BIOME

The BIOlogy Education MEssenger

(An ATBS eNewsletter)

CONTENTS OF THIS ISSUE:

From The Editorial Team.....

The editorial team of Biome – the ATBS eNewsletter is happy to be here with the seventh issue! The Nobel Prizes have just been declared and it is a good time to celebrate by reading some academic content which we believe could be helpful to the teaching community of the biological sciences.

ATBS, as most of you are aware is the Association of Teachers in Biological Sciences. Anyone interested in biology is encouraged to become a member of the association and be a part of a common platform to interact with other like-minded people who would like to improve the teaching of biology. The intent of bringing out these issues of the eNewsletter is to put forth thoughts and ideas of biology teachers across for others in the subject area to comment, deliberate and maybe practice them in your regular biology classrooms.

In this issue we have the second part of the two-part article by Dr. Sasikumar Menon titled 'The Avian Flight of Endurance'. In case you missed reading part one of this article, you can find it in issue 6 at http://www.atbs.in/newsletter.html. Also included is an article bringing to light an interesting twist in the history of cell and molecular biology. This article is titled 'A History of the Correction of the Human Chromosome Number' and is written by Prof. B. B. Nath. The theoretical test items included in the various levels of the Indian Biology Olympiad Programme and the student item response data obtained post the tests is a useful tool to analyse the students' understanding of several basic concepts in biology. The article 'Analysis of Students' responses to a question on renal portal system' by Prof. Rekha Vartak shares some such findings. In the Biology can be Fun section, we attempt to share with you readers some experimental task which students can enjoy doing in the biology laboratory.

We wish that as you read through this issue, each one you will find at least some part/article in the newsletter of direct relevance to you. Do feel free to share your feedback and inputs with us. Wish you all a happy time reading this newsletter!!

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The Avian Flight of Endurance

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❖ Dr. Sasikumar Menon has been a teacher for almost three decades now and has been teaching at the undergraduate as well as postgraduate levels. He is currently the Deputy Director at the Therapeutic Drug Monitoring (TDM) Laboratory in Mumbai. He is also a wildlife and nature conservation enthusiast.

During migration, birds can sustain metabolic rates of 10 to 15 times higher than at resting state. Most of the energy is provided to the working muscles by mobilizing lipids form the extra-muscular adipose tissue. The migratory birds can mobilize, transport and oxidize lipids at rates more than 10 times of what is recorded in mammals. Physiological adaptations for such high rates of lipid mobilizations are not fully clear. *In vitro* studies and other studies on waders like ruffs and sandpipers have shown that birds selectively mobilize the short-chain and unsaturated fatty acids more rapidly than the long-chain and saturated ones. Whereas mammals use albumin-bound non-esterified fatty acids for supplying lipids to muscles, migratory birds rely on circulating lipoproteins which have higher energy carrying capacity as compared to fatty acids. Studies on the western sandpiper (*Calidris mauri*) have shown significant variations in lipoprotein levels during their migration along the Pacific coast. To overcome the hydrophobicity of lipids for efficient transport across the cytosol to the mitochondria, birds like migratory insects and fish, rely on specific muscle fatty acid binding proteins (M-FABPs) that solubilize intracellular fatty acids and accelerate their mobilization. The expression of these FABPs in the muscles is upregulated during migration. Comparative studies have shown that oxidative capacity of flight muscles is higher in migrant species than in sedentary species. Studies on passerines have shown significant increase in triacylglycerols (TAGs) and very low density lipids

(VLDL) during migration as compared to fasting. Fatty acids are taken up by liver which acts as an exogenous fatty acid sink and are re-esterified and released back into the plasma as VLDL. This pathway provides large amounts of fatty acids to muscle through circulation.

