



D. K. GOVERNMENT COLLEGE FOR WOMEN

Nellore, Andhra Pradesh- 524003

Autonomous College, College with Potential for Excellence

Re-accredited with "A" Grade by NAAC



Report on

COLLABORATIVE ACTIVITY OF

D.K.W CUBE NELLORE AND

CUBE , HBCSE, TIFR MUMBAI

CUBE

Collaborative
Undergraduate
Biology
Education



05/Jan/2021

COLLECTION OF EGGS

05/Jan/2021



A MALE FISH



B FEMALE FISH

"Education is not something we do to our students; it must be something we do in collaboration with our students" Brewer & Smith, 2011

CUBE

Collaboratively Understanding Biology Education

The **CUBE (Collaboratively Understanding Biology Education)** programme at the **Homi Bhabha Centre for Science Education (Tata Institute of Fundamental Research)** is a project-based science education program. It is designed to cultivate a scientific attitude within students in a collaborative and conversational learning environment. Formerly called the Collaborative Undergraduate Biology Education program, though most of its participants are undergraduate students and teachers, school students and teachers also join as and when feasible.

In this program, the participants work collaboratively on simple experiments using model organisms, like zebra fishes, fruit flies, earthworms, snails, *Moina*, butterflies, *Hydra*, rotifers, *C. elegans*, and *E. coli*, to observe their features and study biological phenomena, like olfaction, circadian rhythms, regeneration, hypoxia, biodiversity, etc. Students then discuss their observations with their peers, in the lab and on social media.

THE LEARNING OUTCOMES THROUGH CUBE.

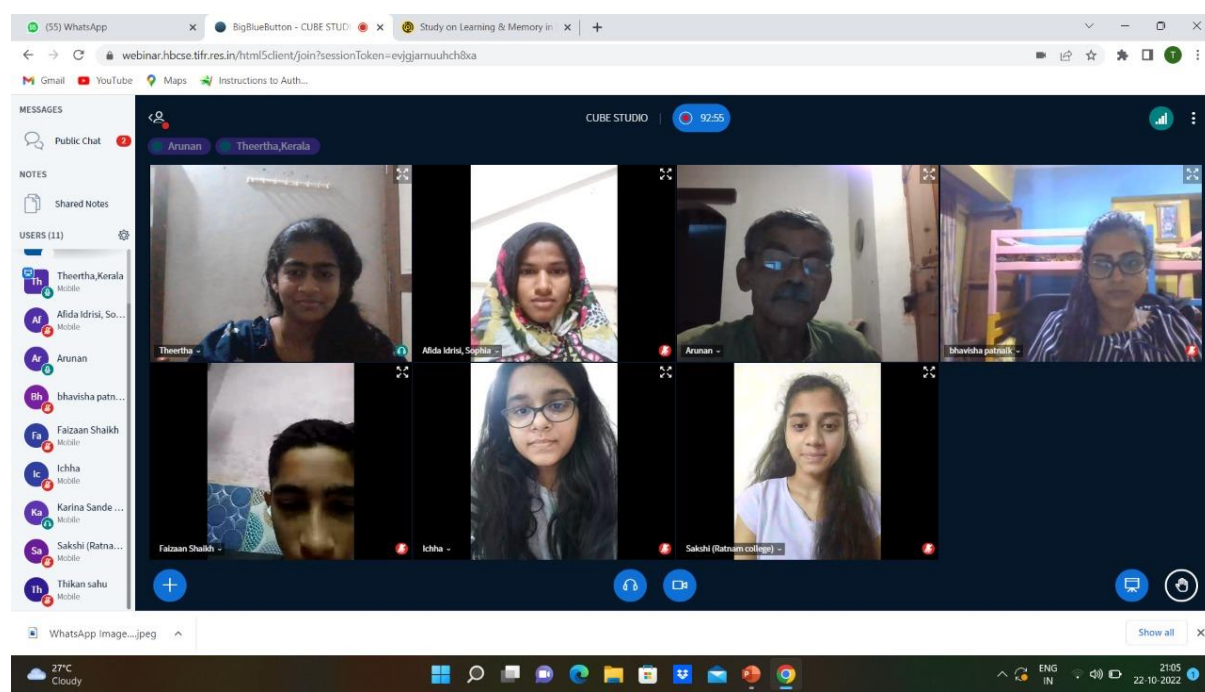
The **CUBE program** is engaging students with feedback mechanisms even during the lockdown. In addition to the home labs, about 30 students from across the country and their mentors meet daily (for about 3 hours) for discussions in an online forum called **CUBE ChatShaala**. The forum runs on the BigBlueButton webinar platform free and open-source software) hosted on HBCSE's server.

