown in Fig. 17 for 2015-2030. Because of the above mentioned water supply priority and preference, water supply delivered to agriculture shows decreased from June and then slight increases in August and again shows decreasing trend till December. This is due to fact that all water will be used to meet out domestic demand and only limited water supply available for agriculture purpose. In winter months, it can be seen that water is delivered due to availability of winter rainfall except in March where low or no rainfall occurs. To meet out the unmet water demand of agriculture and to increase the supply delivered to agriculture, WEAP models needs more refined datasets and perhaps more comprehensive water allocation planning taking into consideration of nearest water resources available if any.

Fig. 18 shows the annual water supply delivered for agriculture. WEAP simulation projected the low annual water supply for agriculture in year 2024 of about 12 thousand cubic meter average; whereas, projected higher water supply of about 36 thousand cubic meter average for years 2020. This is in contradiction with the projected amount of rainfall received in respective years. This is due to fact that second priority was given for agriculture sector (against the first priority to population and livestock) during WEAP model formulation and same water sources (river and groundwater) preference

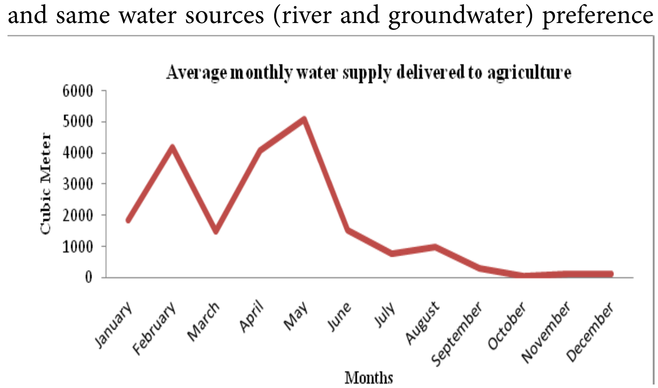

Fig. 17. Average monthly water supply delivered to agriculture

was given but only after satisfying the demands of population and livestock of the watershed. WEAP perform poorly while simulating annual water supply delivery to the agriculture in present case.

Crop Yield

Effect of climate change and water scarcity on the crop production inMohalkhad watershed, was studied using WEAP modeling. Here, authors have to mentioned specially that due to limitation of comprehensive datasets, only selected crops (Fig. 19) including orchards were taken into consideration while simulation modeling of WEAP. The WEAP outputs for selected crops during year 2020 (a dry year) with an annual rainfall of 322.97 mm were compared with those of year 2022 (a normal year) featuring an annual rainfall of 966.38 mm, as

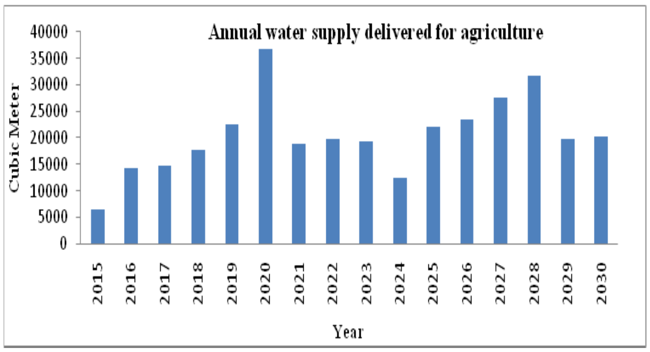

Fig. 18. Annual water supply delivered to agriculture (2015-2030)

presented in Fig. 19. In a dry year, insufficient water supply disrupts crop growth, resulting in a detrimental impact on overall crop production. Conversely, during a normal year, crops receive ample water due to favorable rainfall conditions, leading to positive effects on total crop production. Kharif crops, being sensitive to water availability, exhibit reduced yields in dry conditions. For instance, Tomato yield in the year 2020 is predicted to be 390 tonnes, against the 466 tonnes in the year 2022. On similar account, Maize yield may diminish due to water scarcity to 450tonnes in the dry year of 2020, which in the normal year of 2022 reaching 600 tonnes. This responsiveness of crop yield to rainfall is evident in Fig. 19 for both years, wherein crop yield in the normal year (2022) surpasses that in the dry year (2020). The comparison of crop yields highlights their sensitivity to dry, wet, and normal year rainfall conditions, showcasing variations in yield for respective years (Fig. 20).

In 2020, the lowest crop yield is observed, attributable to the scarcity of rainfall.

CONCLUSION AND FUTURE OUTLOOK

For the evaluation of water supply and demand in Mohalkhad watershed, WEAP model is customized for the watershed using available limited datasets. The possible impact of climate change on water demands and supply (of and for population, livestock and agriculture) and to meet out the unmet water demand from available sources with-in-year and years to come has been studied. The WEAP model was customized using Current Year (2015) data sets and future

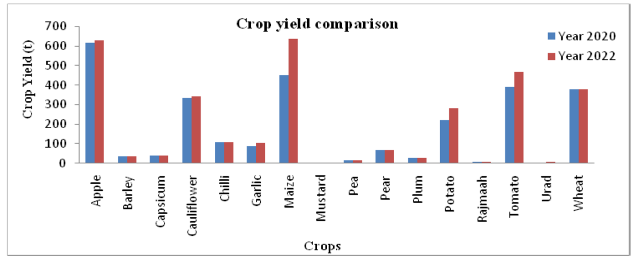

Fig. 19. Comparison of crop yield of selected crops for year 2020 (Dry year) and 2022(Normal year) for Reference Scenario

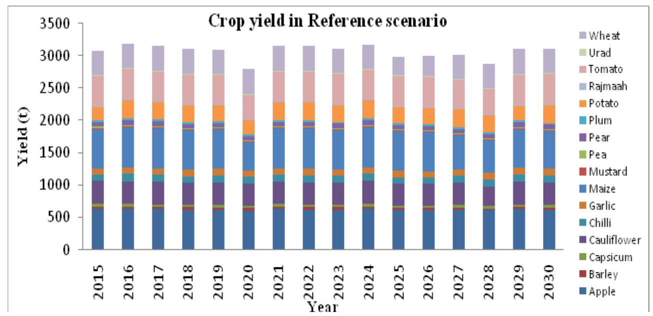

Fig. 20. Crop yields in Reference Scenario in Mohalkhad watershed for 2015-2030

climate datasets (2016-2030) of GCM GFDL-ESM2M under RCP 4.5. This attempt of WEAP model customization for Mohal khad watershed has provided many useful initial results that can be utilized and build upon in future for effective water management. There has been considerable variability seen in rainfall and same has been reflected in the respective results of hydrologic analysis, water allocation and crop yield analysis/outputs of the WEAP modeling. Overall, the customized WEAP model performs satisfactorily with few exception and able to simulate the climatic variation and corresponding water demands and available options to meet out unmet demands of the different sectors. Present study of customization of WEAP model for Mohalkhad watershed is the first ever attempt to data scared Himalayan basins. Due to this reason, the presented customized WEAP model for Mohalkhad watershed has limitations because of the non -availability of some datasets required for the model. To this, default values of WEAP modeling framework has been taken with certain area specific logical assumptions.

The main aim behind this exercise was to demonstrate the first ever use of WEAP modelingto evaluate the water demand and supply in context of climate change in the watershed and to set the platform for future studies. Refined datasets, population growth indexing, improved ranking for water supply preferences&priorities, and additional crop related data may be used to achieve highest modeling efficiency of WEAP simulation and strengthen the interpretation. Further, using PEST calibration method of WEAP model, the calibration and validation part can be improved. The provision of hypothetical water recharge or storage structures and water efficient agricultural practices within the modeling framework can be made in order to demonstrate their impact on water demand and supply scenario so as to develop the best water management practices in watershed in the context of climate change.

ACKNOWLEDGEMENTS

The authors are thankful to the Director, G. B. Pant National Institute of Himalayan Environment, Kosi-Katarmal, Almora, Uttarakhand for providing the facility in the Institute for present work. Authors also like to thanks Mrs. Jyoti Patil, Scientist - D, NIH Roorkee for valuable guidance on WEAP model& in analysing and interpreting the result; and to Dr. Amey Pathak, Prof. IIT Kharagpur for providing the climate model data; as well as to the Statistical, Patwari and Agriculture departments of Himachal Pradesh for providing the required datasets for the present study. The first author also acknowledge the funding support from National Mission on Himalayan Studies (NMHS), Himalayan JRF, MoEF&CC, Govt. of India.

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AEROSOLS, CLIMATE DYNAMICS, AND AIR QUALITY: A COMPREHENSIVE UNDERSTANDING OVER THE NORTHWESTERN HIMALAYAN REGION

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Concerns about pollution, rising greenhouse gas emissions and climate change are shared by people everywhere. Aerosols, which are tiny particles and droplets in the air, play a big role in affecting the temperature in the Himalayan region. They have dual impact on temperature; they can cool the Earth's surface by blocking sunlight but also can warm the atmosphere by absorbing radiation. Additionally, they influence cloud formation and precipitation patterns. So, if we want to do something about climate change in the Himalaya, we need to think about these aerosols. Researchers are working very hard to understand these particles, and it appears that aerosols are and/or will remain a major contributor to climate change across the Himalaya (Ramachandran *et al.*, 2023).

Extensive research is being conducted on the significant role played by absorbing aerosols, such as dust, brown carbon (BrC), and black carbon (BC). Activities such as the open burning of biomass and waste, combustion of household biomass, and combustion of fossil fuels release carbon dioxide, BC, BrC and bromine into the atmosphere. Among absorbing aerosols, studies reveal that BC takes the lead, contributing to over 75% of aerosol absorption over the Himalaya (Ramachandran *et al.*, 2020). By absorbing shortwave solar energy and warming the atmosphere, BC aerosols have a direct impact on the climate. Recent scientific assessments of the 6th Intergovernmental Panel on Climate Change (IPCC) report also estimates that BC is the most absorbing atmospheric aerosol with a best estimate of effective radiative forcing of around +0.107 W m^{-2}, thereby increasing the global mean surface air temperature by 0.063°C for the period 1750–2019 (Szopa *et al.*, 2021).

The ground-based aerosol observations from 2019-2022 were carried out at G. B. Pant National Institute of Himalayan Environment, Kosi-Katarmal (29.64°N, 79.62°E, 1225 m amsl) in the northwestern Himalaya. BC measurement was carried out using an Aethalometer (model AE-33, Magee Scientific U.S.A.) while mass concentration of particulate matter of size less than 10μ, represented as PM10 was obtained using APM 460 NL Respirable Dust Sampler (RDS). APM 411 TE gaseous sampling attached with RDS allows to collect gaseous pollutant samples (for monitoring SO_2, NO_2, etc.) as well as dust samples simultaneously. The measurement of mass concentration of particles of size less than 2.5μ in the ambient air, represented by PM2.5 was obtained using APM 550 MFC fine particulate sampler.

According to the India Meteorological Department (IMD), seasons of the state are characterized as winter (December–February), pre-monsoon (March–May), monsoon (June–September), and post-monsoon (October–November). Monthly variation of those meteorological parameters along with the study site is shown in Fig. 1.

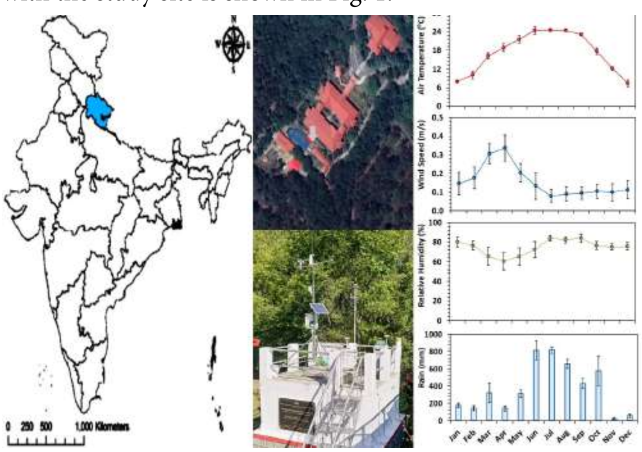

Fig. 1. Study site location along with the meteorological conditions

The maximum temperature observed at the site is about ~25°C during June-July while the winters are usually cold and the minimum temperature of ~8°C is observed in December. The site is influenced by heavy rain during the monsoon period starting from June and lasting till early September and is thus characterized as a wet period. The total amount of rainfall over the entire period was 4490 mm. However, ~60% rain occurred during monsoon (2726 mm), whereas only 381 mm rainfall was recorded during winter in the study period. Key parameters, including PM10, PM2.5, BC, SO_2, and NO_2 and monitored from 2019 to 2022. The concentrations of these pollutants were compared to the standards set by the Central Pollution Control Board (CPCB). PM_{10} was observed to be 45.3 ± 1.8 μg m^{-3}, while $PM_{2.5}$ was 35.7 ± 2.2 μg m^{-3}. Notably, PM_{10} and $PM_{2.5}$ concentrations were found to be below the prescribed limits, ensuring that air quality in the studied area met regulatory standards. Similarly, the concentrations of SO_2 and NO_2 were within the acceptable limit (Table 1). The air quality in the studied area met regulatory standards within the allotted time except for the forest fire days. The PM10 level on forest fire days reached 101.6 µg m-3, exceeding the acceptable limit by 70%. Similarly, the $PM_{2.5}$ level was about 15% higher than the allowed limit.

