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Practical
Plant Physiology
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المرحلة الثالثة – الدراستين الصباحية والمسائية

الفصل الدراسي الاول

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Lab 1

Solutions and Concentrations

Solutions: are mixtures of solute (s) and solvent play important roles in our life, such as: milk, cheese, butter, ink, colored gems and rubber.

Solute: dissolved material (s) could be gas, liquid or solid.

Solvent: Dissolving medium, often liquid frequently **water (H_2O) which is universal solvent, highest polar solvent, colorless, tasteless, odorless, with a boiling point 100 °C and freezing point zero.**

- **Types of solutions according to the solubility of the solute (s) in the solvent:**

1- Unsaturated solution: More solute could be dissolved in solvent which means more solvent particles are available to dissolve solute. Decreasing volume of the un saturated liquid (as by evaporation) could turn it to saturated or super saturated solution.

2- Saturated solution: No more solute will dissolve at a specific temperature.

3- Super saturation solution: contains more of the dissolved solute than could be dissolved by the solvent. result when some condition of a saturated solution is changed, for example increasing temperature, , or increasing pressure**.**

- **Types of solutions according to solute properties**

True solutions	Colloidal solutions	Suspensions
Solute **invisible** neither by naked eye nor by microscope	Solute **invisible** by naked eye , but could be **visible** by microscope	**Easily visible**
Solute do not settle down spontaneously	Solute do not settle down spontaneously	Settle down spontaneously
Components cannot be separated neither by filter paper nor by centrifugation	Mostly can be separated by centrifugation	Components can be separated easily by all means

Concentrations of solutions: Concentration is a way of describing mixture composition **or** it is a way of describing ratio of solute (s) and solvent (s). For example: 1) alloys and 2) soil fertilizers 3) air contains 1% of inert gases we are referring to their concentration as a percentage.

Concentration of solutions could be expressed in different ways:

I- Percentages

A- Weight/ weight (w/w)

There are many examples such as: alloys (سبائك), colored glass and most important example related to plant physiology is percentage of fertilizer in a soil mixture.

Note/ Find out or what is percentage meaning what is the concentration

Q/ Find out percentage of a fertilizer in its mixture with soil, total weight of the mixture 1kg, while weight of soil 600 g.

B-Volume/volume (v/v)

Q/ Aqua regia (الماء الملكي) is a mixture of hydrochloric acid and nitric acid in a ratio 1:3, Find out percentage of nitric acid, total volume 200 ml. while Hcl volume is 50 ml.

Q/ Prepare 5% of ethanol. **Answer/** It could be dissolved either by add 5 ml of ethanol to 95 ml of water.

C- Weight/ volume (w/ v)

For example concentration of a solution containing 5g of solute in 100mL is 5% w/v.

D- Part per million and part per billion (Ppm and Ppb)

"Part per" is used for low and very low concentrations. Generally it is very similar to w/w – 1Ppm in w/w means 1 milligram (mg) of substance per every kilogram (kg.) of sample and v/v- 1ml of SF6 per every 1000 L of hydrogen to make 1Ppm of SF6, in addition it is similar to w/v- 1Ppm $CoCl_2$ means 1 µg of $CoCl_2$ per 1ml water or 1 mg of $CoCl_2$ per 1L water.

To convert % to Ppm or vice versa this equation can use:

$$\boxed{\% = \frac{\text{Ppm}}{10000}}$$

Q / convert 11% of NaCl to Ppm

$$11 = \frac{\text{Ppm}}{10000} \rightarrow = 110000 \text{ Ppm}$$

Or/ $11 \rightarrow 100$

$x \rightarrow 10^6$ ► $x = \frac{11 \times 1000{,}000}{100} = 110000$ Ppm

Lab 2

Concentrations of solutions

II- Molarity [M] (Number of moles of a solute in a liter of a solvent)

$$\mathbf{M = \frac{moles\ of\ substance}{Vol.(liter)}\ (1)} \quad \blacktriangleright \quad \mathbf{mole = \frac{weight}{molecular\ weight}} \quad \blacktriangleright \textbf{ Then}$$

$$\mathbf{M = \frac{weight}{molecular\ weight} \times \frac{1000}{Vol.(ml.)}\ (2)}$$

Q- Prepare 0.5 L of KCl, m.wt.= 294.20 and its concentration= 0.1M?

