Science Last Fortnight

Farmed Edible Oyster

Healthier than wild ones

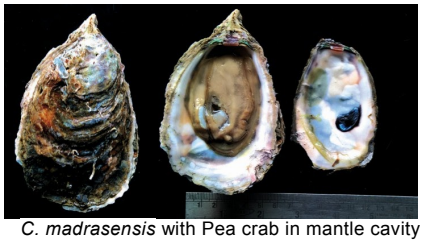
Indian backwater oyster, *Crassostrea madrasensis* (or *Magallana bilineata*) is a commercially cultivated edible oyster species. It is a source of livelihood and empowerment to Kerala's coastal communities. In its native habitat, the oyster is known to have many parasitic infestations. How do they fare in aquaculture conditions?

V. Kripa and team from the Central Marine Fisheries Research Institute, Kochi conducted a comparative study on wild and farmed *C. madrasensis* from the Vembanad Lake in Kerala. They collected oyster samples from natural intertidal reefs and from an aquafarm during the dry summer and the wet monsoon seasons. They also recorded the temperature and salinity of the water.

The presence of parasites, particularly the protozoan parasite, *Perkinsus beihaiensis*, accounts for high intensity infections in the Tuticorin Bay. However, in the population under study, it showed low prevalence. Comparatively higher prevalence of infection was seen during the dry season, which may be due to higher temperature and salinity that favours parasite proliferation, says N. K. Sanil, CMFRI, Kochi.

Histology studies revealed infections by the Pea crab, *Pinnotheres* sp., which damages the gills, the mud worm, *Polydora*, which causes shell blisters, *Cliona* sp. which causes shell porosis, and boring sponge infections that interfere with shell opening and closing, hampering food intake. This affects growth and leads to deformed shells. The researchers were happy to find that the prevalence of these parasites was relatively low in the commercially cultivated population under study.

The mere presence of parasites in a population does not implicate poor population health. Oysters can also serve as intermediate host to commensalistic reef fauna. The researchers examined various infection parameters including prevalence, intensity and the condition index of the infected organisms. A lower value of this index in wild populations may be due to their closeness to the shore which directly exposes them to anthropogenic effluents and greater stress levels, say the researchers.



C. madrasensis with Pea crab in mantle cavity
Image: G. Suja

The results from the study show that culture methods used for the oysters are beneficial for oyster farming. However, periodic surveillance is strongly recommended for better control over the chances of disease outbreaks in cultures, says K. Sunil Mohamed, CMFRI, Kochi.

'Our study provides baseline data to help manage the health of wild and farmed Indian edible oyster populations' says G. Suja, CMFRI, Kochi.

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Resisting Bacterial Resistance

Banana peels to the rescue

T. Ramanathan at the Annamalai University has been searching for molecules that can overcome the virulence of bacteria that are resistant to modern antibiotics. When he came to know that banana leaf and fruit peel have been traditionally used in Africa and South Asia for treating burns and wounds, he and his colleague K. Vijayakumar decided to investigate the claim.

They peeled the fruits of *Musa acuminata*, dried the peels, powdered and extracted the phytochemicals with different kinds of solvents – petroleum ether, chloroform, ethyl acetate and methanol – to search for the active ingredients. Then they tested the extracts on *Pseudomonas aeruginosa*, a bacterium known to be involved in skin/wound infections such as burn wound sepsis, hot-foot syndrome, necrotizing fasciitis, subcutaneous nodules, ecthyma gangrenosum, gangrenous cellulitis and surgical site infections. The bacteria are also known to form biofilms to protect themselves against antibiotics. The researchers found that the methanol extract inhibited biofilm formation.

Now the question was: how does it inhibit quorum sensing?

The duo found that the methanol extract at concentrations above 600 micrograms per millilitre inhibits bacterial growth. So they started testing it at lower concentrations and found that 400 micrograms per millilitre is adequate to inhibit biofilm formation without inhibiting growth. The polysaccharides that are secreted for biofilm formation reduced by more than 80%.

Can the extract disrupt the biofilm even after it is formed? Microscopic examination showed that the mature biofilm was disrupted and the bacteria broke up into microcolonies.

Next the researchers checked the known virulence factors of *P. aeruginosa* – LasA protease, LasB elastase, protease, pyocyanin, alginate and rhamnolipid production. Spectrophotometric assessment showed that these factors were reduced when the bacterium was treated with the methanol extract.

So it was clear that the treatment with the methanol extract is impacting the transcription of the genes. The team followed up with experiments using real-time quantitative PCR. And they found four genes known to be involved with the development of antibiotic resistance in the bacterium. All four genes were downregulated.

