Current Science Reports

Lab-on-a-chip
Detecting fluorides

Fluorides, inadequate or surplus, harm us. Too little causes dental caries and too much leads to dental and skeletal fluorosis. But measuring fluorides is not easy. The instruments are bulky. The process is time-consuming.

Recently, researchers from India, Australia and Saudi Arabia designed a small device to detect fluoride ions using microfluidic technology. Microfluidics reduces analysis time with minimal sample volume. The team synthesised a chemical sensor that changed colour within a minute.

'The colour change is due to amine and hydroxyl groups in the probe,' explains Mahaveer Kurkuri, Jain University, Bengaluru.

The team then fabricated a device with two microchannels of entry. A small amount of the sample in one channel and the probe in the other are joined into an s-shaped microfluidic channel to ensure mixing. The outlet was connected to a light source and a spectrophotometric detector.

The scientists tested the device with various fluoride concentrations. The device could detect 1.5–100 parts per million of fluoride.

Then the device was used to detect fluoride in a mixture of halides. The sensor only responded to fluoride ions.

The team compared fluoride content in different water sources using a standard colorimetric test and the new device. The results were well-matched.

Water board authorities can adopt this simple device to test for fluoride in drinking water.

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Severe Haze Events
Post-monsoon air pollution

Seventh to fourteenth November 2017, a haze engulfed Delhi. Pollutants and particles accumulated in the air, making it hard to breathe. Low visibility forced schools shut and life came to a standstill.

To investigate the event, researchers from the Dayalbagh Educational Institute, Agra took satellite and weather data and ground-based observations from 22 stations in 12 cities spanning Delhi, Punjab, Haryana and Uttar Pradesh. They examined air quality, particulate matter, concentrations of toxic pollutants and air movement.

After 6 November, wind carrying pollutants moved from China and the Arabian Peninsula towards the Indo-Gangetic plains. Meanwhile, to the northwest, large-scale post-monsoon agricultural burning was emitting chloride, nitrate, sulphate and ammonia ions.

The increase in air pollutants raised relative humidity to above 90%, blocking solar radiation. Consequently, daily temperatures dropped. Wind speed reduced. Air stagnated. This decreased visibility to less than half a kilometre. Restricted pollutant dispersal made the air toxic.

After 12 November, the wind moved away from the Indo-Gangetic plain. This dispersed pollutants and partially reduced their concentrations.

The researchers say that action to reduce crop residue burning and vehicular emissions can prevent such severe haze events.

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Mitigating Arsenic Stress
Potassium humate priming in rice

Arsenic in soil inhibits rice seed germination and reduces plant growth. Scientists have been trying to mitigate arsenic phytotoxicity in rice plants. To reduce salt stress in plants, the potassium salt of humic acid, has been used. Can it help remediate arsenic in rice?

Researchers from the Jadavpur University, Kolkata and IIT Kharagpur investigated.

Humic acid consists of various aromatic polymers, residues from microbial metabolism. Seed priming has been used to improve germination and stress tolerance. So, the team primed rice seeds with a potassium humate solution.

Arsenic in soil can have a valency of 3 or 5. The team investigated germination and seedling growth for a popular rice variety, IR64, under both types of arsenic ions. They compared results with those grown without arsenic. Twelve days after seeding, germination and seedling growth were higher in experimental and control samples primed with potassium humate. Total chlorophyll content increased by 27%!

'Potassium humate priming enhances the bioaccumulation of essential macro and micronutrients in rice seedlings,' says Deepanjan Mridha, Jadavpur University.

Arsenic uptake and translocation in primed rice seedlings was reduced by more than 33%, say the researchers. So, oxidative stress and antioxidant activity in the seedlings was also lower.

'Priming seeds with potassium humate can improve rice production in all regions. And minimise arsenic toxicity in arsenic-contaminated regions,' says Tarit Roychowdhury, Jadavpur University.

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Enhancing Rice Yield
Using iron-pulsing

Rice is a staple for most Indians. But yield is nearly 35% less than demand. Research shows that pulsing rice seeds with iron salts can increase yield. But what is the optimum period for pulsing?

Researchers from various institutes teamed up to test. They sterilised rice seeds and pulsed them with ferrous sulphate or ferric chloride for one, two and three days.

Seeds treated with 5 millimolar iron salts for three days performed better in germination percentage and seedling vigour. This was more prominent with the ferrous sulphate solution.

Using a histochemical stain, the researchers detected iron in the pulsed seeds.

'Iron penetrates the seed and crosses even the endosperm,' explains Subhabrata Paul, Presidency University, Kolkata.