During migration, birds alternate between fasting while flying and then feeding upon arrival at stopover sites. Such rapid changes in fasting and feeding states, affect the structure and function of digestive system in migratory birds. Fasting during flight regresses the digestive system and during subsequent feeding, the food intake initially reduces till the digestive system is rebuilt. This time-lag associated with the rebuilding of gut can restrict the energy uptake by the migrant. Migratory birds are known to accumulate fat stores by eating more and by selecting diets higher in total lipid content. Whereas diets with high protein-to-calorie ratios minimize fat storage, those with low protein-to-calorie ratios maximize fat storage. Many migratory songbirds shift from feeding on insects (high protein-to-calorie ratio) to fruits (low protein-to-calorie ratio) during their migration. In a recent study, European robins were caught during their migratory flight through Swiss Alpine passes. The blood levels of protein carbonyls (a marker for oxidative stress) and glutathione peroxidase (an antioxidant enzyme) were measured. The study established that a free-flying migrating passerine during endurance flight is also exposed to a higher oxidative stress like mammals. The results also showed that the migratory bird is able to concomitantly upregulate its antioxidant capacity suggesting that a free flying migrant adapts their antioxidant system to endure the oxidative stress of extraordinary exercise of flight, during migration.

Activities conducted by ATBS during 2018 - 2019!!

- The 13th Annual National Conference on 'Wetlands and Climate Change' was held on 2nd February, 2019 at SIES College of Science, Commerce and Arts, Nerul, Navi Mumbai.
- A Resource Generation Camp (RGC) for generating a question bank for the first stage Olympiad examination, National Standard Examination in Biology (NSEB) was held from 15th till 17th May, 2019 at HBCSE, Mumbai.

The BIOLOGY OLYMPIAD PROGRAMME:

Stage 1: NSEB (National Standard Examination in Biology)

Stage 2: INBO (Indian National Biology Olympiad)

Stage 3: OCSC (Orientation cum Selection Camp)

Indian team of 4 students selected at the end of the OCSC!

Team undergoes training at HBCSE, Mumbai prior to departure!

Studies have also shown that some birds use components of their food to enhance their aerobic capacities by preferential feeding on selected food sources. "Natural Doping", as many authors call it, has been recently studied in semipalmated sandpipers (*Calidris pusilla*, L.) which undertake a non-stop flight across the Atlantic Ocean from Canada to South America by covering a distance of about 4500 kilometres in three days at an average speed of 60 km/hour. During their sojourn at the Bay of Fundy, New Brunswick, Canada, semipalmated sandpipers feed mainly on the small burrowing mud shrimps, an amphipod (*Corophium volutator*) and almost doubles their body weight. The amphipods are rich source of n-3 polyunsaturated fatty acids (n-3 PUFAs) which not only become a source of energy but also enhances the capacity of endurance flight just before the birds commence their trans-Atlantic crossing. The dietary n-3 PUFAs of the sandpipers, mainly contain eicosapentaenoic acid and docosahexaenoic acid. These n-3 PUFAs are incorporated into membrane proteins which causes changes in the membrane fluidity, permeability, n-3/n-6 ratio and the local molecular environment. These changes in turn activate key membrane proteins like carnitine palmitoyl transferase, Na+/K+ATPase, Ca2+/Mg2+-ATPase, ion channels and the insulin receptor. In addition, eicosapentaenoic acid and docosahexaenoic acid are ligands for peroxisome proliferator-activated receptors (PPARs) that regulate the expression of genes controlling lipid metabolism like β-oxidation enzymes and fatty acid binding proteins. Thus dietary supplementation of n-3 PUFAs is a behavioural adaptation to enhance the endurance capacities of the bird during migration. Such preferential coastal stop-overs have been observed in many other migratory bird species and it becomes significant to protect and conserve such habitats to sustain these

migratory populations, especially with the threat of climate change looming large.