Table 1. Status of particulate and gaseous pollutants (2019-2022) in the north-western Himalayan region

Air pollutants	CPCB Standards (Annual, µg m^{-3})	Sample No. (24 h)	Average Concentration Value ± SD (µg m^{-3})	Forest fire days
PM_{10}	60	433	45.3 ± 37.2	101.6 ± 51.2
$PM_{2.5}$	40	409	36.6 ± 22.4	46.0 ± 25.2
BC	-	1186	1.34 ± 1.3	2.9 ± 2.5
SO_2	50	433	2.2 ± 2.3	2.8 ± 4.1
NO_2	40	433	12.1 ± 11.6	15.6 ± 17.8

The mean BC mass concentration was 1.34 ± 1.3 µg m^{-3} and was found to exhibit strong diurnal variability with sharp morning and evening peaks in all the seasons. Notably, the pre-monsoon period exhibited the highest variability in BC levels, reaching up to 13.7 µg m^{-3} shown in Fig. 2. This can be because of increased biomass burning activities, such as forest fires, which intensify during the pre-monsoon months. During forest fire days, BC was found to have increased by ~120% compared to non-forest fire days. Katarmal region is characterized by dense forest especially the chir pine, making it vulnerable to forest fires.

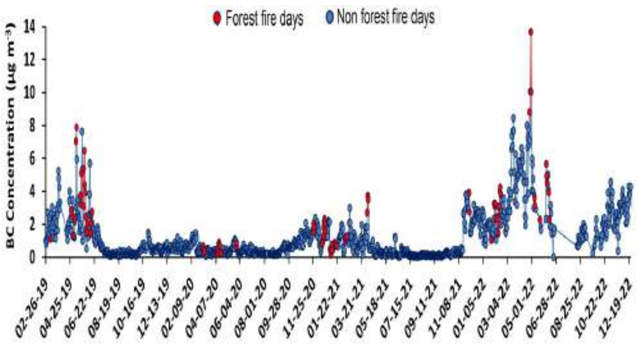

Fig. 2. Daily variation in BC during forest and non-forest fire days

We first use an aerosol optical model: Optical Properties of Aerosols and Clouds (OPAC, Hess *et al.*, 1998) to derive key optical properties for estimating the impact of BC on aerosol radiative forcing (ARF). These properties, including aerosol optical depth (AOD), single scattering albedo (SSA), and asymmetry parameter (AP), are then fed into the Santa Barbara Discrete Ordinate Atmospheric Radiative Transfer model (SBDART, Ricchiazzi *et al.*, 1998) for ARF estimation. The yearly mean variation in ARF at the top of atmosphere (TOA), surface (SUR), and atmosphere (ATM) at the observation site are presented in Fig. 3. During the observation period, the RF_{TOA}, RF_{SUR} and RF_{ATM} in Katarmal varied from −7.6 to −12.2 W m^{-2} (mean = −9.5 W m^{-2}), – 11.6 to −27.9 W m_{-2} (mean = – 20.3 W m^{-2}) and +4.0 to +15.7 W m^{-2} (mean = +10.8 W m^{-2}), respectively. The average RFATM at Katarmal is comparable to Manora Peak (+10.2 W m^{-2}) (Srivastava *et al.*, 2012) and Gulmarg (+13.5 W m^{-2}) (Romshoo *et al.*, 2023). The highest RFATM was observed during April 2022 (+15.7 W m^{-2}) and March 2022 (+14.6 W m^{-2}), implying a stronger atmospheric warming potential primarily due to elevated occurrence of forest fires. Further, the observed annual mean heating rate was 0.3 Kelvin day^{-1}.

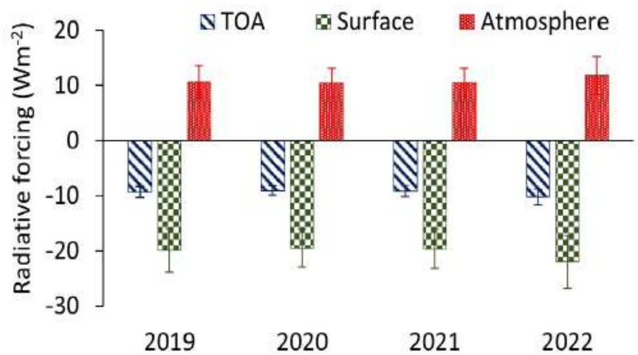

Fig. 3. Yearly variation of BC aerosol radiative forcing at TOA, SUR, ATM at NIHE, Katarmal, Almora.

CONCLUSIONS

The research findings reveal key insights, which are summarized as follows:

1. The annual average BC concentration over the entire study period was 1.34 ± 1.3 µg m^{-3}. Maximum monthly mean BC was 5.94 µg m^{-3} in April, while minimum was 0.09 µg m^{-3} in September.
2. BC aerosols show diurnal variations with prominent peaks in the morning and evening. The highest seasonal concentrations with 2.2 ± 1.9 µg m^{-3} were observed during the pre-monsoon period.
3. PM_{10} (45.3 ± 1.8 µg m^{-3}) and $PM_{2.5}$ (35.7 ± 2.2 µg m^{-3}), along with gaseous pollutants were within the threshold limits. However, PM10 and PM2.5 levels during forest fire days exceed acceptable limits.
4. The mean surface forcing, averaged during the entire period, was found to be +10.8 ± 2.9 Wm^{-2} (heating rate = 0.3 K day^{-1}) for BC aerosols. This value increased by around 45% during the forest fire.

ACKNOWLEDGEMENTS

Our heartfelt thanks are extended to the Director of G.B. Pant National Institute of Himalayan Environment, Kosi-Katarmal-263 643, Almora, Uttarakhand for providing essential facilities that could make the present study possible. The authors acknowledge ISRO GBP-ARFI, NMSHE TF-3 (Phase II), and ISRO GBP-ATCTM for providing the partial financial support to conduct the study. The authors also acknowledge the OMI and MERRA-2 for providing data freely for research use.

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PHYSICOCHEMICAL CHARACTERISTICS AND PLANKTON DIVERSITY OF A THERMAL SPRING IN ARUNACHAL HIMALAYA, NORTH-EAST INDIA

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ABSTRACT

Thermal springs create distinctive and uncommon ecosystems with specific geothermal and physiochemical features. These springs in the Himalayan region are considered a natural blessing, and some remain undiscovered due to their remote locations. Phytoplankton and Zooplankton are fundamental elements in any aquatic ecosystem. The plankton community is a dynamic system that promptly reacts to alterations in the physical and chemical attributes of the water environment and. Thus, present study aims to assess the physicochemical characteristics and abundance of phytoplankton and zooplankton in the Grengkhar hot spring located near the Tawangchu River in the Tawang district. A total of 11 physicochemical parameters of Grengkhar hot spring were analyzed. The hot spring exhibited a temperature (39.2 °C) suitable for plankton growth. Parameters such as pH (6.90), total hardness (147 mg/L), calcium (53.31 mg/L), magnesium (23.50 mg/L), and sulphate (76.50 mg/L) were within acceptable limits, while total dissolved solids (1350 mg/L) and chloride (310.6 mg/L) fell below permissible limits. The study of plankton diversity in the thermal spring identified a total of 14 plankton species. A total of 7 phytoplankton were identified and categorized in four different class viz. Bacillariophyceae, Coscinodiscophyceae, Euglenozoa, and Cynophyceae. Among the phytoplankton, Bacillariophyceae class was the most prevalent (3 nos.) whereas other three remaining individual class shared one each. Among the 7 zooplankton, Copeoda class predominated (4 nos.) followed by Branchiopoda (2 nos.), and Monogononta (1 no.). The physicochemical characteristics of the hot spring found suitable and within the permissible limit. The presence of the phytoplankton and zooplankton diversity of the hot springs signify the fair quality of the water for plankton growth. However, a detailed seasonal investigation on different physicochemical and biological parameters in connection with plankton diversity would be helpful in ecosystem analysis of the thermal spring.

Keywords: Hot spring, Phytoplankton, Zooplankton, Arunachal Pradesh, Eastern Himalaya

INTRODUCTION

Thermal springs are natural groundwater discharges where warm or hot groundwater regularly emerges from the Earth, consistently exceeding the surrounding ground temperature. These springs create distinctive and uncommon ecosystems with specific geothermal and physiochemical features. Thermal springs support a distinct microbial community, potentially harbouring novel microorganisms of scientific and biotechnological importance, due to their extreme conditions (Nanda *et al.*, 2022; Malesevic *et al.,* 2023). The productivity of any aquatic environment relies on the abundance of plankton within it. Aquatic ecosystems host diverse communities that define the ecosystem's characteristics and functionality in terms of sustaining production and the food chain. The plankton community is a dynamic system that promptly reacts to alterations in the physical and chemical attributes of the water environment (Ganie *et al.,* 2018).

Phytoplankton and zooplankton serve as fundamental elements in any aquatic ecosystem (Dash *et al.*, 2012). The phytoplankton population, considered the biological richness of a water body, plays a crucial role as a key link in the food chain (Panda *et al.,* 2012). In aquatic ecosystems, phytoplankton act as the foundation of the food web, providing a nutritional base for zooplankton and, subsequently, other invertebrates (Ganie *et al.,* 2018). Conversely, zooplankton function as primary consumers, playing a critical role in maintaining the foundational levels of aquatic food webs as the second trophic level in most aquatic environments (Dash *et al.,* 2012). Beyond their significance as primary producers and contributors to ecological balance in food webs, phytoplankton also serve as valuable indicators of water quality. Various abiotic or physicochemical factors, such as pH, light, temperature, salinity, turbidity, and nutrients, regulate the abundance and composition of phytoplankton in aquatic ecosystems (Panda *et al.,* 2012). On the other hand, zooplanktons, with their short life cycles, swiftly respond to changes in aquatic environmental conditions, such as pH, colour, odour, and taste, making them useful indicators of the overall health and conditions of their habitats. In specialized ecosystems like hot springs, where physico-chemical parameters differ significantly from other freshwater aquatic environments, studies on plankton diversity in relation to these parameters become particularly significant (Dash *et al.,* 2012). Hot springs in the Himalayan region are considered a natural blessing, and some remain undiscovered due to their remote locations. According to the Geological Survey of India (GSI), the seven geothermal provinces in India boast a total of 400 hot springs. Arunachal Pradesh, situated in the eastern Himalaya, is a part of the larger Himalayan Geothermal province (Chatterjee *et al.,* 2022). Despite global studies extensively exploring phytoplankton diversity in thermal springs, only 28 hot springs in India have been subject to investigation and examination through both culturally independent and culturally dependent studies (Poddar and Das, 2018). Even among the reported hot springs, comprehensive scientific research is lacking for many. Bora *et al.,* (2006) provided chemical parameters for two hot springs, one in the Dirang area of West Kameng district and another in the Kitpi area of Tawang district. Taye and Chutia (2012) briefly reported on the physical and chemical characteristics of hot springs, including Braksar, Thimbu, Tsachu, and Dirang. The diversity of freshwater algae in Arunachal Pradesh was also done by Das and Adhikary (2012). Phytoplankton of foot hill belt of Arunachal Pradesh was prepared by Nath and Baruah *et al.*, (2021), however the study was more focussed on Papumpare district. A recent study by Nanda *et al.*, (2022) focused on the physicochemical characteristics and diurnal temperature variations in the Dirang hot springs. However, there is still a gap in understanding the detailed characteristics of plankton diversity in these hot springs. The present study aims to document the abundance of phytoplankton and zooplankton in the Grengkhar hot spring located near the Tawang-Chu River in the Tawang district of Arunachal Pradesh.

MATERIALS AND METHODS

Study Area

The study was conducted in the Grengkhar village under Tawang district of Arunachal Pradesh. The hot spring is located at 27° 33' 10.9" and 91° 53' 41.62' E in the Grengkhar village in the Kitpi area of Tawang district. Tawang is situated at an elevation ranging from 1800 to 3300 meters above mean sea level in the Eastern Himalayan Region. The thermal spring is located just below the Grengkhar village near the Tawang-Chu River (Fig. 1). The region's topography is predominantly mountainous, experiencing an arid tundra or cool temperate climate in the north, with regular snowfall from November to March each year. Most of the hot springs in the region emanate from a sequence of garnet-bearing high-grade gneiss-migmatite-schist-quartzite within the Se La Group. The local population of Tawang relies on natural water sources for their daily necessities. The geothermal springs (hot springs) are medicinally and culturally significant to the local community. Although the Tawang encompasses the momentous natural beauty for the tourist; owing the folklore and health benefits, the hot springs are also play an important role in the status of the area.

