

Science Last Fortnight

Rock Phosphate

Replacing phosphorus fertilisers

India is a net importer of phosphates, an essential component of NPK fertiliser in agriculture. This expense is a drain on the exchequer.

Low grade rock phosphates, abundant as natural deposits in India, can be used instead of imported chemical phosphates. However, rock phosphates are not as water soluble as chemical fertilisers. Hence uptake by plants is poor.

B. B. Basak from an ICAR institute in Gujarat studied rock phosphates from Udaipur, Jhabua, Purulia and Mussoorie to understand their relative merits for use in agriculture.

He extracted rock phosphate samples with distilled water, neutral ammonium citrate, dilute citric acid and formic acid. And found that water soluble phosphorus was low in all samples. Udaipur rock phosphate had higher amounts of soluble phosphorus than rock phosphates from other regions. Solubility, he discovered, was higher with 2% citric acid.

Basak then conducted pot culture experiments with palmarosa and rye grass to understand the effectiveness of rock phosphates in acid soils. He found higher biomass yield in Ranchi soil where the yield was slightly higher in palmarosa than in rye grass.

The relative agronomic efficiency of Udaipur rock phosphates was found to be high in both crops. The efficiency was higher in Ranchi soil than in Bhubaneswar soil.

Even phosphorus recovery efficiency, reflecting phosphorus uptake, was higher with Udaipur rock phosphates in Ranchi soil. The scientist recommends Udaipur rock phosphate as a good alternative to expensive superphosphates, especially in acidic soils. Moreover, it can be used as input for organic agriculture since rock phosphates are natural deposits.

Basak has also found a correlation between citric acid-based extractants and agronomic effectiveness, with neutral ammonium citrate. So now it is easy to predict the agronomic out-

come by checking the citric acid-based extraction of rock phosphates found in different regions.

DOI: 10.1080/03650340.2019.1571681

Neem Protects Goldfish

Antiparasitic effects

Chemicals used for controlling parasites in aquaculture cause environmental toxicity. Neem leaf, bark and seed are known pesticides. But can neem leaf extracts fight parasitic infestations in aquaculture?

Scientists from the ICAR-Central Institute of Fisheries Education, Mumbai extracted phytochemicals from neem leaves using water, ethanol, chloroform and acetone as solvents. They found alkaloids, flavonoids, saponins and tannins in the extracts. But the acetone extract lacked flavonoids and saponins. And terpenoids were present only in ethanol and aqueous extracts.

The team tested the effectiveness of these solvent extracts on goldfish, *Carassius auratus*. 'Goldfish is most susceptible to the parasite *Argulus*', says Pushpa Kumari, the leader of the research team. *Argulus japonicus* is a louse that infests fish. In the aquaculture industry, it spells disaster.

From sample goldfish, the researchers collected adult *A. japonicus* parasites and treated them separately with each of the organic and aqueous solvent extracts. When exposed to the ethanol extract, all the parasites perished after six hours. But only half of them died when treated with the acetone extract. This, they infer, may be due to terpenoids in the ethanolic extract. Though the ethanolic extract was effective, it was highly toxic to the goldfish because it had phenolic compounds. So they did not use the extract for their *in vivo* study.

For *in vivo* experiments, they used an aqueous extract of neem at different concentrations. They noted 100% antiparasitic efficacy at 3.5 grams per litre.

Since neem leaves are available throughout the year, and aqueous extracts can ward off infestation, aquaculture farmers and aquarium

enthusiasts now have a simple technique to keep fish free of louse.

DOI: 10.1016/j.aquaculture.2019.05.060

Ginger and Turmeric

Organic vs conventional cultivation

The North-East is a major contributor to the production of ginger and turmeric in India. In fact, the Lakadong turmeric genotype from Meghalaya and the Nadia ginger cultivar from Karbi Anglong, Assam have geographical indication tags due to superior quality. But what are the locations that yield the best ginger and turmeric? How can we improve productivity?

