

Current Science Reports

Extracting Extremophiles

Queen Maud Land, Antarctica

The icy crags of Queen Maud Land rise defiantly from Antarctica's frozen wastes. Avinash Sharma and team from the National Centre for Cell Science, Pune travelled to Maitri, the Indian research station there, seeking organisms that survive and thrive in such extreme conditions. The 38th Indian scientific expedition, March 2019, supported by NCPOR Goa, was perfect for the purpose.



Image: Gallery Sand via Wikimedia Commons

In sub-zero temperatures, the researchers scoured glacier sediments for samples. Back at Pune, they tried to culture, and identify extremophiles in the samples. After successfully culturing one bacterium, they identified the species using 16S rRNA gene sequencing. It belonged to the *Marisediminicola* genus. But it was not the same as the *Marisediminicola antarctica* that Chinese scientists had discovered, some years back. It was a distinct species, the second species under *Marisediminicola*, the marine sediment dweller.

Now the team had to name the new species. 'We dedicated it to Subhajt Sen, who lost his life during the 37th Indian scientific expedition to Antarctica in 2017,' says Avinash. So the species was called *Marisediminicola senii*.

Extremophilic microorganisms have unique and versatile metabolic properties with possible biotechnological applications. Genome sequencing and the biochemical and physiological characterization of *M. senii* revealed speciality genes that confer antibiotic resistance, making them a potential organism to study the mechanisms involved.

'The new bacterium's genes have many useful functional categories,' says Kunal Jani, NCCS.

However, they are very sensitive to temperature and environmental changes. So, they have to be cultured and stored carefully for future research to develop industrial applications.

There is also a need to explore how the new microorganism adapts to extreme cold conditions. So the team has preserved the new microbial taxa in one of the world's largest microbial repositories at the National Centre for Cell Science.

'Such preservation provides easy access to newly discovered species for further research, teaching and quality control,' says Swapnil Kajale, NCCS.

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Killari Earthquake Impact

On Tirna river flow

30 September 1993. A devastating earthquake struck Latur District, Maharashtra, killing nearly ten thousand people. The epicentre was at Killari, in the basin of the nearby river, Tirna. In fact, most earthquakes in the region during the last 800 years had epicentres there.

B. V. Lakshmi and team from the Indian Institute of Geomagnetism, Navi Mumbai along with Soumitra Mishra, now in a university in South Africa, recently tackled an interesting problem: since the area is a hub for several seismic fracture zones or faults, how did past earthquakes affect river flow?

They divided the river basin into six sections based on changes in the direction of river flow. And they collected 233 sediment core samples from those sections.

They examined layer after layer of the sediment's magnetic susceptibility – its ability to be magnetised by an external field. This property is not isotropic – it varies along different axes. The technique determines the orientation of magnetic minerals in the soil. So it helps figure out the direction and velocity of river flow.

The researchers observed that the upper part of the basin flows at low to medium velocity. The lower basin, a relatively flat land, also flows at low velocity, but the direction changes. The direction of flow shifts again, from upper to middle basin.

Velocity increases and direction changes once again in the central part of the basin, near Killari town. The researchers attribute this sudden shift to the increase in slope of the river valley. This could be due to movements across the North West–South East fault.

Tectonic movements across this fault may also be the main cause of the Killari earthquake, say the researchers. The team warns that seismic activity across this fault may trigger future earthquakes. Next time, it could well flood the flat downstream part of the Tirna.

To minimize loss of life and property, urban development planning in the region needs to take the possible risks into account.

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Removing Organic Micropollutants

Banking on backwaters

Lake Vembanad is the backbone of Kerala's backwater tourism. More than 16 lakh people bank on the mildly saline water for domestic, agricultural and industrial purposes. Researchers from the Mahatma Gandhi University, and CUSAT, Kerala were concerned at the increase in organic micropollutants in the lake.

Recently, they reported estimating the organic micropollutants in the backwater canal at Arpookara. The team collected surface water samples from three sites in the canal system from January to December in 2015 and analysed them. Out of the eleven organic micropollutants identified, five compounds were known to be environmentally toxic.

The micropollutant most frequently observed and with the highest concentration in the area is dibutyl phthalate – a common ingredient in

plastics, personal care products and food packing materials.