The **ChatShaala** is a conversational learning mode where students talk about their **work progress, challenges, goof-ups, designing experiments, about model organisms, and so on**. Several hot topics in biochemistry, molecular biology, developmental biology and neuroscience too.

CUBE NELLORE GROUP WORKING ON ZEBRA FISH MODEL SYSTEM



STUDENTS PARTICIPATION IN CUBE CHATSHAALA ON BIG BLUE BUTTON



CUBE NELLORE GROUP

B.Sc. Aquaculture students of D.K.Govt College for Women(A),Nellorecollege have been collaboratively working on **Zebra fish** model system from January 2020 to JULY 2022

During lockdown due to covid-19 zebra fish model system was carried out as Home lab work under guidance of M.C Arunan sir ,TIFR,Mumbai as CUBE NELLORE.

OBJECTIVES OF CUBE NELLORE

- To learn about the life cycle of a **zebra fish** ,
- To understand and expertise about maintain zebra fish at home and lab conditions ,
- To treat a diseased **zebra fish** by understanding symptoms and behaviour
- To understand about the diet requirements of a zebra fish .
- To learn about the feeding behaviour of a zebra fish ,
- To acquire knowledge of the role of circadian rhythms in feeding of zebra fish ,
- To observe the swimming patterns of a zebra fish ,
- To check the physical and chemical parameters of water to maintain a zebra fish.

- To investigate and study about toxicants and phenotypic exposure effects on zebra fish
- To experiment and to rear zebra fish through generation studies.
- To publish findings in quality journals.

ABOUT OUR MODEL ORGANISM

ZEBRA FISH



Why Zebra fish as a model system :

Since the 1960s, the Zebrafish (*Danio rerio*) has become increasingly important to scientific research. It has many characteristics that make it a valuable model for studying human genetics and disease.

Key facts:

- The use of zebra fish (*Danio rerio*) as a model organism began in the 1960s.
- The zebra fish is a tropical fish native to Southeast Asia.
- The zebra fish is about 2.5 cm to 4 cms long.
- In its larval stages it is transparent and as it matures to an adult it develops stripes that run along the length of the body and look blue in colour.
- Males are slender and torpedo –shaped usually with a pink or yellow tinge.
- Females tend to be less pink than the males and are fatter due to the eggs they carry
- The complete genome[?] Sequence of the zebra fish was published in 2013.
- Zebra fish have already been used to help unlock a number of the biological processes behind muscular dystrophy, and are an important model for understanding the mechanisms of development and diseases such as cancer.
- Its genome is 1,505,581,940 base pairs in length and contains 26,247 protein-coding genes.

Benefits of the zebrafish:

- The zebra fishes are small and robust.
- They are cheaper to maintain than mice.
- The zebrafish genome has been fully sequenced to a very high quality. This has enabled scientists to create mutations in more than 14,000 genes to study their function.
- Break of daylight triggers mating in zebra fish (many other fish only lay eggs in the dark).
- Zebrafish produce hundreds of offspring at weekly intervals providing scientists with an ample supply of embryos to study.
- They grow at an extremely fast rate, developing as much in a day as a human embryo develops in one month.
- Zebrafish embryos are nearly transparent which allows researchers to easily examine the development of internal structures. Every blood vessel in a living zebrafish embryo can be seen using just a low-power microscope.
- As zebra fish eggs are fertilised and develop outside the mother's body it is an ideal organism for studying early development.
- Zebrafish have a similar genetic structure to humans. They share 70 per cent of genes with us.
- 84% of genes known to be associated with human disease have a zebra fish counterpart.
- As a vertebrate, the zebra fish has the same major organs and tissues as humans. Their muscle, blood, kidney and eyes share many features with human systems.
- Zebra fish have the unique ability to repair heart muscle. For example, if part of their heart is removed they can grow it back in a matter of weeks. Scientists are working to find out the specific factors involved in this process to see if this will help us to develop ways of repairing the heart in humans with heart failure or who have suffered heart attacks.

Designing and construction of aquarium:

We made a glass aquarium of 45x20x20 cms dimensions with 30 litre water capacity, for our model system. For constructing an aquarium, we need 5 clean grease free glass slides of specified measurements and attached them using a silicone gel in form of a glass tank, after drying it for 2 days, we introduced our 15 zebra fishes in which 7 of them are females and 8

of them are males. Then we feed them with artemia as a live feed due to its benefits in fish diet.