Last fortnight, scientists from two ICAR institutes in Meghalaya report testing seven different locations in the North-East for the yield and quality of the two crops.

Using contacts from Krishi Vigyan Kendras at different locations, they enlisted farmers who cultivated turmeric and ginger in the region.

Both are rhizomatous crops and prone to fungal infections. The researchers pitched a farmer-friendly fungus, *Trichoderma harzianum*, as fungicide. As organic manure, they used farm yard manure enriched with phosphorus-solubilising bacteria, vermicompost and neem cakes.

Yield, they noted, was consistently higher in organically cultivated ginger and turmeric at all seven locations. The researchers checked the amounts of active components – oleoresin in ginger and curcumin in turmeric – as a measure of quality. Organically cultivated ginger and turmeric were superior in this respect also.

There was wide variation in the yield and quality of the nutraceutical spices between different locations. Thus the 177 farmers who participated now know whether they should grow ginger or turmeric or both.

Interestingly, differences in temperatures at different altitudes had no significant effect on quality. Thus, rising global temperatures may not influence organically cultivated ginger and turmeric in the region.

DOI: 10.1016/j.indcrop.2019.04.068

Ready-to-eat Rice

Enhanced with iron and folic acid

Micronutrients are essential for healthy cell growth. Red blood cells need iron and the nervous system needs folic acid. Deficiencies of these micronutrients are a public health problem in developing countries, especially among underprivileged children and natural disaster victims.

Can we enrich staple foods with micronutrients? And make them cheaper and more readily available?

Last fortnight, scientists from the Tezpur University, India and the Queen's University, UK reported a solution: ready-to-eat parboiled rice fortified with iron and folic acid. They chose a rice variety called *chokuwa* from Assam. Amylose content in this rice is low. So, after parboiling and dehusking, rehydrating by soaking in warm water is adequate to make the rice soft and ready to eat.

The researchers soaked the rice in iron and folic acid solution for parboiling at 60°C for 90 minutes. After draining and steaming the rice, they dried and milled it to remove husk. Then, they soaked the fortified rice in warm water to ready-to-eat softness and calculated how much iron and folic acid would be released into blood after digestion. Using a three-step process with amylase, pepsin and pancreatin-bile to simulate the human digestive system, the researchers proceeded to test the rice.

When they analysed the digested fraction, they found that about 60% of iron and a lot more folic acid are easily absorbed by the intestines.

Analysing the digested mineral fraction to see how much iron and folic acid was transported to model cells for active effect, they found that nearly 15 to 30 per cent iron and folic acid became bioavailable in the fortified rice against negligible amounts in unfortified parboiled rice.

The activity of digestive enzyme hydrolysing starch to release glucose showed that the glycemic index of the parboiled rice was in the low to medium range.

The lower glycemic index makes it less harmful for diabetics. Higher iron and folic acid content in the fortified parboiled rice can also be a boon for

women and children with anaemia and folic acid deficiency. And this ready-to-eat micronutrient rich rice is faster to produce, say the researchers.

While waiting for varieties that are fortified with micronutrients using genetic manipulation, here is an immediate and easy solution to micronutrient deficiency in our populations.

DOI: 10.1016/j.foodchem.2019.04.044

Human Donor Milk Components

Affected by Holder pasteurisation

Human breast milk provides essential nutrients and immune substances to infants. But, sometimes, infants cannot receive their mother's milk. To ensure the survival of such children, milk banks are often set up in hospitals.

To kill harmful microorganisms in human milk banks, Holder pasteurisation – heating milk to 62.5°C for 30 minutes – is a method used worldwide. Though we know that pasteurisation affects the vitamin C and D content of milk, the impact on the immunological components of human milk has not been adequately investigated.

Last fortnight B. Adhisivam and team from JIPMER, Puducherry reported the macronutrient and immunoglobulin profile of pooled donor human milk before and after pasteurisation.

