Current Science Reports

Upper Alaknanda Basin
Glacier inventory and changes

Rapid mass loss in Himalayan glaciers directly impacts the availability of water in major rivers downstream. Studies show high variability in Himalayan glaciers. While some show mass growth, others show loss. The changes in the glaciers in the upper Alaknanda Basin have not been investigated after 2006. What is the current status of glaciers in the Upper Alaknanda Basin?

To find out, Aditya Mishra from the H.N.B. Garhwal University, Uttarakhand collaborated with researchers from Zurich and the UK. They used satellite data for the snow-free period, September and October, from 1994 to 2020. Approximately 200 glaciers were mapped in 2020. But detecting changes was possible for only 138 due to cloud cover in the 2006 satellite image.

The researchers manually digitised glacier boundaries in the images and, with the help of the glacier outline and digital elevation model, generated data on glacier area, perimeter, minimum, maximum and mean elevation. Glaciers with an area of more than 10 square kilometres were distributed mostly at an elevation range of 4700–5800 metres above mean sea level. Half the glacier area was at an elevation of above 5200 metres.

The total glaciated area in the Upper Alaknanda was around 356 square kilometres. About one-fourth of the glacier area was covered with debris. Debris cover was consistent throughout the elevation range, between 3800 and 5850 metres. During the last 25 years, smaller glaciers lost about 8% of their mass. Larger glaciers lost an area of about 2%. Compared to the data between 1994 and 2006, the length of the large glaciers has increased significantly between 2006 and 2020.

Glacier changes are influenced by climatic factors such as temperature and precipitation as well as topographic factors such as glacier area, length, slope and snout characteristics. Using climate data, the researchers analysed temperature and precipitation. There was an increase of 0.5 degrees Celsius in the mean annual temperature. Rainfall showed a decreasing trend till 2000 and then an increasing trend. Summer rainfall showed an increasing trend while winter precipitation reduced.

'Besides the imbalance between snow mass loss and accumulation, glacier size, the extent of debris-cover and elevation range impact the rate of glacier area loss in the Upper Alaknanda Basin,' says Aditya.

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Seagrass Ecosystem
Natural carbon sink

The ocean acts as a sink for atmospheric carbon dioxide. Besides the carbon dioxide dissolved in water and organic carbon in sediments, oceans contain large quantities of organic carbon. The seagrass ecosystem, for example, is estimated to hold 20 trillion kilograms of organic carbon as sediments and biomass.

Researchers from IIT-Bhubaneswar collaborated with the city-based Siksha 'O' Anusandhan University, the Bombay Natural History Society, and the Srusti Conservation Foundation, Pune to assess the carbon storage capacity of seagrass ecosystems of the Andaman and Nicobar Islands.

India has 16 species of seagrass. For the study, the team selected Pacific turtlegrass, *Thalassia hemprichii,* a dominant and ecologically important seagrass in the intertidal regions of Havelock, Neil and Burmanallah islands of Andaman and Nicobar.

Image: Amrit Kumar Mishra

They assessed the organic carbon stocks in sediment and the biomass of *T. hemprichii* in two different environmental settings: seagrass meadows adjacent to mangroves, and seagrass away from the mangroves.

The total organic carbon stock in the 144-hectare seagrass ecosystem associated with the mangroves was around 1000 megagrams, twice that at sites far away from mangroves.

'Nutrients and organic matter from mangroves help seagrass accumulate organic carbon. This may be the reason for increase in the seagrass canopy and shoot density,' says Amrit Kumar Mishra, IIT-Bhubaneswar.

The researchers calculated the carbon dioxide equivalent storage of *T. hemprichii* meadows by multiplying the total organic carbon stock with a commonly accepted carbon dioxide conversion factor. The carbon dioxide equivalent was estimated to be around 5500 tonnes!

The researchers estimated the social cost of carbon using a country-level approach – 86 US dollars per tonne of carbon dioxide. The carbon stored at the study sites was worth around 40 million Indian rupees.

India has seagrass of various species spread over an area of around 510 square kilometres. The researchers recommend conserving seagrass, especially in mangrove ecosystems.

Implementing the island protection zones programmes of the National Action Plan on Climate Change of the Government of India will benefit the country's carbon reduction plan through the intended Nationally Determined Contribution under the Paris Climate Agreement 2015, say the researchers.

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Vitamin K2
For healthy gut microbes

The gut has many helpful and harmful bacteria. Gut microbiota play a crucial role in maintaining health. Many diseases and even cognitive decline are associated with an imbalance in gut microbiota – dysbiosis. Vitamin K2 is reported to modulate gut microbiota. Could cognitive decline induced by gut dysbiosis be reduced by the vitamin?