Hatching Artemia:



Fig A : Hatched Artemia**Fig B :** microscopic view of a Artemia /Brine shrimp

Fig C : Unhatched artemia cyst

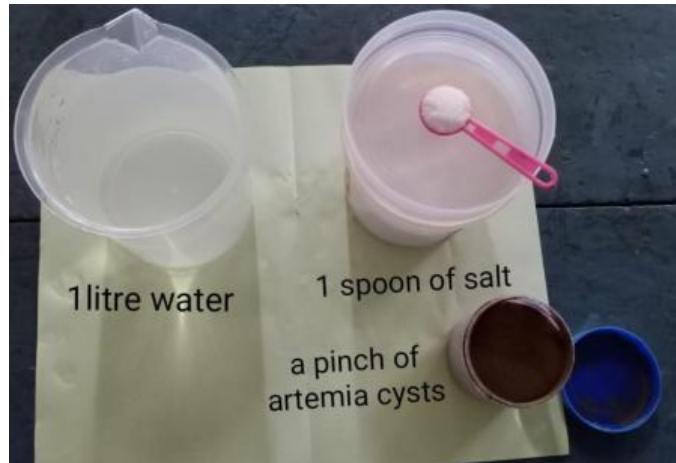
Artemia is a genus of aquatic crustaceans also known as **brine shrimp**. *Artemia*, the only genus in the family **Artemiidae**. *Artemia* populations are found worldwide in inland saltwater lakes, but not in oceans. *Artemia* are able to avoid cohabiting with most types of predators, such as fish, by their ability to live in waters of very high salinity (up to 25%). *Artemia* is used as a rapidly growing food source for farm fish, molluscs, and shrimp. The eggs are collected in bulk from salt lakes and shipped dry to aquaculture farms. The eggs can be stored dry until needed and then easily hatched in bulk as live food

The following steps will achieve optimum brine shrimp hatch rates.

- **Set Up:**
Place hatching cone or similarly shaped vessel in well-lit area. Cone should be semi-translucent for ease of harvesting and light transmission.

- **Add Water:**

Fill cone with water and add salt by 3 gms for 1 litre of water and adjust salinity to



25 ppt (parts per thousand). Optimum hatching temperature is 82°F (28°C).

- **Add Cysts:**

Add cysts at the rate of 0.5 gram per litre.

- **Aerate:**

Provide adequate aeration to keep cysts in suspension.

- **Hatch:**

Depending upon water temperature, cysts should hatch in approximately 18-36 hours.

- **Harvest:**

After hatching brine shrimp, turn off or remove aeration and wait several minutes for the shells and baby brine shrimp (or nauplii) to separate. Newly hatched nauplii will settle to the bottom of the cone or move towards a light source; the shells will float to the surface. Once separated, the nauplii can be siphoned from the bottom with a length of air tubing or gently drained through the bottom of the cone through a valve, if so equipped.

- **Rinse:**

The warm incubation temperatures and metabolites from the hatching medium create ideal conditions for a bacteria bloom. Rinsing of the baby brine shrimp in a fine mesh net or sieve using clean fresh or salt water is important before feeding them to your fish.

- **Clean Equipment:**

Tanks and brine shrimp hatching equipment should be cleaned and disinfected routinely.

Breeding

Zebrafish are usually at optimal breeding condition between ~3 and 18 months of age. Pairwise breeding should not be performed for two consecutive days¹¹; however, in-tank breeding can be performed daily as a tank can hold many fish which reduces the chance of the same pair of fish being bred for two days in a row. Breeding should be undertaken at regular intervals even if eggs are not required. This process will ensure the breeding cycle of the fish is maintained. It is recommended that there are more females than males in a



breeding set-up.

Male zebrafish change their female partners on a daily basis¹² which further supports this recommendation. Furthermore, within our laboratory we initially experienced problems with breeding, however, using more females than males in a breeding set-up helped solve the problem.

Moreover, feeding with a high protein content diet and brine shrimp two-three times a day, mixing fish from different tanks (from different parents), maintaining the temperature of the breeding set-up between 27 and 28 °C, and squeezing the bellies of females with blocked ovary tubes using gentle massage further improved egg production. We recommend keeping a record of fish lines/ origins to avoid in-breeding between siblings. This further improves embryo production.

Keeping a record of the number of embryos laid by fish from each tank is also recommended.