JIPMER has a milk bank that stores breast milk from lactating healthy mothers under sterile conditions. The researchers collected samples from 90 mothers, 5 to 10 days after delivery. They randomly paired and pooled donor milk from thirty for analysis. The researchers used a human milk analyser to calculate the energy content of milk before and after Holder pasteurisation. They observed a reduction in protein by more than 10%, fat by about 25%, and energy content by more than 15%, after pasteurisation. Carbohydrate content did not show any significant difference.

The team also measured IgA and IgG immunoglobulin profiles in the samples. They observed a reduction in immunoglobulin A by 30% and immunoglobulin G by 60%.

Holder pasteurization affects nutrition in human milk. And the immunoglobulins that can protect the newborn are reduced even more. However, Holder pasteurised donor milk is a lifesaver when infants cannot receive mother's milk, say the researchers.

DOI: 10.1080/14767058.2018.1455089

Coronary Angioplasty

Not useful before bypass?

The established treatment for ischemic heart disease is to surgically graft a bypass to the affected coronary artery. Before coronary artery bypass surgery, cardiologists often advise coronary angioplasty. However, research on the issue has been ambivalent. Researchers have reported both the positive and negative effects of prior angioplasty on the outcome of bypass surgery. A clear consensus for action on high-risk populations with severe left ventricular dysfunction is sorely needed.

A team of cardiologists from around the world, including Jyotsna Maddury from the Nizam's Institute of Medical Sciences, Hyderabad now report how coronary angioplasty prior to bypass grafting affects the risk of mortality.

The team had earlier carried out a randomised clinical trial on surgical treatment for ischemic heart failure. There, they compared bypass surgery along with standard medication with treatment by medication alone to see how each affected the risk of mortality. From over 20 countries, the team took around 1200 patients with multi-vessel coronary artery disease and severe left ventricular dysfunction. Patients were randomized to standard medical treatment with or without bypass grafting and followed up for about 10 years. The team found that bypass grafting plus medication is superior to medication alone in reducing mortality in such patients.

For the current study, the researchers retrospectively analysed data from patients in the surgical treatment trial who had undergone percutaneous coronary angioplasty before enrolling for the trial. Of the 610 patients randomised to bypass grafting, 13% had prior coronary angioplasty. From this data, the researchers found that

angioplasty, before coronary artery bypass grafting, did not affect the prognosis for patients with ischemic heart disease and severe left ventricular dysfunction.

In light of this result, Indian cardiologists might need to reconsider advising angioplasty before bypass grafting for patients with ischemic heart diseases.

DOI: 10.1016/j.ijcard.2019.03.029

Diabetic Neuropathy

Half of those with diabetes for more than 25 years develop diabetic neuropathy, a degenerative disorder of the peripheral nervous system. Presently, controlling blood sugar is the only way to control progression to neuropathy.

Hefazat Hussain Siddiqui and a team of researchers from four institutions in Lucknow evaluated the cure of diabetic neuropathy using the root bark extract of *Onosma echinoides*, an herb flagged by ethnobotanists for its anticancer, antiseptic and antihelminthic properties.

The scientists used neuroblastoma cell lines to evaluate the hexane extract of *O. echinoides*.

They grouped the cells with different concentrations of *O. echinoides* extract and different concentrations of glucose. The researchers evaluated the reactive oxygen stress as well as the antiproliferative, and apoptotic activity of *O. echinoides*. They observed more cell shrinkage and apoptosis in high glucose cells than in cells with treated normal glucose and in control groups.

Using high performance liquid chromatography, the team found shikonin in all the extracts. Shikonin, a reddish plant pigment, is reported to protect organs from immune damage. However, the extract requires further clinical trials before it can be used for diabetic neuropathy, says Siddiqui, Integral University, Lucknow.

DOI: 10.1080/14786419.2018.1460838

Caffeine Slows Cancer Growth?