The team also found an increase in hydrolytic enzymes which break and mobilise stored food in the seed.

'Enzymes in starch and protein metabolism increased activity and accelerated germination,' explains Swarnali Dey, University of Calcutta.

The activity levels of antioxidant enzymes showed that the treatment did not induce stress.

The researchers maintained seeds in the pulsing nutrient solution for 14 days and observed that growth was retarded.

'Treating up to safe limits is beneficial but toxic beyond it,' warns Rita Kundu, University of Calcutta.

To check if the benefits of iron-pulsing persist when treated seeds are planted without fertiliser, seeds treated for three days were sprouted and transplanted in a field 21 days later. The team recorded high yield for two consecutive years. 'The grains had higher iron content,' mentions Geetha Gopal, Vellore Institute of Technology, Vellore.

'Iron-pulsing is cost-effective. With little effort, it can increase rice yield,' adds Amitava Mukherjee, VIT.

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Peanut Skin Phenolics

Extracting antioxidants

Peanut skin is a valuable source of antioxidants. But over 750,000 tonnes of peanut skin go waste annually.

Researchers from various institutes decided to evaluate nutraceuticals in four popular varieties: K-6, K-9, TMV-2 and TAG-24.

With high-performance liquid chromatography, they found that all four peanut varieties had high p-coumaric acid, an antioxidant with anti-inflammatory and antibacterial effects.

The team conducted an electrical conductivity test to determine the intactness of the peanut skins since intact skin indicates the presence of high quality antioxidants.

'K-6 peanut skin had low electrical conductivity. So it has high value antioxidants,' says Yanamula Mohan Reddy, Sri Venkateswara University, Tirupati.

But which variety has the highest antioxidant activity? To find out, the team used the Fourier transformed infrared spectra of peanut skin to identify phenolic compounds.

'The skin of all four varieties is high in phenolic compounds,' says Anjali Devi Chintagunta, Vignan's Foundation for Science, Technology and Research.

'The highest antioxidant activity was in the K-9 variety,' says Ashutosh Kumar, Indian Institute of Seed Science.

'Antioxidants from peanut skin can find applications in food industries and nutraceuticals,' says Dinesh K. Agarwal, Indian Institute of Seed Science.

However, extracting phenolics from skin should be cost-effective and safe. The team experimented on various solvent systems to find which one could efficiently dissolve most phenolics. They found that the solvent containing mostly acetone, some water, and a touch of acetic acid would be the best to extract phenolics and worked out the optimum ratios.

Peanut processing industries now have an opportunity to extract value from waste.

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COVID-19 Surveillance

Wastewater-based epidemiology

Quantifying viral genes in wastewater is an efficient and economical approach to monitor infections and community outbreaks in large cities.

Recently, scientists from the Indian Institute of Chemical Technology and the Centre for Cellular and Molecular Biology came together to assess wastewater-based surveillance for COVID-19 infection in Hyderabad city.

They collected sewage samples from all the major sewage treatment plants of Hyderabad for a month during July–August 2020. And they tested the samples using RT-PCR.

An infected person can shed viral materials through faeces for up to 47 days.

'From viral RNA copies in the samples, we used this estimate to calculate the number of infected people in the active phase of infection,' says Hemalatha Manupati, CSIR-IICT.

'However, there is uncertainty due to the difference in the number of viral particles excreted per individual,' adds S. Venkata Mohan, CSIR-IICT.

As per their calculation, infected people in the city could have been between 30,000 and 30 lakhs.

'The large-scale wastewater-based monitoring is effective for screening the intensity of infections only in large cities,' says Rakesh Mishra, CSIR-CCMB, Hyderabad.

A previous study showed that about 500 positive cases out of 10,000 persons are required for detecting the COVID-19 virus in wastewater.

Focused research to enhance the sensitivity and specificity of the method is necessary to relate viral genome count and the prevalence of COVID-19 infection.

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Drugs for Leishmaniasis

Tapping natural products

Leishmaniasis, caused by *Leishmania* parasites, commonly affects the skin. But visceral leishmaniasis attacks organs and is fatal.

Existing drugs, antimonials, pentamidine, amphotericin B and miltefosine, have side-effects and the parasites are developing drug resistance.

So Abdur Rab and team from Jamia Millia Islamia University, Delhi collaborated with researchers from Saudi Arabia to find an alternative. They chose to attack the ergosterol pathway in the pathogen. Since we do not have ergosterol and have cholesterol instead, ergosterol synthesis is a safe target. The researchers targeted *L. donovani*'s sterol C-24 methyltransferase – a key enzyme in ergosterol synthesis.

