Science Last Fortnight

Exploring the Deep-blue

Diversity at the ocean floor

In March 2017, a research expedition from the Centre for Marine Living Resources and Ecology and the Kerala University of Fisheries and Ocean Studies set out aboard the *Sagar Sampada*, a research vessel managed by the CMLRE, to explore living resources in the deep oceanic regions around the Andaman and Nicobar islands. The archipelago is a highly diverse ecosystem and the deep-ocean there has not been fully explored.

The expedition explored the depths around the region for about a month. The scientists used the specially designed High-speed Demersal Trawl net to catch fish on the ocean floor. They used an in-built echo sounder to find the best areas for smooth trawling to avoid net damage.

The scientists on board examined the catch in detail. After preliminary identification, ambiguous specimens were preserved and brought to the laboratory for detailed identification.

One fish, caught at a depth of about 500 metres in the North Andaman region, was particularly intriguing. The fish was beautifully coloured with reddish head fin and body, while the ventral side was whitish. There were white margins near the fins, except around the pectoral fins.

Image: CMLRE

After protracted studies and consultations, the scientists have now reported the identity of the fish: *Owstonia kamoharai*. The genus, Owstonia, is named after Alan Owston, an amateur naturalist from Japan. The species is named after Toshiji Kamohara, a zoology professor at Kochi University, Japan. So the fish is commonly called Kamohara's Bandfish.

The species was previously only reported from the Pacific Ocean. This is a new record of rare deep-sea fish in the Andaman-Nicobar region. The deep-ocean in the region shares many similar characteristics with the deeper parts of the Pacific Ocean. The chances of finding similar species from entirely different geographical regions with similar ecology are high, says Sileesh Mullasseri, KUFOS.

From the same expedition, two other new records of rare deep-sea fish were reported earlier. This is the third in the series. Besides throwing light on the deep-sea genetic diversity of India, these findings are expected to fuel investigation into the bioactive components of marine living resources, says Aneesh Kumar, CMLRE.

The *Sagar Sampada* was commissioned in 1984 and has participated in hundreds of research explorations in Indian waters, discovering many living resources around the Indian Ocean region. It is the only vessel in India capable of catching fish that live at one-kilometre depth in the ocean.

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Urban Black Carbon

Brahmaputra River valley

While all attention is focused on black carbon emissions from the burning of agro-waste in Punjab and Haryana as well as from automobile emissions from Delhi and cities in the Gangetic Plains, N. Barman and S. Gokhale from IIT-Guwahati were busy looking at the issue in the less investigated city of Guwahati, Assam, in the North-East.

During 2016–17, they measured black carbon concentrations using an aethalometer at one minute resolution, at two locations about two and a half kilometres apart: one near the centre of the city and the other closer to the Brahmaputra.

The sizes and composition of black carbon particles from biomass burning and those of black carbon from fossil fuel burning are different. So the researchers could show that, in the city of Guwahati, the major contributor to black carbon is fossil fuel burning. The number of vehicle registrations in the city has been growing rapidly in recent years, point out the researchers.

The concentrations peaked in the morning and at night. Black carbon concentrations seemed to decrease with temperature and solar radiation, but increased with increase in relative humidity. The hydrophobic nature of black carbon may be responsible for this, point out the researchers.

The site near the centre of the city consistently showed higher amounts of black carbon than seen nearer the river. Besides the high density of vehicles in the centre of the city, air movements near the river may also play a role in this difference, say the researchers. The two- to three-fold difference between the two locations narrowed in winter when both locations had similar, high concentrations of black carbon.

The Himalayan mountain belt, with a south-east orientation north of the Gangetic Plains, turns north-east north of Guwahati and then turns south to ensconce the Brahmaputra valley. The transportation and deposition of black carbon in this region is significant. So the researchers examined the phenomenon using the most recent version of HYSPLIT, the Hybrid Single Particle Lagrangian Integrated Trajectory Model.

A part of the deposition is in snow-covered regions and impacts the melting rate of snow. Deposition in the mountains increases from January to May. So, if emissions are controlled during this time, the impact on melting can be controlled, say the researchers.

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Chemicals in Jaggery Making

How much is too much?

Jaggery is considered a healthier alternative to sugar since it contains antioxidants such as polyphenols and flavonoids. Taking cues from the sugar industry, sometimes chemicals are used in making jaggery to enhance its colour and crystalline appearance, as well as to improve shelf life.

How do these chemicals work? How much of these chemicals can be

considered safe to retain the 'healthy' tag for jaggery?