To find out, Sugato Banerjee, National Institute of Pharmaceutical Education and Research, Kolkata collaborated with a team of researchers from the

Birla Institute of Technology, Ranchi. To one group of mice, they administered ampicillin, a broad spectrum antibiotic, known to reduce the population of Gram-negative and Gram-positive bacteria, causing dysbiosis. To another group of mice, they gave vitamin K2 along with ampicillin.

The researchers collected the faeces of the mouse groups to determine the abundance of beneficial gut bacteria. There was a decline in the abundance of these bacteria in mice that were given ampicillin. Mice given vitamin K2 had higher levels of beneficial bacteria.

Did reducing gut microbiota using ampicillin affect cognition? To find out, the team performed behavioural and cognitive studies on the mice. Step down latency in the passive avoidance test, transfer latency in the elevated platform test and tests for a preference for novel objects showed reduced cognition and recognition memory in antibiotic-treated mice. But, in mice treated with vitamin K2, such antibiotic-induced cognitive decline was lower.

In the Morris water maze test, mice given only the antibiotic tended to take more time to find the hidden platform than those that received vitamin K2.

How does vitamin K2 make such differences?

The researchers measured the brain levels of the reduced form of glutathione, a scavenger of toxic oxygen radicals, and the activity of superoxide dismutase, an enzyme involved in defence against oxidative stress. In the brain of antibiotic-administered mice, the researchers found reduced glutathione levels and low superoxide dismutase activity.

When treated with vitamin K2, the glutathione and superoxide dismutase levels returned to normal. So, vitamin K2 seemed to reduce cellular oxidative stress levels in the brain.

The team also found that administering the antibiotic amplified brain malondialdehyde, a marker for neuroinflammation. Co-administration of vitamin K2 reduced neuroinflammation.

Are there any morphological changes associated with antibiotic treatment?

The researchers examined the tissues of the intestine under a microscope. Antibiotic treatment caused damage to the intestinal villi and the crypts present in them, causing barrier damage and increasing intestinal permeability. Administration of K2 normalised the altered intestinal changes.

An examination of the hippocampus, the region of the brain involved in memory and cognition, showed that mice with intestinal dysbiosis had lower neuronal densities in the hippocampus. Vitamin K2 co-administration preserved hippocampal neurons.

The results suggest that vitamin K2 could be used as a nutritional supplement to counter gut dysbiosis-associated cognitive decline.

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Indian Spectacled Cobra

Venom for lung cancer

The Indian spectacled cobra, Naja naja, is a poisonous snake. The snake's venom, however, was found to increase survival in a mouse model of Ehrlich ascites carcinoma. The venom contains many components. The components were separated using ion exchange chromatography and one of the components, NN-32, a peptide in the thirty-second band, was found to be effective against breast cancer cell lines.

Recently, Sangeeta V. Pandit, Savitribai Phule Pune University took a step further and tested the effectiveness of NN-32 against lung cancer. Her Ph.D. scholar, Pratibha Kurkute, and Amol Jadhav from the Institute of Applied Biological Research and Development, Pune selected a lung cancer cell line and a mouse fibroblast cell line for their experiments.

Treatment with NN-32, they found, reduced the viability of lung cancer cells. Normal mouse fibroblast cells were not similarly affected.

Using scanning electron microscopy, the researchers could see apoptotic bodies that led to cell death forming in the lung cancer cells. The cells had shrunk and there were clear zones in between. The cells were getting detached upon NN-32 treatment. Fibroblast cells treated with NN-32, on the other hand, showed round cells with normal morphology.

Using flow cytometry, the researchers confirmed that the snake venom peptide, NN-32, causes early death of lung cancer cells. The fibroblast cells were less susceptible, requiring more than 12 times the concentration of NN-32 to have the same effect.

But how does the NN-32 peptide cause cell death?

The researchers measured the reactive oxygen species levels and mitochondrial membrane potential of the cancer cells. High levels of reactive oxygen species and lower membrane potential were observed in lung cancer cells treated with NN-32. The extreme stress experienced by the cancer cells is responsible for cell death, suggest the researchers.

'NN-32 in the venom of the Indian cobra species shows potential for use in cancer therapy. But further *in vivo* studies followed by clinical trials are needed to use the peptide for treating cancers,' says Sangeeta V. Pandit.

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Treating Brain Tumour

Ellagic acid and naringin

Glioblastoma is a common type of fast growing cancer of the brain and spinal cord. The tumours cannot be targeted by conventional drugs used for treating brain tumours as the drugs do not easily pass through the blood-brain barrier. However, plant metabolites, such as flavonoids, alkaloids and fatty acids, have been found to penetrate the blood brain barrier.