Non-stop flights are common among migratory shorebirds and often involve trans-oceanic crossings and these birds have evolved ways to overcome limitations that otherwise apply to mammals like the overall metabolic rate, lipid catabolism, protein sparing and duration of endurance exercise. The endurance adaptations in these birds mainly include special mechanisms for mobilizing lipid from adipose tissue to the mitochondria of the flight muscles and very high oxidative capacity of the flight muscles to catabolize lipids. Upregulating the expression of FABPs and antioxidant enzymes like glutathione peroxidase additionally optimize the muscle function during endurance exercise of non-stop flights. In summary, it would be rather a surprise, if these well-designed nature's flying machines are unable to accomplish such endurance exercises. Thus, research on the physiology of long-distance migrants has long term implications in the treatment of obesity, in improving the performance of human athletes and in wildlife conservation.

References:

- Gannes L. Z. "Comparative Fuel Use of Migrating Passerines: Effects of Fat Stores, Migration Distance and Diet.", The Auk, Vol. No. 118(3), 2001.
- Gill R. E., Tibbitts T. L., Douglas D. C., Handel C. M., Mulcahy D. M., Gottschack J. C., Warnock N., McCaffery B. J., Battley P. F. and Piersma T., "Extreme Endurance Flights by Land birds Crossing the Pacific Ocean: Ecological Corridor Rather Than Barrier ?", Proceedings of Royal Society B, 2008.

30th INTERNATIONAL BIOLOGY OLYMPIAD (IBO) 2019 AT A GLANCE!

- Venue: Szeged, Hungary
- Dates: 14th to 21st July, 2019
- No. of participating countries: 72
- No. of student participants: 280

- Hedenstrom A., "Extreme Endurance Migration: What Is the Limit to NonStop Flight?", PLos Biology, Vol. No. 8(5), 2010.
- Jenni-Eiermann S., Jenni L., Smith S. and Costantini D. , "Oxidative Stress in Endurance Flight: An Unconsidered Factor in Bird Migration.", PLOs One, Vol. No. 9(5), 2014.
- McWilliams S. R., Guglielmo C., Pierce B. and Klaassen M., "Flying, Fasting, and Feeding in Birds During Migration: A Nutritional and Physiological Ecology Perspective.", Journal of Avian Biology Vol. No. 35, 2004.
- Weber, J. M., "The physiology of long-distance migration: extending the limits of endurance metabolism.", The Journal of Experimental Biology, Vol. No. 212, 2009.

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30th INTERNATIONAL BIOLOGY OLYMPIAD (IBO) 2019 AT A GLANCE!

❖ **Indian Team Results:**

3 Silver medals and 1 Honorable Mention!!

Student Team members:

- **Hardik Gupta**
- **Arunangshu Bhattacharya**
- **Suryadeep Mandal**
- **Akshay Gupta**

TEAM INDIA – IBO 2019

From Left to right: Arunangshu Bhattacharya, Hardik Gupta, Suryadeep Mandal, Akshay Gupta

A HISTORY OF THE CORRECTION OF THE HUMAN CHROMOSOME NUMBER

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A scientific theory or a hypothesis can be falsified and remains to be testable. Many claims in science were subjected to refutability from time to time. If one looks at the history of science, there have been many examples of theories proposed in the past which were disapproved subsequently. For example, spontaneous generation was an obsolete principle concerning life originating from inanimate matter and the hypothesis was proven wrong in the 19th century by Louis Pasteur. Many scientific concepts and findings witnessed alterations over time. For instance, all of us know the story of Copernicus and Galileo and their contributions in astronomy. For centuries, we believed in the geocentric model of the universe and until 1500s, Ptolemy's theory dominated the academia to make us believe that the Earth was stationary and all the planets and the stars revolved around the Earth. It was Copernicus and Galileo who changed the notion. The present article portrays a similar scientific spirit for the discovery of the correct number of human chromosomes.

Many of the current generation of teachers and students may not be aware of the fact that the correct number of human chromosomes was not ascertained until 1955. Ironically, the structure of DNA was published earlier in 1953 marking a golden age of genetics and molecular biology without the authentic information of human chromosome number. In 1956, Joe Hin Tjio and Albert Levan revealed that the correct number of human

Do you know??