Using docking studies, they screened a library of natural compounds with antimicrobial activity against the target enzyme. Gigantol, a compound from *Dendrobium* orchids, showed the best fit with the enzyme.

In-vitro, gigantol inhibited promastigotes, the form of the parasite transmitted by sandflies, and amastigotes, the active developmental stage of the

parasite in humans. The compound induced oxidative stress in promastigotes by generating reactive oxygen species, say the researchers.

Around 60 micromolar gigantol cut down the activity of sterol C-24 methyltransferase by half with no adverse effect on human cells.

'Further studies are needed to understand the antileishmanial activity of gigantol,' says Abdur Rab, Jamia Millia Islamia University.

The pharma industry can now take the research forward to create affordable leishmaniasis treatment.

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Myopic Macular Degeneration

Screening to detect

Half the world will have myopia by 2050, as per global prevalence of myopia reports. One-fifth of those with myopia show degeneration of the macula, the central part of the retina. Unless detected early, this leads to loss of vision. Trained professionals can detect myopia from scanned retinal images, but it takes time and effort. A perfect setting for artificial intelligence to step in.

Vinay Nangia from the Suraj Eye Institute and 38 researchers from nine countries collaborated to muster 225,000 retinal photographs! More than adequate to train and test the system.

To extract pathological patterns in the images, the team developed convolution neural network-based algorithms – two with different criteria to detect eyes with myopia and one to detect eyes with macular degeneration. The algorithms could detect myopic macular degeneration as either present or absent.

Among myopic eyes with growth of new blood vessels, a treatable cause of vision loss, 99% were identified.

The researchers asked six experts to detect problems in 400 images from another dataset and compared the results with those from the algorithms. The algorithms performed faster and better than human graders.

As the retinal photographs, at different locations, were transferred and analysed in real time, this was also a demonstration of implementing block-chain technology in medical practice, say the researchers.

The system can now be used for creating national databases to facilitate early treatment for macular degeneration. A faster, convenient, low-cost and efficient data sharing system for the international medical fraternity to explore and exploit.

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Mosquito Repellent Polyester

Protective textile

Applying mosquito repellents on skin is effective but can cause skin allergies. Why not incorporate mosquito repellents in textiles?

Javed Sheikh and Ankit Singh at IIT Delhi soon realised that the widely-used repellent DEET or N,N-diethyl-3-methyl benzamide is removed by washing. So why not attach mosquito repellents to dyes?

The team selected ethyl anthranilate, a highly volatile mosquito repellent, and coumarins, multifunctional mosquito repellents. These compounds, combined separately with a base of DEET, were synthesised into two dyes and applied to polyester fabric. Ankit Singh then wrapped his arms with undyed and dyed fabric and shoved them into a mosquito-filled cage.

Polyester dyed with coumarins showed 97% mosquito repellency. Polyester dyed with ethyl anthranilate showed 100%. A little dye is effective due to the synergistic effect of the two repellents.

Polyester fabric only treated with DEET showed negligible mosquito repellence after just five washes. But, with the dye, it retained activity even after twenty washes.

'Combining the repellents increased repellence while reducing the doses needed,' says Javed Sheikh.

The dyes also have anti-bacterial, anti-fungal and antioxidant properties which make them good candidates for the textile industry.

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Exotic Tree Plantation

Impact on local biodiversity

Exotic tree species are popular in managed forests because they have commercial or ornamental value and are easy to maintain. But, how do they affect local biodiversity?

Recently, Kiran Choudaj and Varsha Wankhade, from the Savitribai Phule Pune University, investigated the impact on local avian and plant biodiversity. They identified 16 sampling sites across five hills in and around Pune. Six had natural savanna vegetation, and ten had exotic plantations.

The team also sampled bird and plant species diversity at the sites. Plantation sites had higher tree density but were lower in plant species diversity.

'The plantation reduced the growth of small shrubs and ground level plants,' says Kiran Choudaj.

The researchers found 86 different bird species in the savanna vegetation sites – some very specialised in their feeding habits. Exotic plantation sites had only 38 species – less than half the number. Most were omnivores.

More than half the area of these hills is under exotic vegetation now.

'Losing natural savanna vegetation implies losing a lot of plant and avian biodiversity. Savannas take over a century to recover,' says Varsha Wankhade.

The biodiversity of the hills in Pune supports the larger ecosystem there. Public awareness and policy change in forestry practices can help protect local biodiversity.