Fig. 1. Location map and site of hot springs in Tawang, Arunachal Pradesh

Sample collection and physicochemical analysis

The water samples were collected in clean glass bottles and brought to the laboratory for comprehensive physical and chemical analysis, adhering to established protocols (APHA, 2005). Sampling was executed using double-stoppered wide-mouthed bottles, meticulously rinsed with sampling water before collection, and sealed post-sampling. Subsequently, these bottles were promptly transported to the laboratory for the assessment of chemical parameters. The examination of water samples for the presence of various chemicals followed the standard methods for water and wastewater analysis (APHA, 2005). In-situ measurements of temperature, pH, and EC were conducted using the Multi-Parameter PCS Tester (AQUASOL, AM-AL-01, Rakiro). Whereas, sodium was measured using flame photometer (Systronics 128). The parameters including alkalinity, chloride, calcium, and total hardness was measured using the titration method. The total dissolved solid was measured using the titration method. Phytoplankton samples were obtained by filtering the water using plankton net (~45 μm mesh size). These collected plankton net samples were stored in PE bottles and immediately fixed with Lugol's iodine solution. For Zooplankton samples a plankton net (~ 75 μm mesh size) was used for filtering the water and collected in PE bottles. The Zooplankton samples were immediately fixed with the formalin solution (5%). The plankton samples were then brought to the laboratory and allowed to settle overnight. Then the plankton samples were studied with the help of foldscope following the standard methodology (Nanda *et al.*, 2020; Nath and Baruah 2021; Panda *et al.*, 2012). The plankton taxa were identified by consulting the standard literatures, monographs, and other guides available on the internet.

RESULT AND DISCUSSION

The study was conducted on 11 physicochemical parameters of Grengkhar hot spring, and the majority were found to be within acceptable or permissible limits (Table 1). The hot spring exhibited a temperature of 39.2 °C. Parameters such as pH, total hardness, calcium, magnesium, and sulphate were within acceptable limits, while total dissolved solids (TDS) and chloride fell below permissible limits. However, the electrical conductivity (EC) value exceeded the permissible limit (1.5 mS/cm) according to WHO guidelines. The study of plankton diversity in the thermal spring identified a total of 14 plankton species. Among these, 7 were categorized as phytoplankton and 7 as zooplankton based on their characteristics. The phytoplankton comprised 7 genera from 7 families, with Bacillariophyceae class being the most prevalent. In contrast, zooplankton exhibited 7 genera across 5 families, with Copeoda class predominating and contributing four genera (Table 2). A water body functions as an ecosystem characterized by a network of diverse physico-chemical parameters and its biota. The interaction between these physico-chemical parameters and plankton communities constitutes a comprehensive ecosystem. These interactions are subject to complex influences, some of which lead to quantitative changes, particularly during plankton production (Dash *et al.*, 2012). Temperature plays a crucial role in the population dynamics of phytoplankton, with late winter and spring representing optimal seasons for biological production and reproduction. During these favourable periods, zooplankton abundance tends to increase. Conversely, high temperatures can have adverse effects on zooplankton survival, establishing an inverse relationship with water temperature. In the current study conducted in December, where the temperature was recorded at 39.2°C (Table 1), conducive conditions were observed for both biological and biotic reproduction. This resulted in the identification of 7 phytoplankton and 7 zooplankton species (Fig. 2-3).

The pH serves as an indicator of the equilibrium between hydrogen ions and hydroxyl ions in water (Prasanth *et al.*, 2012). The ideal pH range for phytoplankton growth falls between 5.37 and 7.4, a condition affirmed by the current investigation. However, the presence of zooplankton grazing on phytoplankton blooms was found to diminish the phytoplankton count in the aquatic system (Manickam *et al.,* 2017). Lower pH levels negatively impact a species' ability to maintain salt balance, leading to halted reproduction. Most species perish at pH levels of approximately 4 or below and 11 or above. In an alkaline environment, pH values were presumed to be conducive to the proper growth and development of zooplanktons (Dash *et al.,* 2012). Chemical analysis of thermal waters in the Tawang and West Kameng Districts of Arunachal Pradesh indicated that the waters exhibit mild acidity to weak alkalinity. The electrical conductivity (EC) values for hot spring samples ranged from 269 to 3600 µScm-1 (Dutta and Gupta, 2022). The hot springs exhibited elevated conductivity due to the presence of abundant minerals in the water. Dilution of solid substances, in turn, results in reduced EC value, alkalinity, and zooplankton production. Electrical conductivity (EC) proves to be a reliable indicator of water quality, with higher conductivity recorded in the hot spring, suggesting an enrichment of salts in the groundwater. The relationship between total dissolved solids (TDS) and EC is influenced by ion composition, including factors such as calcium, magnesium, chloride, among others (Taylor *et al.*, 2018).

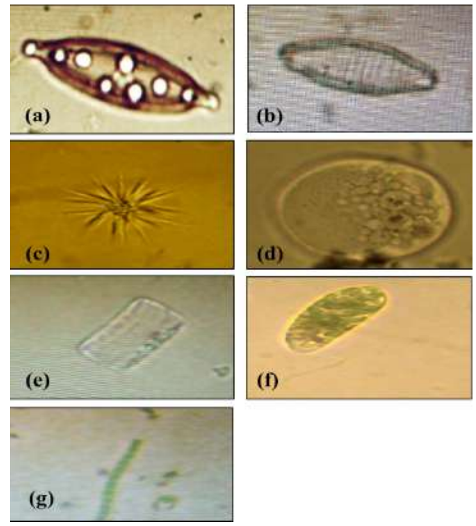

Fig. 2. Phytoplankton diversity in the hot spring (a) Navicula (b) Epithemia (c) Ankistrodesmus (d) Actinocyclus (e)Achnantes (f)Euglena (g)Nostoc

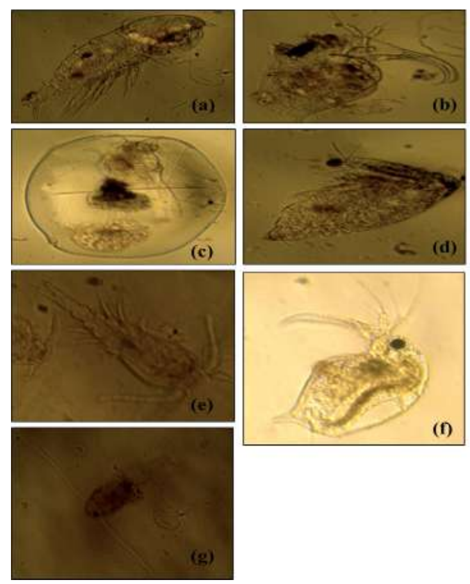

Fig. 3. Zooplankton diversity in the hot spring (a) Nauplius (b) Bosmina (c) Asplancha (d) Nauplius (e) Cyclops (f) Daphnia (g) Diaptomus

Table 1. Physicochemical characteristics of the Grengkhar hot spring in Tawang

Parameters	Grengkhar hotspring	Acceptable limit*	Maximum permissible limit*
Temperature (°C)	39.2	-	-
pH	6.90	6.5-8.5	No relaxation
EC (mS/cm)	2.04	1.5#	-
Total Dissolved Solids (mg/L)	1350	500	2000
Alkalinity as CaCO3 (mg/L)	298	200	600
Total Hardness as CaCO3(mg/L)	147.0	200	600
Calcium as Ca (mg/L)	53.31	75	200
Magnesium as Mg (mg/L)	23.50	30	100
Chloride as Cl (mg/L)	310.6	250	1000
Sulphate as SO4 (mg/L)	76.50	200	400
Salinity (ppt)	1.04	-	-

*As per the Bureau of Indian Standard for Drinking Water (ISO 10500:2012)
World Health Organization guidelines 2017

Table 2. Details of phytoplankton and zooplankton diversity in the hot spring

Class	Order	Family	Genera
Phytoplankton diversity			
Bacillariophyceae	Naivulales	Naviculaceae	Navicula
Bacillariophyceae	Rhopalodiales	Rhopalodiaceae	Epithemia
Bacillariophyceae	Achnanthales	Achnanthaceae	Achnanthes
Coscinodiscophyceae	Coscinodiscales	Hemidiscaceae	Actinocyclus
Euglenozoa	Euglenales	Euglenophyceae	Euglena
Cynophyceae	Nostocales	Nostocaceae	Nostoc
Chlorophyceae	Sphaeropleales	Selenastraceae	Ankistrodesmus
Zooplankton diversity			
Monogononta	Ploima	Asplanchnidae	Asplancha
Copepoda	Cyclopoida	Cyclopidae	Nauplius
Copepoda	Calanoida	Diaptomidae	Diaptomus
Copepoda	Calanoida	Diaptomidae	Neodiaptomus
Copepoda	Cyclopoida	Cyclopidae	Cyclops
Branchiopoda	Anomopoda	Daphniidae	Daphnia
Branchiopoda	Anomopoda	Bosminidae	Bosmina

Hardness, mainly measured by calcium and magnesium content, falls within the permissible range in the current investigation, with a value of 147 mg/l (refer to Table 1). While many aquatic organisms can tolerate a wide range of calcium hardness concentrations, the preferred range is typically between 75 mg/l and 250 mg/l (Dash *et al.,* 2012). Alkalinity, a crucial parameter, influences the dissociation of bicarbonate into carbonates and carbon dioxide, thereby increasing alkalinity. The observed high alkalinity demonstrates a positive correlation with zooplankton diversity, suggesting a potential association between total alkalinity and planktonic yield (Singh *et al.,* 2002). The presence of chloride ions can be attributed to chloride salts, metals, or inorganic compounds. These chloride salts play a crucial role in hot springs, contributing to balneotherapy for individuals and potentially influencing gastrointestinal and bowel-related issues. The elevated concentration of chloride (Cl-) is critical for proper phytoplankton and plant growth, impacting the taste and overall suitability of drinking water (Sherpa *et al.,* 2013; Manickam *et al.*, 2017). Sulfate is a prevalent chemical substance in drinking water, offering properties conducive to balneotherapy, promoting muscle relaxation, and aiding in the treatment of skin diseases. Sulfates also serve as important chemotherapeutic agents, acting as antiseptics and natural drugs (Sherpa *et al.,* 2013). Thus, the hot spring provide good water quality along with the plankton diversity.

CONCLUSION

The present study provides a baseline information concerning physicochemical characteristics as well as phytoplankton and zooplankton diversity of the hot springs. The physicochemical characteristics of the hot spring found suitable and within the permissible limit. The presence of the phytoplankton and zooplankton diversity of the hot springs signify the fair quality of the water for plankton growth. However, a detailed seasonal investigation on different physicochemical and biological parameters in connection with plankton diversity is required for ecosystem analysis of these hot springs.

ACKNOWLEDGEMENTS

The authors are thankful to the Principal, Dera Natung Government College, Itanagar; Director, G.B. Pant National Institute of Himalayan Environment (NIHE), Almora, Uttarakhand and Centre for Earth Sciences & Himalayan Studies, Itanagar, Government of Arunachal Pradesh for providing necessary facilities to carry out the work.

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IDENTIFICATION AND CHARACTERIZATION OF HELMINTHOSPORIUM SPECIES CAUSING DISEASES IN SORGHUM AND SOME LESSER MILLLETS OF BIHAR

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ABSTRACT

Helminthosporium diseases are a major constraint to sorghum production, causing significant yield losses and reducing grain quality including lesser millets in Bihar, India. Identification and characterization of the *Helminthosporium* species causing diseases in sorghum and lesser millets are crucial for the development of effective management strategies. The aim of this study was to identify the various *Helminthosporium* species occurring on sorghum. Determine the range of variability amongst different species and Isolates of *Helminthosporium* as regards morphology, physiology and pathogenicity. The findings of this study provide valuable information on the *Helminthosporium* species causing diseases in sorghum and lesser millets in Bihar and can contribute to the development of effective disease management strategies in the region.

Keywords: *Helminthosporium* species, Pathogenicity, Physiology, Sorghum

INTRODUCTION

Helminthosporium is a genus of plant-pathogenic fungi that causes diseases in several cereal crops, including sorghum and lesser millets in Bihar, India. These diseases have been a major cause of yield loss in these crops, leading to economic losses for farmers. *Helminthosporium* is a genus of fungi that can cause a range of diseases in plants, including sorghum. Sorghum is an important cereal crop that is widely cultivated in tropical and subtropical regions and grown worldwide for food, feed, and fuel. *Helminthosporium* is one of the most common genera of fungi causing sorghum diseases. The identification and characterization of *Helminthosporium* species are essential for the development of effective management strategies. Several *Helminthosporium* species have been reported to cause sorghum diseases, including *Helminthosporium maydis*, *Helminthosporium turcicum*, *Helminthosporium spiciferum*, and *Helminthosporium tetramera*. The symptoms of *Helminthosporium* diseases in sorghum include leaf blight, leaf spot, and stem rot. Morphological characteristics, such as colony morphology, conidiomata, and conidial shape and size, can provide initial clues for the identification of *Helminthosporium* species. However, these characteristics can be variable and overlap among species, making it difficult to distinguish them solely based on morphology. Molecular techniques, such as PCR amplification and sequencing of the ITS region, have been used to identify and characterize Helminthosporium species causing sorghum diseases. In addition to molecular techniques, other methods such as pathogenicity tests, host range determination, and mycotoxin analysis can also aid in the identification and characterization of *Helminthosporium* species. These methods can provide valuable information on the virulence, specificity, and secondary metabolites of the fungi. Once the *Helminthosporium* species has been identified, it is important to characterize its pathogenicity and virulence. Pathogenicity and virulence can vary depending on the *Helminthosporium* species and the sorghum cultivar. Characterization of pathogenicity and virulence can involve inoculating sorghum plants with the fungus and monitoring the disease symptoms. In conclusion, identification and characterization of *Helminthosporium* species that cause diseases in sorghum are essential for developing effective control strategies. Accurate identification of the fungus can help target specific control measures, such as fungicides or resistant cultivars. Characterization of pathogenicity and virulence can provide insight into the mechanisms of disease development and help identify potential targets for control.