The team estimated the ecological risk quotient using algae, daphnia and fish – organisms from three different levels in the freshwater food web. The levels of oxybenzene, chlorophene and dibutyl phthalate posed medium risk, and diethylbenzamide posed low level risk to the target organisms.

The actual risk will be even higher since the risk quotient considers only the target compounds and not the more toxic metabolites, say the researchers. Moreover, the cocktail of micropollutants has greater impact on non-target organisms.

So the team devised a solution for its removal: low-pressure microfiltration using layers of antifouling polyelectrolyte membranes. The system traps contaminants based on surface charge, hydrophobic build-up force and solute characteristics. The presence of ions and surfactants in the canal facilitated the complete removal of the most hydrophobic solute, chlorophene and enhanced oxybenzene removal.

Such baseline information along with a solution can help regulatory agencies initiate mitigation measures to control and manage micropollutants in backwater bodies in other parts of India.

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Smouldering Coal Fields Detection by satellite imaging



Image: TripodStories via Wikimedia Commons

It has been over a century since coal fires started in the Jharia coal fields, Jharkhand. Unchecked, these subsurface fires in coal seams cause health and environmental hazards. Early detection can help douse them.

Satellite thermal imaging can pinpoint high temperature areas on earth.

But can it represent and pinpoint coal fires underground?

This burning question prompted two scientists from the National Institute of Technology, Rourkela, to compare satellite thermal imaging data with field data from the Jharia coal fields at Dhanbad.

They took data from Landsat – Land Remote Sensing satellites, colliery maps, and surface thermal images for 2009 and 2019. They also collected Landsat data of three different ranges of frequencies – near infrared, red and thermal infrared.

Then, they applied atmospheric correction on the thermal infrared data to calculate the top of atmosphere radiance and brightness temperature. Using near infrared and red frequencies, the duo generated a map of emissivity from the land surface. To this land surface emissivity map, they applied the temperatures calculated from the brightness and created a land surface temperature map.

To verify the validity of their method, the researchers collected the colliery boundary maps of the Jharia coal field and land surface thermal images using a thermal camera with global positioning system.

The threshold land surface temperature value for the detection of coal fire pixels was 38°C. There were 22 areas with temperatures more than 38 degrees. And they were indeed fire-affected.

‘Satellite thermal infrared band data failed to differentiate between land surface temperatures above 50 degrees Celsius,’ says A. K. Gorai, NIT Rourkela.

Next, they validated the satellite data with field images collected at the same time. There was not much difference between the two.

Thus encouraged, the team created land surface thermal maps for 2009 and 2019. They superimposed the fire maps and noticed that coal fire zones have increased during the decade, with very few fires extinguished.

‘This is alarming. And needs addressing before the situation worsens,’ says Shanti Swarup Biswal, NIT, Rourkela.

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Peanut Shell Adsorbs Dye from wastewater

Wastewater from carpet, leather, paper and textile industries contains auramine, a carcinogenic dye. A basic dye like auramine can be adsorbed by the abundant cellulose, hemicellulose and lignin found in peanut shells. Arpan Herbert, Upendra Kumar and Prashanth Janardhan from NIT Silchar, Assam recently tested the concept.



Image: 123rf, via Wikimedia Commons

They washed, dried and powdered peanut shells. Perhaps enhancing the ion exchange capacity of powdered shell by treating it with sodium hydroxide solution would make it adsorb and retain dye more efficiently. So, the researchers tested both – powdered shell and alkali treated powder.

Both absorbed the dye. However, when the researchers increased dye concentration from 25 to 250 milligrams per litre, adsorption decreased. This was due to the saturation of available sites in the shells, they say.

When they increased the amount of adsorbent, dye removal increased but adsorption capacity decreased. Five grams per litre was optimal. At this concentration of the powder, maximum adsorption occurred at the end of 20 minutes. The powder adsorbed more than 80% of the dye. The alkali-treated shell powder adsorbed more – nearly 95%.

Temperature and pH also influence adsorption. At low pH values, adsorption was lower due to opposing electrostatic interactions. With increasing pH, adsorption increased. The best results were obtained when pH was neutral. Alkali-treated powder adsorbed 99% of the dye. Shell powder not treated with alkali adsorbed 85%.

In both types of shell, adsorbance increased with temperature rise, suggesting an endothermic reaction. The researchers analysed the thermodynamic favourability of the reaction by calculating Gibbs free energy. It was negative, suggesting the reaction's ability for proceeding on its own – a spontaneous process.