Either (1) $M = \frac{\text{moles of solute}}{\text{Vol.(liter)}} \blacktriangleright$ mole= $0.5 \times 0.1 = 0.05 \blacktriangleright$ mole= $\frac{\text{weight}}{\text{molecular weight}}$

Wt.= mole× m.wt. ► wt.= 0.05× 294.20= 14.710 g from KCl add to a little amount of water then complete the volume up to 0.5 L.

please focus on the unit of vol. (L.) as you deal with the equation (1)

Or (2) $\mathbf{M = \frac{wt.}{m.wt.} \times \frac{1000}{vol.(ml)}} \blacktriangleright$ please here in this convert vol. to (ml) =500 ml

Then apply the equation (2) ► $0.1 = \frac{wt.}{294.20} \times \frac{1000}{500} \blacktriangleright$ wt. will also =14.710g dissolve in a little amount of water and fill up to ((500 ml))

III- Molality [m]= $\mathbf{\frac{mole\ of\ solute}{Kilogram\ of\ (solvent)}}$ **A molality(is the number of moles of a solute dissolves into exactly 1.0 liter water)**. Be careful not to confuse molality and molarity. Molality is represented by a small "m," whereas molarity is represented by an upper case "M." Note that the solvent must be weighed unless it is water. One liter of water has a specific gravity of 1.0 and weighs one kilogram; so one can measure out one liter of water and add one mole of the solute to it. While a molar of a solution is made by placing 1 mole of a solute into a volumetric flask water is then added to the volumetric flask up to the one liter line. That is the distinction between molality and molarity.

Ix- Normality [N] (Number of equivalents of a solute in a liter of a solvent)

$$N = \frac{\text{no.of equivalents}}{\text{volume (Liter)}} \quad \blacktriangleright \quad \textbf{no. of equivalents} = \frac{\text{weight}}{\text{equivalent weight}}$$ ► But most the preparations in lab. Use volumes of solutions in (ml.) then the equation will be

$$N = \frac{\text{wt.}}{\text{eq.wt.}} \times \frac{1000}{\text{vol.(ml.)}} \quad \blacktriangleright \quad \left(\text{eq.wt.} = \frac{\text{M.wt.}}{\text{Valence}}\right)$$

Normality can only be calculated when we deal with reactions, because normality is a function of equivalents**. (Normality = molarity x n)** (Where n = the number of protons (H) exchanged in a reaction). **According to these informations we could assume that (1N=1M) only if the number of H=1.**

Each molecule of hydrochloric acid will dissociate to yield one hydrogen ion and one chloride ion in solution. This means that one mole of hydrochloric acid can yield one equivalent of hydrogen ions. 1 M HCl is equal to 1 N HCl.

Each molecule of sulfuric acid (H_2Po_4) can dissociate to yield two hydrogen ions and a sulfate ion in solution, so one mole of sulfuric acid can form two equivalents of hydrogen ions. The concentration of compounds that do not contain hydrogen ions, such as calcium chloride, calcium chloride (1M is the same as 2 N)because calcium chloride dissociates to give two moles of chloride ions, which could react with two moles of hydrogen ions. The calcium ion is divalent. That is, it has a +2 charge. This +2 charge means one calcium ion could take the place of two hydrogen ions.

Q/ How much the concentration (in M, N, % and Ppm) of a calcium chloride solution if 111g of this salt was dissolved in 1 L. (m.wt.= 111 g/mol.)

1- $\mathbf{M = \frac{wt.}{m.wt.} \times \frac{1000}{vol.(ml)} \blacktriangleright M = \frac{111}{111} \times \frac{1000}{1000} = 1M}$

2- $\mathbf{N = \frac{wt.}{eq.wt.} \times \frac{1000}{vol.(ml.)} \quad \blacktriangleright \quad \left(eq.wt. = \frac{M.wt.}{Valence}\right)}$

First of all eq.wt. = $\frac{111}{2}$ = 55.5 g/mol

Second N= $\frac{111}{55.5} \times \frac{1000}{1000}$ ► N = 2 ► please focus on the data that you had been given wt., volume(1L converted to ml. because the equations of M and N are dealing with volumes in ml.) and m.wt. all same but the N was twice the molar concentration, because of valence=2 (calcium chloride dissociates to give two moles of chloride ions, which could react with two moles of hydrogen ions.)