Table 1. *Helminthosporium* species studied with their locality

Helmintosporium species and isolate No.	Locality	State	Hosts
ISH1	Dhole	Bihar	*Sorghum Harepense*
ISV62	Chianki	Bihar	*Sorghum Harepense*
IDA 1	Sabour	Bihar	*D. aegyptium*
ISV 72	Sabour	Bihar	*S vulgare*
ISH 2	Dhole	Bihar	*Sorghum Harepense*
ISV 3	Dhole	Bihar	*S vulgare*
ISeI 4	Asauni Nabtola	Bihar	*Setaria Italica*

ISeI 5	Ladha	Bihar	*Setaria Italica*
ISeI 7	Paharpur	Bihar	*Setaria Italica*
ISeI 8 A	Babupur	Bihar	*Setaria Italica*
ISeI 8 B	Sabour	Bihar	*Setaria Italica*
ISeV 15	Sabour	Bihar	*Setaria Verti-ciliata*
ISeI 6	Shahabad	Bihar	*S I talica*
IEF 4	Nabtola	Bihar	*Echinochloa frumentaceae*
IEF 7	Ladha	Bihar	*Echinochloa frumentaceae*
IEF 9 A	Babupur	Bihar	*Echinochloa frumentaceae*
IEF 9 B	Sabour	Bihar	*Echinochloa frumentaceae*
*H.homl*11 Luttrell: IDA	Sabour	Bihar	*D. aegyptium*
IPS 6	Paharpur	Bihar	*Paspalum Scrobiculatum*
H.victoriae Meehan and Murphy:	Bagha	Bihar	*Paspalum Scrobiculatum*
IPS 10			

METHODOLOGY

In Bihar, Sorghum is grown in both rain-fed and irrigated areas. The major sorghum-growing districts in Bihar are Darbhanga, Samastipur, Muzaffarpur, Vaishali, and East Champaran. The state government has implemented several programs and schemes to support sorghum cultivation, including the distribution of high-yielding and drought-resistant varieties of seeds, as well as providing financial and technical assistance to farmers. *Helminthosporium* species with isolate occurring on sorghum and the smaller millets in different parts of the Bihar were studied.

Single sporing: Spores were scraped directly from the diseased portion of the leaf lamina on sterilized slides and dispersed by slightly tilting the glides and tapping gently against the working table so that well separated spores could be located under the low power of the microscope. A single spore was picked up directly from the slide with the help of sterilized needle and inoculated on autoclaved PDA slants aseptically. Where the spores were not available on the natural spots, the spots were cut into small pieces along with adjoining healthy portion of the leaf lamina and then washed in several changes of sterile water and placed in moist chamber aseptically. Within 4-5 days numerous conidia developed. There after the same procedure was adopted as in 'A' for isolation of a single spore.

Culture media: A number of culture media were used in various experiments. Their pH was adjusted to 6 or any other desired value by Beckman's glass electrode pH meter. The media were prepared according to the standard formula given by Riker and Riker (1936), Ainsworth and Bisby (1960) and others.

The following culture media were used for laboratory studies: Patato dextros agar, Maize meal agar, onion agar, Sorghum leaf extract agar, Rise meal agar, Richard's medium, czapek's medium, and Glucose peptone medium

Preparation of media: For preparing media, the standard methods described by Riker & Riker (1936), Ainsworth and Bisby (1960) and others were followed twenty and ten ml of medium were poured in plants and test tubes respectively and the latter plugged with non-absorbent cotton. For preparing synthetic and non-synthetic solid media, the constituents were dissolved in distilled water, filtered through gauge, the filtrate squeezed out and the desired volume made up by addition of distilled water. Thereafter 2 percent agar was added for solidification by heating in water bath. These were then thoroughly mixed and autoclaved at 15 lbs. pressure for 25 minutes unless otherwise mentioned, cooled to 45C and then poured into sterilized Petri dishes of 4t diameter.

For preparation of host extract agar medium: 250 g of plant material (dried or green) was cut into small bits, boiled for half an hour in distilled water and 2% agar was added.

In preparation of liquid media: Agar was omitted. The ingredients were dissolved in distilled water separately within the limit of the total volume to be prepared and finally mixed together, 25 ml ofthe medium were pipetted in 100 ml flasks. The flasks were then plugged with non-absorbent cotton. The flask containing liquid media were sterilized in autoclave at 10 lbs. pressure for 30 minutes to prevent hydrolysis. Glassware were cleaned and sterilized. Soils and pots were also sterilized. Seeds were sterilized with0.1% mercuric chloride solutions for two minutes after that seeds were washed several times with distilled Water. Inoculation was performed with 7 - 10 days old culture maintained on PDA were used for Inoculating the medium.

Effect of Temperature: Petri plate containing 20 ml of sterilized potato dextrose agar medium were incubated with 2 mm discs of 7 - 10 days old cultures maintained on PDA and kept at different temperatures ranging from 20 - 400C. Colony Growth were measured at different intervals for 120 hours.

Effect of pH: Isolates were grown in 100 ml flasks containing 25 ml of sterilized Richard's medium adjusted to different pH.

Measurement of Redial growth: In Petri dish culture the linear growth of the mycelium was measured in two directions at right angles to each other and expressed as average diameter of the developing colony in mm. When the growth was irregular the measurement were taken along the largest and shortest diameters and the average was taken as measurement of growth.

Microscopic Growth Features: Amount of aerial mycelium, colour, texture, zonation, saltation including sectoring the other characters of mentionable features were recorded for macroscopic growth features or the cultures.

Measurement of spores: Slides were prepared for recording the range of length and breadth and the septation of 50 conidia in two replications at random. Length, breadth and septation were measured in each case.

Effect of culture filtration: Seeds were soaked for 24 hours in the culture filtration and placed in petri plates lined with sterilized filter papers previously soaked in the cultures filtrate. Percentage of germination and root and shoot growth was recorded at the different intervals.

Record of pigmentations: Munsel color standards were used for recording the color of the mycelium and the culture filtrates.

Spore germination: Spores were collected either from the natural lesions or 7 - 10 days old culture and put in hanging drops on cover slips Incubated over a glass ring fixed on the surface of the slide.

Pathogenicity: For testing the pathogenicity of *Sorghum vulgare, S halepense, zea mays, setaria italic* and other graminicolous hosts against *Helminthosporium* species. The spore cum mycelial suspension from 7 - 18 days old plate culture were used. The spore cum mycelial suspension (nixed in distilled sterilized water with the help of mechanical grinder for 2 minutes) was sprayed on the leaves with the help of a fine sprayer. The inoculated seedling were kept in humid chamber for 48 hours at 28 4^{0}C after which the infection was observed.

RESULT AND DISCUSSION

Studies were made on the range of variability in several species of *Helminthosporium*. Especially those affecting sorghum and the minor millets. Four Isolates of H*elminthosporium turcicum* pass that was ISV 49, ISV 52, ISV 69 and ISV 74 differed in respect of their symptomatology, morphology, cultural characters, physiology, pigmentation, toxicity and pathogenicity. The lesion length caused by different isolates, was different. Longest spots were found In ISV 69 shortest in ISV 74 and intermediate in others. Under laboratory condition, spores from all the four isolates could survive 4 - 5 months. The colony characters varied with the medium and the isolates used. The colour of the cultural filtrates at different pH were also varied with the Isolates. Up to 8th day all the Isolates remained colorless but on 15th day the Isolates ISV 49, ISV 52 and ISV 74 produced coloration, (moderate olive to pale orange yellow) in both the acidic and alkaline sides (with few exceptions) but the Isolate ISV 69 remained colorless through out. The Initial pH 6.10 was found to be optimum for growth. The final pH started towards alkalinity, the pigmentation in acidic medium was generally pale orange yellow whereas it was strictly colorless in alkaline medium. The fungus grows well on PDA and Richard's liquid medium. The optimum temperature for growth was found to be 300C and the optimum pH 6.10.

***Helminthosporium turcicum* pass**.

Symptoms of the disease on *Sorghum vulgare*

The characteristic symptoms incited by all the four isolates ISV 49, ISV52, ISV 69, ISV 74 were found on both the lamina and sheaths. But the lesions on the lamina were most prominent. These lesions were of different sizes varying from small round, oval brownish, narrow to elongated spindle shaped spots along the length of the lesions. They had first water soaked, thin light olivaceous to brown and finally straw colored Centre associated with deeply pigmented (dark brown) margin all-round the spots. In humid weather, the spots

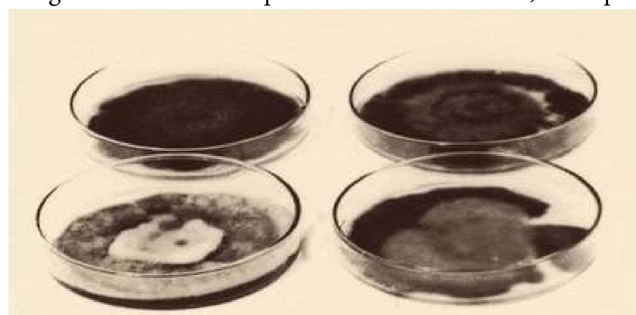

Fig. 1. Colony character of the four isolates of H. turcicum ISV49, ISV74, ISV 52 AND ISV 69 pass on PDA

turned smoky brown especially in the center due to profuse sporulation. This was true for all the four isolates understudy but the size of the spots varied with the isolates.

Viability

The loss in viability of spores of the four Isolates, stored at room temperature in PDA slants was studied. The spores from the four cultures could survive 4 to 5 months. When the percentage of germination was 5, 2, 3.33 and 2.33 for 16V 49, LP' 52, ITV 69 and ISV 74 respectively.

Effect of different solid media on the growth of isolates

Among the four media tried, the isolates grew differently on different media. Isolate ISV-49 grew best on Potato dextrose agar (69,67 mm), followed by Maize meal agar (56.33 mm), Rice polish agar (42.84 mm) and host extract agar (41,33

Fig. 2. Symptoms of *H Tercicum* (ISH1). Symptoms of *H Sorghicola* (ISH2)

mm), Similarly ISV 52, ISV 69 and ISV 74 attained its maximum growth on Potato-dextrose agar, but the radial growth were variable in each case.

Effect of different pH on the mycelial growth of four isolates

The effect of different pH on the growth of isolates ISV 49, ISV 52, ISV 69 and lSV 74 was determined. Richard's medium was taken as the basal medium and its pH adjusted to different levels with the help of NaoH and lactic acid. Twenty five ml of the medium were pipetted into 100 ml Erlenmeyer flasks and plugged with nonabsorbent cotton and autoclaved. The medium was cooled for twenty four hours and later inoculated with different isolates separately and incubated at 29 ± 1o C for 15 days. The mycelial mats were filtered on eighth and fifteenth day in filter paper no. 42 and their dry weight recoded. the results after 15th day of growth (last column) it is observed that the initial and final pH range 6.80 -7.15 supported the best growth of isolates ISV 52, pH range 6.80 -7.35 of isolate ISV 69 and pH 6.10 to 6.70 of isolate ISV 49. In ISV 74 the initial and final pH remained the same i.e. 6.10 on 15th day, though it was slightly higher 6.20 on 8th day. The mycelia weight varied with the isolates. It is concluded that the culture filterates (containing the metabolic products of four isolates of *H. turcicum*) markedly inhibited the germination of seeds and their root and shoot development which varied with the isolates and the pH tried. Germination was affected both in the acidic and alkaline sides. Root and shoot development was affected at all the pH tried, the culture filtrates were more toxic between final pH 7.50 and 8.60.The toxicity was more pronounced on 8th day when the pH was 8.50 and above as compared to 15th day pH 7.50 -8.50. The pH values which were acidic to start with shifted towards alkalinity on the 15th day in almost all cases. The colony characters were variable with the medium used in respect to aerial mycelium and its color, zonation etc. The culture filtrate or either isolate markedly suppressed the seed germination and root and shoot development of scrobiculatum at all the pH tried, most injurious effect was noticed towards alkaline aide, but the inhibitory effect varied with the isolate. The fungus was pathogenic to oats, barley and sorghum. Of several varieties ofscrobiculatum tested for pathogenicity IPS 92, IPS 26 Local were susceptible and IPS 394 resistant. Other varieties, viz., IPS 19, IPS 191, IPS 141, IPS 158, IPS 379, IPS 600 and IPS 606 were resistant to the Isolate. Susceptibility varied with the isolates.