This assists with keeping a track of the best breeding fish tanks and taking measures to improve breeding in the fish not laying eggs.

EGG COLLECTION SET UP FOR COLLECTING ZEBRA EGGS

1. Zebrafish initiate breeding at the onset of light. Fertilized eggs can be obtained either through in-tank breeding or pairwise breeding. While in-tank breeding is more labour-efficient and is implemented for regular embryo collection in our laboratory, pairwise breeding is preferred when genes or mutations are to be screened from individual fish.
2. For in-tank breeding, assemble the in-tank breeder and drop slowly into the fish tank after the onset of light. Alternatively, in-tank breeding set-up can be left overnight in the fish tank.
3. Leave the in-tank breeder for around 15 min to allow the fish to mate before removing the breeder from the tank and collecting the eggs.
4. Pairwise breeding is usually set up late in the afternoon after feeding.
5. Assemble the breeding tank and fill it with aged system water.
6. Transfer one female and one male to opposite sides of the breeding tank. Females can be distinguished from males because of their bigger underbelly.



Fig: Showing the difference between fertilised and unfertilised zebra fish eggs.

7. Males can also be distinguished from females because they are more slender and darker in colour than females. Moreover, males have more yellow colouration in the anal fin compared to females. When in doubt look for the ovipositor in female zebrafish .
8. Remove the divider the next morning shortly after the onset of light. Allow mating to occur undisturbed for 20 min or until sufficient numbers of embryos are laid at the bottom of the tank



ZEBRA EGGS COLLECTED IN HOME LAB.

9. After breeding, return the fish to their tanks. Collect the eggs using a strainer.
10. Wash the embryos thoroughly with system water and Transfer the embryos to a Petri dish
11. Embryos can be observed under a microscope. Fertilized eggs are then separated from the unfertilized eggs using a needle and a pipette .

CUBE NELLORE STUDENTS OBSERVING ZEBRA EGGS UNDER MICROSCOPE



Raising of larvae:

1. Fertilized eggs are kept in a petridish separately undisturbed or in an incubator (~28.5 °C) for 72 hr until the larvae are hatched.



2. We do not feed the larvae because of the presence of yolk sac, as the feed was already reserved for it .
3. Now the larvae are out of the chorion and swimming freely they are ready to transfer to a main fish tank. Larvae need to be fed from 5 days post-fertilization (dpf) and are kept in embryo medium (composition described in Part 3, 10) or system water.
4. Larvae can be kept in round dishes with ~50% or more of water therein changed on a daily basis. The water change should include removing dead or diseased larvae and any other debris.
5. Transfer the larvae gently into a tank containing a small sized baffle (of around 300-400 microns). Dead and diseased larvae should be removed and a few milliliters of



water should be added slowly on a daily basis.

6. After 14 days, larvae tanks can be shelved into the system, and supplied with a small stream of cycling water (1-2 drops per second). As the larvae grow, water flow can be
7. increased. Different sizes of baffles can be used depending on the size of the larvae (e.g. baffle size 300-400, 500, 700-750, and 1000 microns) and a normal plastic baffle should be used for the adult fish.
8. It usually takes 3 months for the embryos to develop into sexually mature adult.

Challenges faced :



Due to fluctuations in water parameters many fishes face a much stress and even dies if conditions becomes more severe. Zebrafish should never be overfed as this may increase the nitrate level in the water, possibly affecting their breeding¹¹, or viability, as some fish may die due to overeating. We recommend providing no more food during any one feeding than a tank of fish can finish within 10 min. It is very important to remove salt from the brine shrimps before feeding them to the zebrafish as excess salt concentration causes death. If more zebrafish eggs are required, fish can be fed three times a day. Cleaning the breeder fish tanks daily also improves levels of egg production.

When feeding on the Aquatic Habitats systems we usually turn off the water pump and air pump to allow the fish to eat the food for 10 min. This decreases the amount of food that is washed into the filters. However, users must be careful to remember to turn on these pumps again afterwards.



Physical and chemical parameters of water :

Parameter	Optimum range
Alkalinity	50-150 mg/L CaCO ₃
pH	6.8-7.5 (6.0-8.5 tolerated)
Temperature	26-28.5 °C
Hardness	50-100 mg/L CaCO ₃
Un-ionized Ammonia	<0.02 mg/L
Nitrate (NO ₃ -) <50 mg/L	Nitrite (NO ₂ -) <0.1 mg/L
Dissolved oxygen	>6.0 mg/L
Salinity	0.5-1 g/L
Conductivity	300 -1,500 µS

Zebrafish Genome compared to Human

As previously mentioned, Zebrafish share a significant percentage of their genes with human beings.