Conventionally used chemo-drugs and radiation therapy fail to control cancer. So researchers have been trying an alternate approach: activating the immune system to counteract

the cancerous growth. However, identifying new molecules that can act on the immune system is a challenge.

There are plant derived chemicals that can stimulate the immune response. Caffeine isolated from coffee beans is known to reduce the risk of cancer. But the mechanism of action remains unclear. How does caffeine affect cancer growth?

Studies have shown that caffeine can bind to the adenosine T-lymphocytes receptor of neuronal cells. T lymphocytes of the immune system also express adenosine receptors. Could this be the target for caffeine in cancer?

Prasanta Kumar Nayak and team at IIT BHU decided to investigate whether caffeine can indeed activate cytotoxic T-lymphocytes. The researchers hypothesized that this can inhibit cancer progression.

So they experimented with mice models where tumour was induced using 3-methylcholanthrene, a carcinogen. After tumour development, caffeine was given through drinking water to one group of mice for eight weeks with a dose equivalent to human consumption. It is estimated that a cup of coffee contains an average of 100 milligrams of caffeine. The other group received drinking water without caffeine. Caffeine-treated mice showed a significant reduction in tumour volume. The researchers say that this anti-tumour activity is perhaps due to the competition of caffeine with adenosine for binding to adenosine receptors.

Caffeine binding to adenosine receptors reduces the expression of the cell surface receptor, programmed cell death protein (PD-1), a key molecule on T-lymphocytes. As a result, there is an increase in cytokine production which allows the T-lymphocytes to attack cancer cells. Thus, the blocking of the adenosine receptor pathway by caffeine enhances the anti-tumour immune response.

More experiments with secondary metabolites from plants that stimulate the immune system will unravel the factors behind oncogenesis to reduce cancer risk.

DOI: 10.1016/j.ejphar.2019.172538

Mirror Therapy For stroke recovery

In stroke, life changes in the blink of an eye but the road to recovery is long and slow. If the lower limbs are affected, the patient's mobility is compromised. The conventional approach consists of physiotherapy – passive movements and weight-bearing training of the affected limb.

Rehabilitation using mirrors to create illusions about the affected part has been explored for phantom limb pain, Parkinsonism and upper limb stroke. The technique relies on the bilateral symmetry of the human body. But such mirror therapy has not yet been explored in detail in patients with chronic stroke affecting the lower limbs.

Kamal Narayan Arya and team from the National Institute for Persons with Physical Disabilities, New Delhi recently reported testing mirror therapy for rehabilitating lower limb functioning after stroke. They included patients between 30 and 60 years suffering with stroke-induced immobility for more than 6 months. And excluded those with pre-existing cardiovascular, neurological and cognitive disorders or with complete immobility.

Seventy-six patients were randomly assigned to conventional physiotherapy or additional mirror therapy. Both groups received 30 physiotherapy sessions over three months.

For the therapy, the team seated patients with a mirror in between their legs such that the affected leg was hidden behind the mirror. The normal leg and its reflection gave an illusion of two functioning legs.

The patients then performed rolling, pedalling, wiping and shifting-pillow movements. The team noted unintentional mirrored movements in the paralytic limb in more than half the patients.

After three months, the researchers compared outcomes between the two groups. And they found significant improvement in movements involving flexion. Though there was not much difference in gait speed, gait deviation improved.

'Optical illusion-based therapy can enhance neuronal recovery', says

Shanta Pandian, Pt Deendayal Upadhyaya National Institute for Person with Physical Disabilities, New Delhi. 'And it is cost effective and safe', adds her colleague, Vikas Kumar.

'Stroke prevalence in India is about one to five people per thousand – greater in urban areas where such interventions are easily set up', says Kamal Narayan Arya, the leader of the research team.

This method to speed up stroke recovery can be easily mirrored around the nation to improve the quality of life for the stroke-affected.