Now the remaining issue was to identify the candidate molecules that might be responsible for the effect. The team fractionated the extract and identified the fraction that showed the effects. They used gas chromatography coupled with mass spectrometry and found twenty different compounds in the fraction. Three of them seemed to be the candidate molecules. So they tested each, individually. One of them

could inhibit biofilm formation effectively. And the molecule was 5-Hydroxymethylfurfural. Adding the other compounds did not show any synergistic action. So 5-Hydroxymethylfurfural is indeed the active ingredient in the banana peel that can stop the bacteria from being virulent, without inhibiting the growth of the bacteria.

Image: Ahmad Fuad Morad via Flckr

Though it might take some time to initiate clinical trials for use in humans, banana peels can be used in veterinary medicine as adjuvant to antibiotics.

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No Till, No Burn

But fertilise with N

Organic matter in soil retains plant nutrients, improves soil aggregation, reduces soil erosion, and enhances water holding capacity. However, when crops are grown, the mineralization of soil organic carbon depletes soil stocks by 30–60% – more than the case with natural vegetation. So soil management strategies are needed to increase soil organic carbon stocks in agricultural lands.

Traditional tillage reduces soil aggregation leading to loss of carbon content in the soil. Stubble burning also tends to reduce soil organic carbon. It is also reported that nitrogen fertilisation helps build up soil organic carbon. But the combination of no tillage, stubble retention and nitrogen fertilisation show inconsistent results in terms of the various fractions of carbon such as stabilized carbon, charcoal and labile carbon. Moreover, long-term studies on the combination are far too few to make farmers confident of adopting these soil management practices.

When Pramod Jha from the Indian Institute of Soil Science, Bhopal went to Australia, he found an opportunity to check – the Hermitage Research Station of the University of Queensland had agricultural plots that were not tilled from 1968, years before Masanobu Fukuoka published 'One Straw Revolution' which initiated organic farming in many parts of the world. Here was a chance to test conventional tillage against no-tillage, retaining stubble against stubble burning and fertilisation with different amounts of nitrogen. With help from Australian colleagues, he set up 12 different treatments in a randomised block design.

Conventional tillage, stubble burning and no-nitrogen fertilisation led to lowest carbon sequestration. There was an increase in soil organic carbon when plots were fertilised with nitrogen per hectare – an 18% increase with 90 kilograms of nitrogen per hectare per year. After 50 years of no-tillage, stubble retention and application of nitrogen, the increase in soil organic carbon was 22.2% more than that found with conventional tillage, stubble burning and no-nitrogen fertilisation.

'Burning stubble increased the charcoal carbon concentration in the soil. It also increased the nitrate–nitrogen concentration. But the stabilized carbon concentration decreased and the metabolic quotient, an indicator of ecological disturbance, was significantly high,' says Pramod Jha.

If farmers in Punjab and Haryana follow these soil management practices, people in the Gangetic plains will breathe easy next winter and India will move closer to the goals of carbon sequestration.

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Persistent Pollutants

Predicting from descriptors

There are hundreds of toxic organic chemicals that pollute the air, water and soil. Some of them are not degraded easily. They persist in the environment and are transported from the location where they are generated. Often, we recognise the problem much later, after a lot of research and only after harm is done. Experimental data on all such chemicals is difficult to generate. Is it possible to theoretically predict the persistence of a chemical based on its structure and composition?

Last fortnight, Pathan Mohsin Khan from NIPER, Kolkata and an international team of collaborators identified 302 persistent organic pollutants and collected available experimental data on their half-life.

They also extracted descriptors of the structures of these molecules focusing on quantifiable descriptors with definite physicochemical meaning. For example, connectivity indices that give topological information of molecular structure, constitutional indices that represent percentage of elements, atom type E-state indices that indicate intrinsic pseudo connectivity and 2D atom pairs that provide spectral information. Since many organic compounds have ring structures, they also took ring descriptors that denote the number of rings, the size and perimeter of the molecule as well as functional group counts and atom-centred fragments. To keep the computation power required to a minimum, they took into account only two-dimensional descriptors and avoided three-dimensional ones.

Now the question was – what are the features of the molecules that make them persistent? There are many algorithms for feature selection. The researchers used a genetic algorithm to select the most relevant features. They selected the descriptors for 227 molecules for training, to select 25 variables that might be helpful for predicting the persistence of organic molecules in air.