The team estimated the cost of the processes. It came to about 70 rupees per kilogram without alkali treatment. And 106 rupees per kilogram for alkali-treated shells. Far cheaper than commercial treatments. Activated charcoal, for example, costs 4000 rupees per kilogram.

Industries using the dye now have an economical solution to their wastewater woes, to strive towards the legally mandated zero emission standards.

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Damask Rose

Elicitor enhances essential oils



Image: David Stang via Wikimedia Commons

Damask rose is highly valued for its essential oil. The oil's composition influences its flavour and odour which determine quality and market price. Growth elicitors such as methyl jasmonate, diphenyl urea and kinetin can enhance yield, and quality. But which works best? At what concentrations?

Meenakshi Thakur and Rakesh Kumar from the CSIR-Institute of Himalayan Bioresource Technology, Palampur investigated. They experimented with 9-year-old bushes. The leaves were sprayed with varying concentrations of the elicitors at the budding stage.

'There were more flowers with a spray of diphenyl urea and the flowers weighed more,' says Meenakshi Thakur, CSIR.

Fresh flower yield from a hectare was more than 1800 kilos!

The team extracted essential oil from the flowers using hydro distillation. The essential oil content was greater in bushes sprayed with methyl jasmonate and diphenyl urea.

The researchers also investigated changes in the chemical profile of the essential oil. They identified 26 volatile compounds of simple and modified unsaturated hydrocarbons such as monoterpenes in the essential oil. The composition of the compounds has characteristic ratios to produce the typical fragrance of the damask rose.

The duo then quantified the chemicals in essential oils from the roses with different treatments. They found that methyl jasmonate enhances the percentage of the monoterpenes while reducing the percentage of the hydrocarbons. With any alteration of chemical percentage, the essential oil's odour also changes.

The variation in the composition of the chemicals due to the elicitor intrigued the researchers. They tried to statistically analyse the variation in the composition of the compounds in response to the elicitors and also the relationship between the compounds. But were unable to arrive at the right recipe. More studies are needed to understand the role of elicitors and environmental conditions in changing the essential oil composition.

One thing is clear: the combination of 15 milligrams per litre of diphenyl urea and 50 millilitres per litre of methyl jasmonate may enhance rose yield and essential oil content.

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By-product to Bioactive *Non-thermal nanofiltration*

Curcumin is extracted from turmeric for pharmaceutical applications. The oleoresin, the mother liquor left after curcuminoid extraction, contains volatile oils, fixed oils and leftover curcuminoids. These can be extracted using hexane. The volatile fraction of the hexane-extracted oil contains all the sesquiterpenoids –

natural bioactive compounds found in plants and insects. How do we recover valuable bioactive volatiles wasted during curcumin manufacture?

Scientists from the Central Food Technological Research Institute, Mysuru and the Academy of Scientific and Innovative Research, Ghaziabad recently came up with a solution: nanofiltration using membranes. They used five commercial polydimethylsiloxane-based nanofiltration membranes. Such membranes are hydrophobic, solvent-resistant and non-polar to experimental materials.

After using hexane to extract turmeric oil from the oleoresin, the researchers made a membrane apparatus. They cut the nanofiltration membranes into circular discs and fitted them into a cell with a porous support. They poured the hexane solution into the cell and stirred it.

The molecular weight of turmeric's volatile oil components is lower than that of non-volatile components. So, non-volatile components are retained by nanofiltration membranes. However, minor non-volatile components with low molecular weight curcuminoids were almost completely retained because of polarity.

The filtered volatiles were mainly monoterpenoids and sesquiterpenoids.

The nanofiltration membranes have high selectivity for volatile oils and increased the content from 75.0% to 97.8%, say the scientists.

They also claim that the colour value and viscosity of the membrane-processed turmeric oil were comparable to that of hydro-distilled turmeric oil. Membrane-processed turmeric oil had higher stability of turmerones than found in hydro-distilled turmeric oil, owing to its higher total phenolic content.

To get premium quality turmeric oil with higher stability of turmerones, nanofiltration offers a non-thermal route for separating volatile constituents – cost and quality factors necessary for adoption by the pharmaceutical industry.

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A Tale of Two Vectors

Anopheles subpictus and *sundaicus*

Anopheles subpictus is a species complex of mosquitoes, found in mainland India, where they're not considered malaria vectors by the national vector control programme. But, in South-East Asia, they are considered potential vectors for malaria. Is it because they are distinct species? Cryptic, not recognised as yet?