3- We have wt. =111g and vol.= 1L must turn to 1000ml.(Then we deal with w/vol.)

111g → 1000 ml.

× → 100 ml. ► ×= 11.1% will be the answer for the third request.

4- Fourth request was, find the concentration in Ppm?

Answer /either 111(g. or part) → 1000 (ml. or part)

× (g. or part) → 10^6 (ml. or part) ► × = 111000 Ppm

Or %= $\frac{\text{Ppm}}{10{,}000}$ ► 11.1 = $\frac{\text{Ppm}}{10{,}000}$ ► Ppm = 111000

x- Dilution

You can make less concentration solution or even a serial of dilutions from a stock solution (with a higher concentration than the rest) by using this equation:

C1 V1 = C2V2

C1 initial concentration

V1 initial volume

C2 Final concentration

V2 Final volume

Q/ How could you prepare 100 ml. of 0.4 M MgSo4 from its stock solution 2M?

C1 V1 = C2V2

2× V1 = 0.4 × 100 ► V1= 20ml. from the stock solution should be taken and complete the volume up to 100ml with D.W.

Q1/ For example, you have 10mL of an unknown substance with a concentration of 0.5M. If you add 50mL, what will the final concentration be.

V1=10mL

C1=0,5M

V2=60mL

C2= x

C2= 0.001M

Q2/ There is two NaoH solutions, solution a concentration is 2M with 500ml. an M.wt.= 40 g/mol., while solution b concentration is 20%, which of these solutions more concentrated, and how could make an advantage from knowing the answer to decide either the following question was formulated correctly or not ?

Prepare 20% NaoH solution from its stock solution 2M and 500 ml. volume. M.wt.= 40 g/mol.

If there is wrong in question correct it appropriately then answer it.

Lab 3

Water relationships

Diffusion

Molecules are in constant motion and tend to move from regions where they are in higher concentration to regions where they are less concentrated. Diffusion is the net movement of molecules down their concentration gradient. Diffusion can occur in gases, in liquids, or through solids. An example of diffusion in gases occurs when a bottle of perfume is opened at the front of a room. Within minutes people further and further from the source can smell the perfume.

1- Experiment / Effect of molecular weight and temperature on the rate of diffusion

Prepare four tubes containing 5% gelatin then split it equally into two sets, add 10 drops of 0.02M Potassium dichromate ($K_2Cr_2O_7$) to set no.1 and 10 drops of 0.02M Janus green ($C_{30}H_{31}ClN_6$) to set no. 2.

Pick one from each set and place it in a refrigerator, while the other tubes should be kept at room temperature for 24 h.

Measure the distance that each of the different dyes diffused down through the gelatin.

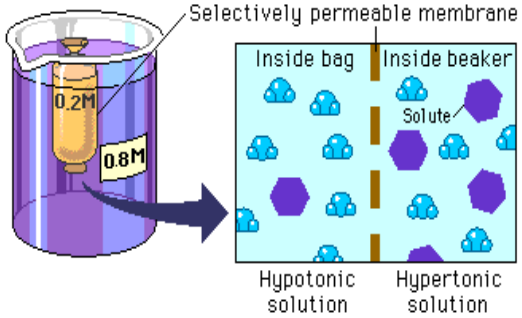
Osmosis

Movement of water molecules through a selectively permeable membrane from a region of its higher concentration to a region of its lower concentration . Osmosis is specialized case of diffusion that involves the passive transport of water. The membrane selectively allows passage of certain types of molecules while restricting the movement of others.

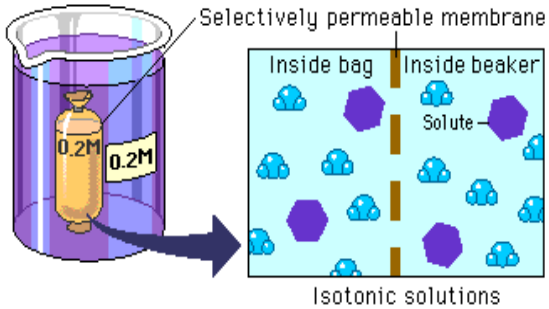
The solute concentration in the beaker is higher than that in the bag, and thus the water concentration is lower in the beaker than in the bag. This causes water to move from the bag (left) into the beaker (right).