DOI: 10.1080/09602011.2017.1377087

Immobilizing Microalgae To treat wastewater

To treat wastewater, we commonly use *Chlorella vulgaris*. But these microalgae get dispersed in water when used as free cells. And harvesting them afterwards poses challenges.

Scientists have tried immobilizing the microalgae with sodium alginate beads. However, nutrient removal rate and algal yield need improvement. Can we tweak the process by optimising the pH, and the concentrations of sodium alginate and calcium chloride to enhance the cleaning of municipal wastewater?

Shantanu Banerjee and colleagues from IIT-Dhanbad decided to evaluate parameters influencing efficiency. They mixed algal suspensions with different alginate concentrations to make immobilized beads. And dropped the suspensions carefully into calcium chloride solution to make small soft beads that harden slowly over 12 hours.

To test efficacy, the researchers collected wastewater samples from a municipal secondary stream in Jharkhand and measured algal growth and nutrient removal with the beads. Algal cells, embedded in alginate beads at a 3% concentration, showed optimal growth. Starting with seven million algal cells per bead, the team found a thirteen-fold yield in 5 days at con-

trolled conditions. Algal growth within the beads can be easily harvested to produce biofuel or fertilisers, making the process economically attractive.

When the researchers analysed the wastewater, they found rapid reduction in ammonium in the initial 24 hours at the optimal concentration. Phosphorus declined sharply in 8 hours and stabilized further. Phosphorus adsorption increased at high pH. And there was higher adsorption of ammonia at normal pH.

Increasing calcium chloride concentration, while making the beads, increased the rate of ammonium removal. This, the scientists say, is due to a greater degree of crosslinking in the alginate beads and increased porosity, leading to more active sites for ammonium ions.

By identifying the factors to be considered when dealing with wastewater with different concentrations of nutrients and by optimising the parameters, the scientists have developed a convenient process for nutrient removal from wastewater. Municipal corporations can now adopt and scale up the process for managing wastewater and producing fertilisers/fuel.

DOI: 10.1016/j.bej.2019.107241

Greenhouse Gas Emission Contribution by colleges

We usually assume that it is industries and automobiles that pollute our environment. But we lack accurate and adequate data on the contribution of institutes of higher education to the overall carbon footprint.

Recently, Sunil D. Kulkarni from the Sir Parashurambhau College, Pune reported developing a method to evaluate his college's carbon footprint. He classified emission sources into direct emitters, indirect emitters and electricity in line with IPCC guidelines. However, he did not take non-academic activities – painting, renovation and refurbishment – into consideration.

Sunil mapped the boundaries of departments and various other areas

of the campus. From water and electricity bills, he calculated emission in terms of CO₂ equivalents using existing conversion factors. He faced a challenge in quantifying indirect emitters, for lack of conversion factors. To overcome this, he derived CO₂ equivalents for those sources from their chemical properties. With questionnaires, he estimated emission due to fuel consumption for transportation. Adding all emissions for 2015–16, he came to an estimate of the net carbon footprint.

For the next two years, he continued to collect data. The average net carbon footprint for three years was approximately 3630 tonnes of CO₂ equivalents!

Sunil's department-wise carbon footprint analysis shows that more than 40 per cent comes from science departments. While humanities contributed 19 per cent, the commerce department contributed only 14 per cent. All others contributed 23 per cent. Sunil attributes the high emission in science departments to higher population, laboratory emissions and waste.

Extending the method to other colleges in Pune from available information, he also estimated their net CO₂ emissions. He estimates that the educational sector contributes around two per cent to overall carbon footprint in Pune.

The study is an eye-opener for educational administrators to take evidence-based decisions to reduce the carbon footprint.

DOI: 10.1016/j.jclepro.2019.05.194

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ACKNOWLEDGEMENT: IISER Pune and the workshop on science writing conducted at the Aravind Eye Care System, Madurai, where most of the reports were written.

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