Image: Joe Athiali via Flickr

Last fortnight, Pankaj Verma, Sanjay Mahajani and Narendra Shah from IIT-Bombay reported addressing the issue. Sodium hydrosulphite or hydros is a chemical commonly used in jaggery production to improve the colour, often in conjunction with phosphoric acid.

To investigate the use of phosphoric acid and hydros in jaggery making, the researchers filtered sugarcane juice, clarified it with milk of lime and added a coagulating agent. They prepared different batches. One had no chemicals at all. In another, the team used 10% acid. In a third, they used hydros, and, in the fourth, they used both acid and hydros. All mixtures were heated and stirred to a semi-solid mass.

Jaggery samples without additives were dark to light brown. The acid-treated product had a light red/brown colour. The hydrosulphite-treated product was light-yellow, whereas jaggery with combined treatment was even lighter. The researchers found that, to get jaggery to retain colour, it is better to add phosphoric acid after the juice is clarified.

Varying the pH of the juice during processing, the team found that jaggery made at a pH of less than 4 was very sticky and could not be solidified. More than a fifth of this jaggery was composed of glucose and fructose. These sugars do not crystallize easily and they hinder the crystallization of sucrose as well. This is why it becomes a sticky semi-solid mass, say the researchers.

The jaggery samples prepared using only phosphoric acid and those with combined treatment contained more fructose and glucose and less sucrose than normal and hydros-treated jaggery. Acid treatment, especially, tends to convert sucrose to fructose and glucose. Fructose is more amorphous in nature than crystalline sucrose, and so acid-treated jaggery samples were less crystalline. Though this makes jaggery feel smoother, the jaggery now has a hygroscopic nature and it tends to dissolve in the absorbed moisture. Jaggery makers use about 5 ml of 10% phosphoric acid per litre of juice. This amount needs to be adjusted to the ratio of sucrose to reducing sugars such as fructose and glucose. Phosphoric acid can be added to keep the pH slightly above 5.2, say the researchers.

Hydros- and combined-treated jaggery samples had lower amounts of minerals, except sodium and sulphur, than found in normal jaggery. The researchers say that sulphur dioxide content was on the higher side in hydros-treated jaggery than in normal jaggery. So, if at all hydros is used, it is safer to use phosphoric acid also to keep the sulphur dioxide content under control, say the researchers.

Microbial analysis of the hydros-treated jaggery showed that it was less prone to contamination, perhaps because the treatment had preservative action, say the researchers. However, under conditions of high humidity, the samples tend to liquefy increasing the risk for contamination by yeast and mould.

When they compared polyphenol and flavonoid contents in the treated samples, they found that the amounts of these phytonutrients were about 20% lower than those seen in normal jaggery.

The chemical treatments do improve the colour, appearance and shelf life of jaggery but make it less nutritious and healthy. So they should be used judiciously keeping nutritional requirements in mind. Normally, half to one and a half kilograms of hydros is used for making about 200 kilograms of jaggery. The optimum amount is 0.6 grams per litre of sugar cane juice.

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Preserving Edible Bamboo

Fermenting is best

Bamboo shoots are a delicacy. Besides being low in cholesterol, bamboo shoots are a rich source of amino acids, vitamins, minerals, carbohydrates and phytosterols. However, bamboo also has toxic cyanogen glycosides. Traditionally, the shoots are detoxified by various methods before consumption. However, though the methods make bamboo shoots safe to eat, they can also alter the levels of beneficial phytochemicals.

Recently, C. Nirmala and fellow researchers from the Punjab University, Chandigarh in collaboration with researchers from the North Eastern Hill University, Shillong made a comparative study of juvenile shoots of bamboo processed by three different methods – boiling, brine preservation and fermentation.

For their experiments, the team chose shoots from *Dendrocalamus hamiltonii,* with higher than safe limits of cyanogen glycosides. The phytochemical analysis of the shoots showed that cyanogenic glycoside was least in boiled shoots, followed by fermented, brine-preserved and fresh shoots.

The scientists then investigated the impact of processed and unprocessed bamboo shoots on the antioxidant defence system when consumed. They took 25 mice, divided them into groups and fed them fresh, boiled, fermented, and brine-preserved bamboo shoot extracts and the controls with no bamboo shoot extract. Assessing the impact of consuming the various extracts on the levels of glutathione, a proxy for stress levels, the scientists found that the increase in glutathione was much higher in treated mice than in the control, indicating that bamboo shoots stimulate the antioxidant defence system. The team also checked for antioxidant enzymes such as glutathione reductase, glutathione peroxidase, superoxide dismutase and catalase. Boiling reduced the level of antioxidant enzymes more than did brine-preservation and fermenting.