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A Tale of Two Habitats

Indian rock agama

Maria Thaker and her group at IISc Bengaluru were surprised to notice a size difference between rural and urban specimens of the South Indian rock agama, *Psammophilus dorsalis.*

To verify and quantify the phenotypic changes, Shashank Balakrishna and Madhura Sham Amdekar went lizard catching in the Bengaluru suburbs and on the nearby Antharagange forest ranges.

They measured 120 lizards. Urban agamas were indeed bigger than village cousins.

'There is also a difference in limb sizes,' says Shashank. Urban lizards

had shorter limbs, which could potentially help them manoeuvre tight spaces.

Bigger size and shorter limbs make the lizards susceptible to predation. Especially the males that are coloured bright yellowish orange and tend to climb conspicuously atop rocks.

'More tail injuries to city agamas suggested that they face greater predation,' says Madhura S. Amdekar.

But this hypothesis needed to be tested. So, the group made dummy lizards replicating rock agama colours. During breeding season, male agamas show different colour patterns involving yellow red and black when they entice females or are aggressive to other males.

The researchers placed dummy lizards at both locations. Urban male dummies were attacked most.

'Apart from predators, city lizards also face human disturbances,' says Maria Thaker, pointing out other selection pressures.

The lizard is a curiosity. From a sand-loving genus (*Psammophilus*) emerges a species that inhabit rocky outgrowths. The colours on the dorsal side (*dorsalis*) serve multiple purposes, from social communication to thermoregulation and reducing predator attention. And now the phenotype is adapted to city life, full of vertical walls.

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Rainwater Harvesting

Case study of village school

Children in a school in Berambadi village, Karnataka were facing a water crisis. Supply from a nearby borewell depended on erratic electricity supply. Meanwhile, most annual rainfall went waste as surface runoff.

Researchers from the Ashoka Trust and IISc Bengaluru collaborated with researchers in the UK to try harvesting rainwater there. The team estimated water demand, using flow meters at washbasins, kitchens, toilets, etc. This provided the clue for the volume of containers to store rainwater. Accordingly, the team constructed surface and underground tanks.

They created slopes on roofs to direct rainwater to drain towards the outlet. And before the rainy season, they ensured clean roofs.

A metal mesh at the outlet plumbing prevented leaves and debris from entering storage containers. To monitor water levels, the researchers installed a water level indicator.

After the rains, the water quality was tested. It was good. But, over time, microbial contaminants increased. So the researchers used sodium hypochlorite, a low-cost way to chlorinate water. Regularly disinfecting the stored water made it safer.

The rainwater harvesting system reduced pressure on the existing water supply by up to 25% and assured water around the clock.

The Berambadi model can be emulated elsewhere to keep children safe from water-borne diseases. It will also ensure better sanitation in schools.

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Plant Microbial Fuel Cells

Wastewater treatment

Domestic wastewater has high amounts of chemical energy which can be converted to electricity using microbial fuel cells. And, in the process, the water is cleaned, without operationally expensive water treatment. The redox gradient necessary to produce electricity is maintained by connecting an anaerobic system to an aerobic system. This interface is naturally seen in wetlands. So, why not construct a wetland system of microbial fuel cells?

Somil Thakur and Bhaskar Das from the Vellore Institute of Technology realised that a single stage microbial fuel cell becomes less productive in time, especially for high strength wastewater. So they incorporated a two-stage microbial fuel cell in a constructed wetland.

For fuel cells, they used two PVC pipes, each about half a metre long, with separate anode and cathode zones. Graphite plates acted as electrodes.

The fuel cells were connected such that effluent from the first stage enters the second stage as influent in the upward direction.

Actively digesting semi-dry sludge from a water treatment plant was inoculated into the anodic zone to acclimatise the system. The cathodes were kept in the open air for channelling oxygen diffusion and were separated from the anodes by layers of sand and gravel. Glasswool was used to stop the intermixing of filter media between anode and cathode zones.

In the constructed wetland, the researchers used *Canna indica*, a plant with a well-developed root system that helps create an aerobic environment, to maintain the redox gradient. The species is tolerant to saline environments.

The researchers estimated the system's performance based on energy recovery and pollutant removal efficiency at both stages. In the first stage electricity generation declined due to organic loading and blockage of filters. But the chemical oxygen demand was reduced considerably. The second stage received organic matter in mostly dissolved form and provided more consistent electricity.

Interestingly, biofilm formation improved electricity flow.

Optimising wastewater flow as per substrate concentration and declogging filters are the challenges remaining to upscale and make the system sustainable.

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