Here are a few questions about some animals which have certain interesting facts associated with them!!

1. **Which living creature was the first to have orbited space?**
2. **Which bird is the national emblem of the United States?**
3. **What makes the Walrus different from other seals?**

……Answers on page numbers 9, 10 and 12!

chromosomes was 46 and not 48 as it was an established 'fact' that had prevailed for almost three decades from 1920s to 50s of the 20th century. The period of "incorrectness" of chromosome number in humans witnessed episodes of propositions, counter arguments and debates, especially during the second decade of the last century. Investigators came out with varied claims for human chromosome numbers. The famous geneticist Thomas H. Morgan also did not rule out the possibility of natural variation in the chromosome number in humans. Hans de Winiwarter added more confusion when he reported differences in the chromosome number from samples obtained from Caucasian and African humans. Nevertheless, the whole controversy came to an end when Theophilus S. Painter reported 48 as the diploid number in humans irrespective of racial differences. Painter had a professional training under the famous cytologist Theodour Boveri and had a strong presence in the field of cytogenetics in his time. He became famous for his seminal work in *Drosophila* cytogenetics. Painter could convince his contemporaries about the importance of fresh samples for chromosome analysis and the necessity of immediate fixation of samples in order to avoid aggregation of chromatin materials. The sophistication of animal cell and tissue culture techniques were non-existent in the 1920s. *Due to the hurdles of culturing human samples of rapidly dividing cells, many cytologists used to wait near the gallows to obtain the testis sample of an executed criminal at the earliest possible attempt to fix the post-mortem tissue!!* During this period of uncertainty regarding diploid chromosome number of humans, Painter's publication in 1923 in the Journal of Experimental Zoology brought an end to the debate and his reported number 48 as the count of human chromosomes remained as a "text book material" for the next three decades or so. Surprisingly, no one felt the necessity to re-examine Painter's claim till Tjio and Levan made a seminal discovery in 1955

(published in 1956) and corrected Painter's erroneous count of the human chromosome number.

Albert Levan at the Institute of Genetics of the University of Lund, Sweden pioneered the use of colchicine for chromosome preparation for arresting the dividing cells in the metaphase stage when chromosome morphology could be conspicuous for viewing and counting. Levan switched over from plant to animal cytogenetics with a special focus on human cancer cells for improving chromosome analysis technique. However, he was intrigued with the correctness of chromosome number of control healthy human subjects. At this juncture, Levan's encounter with Tjio took place which led to a golden chapter in the history of human cytogenetics.

Joe Hin Tjio of Chinese descent, was born in Indonesia in 1919. He had a training in agriculture and a specialization in plant cytogenetics. In 1942, during the turbulent period of Second World War, Java was invaded by the Japanese army and Tjio was arrested and tortured by the Japanese army to gather local military information. At the end of the Second World War, he moved to Holland and was awarded a fellowship to study in Europe. Eventually his fate took him to University of Lund in Sweden where he met Levan. Tjio's collaboration with Levan prioritized authentication of Painter's data and to obtain unambiguous image of normal human karyotype using advanced methodologies developed in Levan's laboratory.

The 1940s and 50s witnessed rapid technological advancement in cytogenetics. During Painter's time, sample tissues used to be fixed and embedded in paraffin followed by sectioning. These thin serial sections were subsequently stained and assembled to reconstruct the nuclei for interpretation of full complement of chromosomes. This tedious process was later simplified using the 'squash' as well as 'colchicine-hypotonic treatment-based'

Answers and more to 'Do you know?'