Recently, Ankita Sindhania and team at the National Institute of Malaria Research, New Delhi decided to decipher this perplexing status of *Anopheles subpictus*.

Previous studies, based on inversions of parts of the X-chromosome, show that there are four sibling species of *Anopheles subpictus*, provisionally named A, B, C and D.

Anopheles sunaicus, a close cousin of *subpictus*, is a potent malaria vector. *A. sunaicus* also has four sibling species acting as vectors at different geographical locations. Since *Anopheles sunaicus* and *Anopheles subpictus* are so similar, are we unable to distinguish between the sibling species of the two mosquito species?

Ankita and team broadened their interest. They collected adult females of *subpictus* and *sundaicus* from different parts of India, the Andaman and Nicobar Islands, and Sri Lanka, with support from collaborators.

A common method to differentiate mosquito species is by counting ridges on eggs. The eggs of species A, C and D of *subpictus* were similar but those of sibling species B seemed to be distinctly different.

'This uncertainty could only be solved by genetic investigations,' says Ankita. They characterised nuclear ribosomal DNA, a quicker way to sort out lineage than using mitochondrial DNA. And found two molecular forms of *sundaicus* and three of *subpictus*.

The sibling species A of *subpictus* is distributed over the mainland and B and C over coasts and islands. The two molecular forms of *sundaicus* turned out to be *Anopheles epiroticus* and *Anopheles sunaicus* species D.

Cytological analysis confirmed the results.

It showed two distinct groups: a *sundaicus* group with molecular forms of *sundaicus* and *subpictus* B and C.

Anopheles subpictus A was the lone member of the *subpictus* group. Studies on the intra genomic sequences and alleles of *subpictus* form A showed that it has a distinct haplotype.

'The results from our molecular studies agree with the location of the mosquitoes and their breeding habits,' says Ankita.

Anopheles subpictus A is distributed in the mainland and breeds during the monsoon. But *subpictus* B peaks twice. The species breeds even after the monsoon and mostly occurs in coastal regions along with the *sundaicus* species.

These molecular results, combined with breeding habits, show that *subpictus* A and B are distinct. *Anopheles subpictus* B should be a member of the *sundaicus* complex.

Anopheles has more than 400 distinct species. Species diversity and the bewildering number of sibling species make vector control complicated.

'To control the disease, we need to understand the vector's behaviour and habitats. These factors vary between species,' says O. P. Singh, National Institute of Malaria Research, Delhi.

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Multiple Pregnancies

Impact of COVID-19

Pregnant women with COVID-19 have high probability of delivering prematurely. Evidence suggests that pregnancies with pre-existing medical conditions are also more liable to severe health complications. However, pregnant women with confirmed infection experience fewer symptoms than those who are not pregnant.

But what if there is more than one baby in the womb? Multiple gestational pregnancies are generally associated with higher risk of both foetal and maternal complications. Does COVID-19 infection make mul-

iple gestational pregnancies more complicated?

Researchers from the ICMR-National Institute for Research in Reproductive Health and the Topiwala National Medical College, Mumbai investigated. They took data from the BYL Nair Charitable Hospital, a dedicated COVID-19 hospital in Mumbai. Data from 4 April to 10 September 2020, and pre-pandemic data from 1 April 2019 to 31 March 2020 included 859 pregnant women with singleton pregnancy and 20 with multiple foetuses.

They found that the trend of pre-term deliveries was similar for multiple pregnancies during both periods. But the incidence of pregnancy complications like pre-eclampsia and eclampsia was higher in SARS-Cov-2 infected mothers with multiple gestations. In such women, during the pandemic, the frequency of admission to neonatal intensive care units was also higher.

However, contrary to research reports on single foetus pregnancies, premature delivery among multiple gestational pregnancies with COVID-19 was comparatively lower.

'Women with multiple gestational pregnancies and COVID-19 need special attention with a multidisciplinary approach to maternal and neonatal care,' says Niraj N. Mahajan, BYL Nair Charitable Hospital.

There was also a higher incidence of twin births – about 34% – than reported in other studies from India.

This could be because the hospital was dedicated to COVID-19 maternity care and, thus, received all referral cases from Mumbai city and nearby regions, they say.

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