To deal with 25 variables is difficult. So what is the best combination of descriptors among the selected variables? To find out, they used best subset selection and homed in on six two-dimensional descriptors. Using partial least squares regression they extracted three latent variables from the six two-dimensional variables.

They tested the theoretical model using the descriptors for the remaining 75 molecules out of the 302 pollutants.

'This is a simple, but robust model', says Pathan Mohsin Khan, NIPER, Kolkata.

'Hydrophobicity, presence of hydrogen bond acceptor atoms, number of halogen atoms and presence of R–CH-X fragments in the molecules are the main factors that increase the half-life of organic chemicals in the air', says Kunal Roy, Jadavpur University.

Now, with this model, it is easy to predict the atmospheric persistence of chemical compounds quickly, using only the knowledge of the chemical structure of the compounds – a timely alternative to costly and time-consuming experimental methods.

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Fishing for Heavy Metals

Scaling up remediation

Poisonous heavy metals like cadmium and lead, discharged from factories, mines, batteries, paints, etc., contaminate rivers and soils. These heavy metals enter the food chain, causing harmful effects to living organisms including humans. Accumulation of lead and cadmium causes damage in kidney, liver, bone and the reproductive system.

Recently, Divya Pal and Subodh Kumar Maiti from IIT Dhanbad proposed an easy way to remove the heavy metals from a highly contaminated aquatic pond in the vicinity of the coal city, Dhanbad. The team used fish scales as absorbent material to immobilize cadmium and lead in the sediments.

Image: Rajesh Dangi via Wikicommons

Fish scales are easily available waste. The scales contain collagen fibres and hydroxyapatite besides some calcium compounds. Both collagen fibres and hydroxyapatite are known to be biosorbents and immobilize dyes and metals.

However, raw fish scales could absorb less than 50% of cadmium and lead metal ions. The researchers thought that, if unwanted minerals and organic matter are removed from raw fish scales, efficiency might increase. They tried different methods. Treating with alkali, treating with acid, hydrothermal treatment… Ultimately they realised that hydrothermal treatment along with acid for three hours at 150 degrees Celsius is the best.

The material is highly porous with a surface area of more than a hundred square metres per gram. Biosorbents with particle size of about 300 micrometres were most effective at pH 6.89, very close to the neutral pH.

The research team found that sediments treated with this material can immobilize more than 90% of cadmium and lead metal ions. Once adsorbed, the metal ions did not leach out easily.

An estimated 18 to 30 million tonnes of fish waste is generated annually and mostly discarded or dumped in the land or sea leading to environmental and health problems, and undesirable odours from dumping sites. Using fish scales to remove heavy metals from contaminated water offers a viable, cheap and environmental-friendly approach to reduce toxic metals and also minimize the impact of fisheries on the aquatic environment.

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Spirulina platensis

For organic dye degradation

Organic dyes released from the textile, paper, pharmaceutical, printing, leather and cosmetic industries pollute water. While photo-catalysts can be used to degrade many of these polluting chemicals, the metal-oxide nanoparticles used for the purpose leave residues in water.

Reports show that many algae are also capable of degrading dyes. These algae accumulate the toxic substances which then need to be harvested. But it is known that some blue-green algae, such as *Spirulina platensis*, also secrete photosensitive proteins. And this is available as waste from raceway ponds where the organism is cultivated for nutraceutical uses. Can we use the photosensitiser from the waste of algal cultivation to remediate water polluted by dyes?

M. Chamundeswari and P. Saravanan from the St Joseph's College of Engineering, Chennai set the problem to their research scholar, Sharmila. The team collected the water from the raceway ponds at three different companies. The water was greenish and contained some amount of microalgae. They filtered the algal cells out to test the cell-less water.

The team selected three different dyes: methylene blue, malachite green and Congo red, released from industries. They mixed the dyes with the *S. platensis*-cultivated wastewater in different concentrations and kept these mixtures in the dark as control and in the presence of sunlight under different temperature ranges, for three hours and kept sampling the water every 30 minutes. From the absorbance levels they calculated the percentage of dye degradation.

'Methylene blue and malachite green took only one hour to degrade. But Congo red took three hours. The mixture of organic dyes was completely decolourised and degraded under sunlight within three hours', says Sharmila, St Joseph's College of Engineering, Chennai.

In control experiments – in dark conditions – this was not the case.

The wastewater from the raceway ponds contained phycobiliproteins along with traces of metal ions. In the presence of sunlight, phycobiliproteins form complexes with metal ions and become active in dye degradation, says Chamundeswari.