Pathogenicity

Twenty one days old seedlings of the variety sabour local were sprayed with mycelial cum spore suspension of *H. setariae* using isolates ISeI 4, ISeI 6, ISeI 7, 1Se1 8A and ISeI 8B and kept inside humid chamber for 48 hours, later the pots Were kept out side. Within 48 hours reddish flecks developed on the leaf lamina in case of each isolates. Consistent isolations from these spots in all the isolates yielded the same pathogen.Twenty one days old seedlings of the variety sabour local were sprayed with mycelial cum spore suspension of *H. setariae* using isolates ISeI 4, ISeI 6, ISeI 7, 1Se1 8A and ISeI 8B and kept inside humid chamber for 48 hours, later the pots Were kept outside. Within 48 hours reddish flecksdeveloped on the leaf lamina in case of each isolates. Consistent isolations from these spots in all the isolates yielded the same pathogen.

Germination

Germination of spores of *H. sateriae* from *E. frumentaceae* was taken asintervals of 1,2,3,4 and 5 hours at 25°C. Differences were observed in the percentage of spore germination and the growth of the germ tubes. The vigour of the (germ tubes) mycelium was noted at the end of 25 hours in different juices of graminicolous hosts and water. Germination was unipolar bipolar as well as interseptal and was quickest and best in distilled water followed by barley juice, tap water, Johnson grass Juice and wheat juice. The growth and vigour of germ tubes was best in barley juice. Mycelium was slender and weak in distilled water.

CONCLUSION

In conclusion, this study on the identification and characterization of *Helminthosporium* species causing diseases in sorghum and some lesser millets of Bihar may have important implications for the development of strategies to manage fungal diseases in these crops.

Fig. 3. *H. sorghicola* on *Sorghum vulgare*

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WATER QUALITY INDEX OF SPRING WATER IN THE KOSI WATERSHED OF KUMAON HIMALAYA

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ABSTRACT

The local communities in the Indian Himalayan region (IHR) depend on springs for drinking, domestic, and agricultural purposes. The varied geology and topography of the Himalayan region give rise to numerous springs, locally known as Naula, Dhara, and Bawdi. Naulas in the Kumaun Himalaya also holding significant cultural and spiritual importance for the community. Such five Naulas (Springs) in five villages viz. Jyoli, Bisra, Kharkuna, Kujyadi, and Dilkot situated in the Upper Kosi watershed in Almora district of Uttarakhand were studied for their physicochemical characteristics and water quality index. The pH (6.5 to 7.1) and total hardness (29.3 to 95.9 mg/L) was found well within the desirable limit. EC (291.4 µS/cm), TDS (213.3 mg/L), and Alkalinity (283.8 mg/L) was found highest in Kharkuna. The cations and cations were also present within the desirable limit as per BIS norms. The WQI value of the all the five springs varied from 17 to 34 and found in A grade with excellent quality. The present study will be a baseline information for further investigation on the ecosystem health of these springs.

INTRODUCTION

Millions of people in the Himalayan region rely predominantly on springs to fulfil their water requirements. Both rural and urban communities in the Indian Himalayan region (IHR) depend on these springs for drinking, domestic, and agricultural purposes. The springs in the hilly terrain consist of seepage water that traverses through shallow weathered and fractured zones. The varied geology and topography of the Himalayan region give rise to numerous springs, locally known as Naula, Dhara, and Bawdi. Naulas and Dharas constitute the primary types of springs in the Uttarakhand Himalaya. Approximately 77% of Uttarakhand's population resides in the high-altitudinal hilly region, with 90% relying on natural spring water for their daily needs. Moreover, in the Kumaon region of Uttarakhand, 60% of rural inhabitants depend on natural springs for their water supply (Chauhan *et al.,* 2020; Chhimwal *et al*., 2022). In the Kumaun region of Uttarakhand Himalaya, local communities rely on spring water harvested through small step-wells known as Naulas to meet their water needs. Most of these Naulas were constructed during the Katyuri and Chand dynasties between the 7th and 8th centuries, holding significant cultural and spiritual importance for the community, contributing to their conservation and cleanliness (Sinha *et al*., 2021). However, various factors such as deforestation, changes in land-use patterns, sanitation practices, population growth, and climate change are increasingly affecting the availability, quality, and quantity of spring water in the region (Chhimwal *et al*., 2022).

The quality of water plays a crucial role in sustaining life on Earth, influenced by water sources, storage techniques, and treatment methods applied (Chauhan *et al*., 2020). Notably, in Himalayan villages, spring water is often used without prior treatment (Jasrotia *et al.*, 2018). Hence, the current study aims to evaluate the water quality of five springs in the Jyoli cluster in the Almora district of Kumaon Himalaya. This assessment seeks to determine the potability of the water, its safety for human contact, and its impact on ecosystem health.

MATERIAL AND METHODS

Study Area

The study was conducted in five villages viz. Jyoli, Bisra, Kharkuna, Kujyadi, and Dilkot situated in the Upper Kosi watershed in Almora district of Uttarakhand. The study area located between 29° 36'38" and 29° 38'13"N latitude and 790 34'40" and 790 36'35"E (Fig. 1). There are around 250 households in these five villages with a total population of about 1500. The per day water consumption of the village cluster ranges between 2960 litres for Kharkuna village to 17685 litres for Kujyadi village. Total water consumption of these villages is around 42000 litres/day (Negi *et al*., 2020). The selection of five springs for investigation was based on their diverse uses, including drinking water, agricultural purposes, household practices, and livestock uses.

Sample collection and physicochemical analysis

Spring samples from five different locations were collected and brought to the laboratory for analysis of various physicochemical parameters (Fig. 2). This included the determination of pH, electrical conductivity (EC), total dissolved solids (TDS), total hardness (TH) represented as $CaCO_3$, and the concentrations of calcium (Ca^{2+}), magnesium (Mg^{2+}) Sodium (Na^{+}), potassium (K^{+}), chloride (Cl^{-}), sulphate (SO_4^{2-}), and nitrate (NO_3^{2-}). The pH, EC and TDS were measured on-site using AQUASOL, AM-AL-01 (Rakiro), while Na^{+}, and K^{+}, were measured using flame photometer (Systronics, 128). The parameters including alkalinity, Cl^{-}, Mg^{2+}, Ca^{2+} and TH were measured using the titration method. The SO_4^{2-} and NO_3^{2-} were measured using spectrophotometer (Shimadzu, UV-2600). The chemical analysis was conducted following the APHA methods.

Fig. 1. Design of the Naula (Spring well) in the study area

Water Quality Index

The evaluation of water quality for drinking purposes includes the application of the Water Quality Index (WQI), a robust mathematical tool introduced by Horton in 1965. This index functions as a comprehensive model, effectively condensing extensive water quality data into a single numerical value. The WQI calculation method involves examining various water quality parameters such as cations, anions, pH, electrical conductivity (EC), and total dissolved solids (TDS). The process of computing WQI includes assigning weights (wi) to each water quality parameter, determining relative weights (Wi), and calculating a quality rating scale (Qi) as depicted in Equation (1). Qi is further defined in Equation (2), incorporating the concentration (Ci) of each chemical parameter (i) in each water sample (mg/L), the total number of parameters (n), and the Indian drinking water standard (Si) for the chemical parameter, as outlined by the Bureau of Indian Standards (BIS, 2012).

$$WQI= (\sum WiQi)/(\sum Wi) \qquad (1)$$
$$Qi=Ci/Si * 100 \qquad (2)$$

RESULTS AND DISCUSSION

A total of 12 physicochemical parameters of five springs were analysed for the water quality index of five different springs viz. Jyoli, Bisra, Kharkuna, Kujyadi, and Dilkot. Notable differences appeared in important factors like pH, EC, and TDS (BIS, 2012). The pH was found well within the desirable limit and ranges between 6.2 to 6.7 amongst the different springs. The varying EC values ranging from 53.7 to 346 µS/cm indicate low to moderate mineral content,with highest recorded in Kharkuna (346 µS/cm) and lowest in Bisra (53.7 µS/cm). Notably, theTDS is directly correlated with the conductivity and therefore TDS values were found in proportion; as TDS increases, EC also increases (Abdulwahid, 2013). Consequently, the TDS values in all five springs were within the desirable limit set by the BIS, with the highest value observed in Kharkuna (251mg/L). The alkalinity was found to be within the desirable limit for all springs except for Kharkuna, where it measured 286 mg/L; however, it remained under the permissible limit. The total hardness of all five springs was also within the desirable range. Additionally, calcium levels ranged from 13.7 to 48.8 mg/L, magnesium from 7.23 to 15.7 mg/L, chloride from 6.96 to 11.6 mg/L, potassium from 4.66 to 7.07 mg/L, sodium from 5.82 to 10.2 mg/L, nitrate from 0.29 to 3.17 mg/L, and sulphate from 0.08 to 0.34 mg/L, all falling within the desirable limits as specified in Table 1. The Ca^{2+} levels remain below the permissible threshold of 75 mg/l. The highest acceptable limit for Mg^{2+} concentration is 30 mg/l, with a permissible limit extending to 100 mg/l according to BIS/ICMR guidelines. The primary origins of Na and K in water arise from the weathering of feldspar minerals and clay composition (Thivya *et al*., 2015). In freshwater, Na generally exists in lower concentrations when compared to Ca^{2+} and Mg^{2+}. The prevalence of Na results from sodium compounds presents in various rocks and soils, readily dissolving and releasing sodium into groundwater (Ram *et al*., 2021). The Cl-concentration remains well below the permissible limit of 200 mg/l (BIS 2012).

The utilization of the Water Quality Index (WQI) is an effective method for capturing the combined influence of individual parameters on the overall water quality. Determining the overall suitability of water for human consumption based on a specific water quality variable alone is challenging. WQI, condenses a wealth of information on diverse water quality parameters into a single numerical value (Tyagi *et al*., in 2013). The Water Quality Index is further divided into five classes, each corresponding to a specific range of WQI values (Ramakrishnaiah *et al*., 2009). This classification system provides a straightforward approach to interpreting water quality assessments, facilitating clear communication of the overall status of water quality. The WQI values for selected five springs are presented in the (Table 2). All springs including Jyoli, Bisra, Kharkuna, Kujyadi, and Dilkot demonstrate “Excellent” water quality, earning them a grade A. This indicates their reliability and high suitability as water sources. The variation in the WQI over the five different locations (Jyoli, Bisra, Kharkuna, Kujyadi, and Dilkot) is shown in Fig (3).

CONCLUSION

The study provided a detailed information on physicochemical characteristics of five springs with their water quality index signifying their suitability for the human consumption. The present study will be a baseline information for the further studies on these springs. However, further investigation

Table 1. Physicochemical characteristics of five springs

Parameter	BIS standards for Drinking (IS 10500: 2012)						
	Desirable limit	Max. permissible limit	Jyoli	Bisra	Kharkuna	Kujyadi	Dilkot
pH	6.5	No relaxation	6.52±0.50	6.26±0.59	6.67±0.42	6.38±0.50	6.38±0.60
EC (µS/cm)	-	-	82.5±15.9	53.7±20.3	346 ±93.3	80.0±29.3	64.0±27.1
TDS (mg/L)	500	2000	79.4±7.74	63.2±11.8	251±33.5	69.9±4.41	58.7±7.44
Alkalinity (mg/L)	200	600	197±96.3	164 ±63.7	286 ±134.	144±54.8	155 ±77.8
Total Hardness (mg/L)	200	600	76.8±32.5	63.6±36.1	197±82.7	93.1±47.6	80.8±44.7
Calcium (mg/L)	75	200	14.7±4.95	13.7±6.99	48.8±19.8	17.3±10.6	16.4±11.2
Magnesium (mg/L)	30	100	8.48±3.00	7.23±5.27	15.7±8.87	12.6±5.56	10.4±5.1
Chloride (mg/L)	250	1000	9.52±3.48	6.96±3.02	11.6±4.95	7.69±3.46	7.29±2.26
Potassium (mg/L)	-	-	5.44±3.02	4.88±1.56	7.07±1.61	5.17±2.72	4.66±1.82
Sodium (mg/L)	-	-	8.29±1.18	5.82±1.69	10.2±2.18	6.09±1.83	7.78±1.88
Nitrate (mg/L)	45	No relaxation	1.34±0.56	0.29±0.02	3.17±1.60	1.31±0.50	0.30±0.03
Sulphate (mg/L)	250	400	0.23±0.02	0.11±0.01	0.34±0.04	0.13±0.02	0.08±0.01

Table 2. Water Quality Assessment of springs in different locations

WQI range	Type of water#	Site	WQI	Quality	Grade
<50	Excellent	Jyoli	18	Excellent	A
50 to 100	Good	Bisra	14	Excellent	A
100 to 200	Poor	Kharkuna	43	Excellent	A
200 to 300	Very Poor	Kujyadi	16	Excellent	A
>300	Not suitable for drinking purpose	Dilkot	15	Excellent	A