Types of Solutions Based on Solute Concentration

Everything in the entire world wants to flow from high concentration to a low concentration. The terms hypotonic, hypertonic, and isotonic are used to compare solutions relative to their solute concentrations. Plant's exterior environment could be described in three ways: hypertonic, hypotonic, and isotonic. **1)** A **hypertonic solution** has a lesser concentration of water outside the cell compared to inside the cell, so water rushes out of the plant cells, causing it to **wilt**. This process is called **plasmolysis**. **2) Hypotonic solution** is the opposite of a hypertonic one, a greater concentration of water outside rather than inside a cell causes water to rush into the cell, making it **swell**, or become **turgid.** **(3) An isotonic solution** is one where a plant and its environment have the same concentration of water, so water moves in and out at the same rate causing **no change** in the plant.



In the illustration, the solution in the bag contains less solute than the solution in the beaker. The solution in the bag is **hypotonic** (lower solute concentration) to the solution in the beaker. The solution in the beaker is **hypertonic** (higher solute concentration) to the one in the bag. Water will move from the hypotonic solution into the hypertonic solution.



In this illustration the two solutions are equal in their solute concentrations. We say that they are **isotonic** to each other.

2/ Plasmolysis's experiment

Peel a thin sliver of a plant leaf called ***Tradescantia pallida*** soak it in a hypertonic solution, after a few minutes examine it under a microscope, you can watch the cells start to shrink, because the protoplasm of the cell (all living and non-living cell's component except cell wall) start to peel away from the cell walls, and the plant cells start to shrivel and shrink away. The plasmolysis could be reversible (the cells back to the normal status) if the sliver place translocates from the hypertonic into a hypotonic solution as soon as possible. But if the leaf sliver remains for longer period then that type of plasmolysis could be **irreversible**.

Imbibition

The phenomenon by which the living or dead plant cell absorbs water by surface attraction . Two conditions have great impact on imbibitions:

1- Nature of the absorbent surface (seed coat and nature of colloids composing the seed).
2- Mobility ratio (depends on water potential gradient and temperature both have a direct proportion to imbibitions, why?).

Different types of organic substances have different imbibing capacities. Proteins have a very high imbibing capacity, starch less and cellulose least. That is why proteinaceous pea seeds swell more on imbibition than starchy wheat seeds.Imbibition of water increases the volume and weight of the imbibant, which results in imbibitional pressure. This fact can be demonstrated by the splitting of rocks by inserting dry wooden stalks in the crevices of the rocks and soaking them in water, a technique used by early Egyptians to cleave stone blocks.

3/ Experiment

Estimate the variation of the imbibition rate by different angiosperm seeds (beans and chick pea)

Weigh 25 seeds from each of the pea, bean and chick pea (initial weigh) and soak those seeds in a beaker containing 100 ml. of water separately for 30,60 and 90 min.

At the end of this duration, first measure water volume that remains in each beaker (observe the differences). Second weigh each group of seeds (final weigh) after those seed being dried using a filter paper.

Calculate the percentage of imbibided water using this Formula:

$$\textbf{of Imbibided water} = \frac{\textbf{Final weigh}}{\textbf{initial weigh}} \times 100\%$$

Arrange your results and data in a table to manifest the distinctions in imbibitions rate between the experimented seeds.

Lab 4

Water potential

The capacity of water molecules to move from an area of higher water potential (contains a higher number of free water molecules) to an area of lower water potential.

Water potential is affected by two factors: **pressure and the amount of solute**. Distilled water has the greatest potential which is zero. As solute is added to distilled water with no outside pressure being applied to it, the water potential of that solution drops, because the water in that solution is less likely to move, because number of free water molecules will decrease as the solute is added and forms free ions bind with water molecules. Water potential (Ψ) is actually determined by taking into account two factors - osmotic (or solute) potential (Ψ_S) and pressure potential (Ψ_P). The formula for calculating

$$\Psi_w = \Psi_S + \Psi_P + \Psi_g$$ (Ψ_g, gravity usually ignored).

Osmotic potential is directly proportional to the solute concentration. If the solute concentration of a solution increases (more negative value), the water potential decreases (also more negative value), please focus on this hint the minus of osmotic potential is not mathematically sign but a clue indicates that dissolved solute reduces water potential, for instance, -0.1 Mpa. And – 0.7 Mpa , -0.1 is the higher water potential valuethan -0.7 but if the osmotic potential is intented the -0.7 will be the highest . Therefore, the more solute that is added to a solution, the more negative its osmotic potential gets as Ψ_S increase and also more negative Ψ_w gets as its value decrease.