So N. V. Anoop and T. R. Arunraj, Nitte (Deemed to be University), Mangalore decided to explore plant metabolites that could be used to treat glioblastoma.

Most drugs presently used for treating glioblastoma target the DNA of cancer cells. Poly ADP-ribose polymerase or PARP-1 is an enzyme involved in DNA repair. Cancer cells over-express this enzyme to survive signals that induce cell death. The cancer cells turn drug resistant and the therapy becomes inefficient after a few doses. Inhibitors of this enzyme prevent DNA repair, leading to the accumulation of DNA damage and cell death. So the team decided to search for and identify phytocompounds that can inhibit the enzyme and help in preventing drug resistance.

They downloaded data on natural compounds with reported anticancer activities from the Naturally Occurring Plant-based Anticancerous Compound-Activity-Target DataBase. Using

Chem-Sketch, free software for drawing chemical structures, they created a two-dimensional structure of each natural molecule.

With the help of molecular docking studies, they analysed the interaction of about forty phytocompounds and the enzyme. Ellagic acid, a polyphenolic compound found in various fruits, nuts and vegetables, and naringin, a flavonoid compound found in citrus fruits, had stable interactions with the enzyme.

Now, *in-vivo* and *in-vitro* studies using these compounds need to be taken up to investigate their potential as an adjuvant in treating glioblastoma.

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Computer-aided Cancer Detection

Selecting the best model

Skin cancers are the most common worldwide. Detecting skin cancers by examining biopsy samples under microscopes takes time and is open to bias and error. To automate cancer detection, deep learning techniques for image classification can be used. In the last few years, many different convolutional neural networks have been developed to extract features and to classify images.

AlexNet, one of the first models, has five convolutional layers and three fully connected layers.

VGGNet consists of 19 layers – a series of convolutional layers, followed by pooling layers where the results from the previous convolutional layer are pooled to reduced complexity, followed by fully connected layers.

Inception, another model, has numerous parallel convolutional layers with various filter sizes useful to capture features at multiple scales and resolutions.

The three models that were developed later had more drastic changes. Residual Network or ResNet has a series of convolutional layers with batch normalisation and activation functions arranged as two components: identity shortcut connection and residual blocks.

In DenseNet, each layer receives direct input from all previous layers, a concatenation of all previous feature maps as input, allowing feature maps to be reused and combined.

Graph Neural Networks is useful to model and analyse graph-structured data.

The results from applying these models vary depending upon available data, the complexity of the images and the network architecture.

Which deep learning method is the best to detect skin cancers from biopsy images?

Dhatri Raval and Jaimin Undavia from the Charotar University, Gujarat recently compared the performance of the six convolutional neural networks models to recognise and classify skin malignancies. They downloaded skin cancer images from available datasets – an international skin imaging collaboration dataset with over 25,000 images, the HAM10000 dataset with more than 10,000 images of skin lesions and the PAD-UFES-20 dataset with more than 2,000 images.

To improve the quality of the datasets for use in different models, the researchers had to resize and normalise the images. To increase the number of images, they used rotation and other transformations of the images.

After they trained the models, the researchers used parameters such as training accuracy, training loss, validation accuracy and validation loss for comparing the results from the six different models.

The DenseNet model scored better than the other convolutional neural network models.

Encouraged by their experience, the researchers tried to compare the models to classify oral cancers. However, they could get only one dataset with about 25,000 clinical and histopathologic oral cancer images.

'Deep learning algorithms, including convolutional neural networks, need large datasets for training,' says Jaimin.

'But it is tempting to test the trained DenseNet model in clinical settings to detect and diagnose skin cancer,' adds Dhatri, his colleague.

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3D Tumour Model

Reducing animal experiments

The conventional method for studying cancer is to grow tumour cells in 2D *in vitro* cultures. But these cultures have only side-to-side cell contact and lack the network with cells in all three dimensions as seen in tumours. So, researchers had to resort to animal models to mimic actual three-dimensional tumour microenvironments. However, animal models are difficult to handle. Moreover, animals do not have the same immune composition as humans. These problems can be overcome by the recently developed 3D cell culture techniques.

Can we create an easy and cost-effective *in vitro* 3D tumour model that can effectively mimic *in vivo* conditions in humans?

To investigate, Prakriti Tayalia and team, IIT Bombay, grew breast cancer cells on agarose-coated plates to avoid adherence to the surface and to facilitate spherical clustering. After incubation, these cells formed spheroids. The researchers created such spheroids from hot and cold types of tumour cells. Hot tumour cells are recognised and thus infiltrated more easily by immune cells. Cold tumours, on the other hand, are not as easily infiltrated.