After weighing the pros and cons for consumption, fermentation appears to be the best way to preserve bamboo shoots while retaining the pharmaceutical value, says C. Nirmala.

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Fighting Fungal Infections

Target in sight, aim....

Many fungi switch between yeast form and hyphal form in response to the availability of nutrients and to ambient temperatures. Most pathogenic fungi take the hyphal form when they become virulent. If we can control the switch, we can also render fungi harmless. So Mukund Deshpande at CSIR-NCL has been investigating the genetic, epigenetic and biochemical parameters of dimorphism in fungi for decades.

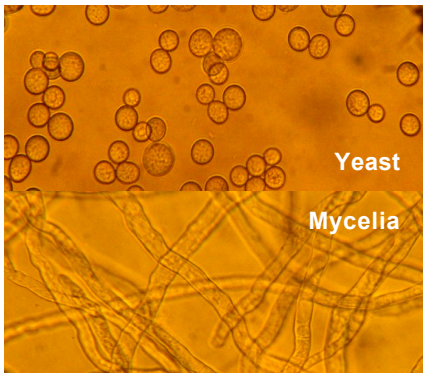

Image: Ejaj Pathan

Mukund and team selected *Benjaminiella poitrasii* for their experimental investigations as the fungus is not pathogenic to people in the lab but shows dimorphism – a safe model to play with. And they soon realised that the enzyme, glutamate dehydrogenase, was a key to the solution.

Now, at the end of his time in NCL, Mukund has come up with the most crucial clues to solve the mystery, with support from Narayan S. Punekar from IIT Bombay and Rajendra Prasad from the Amity University, Gurugram as well as from his Ph D scholars, past and present.

The first clue came a few months ago. Though the fungus takes the mycelia form at lower temperatures and sugar availability and thrives as yeast with adequate sugar availability and under warmer temperatures, these factors were red herrings. The key difference is actually the differences in the way the fungus mobilises nitrogen. Nitrogen is necessary for the synthesis of chitin in the cell walls of the mycelial form.

The team found that both mycelia and yeast forms had a glutamate dehydrogenase enzyme that was dependent on nicotinamide adenine dinucleotide for activity. But there was another type of glutamate dehydrogenase that was dependent on nicotinamide adenine dinucleotide phosphate. And this enzyme differed in the two forms of the fungus – one was seen only in the yeast form and the other in the mycelia form.

Through a series of genomic, transcriptomic and biochemical experiments, the team could establish that this is indeed the switch that determines the form. To clinch the argument, they transplanted the gene involved into a fungus found only as yeast. And it grew mycelia.

Fungal infections are notoriously recalcitrant to treatment. The medicines used are mostly toxic. But, now, with this discovery, the team has uncovered the Achilles heel of fungal infections. The target is in sight. The bullet that inactivates the identified enzyme is the only missing link. And, given the new biotechnological tools, that is a piece of cake.

Meanwhile, Mukund has teamed up with industry to produce low molecular weight chitosan, useful for agricultural and medical applications, from the chitin of *Benjaminiella poitrasii*.

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Deep Wound Healing

Without leaving scars

Skin possesses the capacity to heal minor bruises and injuries. But deep wounds from accidents and medical procedures need intense care for healing. Skin grafting, the common technique used for healing deep wounds, has limitations. It depends upon the availability of donor sites. After surgery, there is a high risk of infection and, often, there are post-surgical scars. While skin grafting heals the wound, the different layers of skin lose their original composition, leading to partial loss of function or joint movement.

So Debrupa Lahiri and others from IIT Roorkee and the Maharishi Markandeshwar University decided to engineer a tri-layer scaffold that mimics skin tissue as a solution.

The first layer should have low porosity and high hydrophobicity. The team engineered this layer by casting a layer of polycaprolactone, biodegradable polyester. The second layer should be slightly more porous and less hydrophobic. The researchers engineered this layer by electrospinning polycaprolactone on top of the semi-dried first layer so that the two layers adhere together. The third layer should be able to hold copious amounts of moisture. So the researchers chose gelatin for the third layer. The prefabricated double layer was spread over gelatin. After freezing the gelatin solution, the researchers froze the tri-layer scaffold and removed water content by lyophilisation. This created suction pressure which improved the adherence between the second and third layers. Now the structure of the tri-layer scaffold resembled three layered skin under the microscope.

After checking whether cells can survive on the scaffold, and whether keratinocytes and fibroblasts can proliferate on it, the team conducted *in-vivo* experiments on rats to check the effectiveness of the scaffold in facilitating wound healing. In untreated animals, the wounds showed less than 20% closure in 15 days. Wounds treated with Neosporin showed 60% healing. But, in wounds treated with the tri-layer scaffold, 90% healing was seen in 15 days.