4/ Experiment

Chardakov Method of determining water potential

Background Informations:

The Chardokov method provides a quick means to determine plant tissue water potentials. This method depends on the change in density in a solution that occurs after a tissue has been immersed in it. The solution gains or losses water depending on the water potential of the tissue. If the density of a solution does not change (no net movement of water) then this solution has the same water potential as the tissues that were incubated in it. It is assumed that solute movement between tissue and solution is negligible.

Method:

1. Dispense 10 mL of a sucrose solution (0.1 - 0.8 molal) into two sets of tubes each containing eight labeled test tubes.
2. Use a cork borer to prepare at least 24 uniform tissue samples from the potato. Cut them to the same length (4 cm) with a razor blade. Work quickly to minimize evaporation and keep the tissue wrapped in a moist towel.
3. Put two or preferably three potato cores in the tubes of one set . If necessary, add more of the appropriate solution to completely submerge the cores but the final volume in each tube must be the same. Add one drop of 0.1M methylene blue to the tubes of second set.
4. Incubate the cores for at least 1.5 h, preferably longer. Periodically swirl the containers. Get rid of potato cores at the end of duration.
5. Using a Pasteur pipet, remove a small amount of dyed solution from each tube in the second set to its match tube (same

concentration) in the set which its tubes previously had tissue sections in it, Immerse the pipette until the tip is approximately at the center of the tube.

6. Slowly release a drop of the methylene blue solution from the pipette and note whether the drop of the dye sinks, disperses, or floats to the surface in this solution.
7. Record your results in a table and repeat this procedure for each of the sucrose solutions. Be sure to use a different pipet for each dye stock.

Table : Response of drops (float, sink, hover) when placed in solutions in which potato cores have been incubated

[Sucrose](molality)	Drop Response
0	
0.1	
0.2	
0.3	
0.4	
0.5	
0.6	
0.7	
0.8	

Lab 5

Separation of plant pigments

Introduction:

Pigment is any substance that has the ability to absorb light. The pigments of plant involved in photosynthesis are:

a- Chlorophylls

1. Chlorophyll a : greenish blue in color with chemical formula $C_{55}H_{72}O_5N_4Mg$.
2. Chlorophyll b: greenish yellow with chemical formula $C_{55}H_{70}O_6N_4Mg$.
3. Chlorophyll c.
4. Chlorophyll d.

b- Carotenoids:

1- Carotens: orange in color with chemical formula $C_{40}H_{56}O$.
2- Carotenols: red in color .
3- Xanthophyll: yellow in color with chemical formula $C_{40}H_{56}O_2$.

c- Phycobillines :

1. Phycoerthyin : red in color.
2. phycocyanin: blue in color.

Carotenoids and phycobillins involved indirectly in photosynthesis through:

1. Protect the leaves from absorbed sunlight.
2. Increase the absorption spectrum of light.

Chlorophylls a and b are the main pigments involved in photosynthesis. The main differences between chlorophyll a and b are:

Chlorophyll a	Chlorophyll b
Chemical formula $C_{55}H_{72}O_5N_4Mg$	Chemical formula $C_{55}H_{70}O_6N_4Mg$
Appeared after chlorophyll b on oaper chromatography(why)	Appeared before chlorophyll b on oaper chromatography(why)
Greenish blue in color	Greenish tallow in color
the chemical structure contains (CH_3) group	the chemical structure contains (CHO) group

The chlorophyll pigment composed of two parts:

1. Tail (phytol): consists of long chain of carbon atoms which have been attached by H and O atoms.
2. Body (porphyrin) contains Mg atom in the center of the body which is surrounded by hexagonal or pentagonal rings.

Separation of the pigments :

1. Take 5 gm from fresh spinach leaves after removing the midrib (why) and placed in-porcelain mortar. Containing 10 ml of 80% acetone with little amount of $CaCo_3$ (why)?
2. Homogenized the leave tissue gently to avoid the destruction of the plastids.
3. Filter the extract using cheese cloth to remove the crushed leave residues.
4. Place the filtrate in separating funnel containing 10ml of ether (why) and shake the funnel.