The researchers compared both the hot and cold 3D tumour models with 2D tumour cultures. They found that both 3D tumour models preserved their cancer characteristics – stem cell-like transition from epithelium, the outer layer, to mesenchyma, a less differentiated form of cells. Moreover, just as in real tumours, the cores of the spheroids were under-oxygenated and necrotic or damaged.

The hot and cold 3D tumours retained the differences in their properties, as seen in the expression of immunosuppressive molecules and cytokines such as interleukin 10 and the transforming growth factor. The antigen-presenting capacity of the two tumour models was also different.

Compared to 2D cultures, the spheroids in 3D cultures showed higher resistance to chemotherapy. These properties of 3D spheroids are similar to those of cancers *in vivo*.

'2D models can give false or exaggerated results when testing cancer therapies. Our *in vitro* 3D model closely resembles the *in vivo* tumour microenvironment. So it is a better platform to test cancer therapeutics,' argues Prakriti Tayalia, IIT Bombay.

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Transgenic Cell Lines

Adding DNA methylation gene

Methylation of cytosine residues in DNA is an important epigenetic modification. The process is catalysed by a family of enzymes, DNA-methyltransferases. The methyltranferase 1 gene, *Dnmt1*, encoding the sole maintenance methyltransferase is overexpressed in neurological diseases such as schizophrenia as well as in many cancers. *In vivo* studies overexpressing this gene are not possible, since its overexpression leads to midgestational lethality.

So K. Naga Mohan and his team from BITS Pilani, Hyderabad decided to use an *in vitro* approach to explore the function of the gene, by creating a transgenic cell line that overexpresses *Dnmt1*. They incorporated the gene construct into mouse embryonic stem cells, using electroporation – a method to make holes in the cell surface and allow small gene constructs such as plasmids to enter the cell. The researchers inserted the gene construct into a stem cell line and confirmed that the number of copies of the gene in the transgenic cell line was nearly twice that of the normal stem cells where the gene had not been inserted. The transcription of DNA into RNA and the amount of DNMT1 protein were also doubled in the transgenic cell line, confirming the expression of the inserted DNA methyltransferase gene.

The transgenic cell line showed normal morphology under a microscope. The team found that the engineered stem cells had the normal number of chromosomes. Chromosome integrity was also maintained. After the *Dnmt1* gene was incorporated into the stem cells, the cells showed the potential to differentiate into different cell types. The cells differentiated at a normal physiological rate into ectoderm, mesoderm and endoderm.

'This cell line could serve as an *in vitro* model for studying neurodevelopmental defects caused by increased levels of the *Dnmt1* gene,' say Sumana Choudhury and Anuhya Anne, BITS Pilani.

'These cells can also be used to identify tumour forming potential in immunocompromised mice,' says K. Naga Mohan.

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Poor Mental Health

Asian undergraduates

Many university students around the world are found to have poor mental health. The situation is particularly worrisome for first-year undergraduate students as they face the transition from high school to university, the move to a new location, and the disruption of social networks.

The COVID-19 pandemic and related lockdowns exacerbated this problem. Most studies on student mental health are from Europe and the United States. But what about the mental health of first-year students at Asian universities during COVID-19?

Azizuddin Khan from IIT-B collaborated with a team of international researchers to study 3300 first year undergraduate students from Vietnam, China, Indonesia, Iraq, Japan, Malaysia, Saudi Arabia, Taiwan, and India. From India, 293 students from IIT-B participated in the survey.

To collect information about their mental health, the team gave the students a self-reporting questionnaire developed by the WHO. Half the respondents reported poor mental health. A quarter reported having suicidal thoughts. Fifteen per cent reported feeling lonely. In Japan and China, the prevalence of poor mental health was less than 20%. But more than 70% of students from Saudi Arabia, Iraq and India had poor mental health.

To measure the extent of the role of loneliness in mental health, the researchers used the University of California, Los Angeles Loneliness Scale.

They used the Oslo Social Support Scale to measure the role of social support in alleviating mental health problems.

Female students, students older than 26, and lonely students had higher odds of experiencing poor mental health. Students who had even moderate social support showed better mental health.

The researchers attribute the high prevalence of poor mental health among students during the second wave of COVID-19 to impaired daily routines, reduced physical and social interactions and possible financial concerns.

To reduce mental health problems among students, the researchers suggest that universities provide facilities for physical activity and actively encourage healthy social interactions among students.

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