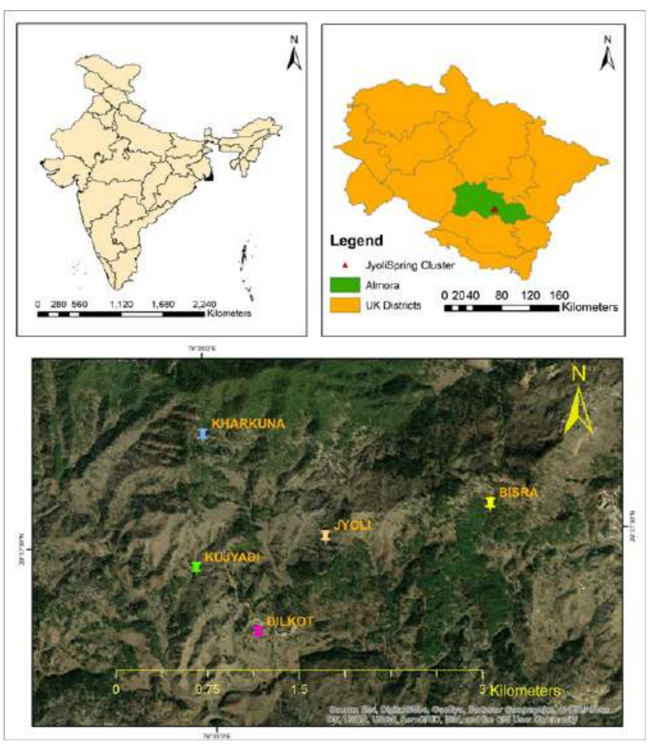

Fig. 1. Map of the Study area and sampling sites

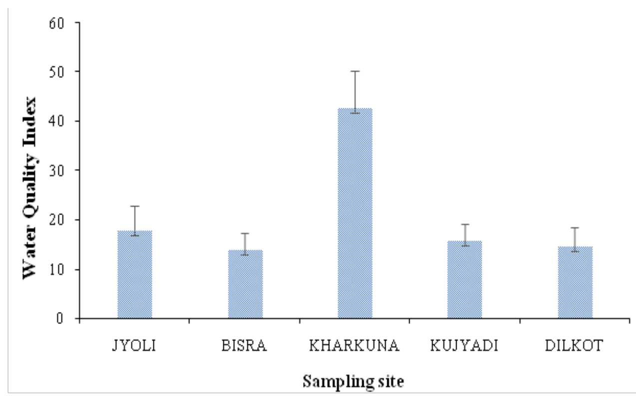

Fig. 1. Water quality index of the five springs of the study area

is required for the ecosystem health of these springs.

ACKNOWLEDGEMENTS

The authors are thankful to the Director, G.B. Pant National Institute of Himalayan Environment (NIHE), Almora, Uttarakhand, for providing the necessary facilities. Further, the authors are also thankful to the local communities for their cooperation during the sampling.

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INFLUENCE OF ESTABLISHMENT TECHNIQUES AND FOLIAR NUTRIENT SPRAY TIMING ON GROWTH, YIELD PARAMETERS, CROP PRODUCTIVITY, QUALITY, AND ECONOMIC VIABILITY OF SOYBEAN (GLYCINE MAX L.) IN THE TARAI REGION OF UTTARAKHAND

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ABSTRACT

A field study was undertaken to investigate the impact of different establishment methods (flat bed and ridge sowing), foliar nutrition treatments (NPK and seaweed extract), and application timings (vegetative + pod filling stage and flowering + pod filling stage) on the soybean crop's response. The experiment comprised nine treatments in a factorial randomised block design with a single control replicated three times with variety PS-1225. The flatbed sowing with conventional practices was treated as standard control. The result revealed that the growth characters viz., plant height, dry matter production Stem, leaf potassium status (1.84%), were higher under flatbed method of sowing. Flatbed method of sowing also recorded the highest biological (6263 kgha^{-1}) and straw yield (4304 kgha^{-1})while, chlorophyll content (42 %), yield attributing characters (i.e., grains per pod, grains per plant, grain yield (2093 kgha^{-1}) and harvest index were observed highest in plants sown under raised bed system. Among foliar spray nutrients, 2 % NPK (water soluble fertilizer) was found superior to sea weed extract in all respects. As compared to standard practice of soybean cultivation (recommended NPK), all the treated plots produced higher growth attributes, chlorophyll content, yield attributes, yield and economic returns. Thus, in northern India sowing on ridges with two foliar spray of 2 % NPK at flowering and pod filling stage can augment the performance of soybean in tarai region of Uttarakhand.

Keywords: Soybean, Sea weed extract, Yield attributes, Water soluble fertilizers, Ridged method

INTRODUCTION

India's and the world's most important oilseed crop is soybean (*Glycine max* L.). This Chinese crop was brought to India by the US in the late sixties and is now grown on 11.8 million ha with 13.5 million tonnes of yield (Director's evaluation, 2020-21). The "Golden bean" is soybean contains 18-20% oil, 40% protein, 30% carbs, 4% saponins, and 5% fibre. It's called "poor man's meat" due to its high protein content. Humans consume little soybean directly due to its beany taste and high anti-nutritional content. Sprouting grains contain thiamine, riboflavin, and vitamins A, B, D, E, and K. Introduced soybean is photoperiod and temperature sensitive. Rained soybean has 2.1 t/ha potential compared to the national production of 1.2 t/ha (Agarwal *et al.*, 2013). In North India's kharif season, soybean crop yields are low due to surplus moisture during vegetative growth and moisture deficit during reproductive growth. The rainy months (July, August) coincide with vegetative crop growth, causing excess moisture and inadequate root formation. Early monsoon departure (mid-September) induces moisture stress during reproductive stage, which impairs reproductive organ development.

Bad drainage and aeration reduce plant nutrient availability and microbial activity due to excessive rain. Faster water disposal can mitigate the effects of inadequate drainage. Ridge or bed planting can speed drainage. The deeper furrows between ridges or beds gather rainwater and convey it to the major outlets. Ridge-grown soybeans produced more than flatbed-grown ones (Patil *et al.*, 2009). Poorly or partly filled soybean pods show poor source sink relation. Soybean leaf senescence begins before pod maturity, breaking source-sink interaction and resulting in unfilled pods and wrinkled seeds. Soybean productivity requires external support for translocation. By reducing stress, foliar nutrition spray boosts crop growth fastest. Foliar application directs nutrients to food synthesis, eliminating waste and reducing fertiliser use. Foliar nutrients can control it. Later nutrient spray delays leaf senescence and increases yield (Sharifi *et al.*, 2018). At 45-50 days after sowing, soybean crop active nodulation slows, and foliar nutrition application improves growth, seed yield, and quality (Sharifi *et al.*, 2018).

Nitrogen, phosphorous, and potassium are essential nutrients for plant activities. Nitrogen helps crop plants flourish. Healthy above-ground plant tissues contain 3–4% nitrogen.

Nitrogen is essential because chlorophyll, which uses sunlight to make carbohydrates from water and carbon dioxide, contains mostly nitrogen (i.e. photosynthesis). Nitrogen starter can boost soybean yield in early growth (Osborne and Riedell, 2006). Phosphorus is needed as a plant structure and as a stimulant for several key activities such flower formation and seed production, uniform and faster crop maturity, crop quality improvements, and disease resistance. Plants use phosphorus to develop seeds. Thus, phosphorus helps seed development and filling, improving yield. Grain legumes need it for nodulation and seed protein (Abidi *et al.*, 2001; Guhey *et al.*, 2000). Plants need potassium for builds vascular bundles, which house xylem and phloem tubes, the stringy component of plant stalks that transports water and nutrients. Potassium increases nitrogenase activity, which accumulates uricides in pod walls and partitions above-ground nitrogen to seeds, increasing seed yields. Singh *et al.*, (2018) observed the foliar NPK (19:19:19) @ 2% enhanced soybean productivity.

Noda, (1990); Panda *et al.*, (2012) found that seaweed is mineral-rich (Ca, Mg, K, Cl, S, P, I, Zn, Cu, etc.) due to their high levels of organic matter, micro and macro elements, fatty acids, and growth regulators, liquid fertilizers from seaweeds can be used to fertilise agricultural crops. Fertilizing twice or thrice at full-flowering and pod-setting reduced flower abortion. Fertilizing at the first-flowering, full-flowering, and pod setting stags reduced pod abortion, but unfertilized plants had the most aborted grains (Oko *et al.*, 2003). Seaweed extract foliar sprays during reproduction increased soybean productivity (Guerreiro *et al.*, 2017). Considering the above facts, the present study was done with following objectives, to analyse the effect of establishment methods, foliar spray and spray schedule on different growth parameters and yield of soybean crop.

MATERIAL AND METHODS

Experimental Site: The field experiment was conducted at E2 Block of Norman E. Borlaug Crop Research Centre of G.B. Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar (Uttarakhand) during kharif 2021 (29.02^0N latitude and 79.30^0E longitude, 243.84m above MSL). Pantnagar has sub-humid, sub-tropical climate with hot and dry summer and severe cold winters. The average annual rainfall of Pantnagar is about 1432 mm.

Soil Characteristics: The soil of the experimental site comes under the soil order Mollisols(Deshpande *et al.*, 1971). Soil texture was silty clay loam, high in organic carbon (0.72%) with a pH of 7.3, low in available nitrogen (218.6 kg ha^{-1}) and medium in availability of phosphorus (12.4 kg ha^{-1}) and potassium (149.6 kg ha^{-1}).

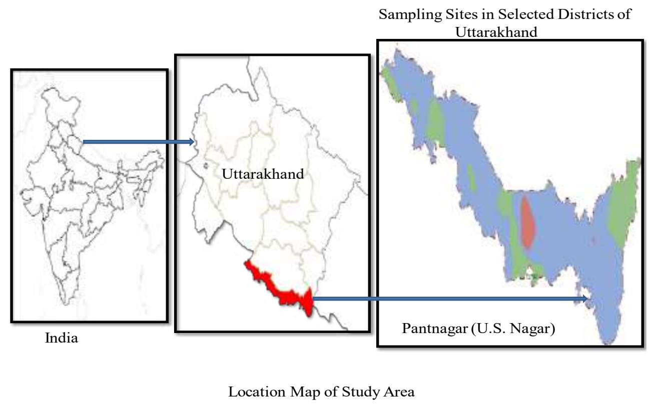

Fig. 1. Map of Experimental sites

Experiment Details: The experiment was laid out in Factorial Randomized Block Design with one standard practice having total nine treatments with three replications consisting of two establishment methods, three foliar nutrition and two spray schedules were studied (T1-Standard practice, T2-Flat bed sowing with spray of NPK @ 2% at vegetative and pod filling stage, T3-Flat bed sowing with spray of seaweed extract @ 0.167% at vegetative and pod filling stage, T4-Flat bed sowing with spray of NPK @ 2% at flowering and pod filling stage, T5-Flat bed sowing with spray of seaweed extract @ 0.167% at flowering and pod filling stage, T6-Ridge sowing with spray of NPK @ 2% at vegetative and pod filling stage, T7-Ridge sowing with spray of seaweed extract @ 0.167% at vegetative and pod filling stage, T8-Ridge sowing with spray of NPK @ 2% at flowering and pod filling stage, T9-Ridge sowing with spray of seaweed extract @ 0.167% at flowering and pod filling stage).The uniform application of 20 kg ha^{-1} nitrogen, 60 kg ha^{-1} phosphorus, 40 kg ha^{-1} potassium and 30 kg ha-1sulphur were done at the time of sowing with NPK, MOP and bentonite sulphur. Foliar application of 2% NPK (19:19:19) and 0.167 % seaweed extract is done twice in plots. According to the treatment, foliar application was done in the plots either at vegetative and pod filling stage or at flowering and pod filling stage. In control plots, conventional cultivation practice was followed and water spray is done instead of nutrient spray at vegetative, flowering and pod filling stages.

2.4Soil Moisture Status in Ridge and Flatbed at Different Dates.

Soil moisture content was calculated in soils under flatbed and ridge conditions during different dates. Six observations were taken during the entire crop period three of which were during dry conditions, where high temperature, low relative humidity and no rainfall was recorded for two-three days and rest three observations were taken one day after the rains. Soil moisture status in both the conditions (Fig. 1) was calculated using the given formula:

Soil moisture content (%)=(W2-W3)/(W3-W1)×100

where,

W1 = weight of container

W2 = weight of container with moist sample and
W3 = weight of container after drying the sample

Observations: Biometrical observations were recorded at different stages (30, 40, 55, 66 and 80 DAS and at maturity). All the precautionary measures were adopted to eliminate sampling error. The observed parameters were plant height (cm), plant dry matter production (g), number of trifoliate leaves, number of branches and chlorophyll content (%) with SPAD meter (model: CHL PLUS), leaf nitrogen, phosphorous and potassium content, number of pods per plant, number of grains per pod, number of grains per plant, weight of hundred grains, biological yield, grain yield, straw yield and harvest index.

ECONOMICS

Cost of cultivation: In order to work out the cost of cultivation, record of different inputs used and operations performed during the crop cultivation was maintained. The local charges of different operations/ labour and prevailing market prices of inputs were used to compute cost of cultivation and was reported as Rs./ha.

Gross return: Gross return was worked out using the minimum support price of soybean grain yield and local price of the stover. It was reported as Rs./ha.

Net return: It was worked out by deducting the cost of cultivation from gross return and was reported as Rs. /ha.

Benefit Cost ratio: It was calculated by dividing the net return by cost of cultivation of the respective treatments.