Based on this understanding, studying the genetic material of Zebrafish can help better in understand of various diseases and conditions in human beings and even possibly discover how to treat such diseases.

Genetic mutations in human beings have been associated with various conditions. However, a researcher may wish to determine whether any loss of function of the gene does indeed cause a condition observed in an individual.

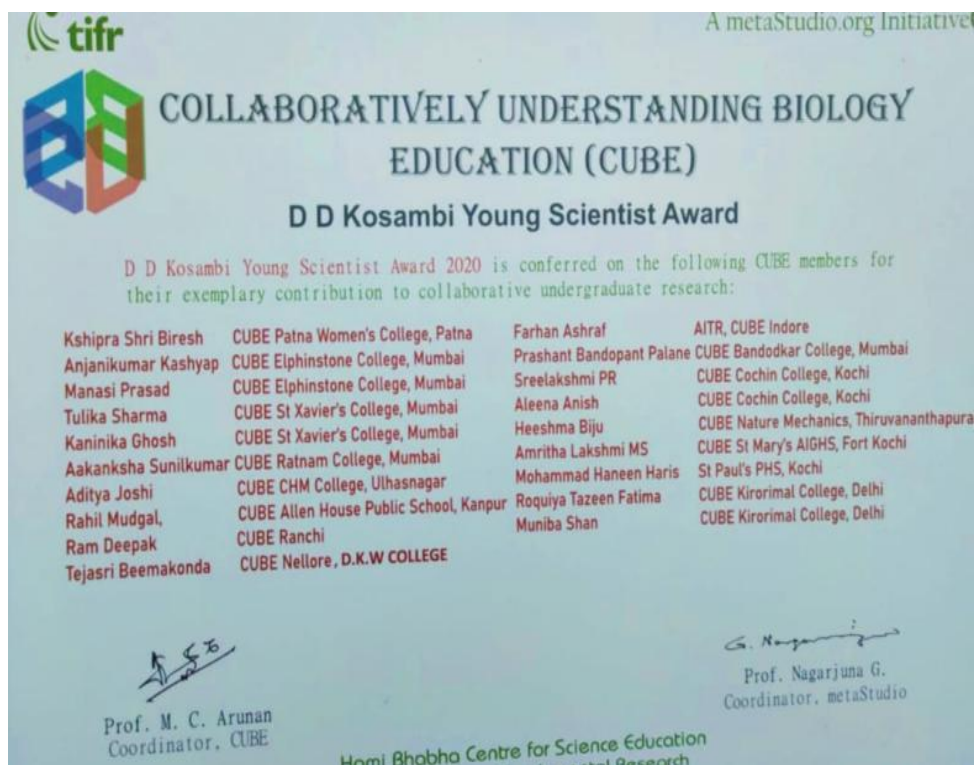
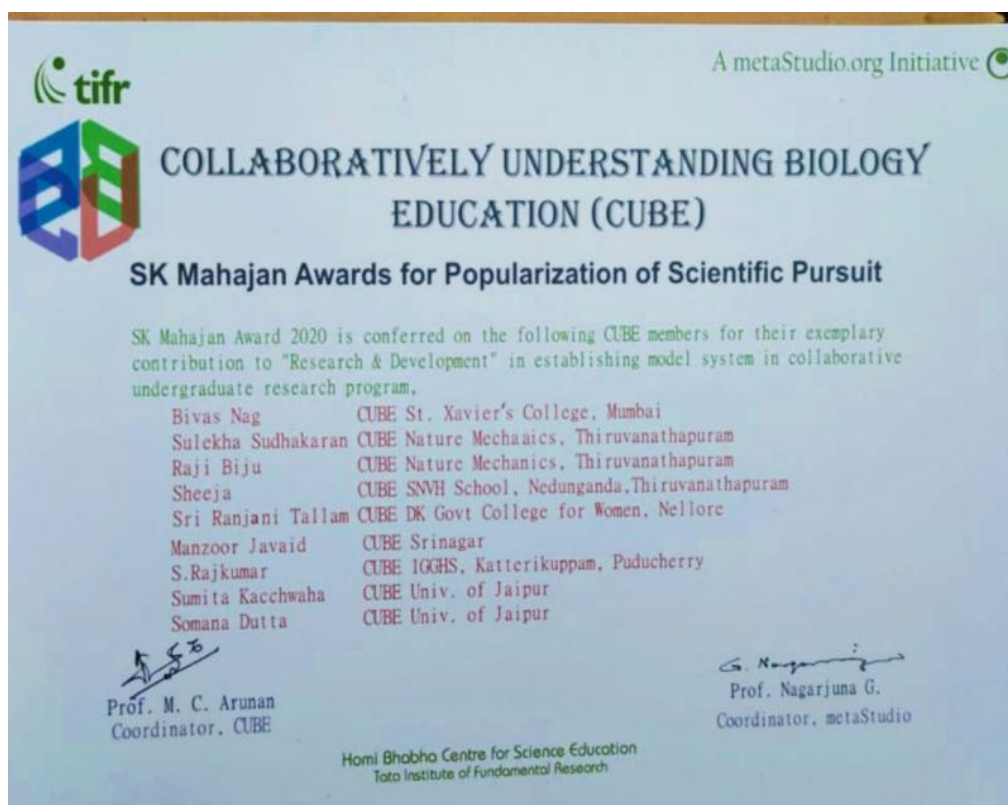
“potential scientists should be tapped at an early age onwards, from the academic hinterlands spread across the country.”