1. A dog named 'Laika'

- 'Laika' is the first living creature who was put on board 'Sputnik -2' on 3 November, 1957 by the Soviet Russia USSR to orbit the world.
- Laika was a mix-breed, mostly Siberian husky.
- Scientists assumed that a stray dog would be habituated to withstand harsh conditions including starvation and cold.
- As part of training, Laika and two other dogs were kept in restricted cages and were fed special food.
- Laika's trip however ended up as a one-way trip and Sputnik 2 burned up in the upper atmosphere in April 1958.

chromosome spreading technique. Levan himself was the pioneer to introduce colchicine for chromosomal work. Previously, T.C. Hsu popularized hypotonic treatment for chromosome spreads. Moreover, Levan got access to rapidly growing fetal lung fibroblast cell culture from Prof. Rune Grubb in Lund. Availability of human fetal tissues was nearly impossible elsewhere and owing to the fact that abortion was legally allowed in Sweden, Levan could make use of cultured human embryonic cells. All these technological advances proved to be congenial for Tjio to attain his goal. Additionally, Tjio's mastery over photomicrography and documentation of chromosome images came handy for the historical discovery made in later years.

Tjio was known both for his extraordinary tenacity and his nocturnal working habits in the laboratory which was on record in the institute's archive. On December 22nd 1955, Tjio completed counting well resolved chromosome spreads obtained from 265 samples at 2.00 am and he came to a final conclusion that human cells have 46 chromosomes and not 48. In 1956 Tjio and Levan published this historic work in the journal *Hereditas* [Tjio J.H. and Levan A. (1956) The chromosome number of man. *Hereditas* 42: 1-6] refuting the earlier claim of 48 as the diploid number in human cells which remained uncontested for nearly 30 years. Tjio and Levan's story is an inspiration to all practitioners of science. We should learn how to explore any genuine 'doubts' arising in scientific propositions and to garner the courage to question what might be an accepted dogma.

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Answers and more to 'Do you know?'

2. The Bald Eagle

- The scientific name of the bald eagle is *Haliaeetus leucocephalus*!
- It is a bird of prey found in North America and has two known subspecies.
- It is found near large bodies of water and mainly feeds on fish. It is an opportunistic feeder and captures fish as it swoops down and snatches the prey with its talons.
- The bald eagle is known to build the largest nests among the birds found in North America and its nests may measure upto 13 ft deep x 8.2 ft wide and 1 metric ton in weight.
- The bald eagle is not actually 'bald' as the name suggests but its name is derived from it having a white head. Thus the adult is mainly brown with a white head and tail.

Analysis of students' response to a question on the renal portal system

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Problem solving skills of students can be broadly classified as lower order skills and higher order skills. Lower order cognitive skills (LOCS) usually include students' memory and recall skills. However, it is desirable that students acquire higher order cognitive skills (HOCS) which include understanding the concepts and application of concepts to solve problems, not just the recollection and comprehension of basic facts. One of the best ways to help students develop HOCS is to make questions involving such skills a part of their course work or assessment.

HOCS can be tested in various ways such as such as asking for interpretations of graphs, analysis of data given or giving a novel situation or a case study. The question given below is an example of a real life situation and its interpretation.

In the regular biology course work, students learn about circulatory systems, the renal portal system of vertebrates being one of them. Veins are the blood vessels that carry deoxygenated blood from an organ to the heart. In vertebrates (other than mammals), some veins before emptying in the heart, divide into capillaries into some other organ, the vein emerging from this organ, then empties the blood to the heart. This type of circulatory system is called a portal system. The question given below pertains to the renal portal system of a reptile.

Answers and more to 'Do you know?'

3. Long canine teeth called 'tusks'

- Seals and walruses belong to a group of marine mammals called pinnipeds.
- Pinnipeds in Latin means 'feather – footed'.
- Walruses belong to the family Odobenidea while seals belong to either Phocidae or Otaridae.
- Both male and female walruses possess tusks and they can grow to lengths of about 3 feet.
- These tusks are used for self-defense against predators; males use them to fight against rival males and walruses also use the tusks as ice-picks to lift themselves out of the water.
- Walruses do not have visible ear flaps and they can walk on their flippers by rotating the hind flippers under their body.