Statistical Analysis: The data were statistically analyzed using analysis of variance (ANOVA) as applicable to Factorial RBD (Rangaswamy, 2015). Significance of differences among different treatments was tested using the standard F test. LSD values were calculated for those parameters which exhibited significant differences(Dass, 2016).

RESULTS AND DISCUSSION

Plant height: In all the stages except at 30 DAS the plant height was affected significantly by establishment methods (Table 1). The crops sown on flat bed was significantly taller than that sown on ridge by 7.9%, 8.2%, 5.8% and 3.9% at 40, 55, 66, and 80 DAS, respectively. A good amount of rainfall was received in the first fortnight after sowing, which caused higher soil moisture content in flat sown crop as compared to ridge sown crop resulting into taller plants (Fig. 1). In contrast, ridge sown crop experienced lower soil moisture content, thus attained lower plant height than flat sown crop. At 55 DAS, a significant increase of 3.6% was recorded in plants where spray was done at vegetative stage (30 DAS) over the plots which were not treated. Foliar nutrition with NPK @ 2 % resulted in significantly higher plants over the foliar nutrition with 0.167 % seaweed extract at 80 DAS and this increase was of 3.1%. Plants in treated plots recorded more plant height over the standard practice plots and the difference was significant at 55, 66 and 80 DAS with magnitude of 3.5%, 2.9%, and 3.8%, respectively. There was no significant difference in plant height between flat sown and ridge sown crop reported byJadhav *et al*., (2008) and Nivrutti (2010). Foliar spray at reproductive stages leads to increase in plant height observed by Gan *et al*. (2003) and Sharifi *et al*., (2018).

Dry matter production: Flat sown crops wererecorded more stem dry matter and leaf dry matter by 9.2 and 7.9% as compare to ridges method (Table 1). Application of nutrients at flowering and pod filling stages increased leaf dry matter significantly by 9.6% over nutrient application at vegetative and pod filling stages. Sharifi *et al*., (2018) also reported that an increase in plant height and plant dry weight when spray on crops was done at flowering and pod filling stage. While NPK application was found superior to sea weed extract with a significant increase of 4.5% in stem dry matter. Haq and

Table 1. Effect of Establishment Methods, Foliar Nutrition, and Spray Schedule on Plant height and Plant dry matter in Soybean

Treatments	Plant height (cm)					Dry matter production(g plant^{-1})	
	30 DAS	40 DAS	55 DAS	66 DAS	80 DAS	Stem dry matter	Leaf dry matter
Establishment methods							
Flat	23.6	45.7	74.9	96.6	99.2	36.55	30.63
Ridge	23.4	42.0	68.7	91.0	95.3	33.17	28.22
SEm ±	0.23	0.48	0.53	0.94	0.52	0.60	0.84
CD 5%	NS	1.2	1.3	2.3	1.3	1.48	2.07
Spray schedule							
Vegetative + pod filling	23.6	44.2	73.1	92.5	95.8	34.56	27.94
Flowering + pod filling	23.3	43.5	70.5	95.1	98.7	35.16	30.92
SEm ±	0.23	0.48	0.53	0.94	0.52	0.60	0.84
CD 5%	NS	NS	1.3	2.3	1.3	NS	2.07
Foliar nutrition							
NPK	23.5	43.9	71.8	95.0	98.8	35.66	29.69
Seaweed extract	23.4	43.8	71.7	92.7	95.7	34.06	29.17
SEm ±	0.23	0.48	0.53	0.94	0.52	0.60	0.84
CD 5%	NS	NS	NS	NS	1.3	1.48	NS
Standard practice v/s Treatments							
Standard practice	22.6	43.0	69.3	91.1	93.6	31.33	25.17

Treat-ments	23.3	43.9	71.8	93.8	97.2	34.86	29.43
SEm ±	0.34	0.72	0.79	1.00	0.78	0.90	1.26
CD 5%	NS	NS	1.9	2.5	1.9	2.22	3.11

Mallarino (2000) also reported that NPK spray leads to increase in dry matter.

Number of trifoliate leaves plant^{-1}

Flat sown crop recorded higher number of trifoliate leaves over ridge sown crop with a significant increased by 17.9%, 10.1%, 13.1% and 11.4% at 40, 55, 66 and 80 DAS, respectively (Table 2). Nutrient spray at vegetative stage supported the plant growth initially but nutrient requirement at flowering stage exceeds that at earlier stage and thus an increment of leaves was seen when spray was done at later stages. Spray with NPK resulted in significant increase on number of trifoliate leaves per plant over spray of seaweed extract at 66 and 80 DAS. Odeleye *et al.*, 2007 recorded higher crop growth in soybean crop with spray at early pod filling stage.

Number of primary branches plant^{-1}

Number of primary branches was affected by establishment method where flat sown crops had a greater number of branches per plant as compared to ridge sown crops (Table 2). The number of branches increased by 16.6% at 55 DAS and 11.9% at 80 DAS in flat sown crop as compared to ridge sown crop. Spray schedule also affected number of primary branches significantly at 80 DAS where an increase of 1.13 branches plant-1was recorded in plots sprayed at flowering and pod filling stage. Treated plots were superior over standard practice for number of primary branches at 66 DAS. At this stage number of primary branches increased by 1.08 which equates to an increase of 14.9%.Similar results were observed by Mannan, 2014 and Sharifi *et al.*, 2018.

Chlorophyll content in leaves

The SPAD readings were affected by establishment method where plants sown on ridge had more chlorophyll content than plants sown in flat bed. SPAD readings were also affected significantly by spray schedule at 40 and 80 DAS. When foliar sprays of nutrients were applied during the vegetative and pod-filling stages, the chlorophyll content was 2.8% higher at 40 DAS, and it was 2.9% higher at 80 DAS when the sprays were used at the flowering and pod-filling stages.Foliar nutrition with NPK showed its superiority over seaweed extract. Similar results were reported by (Sharifi *et al.*, 2018).

Leaf nitrogen, phosphorus and potassium status

Leaf phosphorus and potassium content was significantly af-

Table 2. Effect of Establishment Methods, Foliar Nutrition, and Spray Schedule on Number of trifoliate leaves, Primary branches and leaf chlorophyll content in Soybean

Treatments	Number of trifoliate leaves plant^{-1}					Number of primary branches plant^{-1}			Chlorophyll content (%)		
	30 DAS	40 DAS	55 DAS	66 DAS	80 DAS	55 DAS	66 DAS	80 DAS	40 DAS	66 DAS	80 DAS
Establishment methods											
Flat	8.2	18.7	36.0	55.9	62.4	5.2	7.3	8.8	41.44	39.90	41.58
Ridge	8.0	15.4	32.3	48.6	55.3	4.3	7.1	7.7	41.45	41.99	41.99
SEm ±	0.43	0.41	1.31	1.36	0.77	0.30	0.28	0.23	0.29	0.42	0.50
CD 5%	NS	1.0	2.2	3.8	2.7	0.8	NS	0.6	NS	1.04	NS
Spray schedule											
Vegetative + pod filling	8.1	18.2	36.8	48.8	55.4	5.0	7.2	7.7	42.04	40.71	41.16
Flowering + pod filling	8.1	16.0	31.6	55.8	62.2	4.6	7.3	8.8	40.85	41.17	42.40
SEm ±	0.43	0.41	1.31	1.36	0.77	0.30	0.28	0.23	0.29	0.42	0.50
CD 5%	NS	1.00	2.2	3.8	2.7	NS	NS	0.6	0.73	NS	1.23
Foliar nutrition											
NPK	8.4	17.1	34.2	54.5	60.9	5.0	7.4	8.4	41.77	41.02	42.57
Seaweed extract	7.7	17.1	34.1	50.1	56.8	4.5	7.1	8.0	41.12	40.87	41.00
SEm ±	0.43	0.41	1.31	1.36	0.77	0.30	0.28	0.23	0.29	0.42	0.50
CD 5%	NS	NS	NS	3.77	2.7	NS	NS	NS	NS	NS	1.23
Standard practice v/s Treatments											
Standard practice	7.7	12.6	31.7	46.0	49.7	4.1	6.1	7.5	41.30	37.48	40.44
Treatments	8.1	17.1	34.2	52.3	58.8	4.8	7.2	8.2	41.45	40.94	41.78
SEm ±	0.64	0.61	1.96	1.44	1.15	0.46	0.30	0.34	0.44	0.45	0.75
CD 5%	NS	1.5	3.3	4.0	4.1	NS	0.7	NS	NS	1.10	NS

Seaweed extract	7.7	17.1	34.1	50.1	56.8	4.5	7.1	8.0	41.12	40.87	41.00
SEm ±	0.43	0.41	1.31	1.36	0.77	0.30	0.28	0.23	0.29	0.42	0.50
CD 5%	NS	NS	NS	3.77	2.7	NS	NS	NS	NS	NS	1.23
Standard practice v/s Treatments											
Standard practice	7.7	12.6	31.7	46.0	49.7	4.1	6.1	7.5	41.30	37.48	40.44
Treatments	8.1	17.1	34.2	52.3	58.8	4.8	7.2	8.2	41.45	40.94	41.78
SEm ±	0.64	0.61	1.96	1.44	1.15	0.46	0.30	0.34	0.44	0.45	0.75
CD 5%	NS	1.5	3.3	4.0	4.1	NS	0.7	NS	NS	1.10	NS

Table 3. Effect of establishment methods, foliar nutrition, and spray schedule on leaf nitrogen, phosphorus and potassium status

Treatments	**Nitrogen content (%) in leaves**				**Phosphorus content (%) in leaves**				**Chlorophyll content (%)**			
	40 DAS	**55 DAS**	**66 DAS**	**80 DAS**	**40 DAS**	**55 DAS**	**66 DAS**	**80 DAS**	**40 DAS**	**55 DAS**	**66 DAS**	**80 DAS**
Establishment methods												
Flat	2.85	3.01	3.13	3.11	0.65	0.70	0.97	1.33	1.14	1.26	1.59	1.61
Ridge	2.87	3.03	3.20	3.32	0.77	0.71	1.01	1.40	1.18	1.57	1.78	1.84
SEm ±	0.03	0.03	0.05	0.02	0.03	0.02	0.05	0.03	0.04	0.14	0.04	0.02
CD 5%	NS	NS	NS	NS	0.07	NS	NS	NS	NS	NS	0.11	0.06
Spray schedule												
Vegetative + pod filling	2.93	3.04	3.11	3.12	0.74	0.72	0.97	1.36	1.23	1.57	1.63	1.62
Flowering + pod filling	2.79	2.99	3.22	3.31	0.69	0.69	1.01	1.36	1.09	1.25	1.75	1.83
SEm ±	0.03	0.03	0.05	0.02	0.03	0.02	0.05	0.03	0.04	0.14	0.04	0.02
CD 5%	0.08	NS	NS	0.06	NS	NS	NS	NS	0.10	NS	0.11	0.06
Foliar nutrition												
NPK	2.91	3.06	3.19	3.25	0.71	0.72	1.02	1.41	1.19	1.48	1.76	1.79
Seaweed extract	2.80	2.97	3.14	3.18	0.71	0.69	0.96	1.31	1.13	1.34	1.62	1.67
SEm ±	0.03	0.03	0.05	0.02	0.03	0.02	0.05	0.03	0.04	0.14	0.04	0.02
CD 5%	0.08	0.08	NS	0.06	NS	NS	NS	0.07	NS	NS	0.11	0.06
Standard practice v/s Treatments												
Standard practice	2.83	2.86	2.97	3.01	0.55	0.63	0.78	1.06	0.92	0.94	1.48	1.56
Treatments	2.86	3.02	3.16	3.21	0.71	0.70	0.99	1.36	1.16	1.41	1.69	1.73
SEm ±	0.05	0.05	0.06	0.03	0.04	0.03	0.05	0.04	0.06	0.22	0.05	0.04
CD 5%	NS	0.12	0.14	0.09	0.11	0.07	0.12	0.10	0.15	NS	0.11	0.09

fected by establishment method. With an increase of 15.6 % in leaf phosphorus content at 40 DAS and 10.5 and 13.0% increase in leaf potassium content at 66 and 80 DAS in ridge sown crops over plants sown in flat bed (Table 3). Spraying of nutrients at different schedules recorded a significant increase in leaf nitrogen and potassium content. When foliar sprays of nutrients were applied during the vegetative and pod-filling stages, the nitrogen content was 4.8% while potassium content was 11.5% higher at 40 DAS, and nitrogen content was 5.6% higher at 80 DAS while potassium content was 7.0 and 12.5% higher at 66 and 80 DAS when the sprays were used at the flowering and pod-filling stages. Haq and Mallarino, 2000 also reported an increase in tissue NPK content with foliar fertilization at R2 growth stage. Similarly, NPK spray recorded a significantly higher leaf nitrogen, phosphorus and potassium status.