OUTCOMES

1. Students and faculty are learning about various model systems and gained lot of knowledge and expertise.
2. Working with different people around the country gave diverse set of experiences in collaboration and communication.
3. Faculty and students received appreciation awards and certificates from CUBE

CERTIFICATES RECEIVED FROM TIFR , MUMBAI





Collaboratively Understanding Biology Education(CUBE)

DD Kosambi Young Scientist Award 2022

is conferred to the following CUBE members for their exemplary contribution in Collaborative Research

Enas Shirin Fatma	Hira Public School, Tando, UP	Dhanraj Bhaskar Tribhuvan	Elphinstone College, Mumbai
Ushale Neha Hirahar	Elphinstone College, Mumbai	Mohammed Owais R. Pinjar	NES Ratnam College, Mumbai
Neha Ravaji Rane	RPD Jr College, Sawantwadi, Sindhudurg	Gaurav Mhaprakhar	KISSC, Somaiya Vidyavihar, Mumbai
Pritesh Shivram Parab	Elphinstone College, Mumbai	Anindita Mandal	KISSC, Somaiya Vidyavihar, Mumbai
Sakshi Vishwas Beloshe	NES Ratnam College, Mumbai	Parvinder Shreejit Sethumadhavan	KISSC, Somaiya Vidyavihar, Mumbai
Archita Goddu Rajphar	NES Ratnam College, Mumbai	Amey Kumar Mourya	NES Ratnam College, Mumbai
Ashrita Patra	St. Xavier's college, Mumbai	Iram sayyed	SSEH school, Mumbai
Meghraj V Kamath	MHSS, Goa	Faizan Martin Shaikh	Adarsha Vidyalaya, Mumbai
Rati Chaturvedi	ANIMS, Portblair	Ishita Sonowal	Elphinstone College, Mumbai
Manasi Ramu Prasad	Elphinstone College, Mumbai	Seethalakshmi C. R	SN College Nattika, Thrissur
Rehel Turkey	DSPMU, Ranchi	Tejasri Beemakonda	D.K. Govt Degree College, Nellore
Man Masish Beck	DSPMU, Ranchi	Vyom Gupta	Allen House Public School, Kanpur
Bhawna Solanki	Ramjas College, University of Delhi	Rahul Mudgal	Allen House Public School, Kanpur
Azamiya	University of Kerala, Kariavattom campus	Thikan Sahu	Chaitanya College, Pamgarh
Srujal Jain	Elphinstone College, Mumbai	Aarushi Chitkara	St. Xavier's college, Mumbai

Prof. M. C. Arora
Coordinator, CUBE

STEM CHALLENGES
A metaStudio.org Initiative

Prof. Nagayana G.
Coordinator, metaStudio

Kishore Bharati

CUBE Awards 2022

Inbox



Kishore Bharati 2 days ago

to sharibenas1090, ubaleneha01, ...

Dear all,

We are glad to announce that you are being felicitated with 'CUBE DD Kosambi Young Scientist Award 2022' for your exemplary contribution in collaborative research.

We wish you all the success in continuing this endeavor in science and look forward to your active participation in CUBE initiatives.

Regards,
CUBE_Kishore Bharati



Principal
D.K. Govt. College for Women (A)
NELLORE.