Question:

An antibiotic was found to be very effective in a mammal. A veterinary student used the same dose for a reptile with a similar body weight and injected it into its hind limb. Even after repeated trials, it was found to be totally ineffective. What could be the most probable reason?

Options:

a. The absorptive surfaces of the two animals vary greatly. As a result the minimum effective concentration of the drug could not be reached in the blood of the reptile.

b. The body temperature of the reptile is quite low as compared to the mammal. As a result, minimum effective concentration of the drug could not be reached in the blood.

c. The low metabolic rate of the reptile hindered the rate of diffusion of the drug in the body. Hence, minimum effective concentration of the drug could not be reached in the blood.

d. The drug must have got excreted from the body of the reptile before it could reach the minimum effective concentration in the blood.

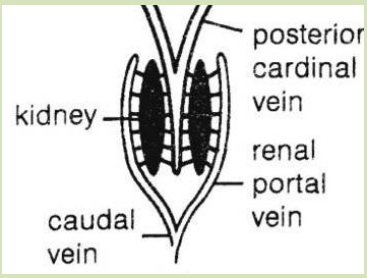
Note: Figures of the renal portal systems of fish and reptile are given on page 13 for reference (Figures in the box).

The question states that an antibiotic when injected into the hind limb of a reptile proves ineffective. In order to find the most plausible explanation, several statements are provided and the students are expected to choose the option that best explains this finding.

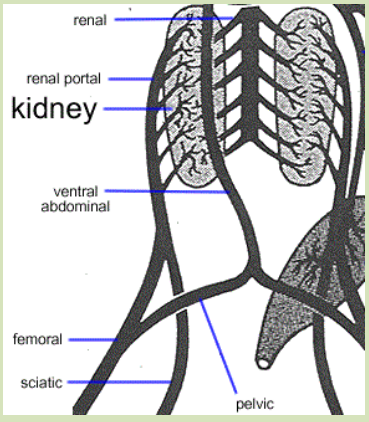
The first option deals with insufficient absorptive surface of the animal. The absorptive surface of the animals of similar body weight cannot vary greatly as it is the basic requirement for all basic body functions and survival.

Figure:

Renal Portal System of Fish:



Renal Portal System of Reptile:



The second option involves body temperature of the animal as an important factor. However, the minimum effective concentration of a drug will depend on the body size of an animal and not on the temperature; hence option 'b' cannot be true.

As stated in option 'c', if the low metabolic rate of a reptile hinders its absorption, it will also hinder its excretion and this will have no net effect on the availability of the drug in the body. Thus option 'c' also does not hold true.

As indicated in option 'd', the blood from the hind limb and tail of a reptile reaches kidneys before reaching other parts of the body. It is therefore most likely that the injected drug gets excreted through kidneys before it reaches optimum blood concentration. Thus option'd' is the most appropriate one.

An analysis of the students' responses showed that among a total of 268 students, only 41 students (15.3%) could answer the question correctly. This question was included in the stage II theory paper of the Indian National Biology Olympiad (INBO) which is a nation – wide exam held for about the top 300 students appearing for the first stage exam. It is interesting to note that although the students study the renal portal system in the pre-university classes, about 85% of the students could not analyse a new situation given to them. Thus the students may exhibit competency in lower order cognitive skills, however, the skill to apply prior knowledge to logically analyse new situation needs to be further strengthened.

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Biology can be fun……..

Separation techniques are regularly used in Biochemistry and Cell Biology Labs for separating, isolating and purifying various components in a given mixtures. The basic separation techniques can be demonstrated and carried out in a regular school lab without the use of any sophisticated instruments. We have included here simple activities in adsorption chromatography and partition chromatography.

REQUIREMENTS:

For Activity 1:

- Regular white chalk
- Toothpick/arrowhead
- Petri plate or flat container
- Water
- Black ink

(Note: Avoid using dustless or dust-free chalks since they do not give good results)

Activity 1: Adsorption chromatography

You are given a white chalk as an adsorbent.