Number of pods per plant

Pods per plant were affected significantly by establishment method and spray schedule (Table 4). Ridge sowing (122 pods per plant) produced significantly higher pods per plant than flat sowing (113 pods per plant). Higher moisture availability in flat bed condition led to excessive vegetative growth. It led to crowding in the phyllosphere as more foliage growth took place and thus photosynthesis capacity in the flat bed condition was less as compared to ridge sown crops where proper aeration was present as number of leaves per plants was comparatively less. This led to better photosynthesis in ridge sown crops which could be the reason of higher number of pods per plant in ridge sown crops. Jadhav *et al.*, 2008 obtained similar results. Spraying at flowering and pod filling stage produced significantly higher number of pods per plant as compared to spray at vegetative and pod initiation stage. Nutrient demand is high at flowering and pod development stage due to formation of sink and translocation of photosynthates from source to sink. This demand is met by spray of nutrients at these stages and hence the number of pods per plants is significantly higher as compared to spray at vegetative and pod filling stages.

Number grains per pods

Ridge sowing produced 14.6% higher number of grains per pod than flat sowing.(Table 4). Comparatively less foliage development in ridge sown crops than flat sown crops led to higher photosynthetic efficiency which in turn resulted in higher number of grains per pods in ridge sown crops. Spraying at flowering and pod filling stage produced 7.8% higher number of grains per pod as compared to spray at vegetative and pod filling stage. As spray at flowering and pod filling stage facilitates development of sink and translocation of photosynthates by meeting nutritional demand of the crop during these stages therefore leads to an increase in number of grains per pods. Basediya *et al.*, 2018 also reported that yield attributing characters were found better in ridge sown crop than flat sown soybean crops. NPK spray was found superior, with a significant difference of 0.17 grains per pod (7.5 %) over seaweed extract. NPK spray was able to meet the nutritional demand of the crop at different stages of growth better than seaweed extract and therefore resulted in higher number of grains per pod.

Number of grains per plant

Number of grains per plant was affected significantly by establishment methods, spray schedule and material of spray (Table 4). Ridge sown crops have a significantly higher number of grains per plant by a margin of 7.2 % over flat sown crops. Similar results were obtained by Verma *et al.*, 2020 and Dhakad and Verma, 2014. Spray at flowering and pod filling stage produced significantly higher number of grains per plant as compared to spray at vegetative and pod filling stage. As the nutritional demand of the crop was met by spray at flowering and pod filling stage, more photosynthetic product was produced and translocated to sink. Hence, number of grains per plants was more. NPK spray also recorded a significant difference over seaweed extract. These results were comparable to those recorded by Mandić *et al.*, 2015.

Weight of hundred grains

Weight of hundred grains was affected significantly by spray schedule and material of spray (Table 4). Spray at flowering and pod filling stage produced significantly higher weight of hundred grains as compared to spray at vegetative and pod filling stage. Mannan, 2014 and Sharifi *et al.*, 2018 also recorded an increase in soybean seed index with foliar spray at flowering and pod filling stages. NPK spray recorded a significant difference of 4.0 % in hundred grain weight over seaweed extract spray. Singh et al., 2018also reported an increase in yield attributing characters by spray of 2 % NPK.

Yields

The yields (biological, straw and grain) were affected significantly by establishment method (Table 4). Plants sown under flatbed conditions produced significantly higher biological and straw yield (6263 kg ha^{-1} and 4304 kg ha^{-1}) than crops on ridges (5670 kg ha^{-1}and 3577 kg ha^{-1}), while, theridges method produced significantly higher grain yield (2093 kg ha^{-1}) than plants sown under flatbed conditions (1958 kg ha^{-1}). For biological yield spray schedules, spray done at flowering and pod filling stage recorded numerically higher biological yield (6052 kg/ha) over spray done at vegetative and pod filling stage (5918 kg/ha). For straw yield spray schedules, spraying done at flowering and pod filling was found superior over the sprays done at vegetative and pod filling stages. For biological yield different foliar nutrition used, NPK showed its superiority over seaweed extract by a margin of 1.6 % however the difference was not enough to be significant. Similar results were reported by Singh *et al.*, (2012) and Gupta *et al.*, (2018), (Verma *et al.*, 2020). Foliar nutrition of NPK recorded significantly higher grain yield over foliar spray of seaweed extract. The magnitude of difference between the two was 4.1% (85 kg ha^{-1}). Das and Jana (2015) also recorded an increase in grain yield with NPK spray.

Harvest index

Harvest index of crops sown on ridges (37.0 %) was more than crops sown under flatbed conditions (31.3 %) (Table 4). The crop sown under flatbed conditions grew vigorously due to heavy rainfall during some stages of crop growth and a high number of rainy days (30). After flowering, partial or complete lodging of the crop was observed due to excessive growth. As a result, the lower leaves turned yellow and died prematurely. All of this contributed to fewer filled pods and smaller pod sizes, most likely due to less photosynthates formation and poor translocation. This is evident by the lower harvest index in crops sown in flat bed conditions. Spraying during flowering and pod filling stages resulted in a 3.5% higher harvest index than spraying during vegetative and pod filling stages. Singh et al., 2018 also recorded higher biological, stem and grain yield with spray at flowering and pod filling stage.

ECONOMICS

Cost of cultivation

Cost of cultivation was numerically higher under ridge method of sowing (Rs. 30047 ha^{-1}) as compared to flatbed method (Rs. 29347ha^{-1}) (Table 5). Cost of cultivation of foliar nutrition with NPK (Rs. 29802ha^{-1}) was numerically higher

Table 4. Effect of establishment methods, spray schedules and foliar nutrition on yield attributing characters, yield and Harvest Index

Treatments	Pods/plant	Grains/pod	Grains/plant	100 seed weight(g)	Biological yield (kg/ha)	Grain yield (kg/ha)	Straw yield (kg/ha)	Harvest index
	40 DAS	55 DAS	66 DAS	80 DAS	40 DAS	55 DAS	66 DAS	80 DAS
Establishment methods								
Flat	113	1.97	149	9.71	6263	1958	4304	31.29
Ridge	122	2.31	161	9.82	5670	2093	3577	36.98
SEm ±	4	0.05	2	0.05	86.09	32.35	81.41	0.65
CD 5%	9	0.11	4	NS	213	80	201	1.60
Spray schedule								
Vegetative + pod filling	106	2.05	150	9.51	5881	1962	3919	33.53
Flowering + pod filling	129	2.22	160	10.02	6052	2089	3962	34.74
SEm ±	4	0.05	2	0.05	86.09	32.35	81.41	0.65
CD 5%	9	0.11	4	0.13	NS	80	NS	NS
Foliar nutrition								
NPK	119	2.22	161	9.96	6015	2068	3947	34.65
Seaweed extract	117	2.05	150	9.57	5918	1983	3935	33.62
SEm ±	4	0.05	2	0.05	86.09	32.35	81	0.65
CD 5%	9	0.11	4	0.13	NS	80	NS	NS
Standard practice v/s Treatments								
Standard practice	99	1.70	148	8.50	5529	1761	3768	31.97
Treatments	118	2.14	155	9.77	5966	2026	3941	34.14
SEm ±	5	0.07	3	0.08	129.14	48.52	122.11	0.97
CD 5%	13	0.17	7	0.19	319	120	NS	2.41

as compared to seaweed extract (Rs. 29591ha^{-1}).

Gross return

Ridge method of sowing provided significantly higher gross return (Rs. 90711ha^{-1}) than flatbed method of sowing (Rs.85058ha^{-1}) (Table 5). Two sprays at flowering and pod filling stages resulted in significantly higher gross return than the two sprays at vegetative and pod filling stages resulting in a difference of Rs. 5491ha^{-1} which was equivalent to an increase of 6.1 %. Similar results were reported by Mannan, 2014. Spray with NPK recorded significantly higher gross return of Rs.3665ha^{-1}over spray of seaweed extract which was equivalent to increase of 4.2 %. Singh et al., 2018 also recorded a significantly higher gross return with NPK spray.

Net return

Ridge method of sowing resulted in significantly higher net return (Rs. 60664ha^{-1}) than flatbed method of sowing (Rs.55712ha^{-1}) (Table 5). The difference between the net return of two method of sowing was Rs.4952ha^{-1}which was equivalent to an increase of 8.2 % over flatbed method. Two sprays at flowering and pod filling stage (Rs. 60934ha^{-1}) resulted in significantly higher net return than two sprays at vegetative and pod filling stage (Rs. 55443ha^{-1}) resulting in a difference of Rs.5491ha^{-1} which was equivalent to an increase of 9.0 %. NPK spray on crops resulted in significantly higher net return over crops on which seaweed spray was done. The difference in net return was of Rs.3454/ha which was equivalent to an increase of 5.8 %.

Benefit-cost ratio

BCR was affected significantly by spray schedule (Table 5). Whereas, with respect to foliar nutrition, NPK showed its over Seaweed extract but the difference between BCR of material of spray was not significant. Spraying at flowering and pod filling stage (2.05) resulted in significantly higher BCR than spraying at vegetative and pod filling stage (1.87). Spraying at former stages resulted in an increase of 9.0 % (0.18) than at later stages. Crops sown on ridges (2.02) have higher BCR than flat sown crop (1.90) but the difference between BCR of the two methods of sowing is not enough to be significant. Although there was non-significant difference in BCR as straw yield in crops sown under flatbed recorded higher straw yield. However, sowing on ridges should be preferred to ensure good germination as it is a rainy season crop where rainfall is unpredictable.

Although vegetative growth was better under flatbed conditions because water is required for cell division and maintaining cell turgidity and nutrients in soil system reach plant root surface using water as a medium. Due to lower soil moisture in ridge sown crop (Fig. 2), water uptake by plants was less as compared to flat sown crops resulting in lower physiological development of the plant due to lower nutrient uptake and less turgid plants and less cell division hence resulting in lower plant height, dry matter, number of trifoliate leaves and primary branches. In the present study increase of vegetative growth in flat sown crop may be due to better soil moisture which can be attributed to variable rainfall received during the early phase of the crop. But excessive vegetative growth in flat-sown crops resulted in crop lodging, which caused the plant part in contact with the ground to decay and rely on the upright portion of the plant for nutrient demand resulting in less leaf chlorophyll content. Excessive foliage growth in flat bed crops hampered light penetration deep within the crop canopy, resulting in poor photosynthesis. Excessive moisture supported vegetative growth, resulting in less photosynthetic translocation into the plant's reproductive parts. As a result, grain yields in flat-sown crops were lower Foliar application of 2% NPK (WSF) applied at flowering and pod filling stages significantly improved the growth and yield of soybean crop. Nutrient spray done at vegetative stage was able to meet the initial requirement of the crop but was not able to support crop at later stage and therefore its supply at flowering and pod filling stage resulted in more number of trifoliate leaves at latter growth stages. Nitrogen, phosphorus and potassium are the major nutrient required by the plant in large quantities therefore their uptake resulted in taller plants than seaweed extract. Even though seaweed extract has more

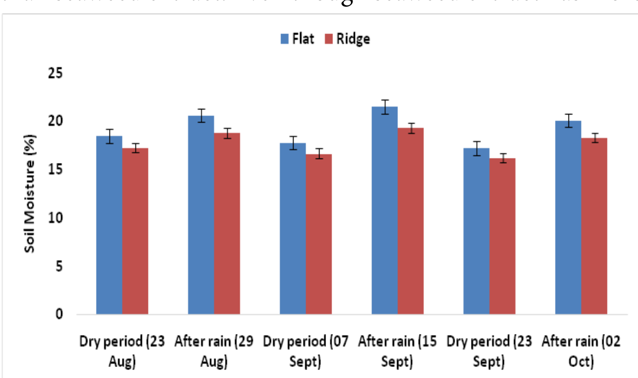

Fig. 2. Soil moisture status (%) in ridge and flat bed at different dates

Table 5. Effect of establishment methods, spray schedules and foliar nutrition on economics of experiment

Treatments	Cost of cultivation (Rs. ha^{-1})	Gross return (Rs. ha^{-1})	Net return (Rs. ha^{-1})	B:C Ratio
Establishment methods				
Flat	29347	85058	55712	1.90
Ridge	30047	90711	60664	2.02
SEm ±	-	1390	1390	0.05
CD 5%	-	3433	3433	NS
Spray schedule				
Vegetative + pod filling	29697	85139	55443	1.87
Flowering + pod filling	29697	90630	60934	2.05
SEm ±	-	1390	1390	0.05
CD 5%	-	3433	3433	0.12
Foliar nutrition				
NPK	29802	89717	59915	2.01
Seaweed extract	29591	86052	56461	1.91
SEm ±	-	1390	1390	0.05
CD 5%	-	3433	3433	NS
Standard practice v/s Treatments				
Standard practice	27252	76462	49210	1.81
Treatments	29697	87885	58188	1.96
SEm ±	-	2085	2085	0.07
CD 5%	-	5149	5149	NS

balanced nutrients concentration, but it could not meet the nutrient demand of crops and thus could not match with the plant height under NPK sprayed crop.

Based on the findings of present study, it may be concluded that ridge sowing is viable establishment method for soybean during rainy season. Foliar application of 2 % NPK (WSF) applied at flowering and pod filling stages significantly improved the growth and yield of soybean crop.

ACKNOWLEDGEMENT

The authors are thankful to the department for providing the required research facilities. I gratefully express my deep sense gratifies to my respected senior, at G.B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India for his keen interest, valuable guidance, and constructive criticism throughout the pursuit of the present research paper and vital suggestion during preparation of this manuscript.

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