5. Add 10 ml of distilled H_2O gently in the inner surface of the funnel to avoid the formation of white emulstion layer.
6. Mix the mixture by shaking the funnel and then allowed to stand for appropriate time. You find two layers in the funnel, upper layer with petroleum ather pigments. Lower layer with water CaCO3+ debis and proteins. Discard the lower layer and place the upper.
7. Place the petroleum ether extract on the appropriate place as shown in the figure using micropipette. Try to concentrate the extract on the place by adding enough extract (20 drops) at different times to ensure the dryness of the place before each addition (Why).
8. Try to conduct your work in a cold place to inhibit the destruction of the chlorophyll by the chlorophyllase enzyme present in the extract.
9. Place the strip of paper chromatography in glass jar containing 10 ml of petroleum ether. The solution will move up on the paper by capillary action causing movement the pigment upward to distances depend on the type at pigment.
10. After a definite time remove the paper from the jar and mark the paper to solvent flow immediately by using pencil Why? allowed the paper to dry then determine the distance of flow of each pigment.
11. Finally, determine the relative of flow (Rf) of each pigment by the following equation:

$$RF = \frac{Flow\ distance\ of\ the\ pigment}{flow\ distance\ of\ the\ solvent}\ x\ 100$$

Lab 6

Hill reactions

Introduction

Photosynthesis process is composed of two major reactions the light reaction and the dark reaction. In the light reaction, the photons of light exited the pigment systems (composed of different types of chlorophylls arranged in a geometric model) and changed them from stable form to exited from within a very short period of time as in the following demonstrations:

Chlorophyll + light→ chlorophyll* (10^{-9} second)

Stable form exited from

Chlorophyll* + X → X* +chlorophyll (10^{-5} second)

exited form stable form

The electrons with high energy transfers though receivers and donor compounds between the pigment systems and was able to reduce ADP to ATP and the $NADP^+$ compound (oxidized form) to $NADPH_2$ (reduced form) by the protons (H+) resulted from H_2O ionization as in the following equation:

$$NADP^+ + H_2O + light \xrightarrow{Chlo.} NADPH_2 + ATP + O$$

The ATP and $NADPH_2$ produced by light reaction are utilized to fix CO_2 to produce different types of carbohydrates by the Calvin cycle reaction reactions (dark reactions) as in the following equation:

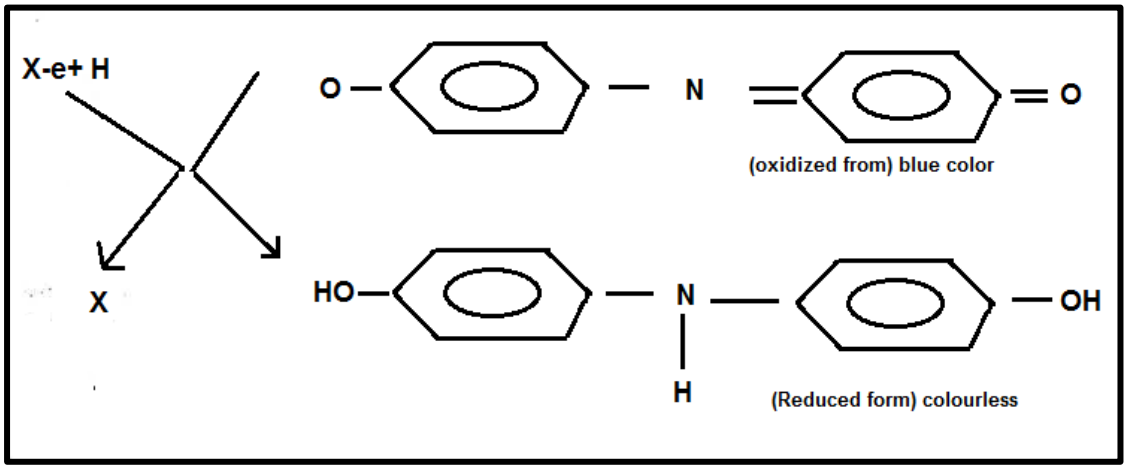
Thus it can be stated that photosynthesis is a water oxidation and CO_2 reduction process which synthesizes carbohydrates in presence of chlorophyll and light.

The presence of electron and its energy inside the chlorophylls of pigment system I and II leads to oxidize the pigments. (lose of its color) and the pigments will restore its color (reduced form) after the electron and its energy leaves to receiver and donor compounds located between the pigment systems.