1. Make a circular groove/notch 1cm above the bottom edge of the chalk with a toothpick.
2. Spread the black ink from the sketch pen directly in the notch uniformly.
3. Take a Petri dish and put 10 ml water in it.
4. Stand the chalk in it with ink mark just above the water level.
5. Observe the separation. Remove the chalk from Petri dish when the solvent has reached a level of 1 cm. below the top.

Draw the chromatogram obtained.

The colour component with greatest adsorption is _____.

The order of colours/ink components with decreasing adsorptivity is:

_____> _____>_____>_____

REQUIREMENTS:

For Activity 2:

- Indicator 1: Methyl orange
- Indicator 2: Thymol blue
- Indicator 3: Sudan red
- Glass test tubes
- Droppers
- Water
- Diethyl ether
- Petri plate or flat container
- Whatman (No. 3) filter paper strip
- Mixture of thymol blue and Sudan red

Activity 2: Partitioning of indicators in various solvent systems.

(A) You are given 3 different indicators.

1. Methyl orange
2. Thymol blue
3. Sudan red

Procedure:

1. Take 2 ml of water in a test tube.
2. Add to it 2 ml of diethyl ether. (Collect from the supervisor)
3. Add 1 drop of indicator 1 to the above two-phase system to check its solubility. Fill the results in the table.
4. Repeat the procedure for the other 2 indicators.

Chemical	Solubility in water	solubility in ether
1		
2		
3		

(B) You are given a mixture of thymol blue and Sudan red. You have to suggest a method/solvent/solvent system to separate them by paper chromatography. Ask for the materials that you require.

Draw the chromatogram & describe the method you selected with reasoning in not more than 3 sentences.

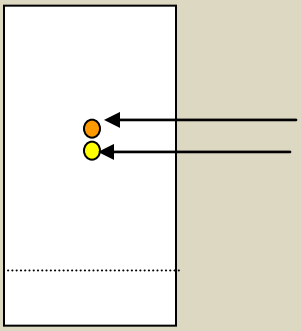
A chromatogram is shown below.

REQUIREMENTS:

For Activity 3:

- Green leaves
- Mortar and pestle
- Acetone
- Eppendorf tube
- Capillaries
- Whatman (No. 3) filter paper
- Glass beaker
- Aluminium foil
- Petroleum ether
- Acetone
- Distilled water
- Test tubes
- Conical flask

What kind of solvent will give this type of separation?



Mobile solvent :_____

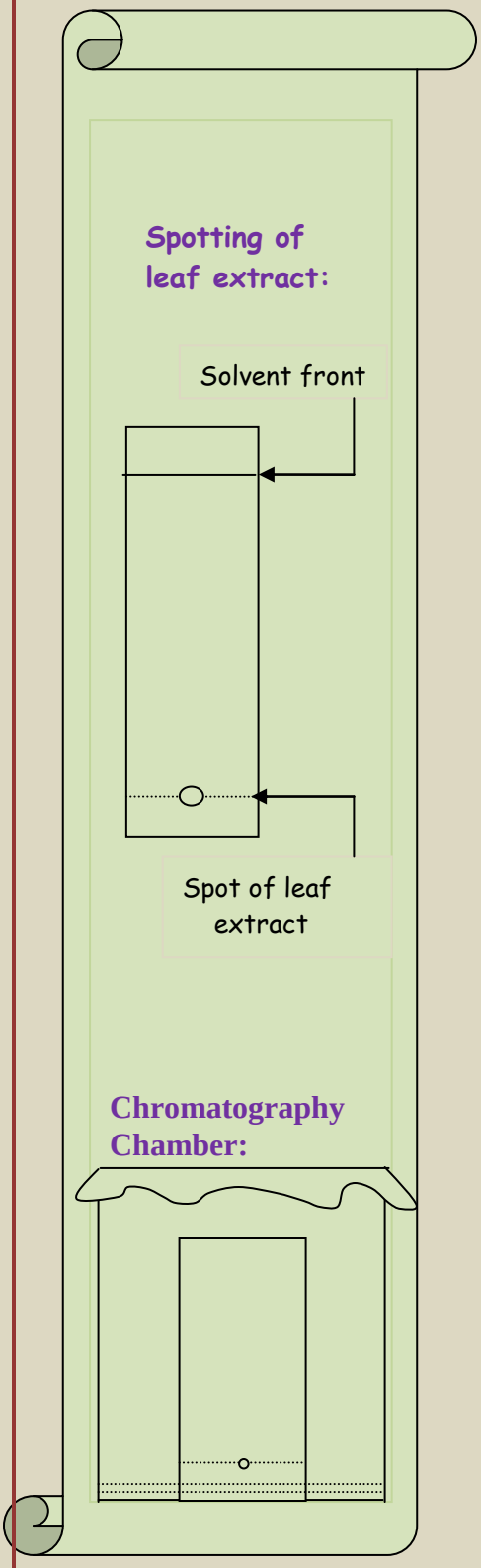
Activity 3: Separation of Plant Pigments By Paper Chromatography