The scaffold keeps each layer of skin in its position while the tissue underneath grows. As the skin regenerates, the scaffold degenerates, healing the wound and keeping the different layers of skin intact and functional. Once the wound is healed, there is no need for surgery to remove the scaffold as it is biodegradable. Under physiological conditions, about 75% of the weight of the scaffold is degraded in the first few days.

The results suggest that the tri-layer scaffold helps deep wounds heal 70% faster without the risk of infection and restores the original composition and properties of each layer of skin. It leaves no scar and full functional regeneration of the skin layers is achieved in the healed wounds.

Further testing and clinical trials are required before the artificial tri-layer becomes available in the market.

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Fractal Dimension of Eye Vessels

Identification of at-risk people

The blood vessels of the brain and the retina share common embryological origins and have comparable anatomy and physiology. So, retinal vasculature can be an indicator of, not only diabetes and diabetic retinopathy, but of hypertension, cardiovascular disorder, stroke and dementia as well.

Naren P. Rao from NIMHANS Bengaluru hypothesised that retinal vasculature can also indicate schizophrenia and bipolar disorder. There have been reports that the diameter of the small veins or venules of the eye in psychotic patients is greater than normal. The ratio of the arterioles and venules has also been reported to be marker for those who are genetically at risk for psychosis. But Naren felt that it would be more accurate to examine the fractal dimension of retinal vasculature.

It has long been established that retinal vasculature is a fractal – the branching process is self-similar, a characteristic of fractal structures. The fractal dimension of the vasculature depends on the number of bifurcations, angles of bifurcations, and the length of vessels between two successive bifurcations. So Naren and team took the help of Abhishek Appaji from the B.M.S. College of Engineering, who specialises in medical electronics. Researchers from the eye clinic at the Maastricht University, the Netherlands also came in to help.

Bhargavi Narendra recruited volunteers from those who came to the NIMHANS clinic. A hundred cases each of confirmed schizophrenia and bipolar disorder, but without any other comorbidities, especially those related to cerebrovascular disorders. They also had volunteers without psychiatrically diagnosed conditions as control.

The volunteers were acclimatised to darkness to dilate their pupil naturally and retinal images of the volunteers were taken using a non-mydriatic fundus camera. The quality of images was not good enough in some cases. After removing those cases, the team used automatic tracing to extract vasculature structure. And then there was automatic repeated counting of the vessels in squares of different sizes to calculate the fractal dimensions of the images.

The fractal dimension of retinal vasculature in bipolar and schizophrenia patients was higher than that in healthy volunteers.

Most eye clinics now have such imaging technologies. Automated analysis of fractal dimensions can help eye clinics identify people at risk for cerebrovascular and psychiatric illnesses.

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Fake News Detection

Automating the process

It is easier to distribute fake news via social media than through traditional mainstream media that tend to check and validate news before distribution. So, fake news production from religious, commercial and political propaganda units has become active, to influence, persuade and brainwash the public through social media.

How can the public differentiate between real and fake news? Can we develop an automatic process for detecting and flagging fake news?

Dinesh Kumar Vishwakarma and team from the Delhi Technological University have now come up with an improved way to automate fake news detection.

Theirs is a four-step approach. First, text extraction: a maximally stable external region in the image is identified using an algorithm for extracting text of all sizes and fonts using optical character recognition. The extracted text is then refined in the next step. Multiple occurrences of the same word are removed and unique entities extracted. Invalid words and spelling errors are also dealt with. The names of any media house are also removed to reduce bias. The refined text is then used for search through Google, a step that is automated by Selenium, a suite of tools. The related web links are ordered and classified into reliable or unreliable sources.

The final stage of processing has a summarizer that uses Python's natural processing tool and a title checker to calculate the Reality Parameter. While regional, national and international news gets reported by many news agencies, local news is often sparsely reported. This posed a problem in fake news identification. The researchers defined the reality parameter as more than 40 for news to be real. If it is less, then consider it fake.

Often traditional media mentions news items from elsewhere to make their readers aware of specific cases of fake news. Automatic scraping of text also identified these as fake. So the researchers tweaked their algorithm to take into account specific keywords that occur in such cases of false negative result.

The researchers then compared their method with other rumour detection methods.

The method detects fake news items with 85% accuracy and has outclassed other existing state-of-the-art systems.

Though the accuracy is best for international news and goes down in detecting regional and local news, if social media platforms incorporate the technique to identify fake news, propagandists and mischief makers will find it difficult to use social media for their nefarious activities.

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