The British scientist hill (1938) found that when a water solution containing plastids and Mn^{++} and Fe^{+++} exposed to light in the absence of CO_2, O_2 is liberated and the ions of Mn and Fe were reduced. The liberation of o2 continued with the continued of photosynthesis processes.

The hill experiment is clearly indicated that the protons of H_2O is responsible for the reduction of $NADP^+$ in light and the O_2 liberated is a result of H_2O ionization.

In vivo $NADP^+$ is the receiver of the electron and protons (H^+) and it is colored in chloroplast. In vitro, in order to prove Hill reactions, we have use electron receiver compound such 2, 4- di chloro phenol indo phenol (DPIP) to substitute the $NADP^+$. This reagent changes its color from blue to colorless when it receives the electrons as showing in the following diagram.



First experiment: Hill reactions with time

Procedure:

1. Prepare 3 test tubes each contained:

   0.5 ml chloroplast extract + 2.5 ml DPIP + 2.5 ml buffer

2. Read the optical density (OD) of the mixture at 620 nm using spectrophotometer as follow:

a- Read the OD of first tube after 5 minutes

b- Read the OD of second tube after 10 minutes

c- Close the third tube just after prepare the mixture and read the OD after 15 minutes

After you read the OD of each tube, you add small amount of ascorbic acid (Vitamin C) to all tubes and take the observation.

Note: Ascorbic acid is able to reduce DPIP and change it to colorless.

Second Experiment: Effect of some physical and chemical factors (dark, boiling and inhibitors) on Hill reaction.

Procedure:

1 - Take 5 test tubes and add to each of them the materials listed in the following table:

MATERIAL	treatments				
	Tube#1 (control)	Tube#2 (test)	Tube#3 (dark)	Tube#4 (boiling)	Tube#5 (inhibitor)
Buffer	2.5ml	2.5ml	2.5ml	2.5ml	2.5ml
DPIP	……..	2.5ml	2.5ml	2.5ml	2.5ml
Atrazine	…….	…….	…….	…….	2.5ml
Chloroplast	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml

Leave all tubes for 10 minutes, centrifuge them at 300 rpm for 15 (why?) and read the optical density at 620 nm then present your results in a table.

Note: The optical densities of the treatments are expected to be close to those presented in the following table:

treatments				
(control)	(test)	(dark)	(boiling)	inhibitor)
0.2	0.25	0.4	0.45	0.6

Q. Why does the inhibitor show the highest OD?

Notes: Expose of chlorophyll to dark does not exited (Why)

1 - Control treatment shoed low OD due to absence of chlorophyll

2 – Boiling cause chlorophyll destruction but there is a probability of releasing some electrons.

Lab 7

Transpiration

It is the loss of excess amount of absorbed water in a vapor status especially in leaves but also in stems, flowers and roots as the rest were consuming in several biological processes in plant body. Leaf surfaces are dotted with openings which are collectively called stomata. The stoma are bordered by guard cells (together known as stomata) that open and close the pore, the opening of the stomata allow the diffusion of carbon dioxide gas from air to plant tissue for photosynthesis. Transpiration also cools plants, changes cell's osmotic pressure, and enables mass flow of minerals and water from roots to shoots. **Guttation** is another process in which a plant get rid of excess water amount (in a liquid status) outward through hydathodes which are dispersed on leaves margins , this process is carried out when the transpiration is unavailable. Guttation usually accompanies with burning the edges of leaves, because of minerals which come out with water and remain on leaves after the water being vaporized.

Transpiration cannot occur without that streaming or the flow up of water from root passing stem then entering leaves tissues, this flowing of water through this path fulfills depending on several biological and physical conditions:

1- **Root pressure**: is caused by active distribution of mineral nutrient ions into the root xylem. They accumulate in the root xylem and lower the water potential. Water then diffuses from the soil into the root xylem due to osmosis. Root pressure provides a force, which pushes water up to the stem, but it is not enough to account for tallest trees. The maximum root pressure measured in some plants can raise water only to about 7 meters, and the tallest trees are over 100 meters tall.

2- **Cohesion** of water molecules to one another and **adhesion** of these molecules to walls of xylem cells, both can overcome gravity pull down force but only for very short distance.