The following procedure can be used to separate pigments present in leaves using paper chromatography.

Procedure:

1. Preparation of leaf extract: Cut the given leaves (2 nos.) into fine pieces. Avoid the midrib region. Transfer these pieces into a clean mortar and start macerating. Add 5 ml of acetone intermittently in small fractions and macerate well. Transfer the solvent into which the pigments have been extracted into a clean tube. Avoid transferring the particulate leaf material into this tube. Stand the tube so as to settle any particulate material in the tube. The supernatant is to be transferred to a clean Eppendorf vial. This extract should be used for spotting in step 3 as well as for TLC in task 4.

2. Preparation of chromatography chamber: A solvent mixture of petroleum ether and acetone (90%) in proportion 100:12 is to be used as the solvent system. The solvent system for running your chromatogram will be provided by us. Prepare 28 ml of the mixture.



Pour 15 ml of the solvent mixture into a dry beaker of 250 / 500 ml capacity.
Immediately cover the beaker tightly aluminum foil. Keep the beaker aside undisturbed. This will allow the chamber to saturate with the vapours of the solvent. Proceed to step 3.

3. Spotting of leaf extract: Use Whatman chromatography paper No.3. Draw a faint pencil line with a ruler one cm. from the bottom edge of the paper. Mark the centre of this line with a faint pencil mark. Do not use eraser on this paper. You are provided with a capillary tube. Dip this capillary in the filtrate obtained in step 1 and allow the extract to rise in tube. Spot a small fraction of the pipetted amount on the pencil mark, gently blow to dry, repeat spotting till the entire pipetted amount is spotted. Once again, pipette out the extract and repeatedly load the same spot 10-15 times. See that the diameter of the spot does not exceed 4-5 mm. After the spotting is over, allow the entire spot to air-dry for another 5 min.

4. Development of chromatogram: Hold the upper corner of the paper with forceps and dip the paper in the solvent of the chromatography chamber prepared in step 2 as shown above.
    - **Make sure that the spot is just above the solvent level.**
    - **Immediately cover the beaker with foil.**
    - **Leave the chamber undisturbed once the paper is placed inside.**

The process of separation of pigments has started. This will take about 20 min. Allow the solvent to rise upto a level of about 1 cm below the top edge. Remove the paper from the beaker. Immediately mark the 'solvent front' (the level

to which solvent rise) and allow to air dry for a few seconds.

From the formula given below, calculate the Rf values of various bands of pigments. If the bands are diffused or broad, consider the upper front for calculations.

Make a table and fill in the Rf values of the various pigments separated.

Rf value of a pigment = distance (cm) traveled by the pigment ÷ distance (cm) traveled by the mobile solvent (Solvent front).

The order of the pigments obtained beginning with the most non-polar is:

_____

_____

_____

---------- *Biology Olympiad Cell*

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Recreation Corner .....

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