It is a simple, light-induced dye degrading method, without any chemicals. We are using wastewater from one industry to treat the wastewater from others, says P. Saravanan.

'This cost effective method could open new doors for sewage treatment plants', adds M. Chamundeswari.

The amounts of the photosensitive proteins in the wastewater from the three companies varied. So it is

important to standardise and to investigate further, says Sharmila.

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Wastewater Woes

Sandy solution

One of the first water purification techniques taught to us in school was using sand and activated charcoal filters. Now, researchers from the Shiv Nadar University in UP have added ultrasonication to this simple idea for cleaning wastewater from paper mills.

The researchers did not use sand from river beds. Instead they took sand obtained while mining a bore well in Ghaziabad, from a confined aquifer about 55 metres below. The thick greyish-black sand had clay as well as carbon nanotubes along with a cubic atomic network mesh of low atomic number elements – silicon, calcium and aluminium – useful in adsorbing heavy metal contaminants.

The researchers used secondary treated paper-mill wastewater as model pollutant sample. They mixed the wastewater sample with sand samples and sonicated this mixture using a 30 kilohertz frequency for two hours and allowed it to sediment.

Ultrasonic waves create successive regions of positive and negative pressure causing nano-to-micro-sized air bubbles whose volume increases on approaching the compressed region. When the bubbles collapse, they release high pressures of about 400 bars and increase the local temperature to about 4500 degrees Celsius.

These hotspots increase the mechanical force needed to break the closed ring structures in tannins and lignins present in wastewater, accelerating degradation, say the researchers.

The sonication released gases and these were absorbed by the carbon nanotubes.

The heavy metals coagulated and sedimented with the sand grains. The size of the sand grains before absorption was 100 to 350 nanometres. After adsorption the grains were more than 800 nanometres. 'The cubic atomic network meshes of silicon, calcium and aluminium have a role to play in the removal of heavy metal ions,' says Vaibhav Shrivastava, Shiv Nadar University, Uttar Pradesh.

The wastewater, which was highly alkaline, shifted to a near-neutral pH and salinity decreased to near potable quality. The greyish colour of the wastewater reduced by about 95% and chemical oxygen demand decreased by nearly 90%.

The researchers now propose a low-cost prototype that can be useful as an economic solution for wastewater treatment.

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A Prey–Predator Model

Refuge for prey, food for predator

More than a hundred years ago, Alfred Lotka, a physical chemist, examined prey–predator systems and found a curious phenomenon. When predators eat prey in large quantities, the prey population falls; the low availability of prey impacts predator population, which, therefore, start falling; when the predator population falls, the prey population increases, which, in turn, increases the predator population. This oscillating steady state was treated mathematically by Vito Volterra, a physicist and mathematician. Ever since, the Lotka–Volterra model has attracted researchers from ecology, environment and other fields.

But there is a problem with the model for practical applications. In reality, predators are not dependent on only one prey for food. And, often, prey has refuge where the predator cannot get to it. Moreover, the populations of both prey and predator are impacted by environmental changes.

G. P. Samanta, now a professor of mathematics at the Indian Institute of Engineering Science and Technology, Shibpur has been studying prey–predator models since the late eighties. Recently he, along with his research scholar, Amartya Das, came up with a prey–predator model that is closer to reality.

Additional food can, of course, distract the predator from prey leading to an increase in prey populations. Additional food can also lead to an increase in predator population leading to reduction in prey population. This depends on the quality and quantity of additional food. Similarly, if the refuge for prey is fool-proof, it can even lead to extinction of the predator, in the absence of any additional food. Besides the availability of additional food for the predator and refuge for the prey, prey–predator populations are threatened by pathogens and other environmental factors that impact the populations of both. These aspects were introduced as stochastic noise in the model, since all the factors involved are not known. The researchers then analysed the issues of extinction and persistence under various conditions mathematically. They used MATLAB for the numerical simulation of their model.

Thus they could work out the outcomes from various scenarios. 'If the prey population gets total refuge, predator population can be led towards extinction,' says Samanta. 'And, if prey population has no refuge, predator population can increase,' adds Amartya.

The prey–predator model with prey refuge as well as additional food for the predator showed that a stable coexistence is possible when there are more prey refuges. However, beyond a point, increase in prey refuge signals the extinction of the predator population. The model shows that a prey–predator system where prey refuge is dependent on the biomass of predators is more conducive to a steady state where both predators and prey can coexist and survive.

This model is better than the Lotka–Volterra model in complex situations as in research where predators of agricultural pests are released to bring down the pest infestation of crops.

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