3- **Transpiration pull force**: The upward transpiration pull force on the sap in the xylem causes draining of the sap (water as major component) from lower parts tissues according to the water

potential gradient creates once the emptying of leaves cells from water by transpiration.

This table summarizes the factors that affect the rates of transpiration.

Feature	How this affects transpiration
1-No.of stomata	More stomata will provide more pores for transpiration.
2-Light	Stomata are directly related to the rate of transpiration, and these small pores open especially for photosynthesis. Whilst there are exceptions for this (such as night or CAM photosynthesis), in general a light supply will encourage open stomata.
3-Temperature	An increased in temperature will increases evaporation rate that will increases transpiration rates.2) Increased kinetic energy of water vapor particles aids diffusion out of the leaf.
4-humidity	A dry external surrounding will make a water potential gradient, and increase the rates of transpiration.
5-Wind	If the water potential gradient from inside to outside the leaf is slightly less, due to the accumulation of water vapor there that will reduce transpiration rate, If there is a wind, that will blow away the vapor and the gradient remains higher.

Experiment 1

Measurement of the transpiration rates under different conditions by using Potometer

Potometer is a device used for measuring the rate of water uptake of a leafy shoot. The causes of water uptake are photosynthesis and transpiration.

Procedure:

1- Cut a leafy shoot from a plant under water. This prevents the xylem from taking up any air. Wetting the leaves themselves will alter the rate of transpiration.
2- Flood the photometer with water to eliminate any air bubbles, then close the reservoir tap.
3- Insert the base of the leafy shoot into a bung, grease the bung with plenty of petroleum jelly (Vaseline) and fit it in the device.
4- Leave the end of the capillary tube out of the water until an air bubble forms then put the end into a beaker of water.
5- Set up the conditions of the experiment. Alterations to lighting (placing the plant in bright light or shadow), wind (directing a fan at the plant), and humidity (placing the plant in a humid chamber) are typical.
6- Measure the distance that the drop takes at regular timing intervals (5, 10, 15, 20, 25, 30 min.) under the mentioned conditions. Figure out the distinctions in the light of the results.

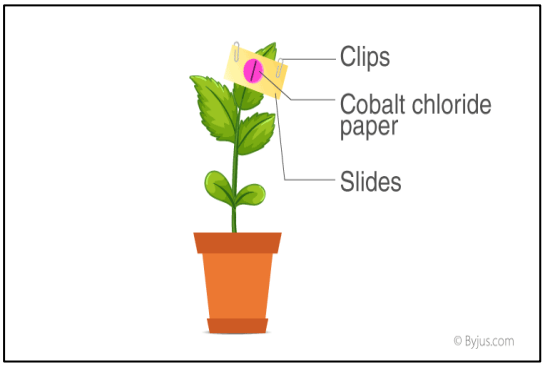
Experiment 2:-

Demonstration of unequal transpiration using cobalt chloride paper

- **Aim of the experiment:** To compare the rate of transpiration from upper and lower surfaces of
  a leaf by using cobalt chloride paper
- **Requirements:** A well-watered dicot plant, 3%solution of cobalt chloride, filter paper, slides, forceps, slides, clips, and Vaseline.
- **Procedure:**

1. Soak two small equal discs of white filter paper in 3% solution of cobalt chloride.

2. Remove the excess of cobalt chloride solution. The soaked filter paper turns pink. Now it is called cobalt chloride paper.



3. Dry the cobalt chloride paper with a hair drier. They turn blue.
4. Place one such cobalt chloride on the upper epidermis of a leaf of a dicot plant and one cobalt chloride paper on the lower epidermis opposite to each other.
5. Press these filter paper pieces with clean glass slides. Clip the two slides together with two separate clips and smear little wax around the glass slides to keep the set up air tight.
6. Compare the time taken for the blue colored filter paper to change into pink.

- **Result:** The filter paper kept on the lower surface turns pink faster than one kept on the upper surface.
- **Inference:** The experiment confirms the unequal rate of transpiration is more on the lower surface of leaf than on the upper surface of leaf.

Experiment 3:- Wilting of leaves

1. Choose 4similar leaves of a dicot plant.
2. Smear both surface of leaf 1 with oil.
3. Smear only the upper surface of leaf 2with oil.
4. Smear only the lower surface of leaf 3 with oil.
5. Let leaf 4 hang freely in the air as a control without smearing it with oil.

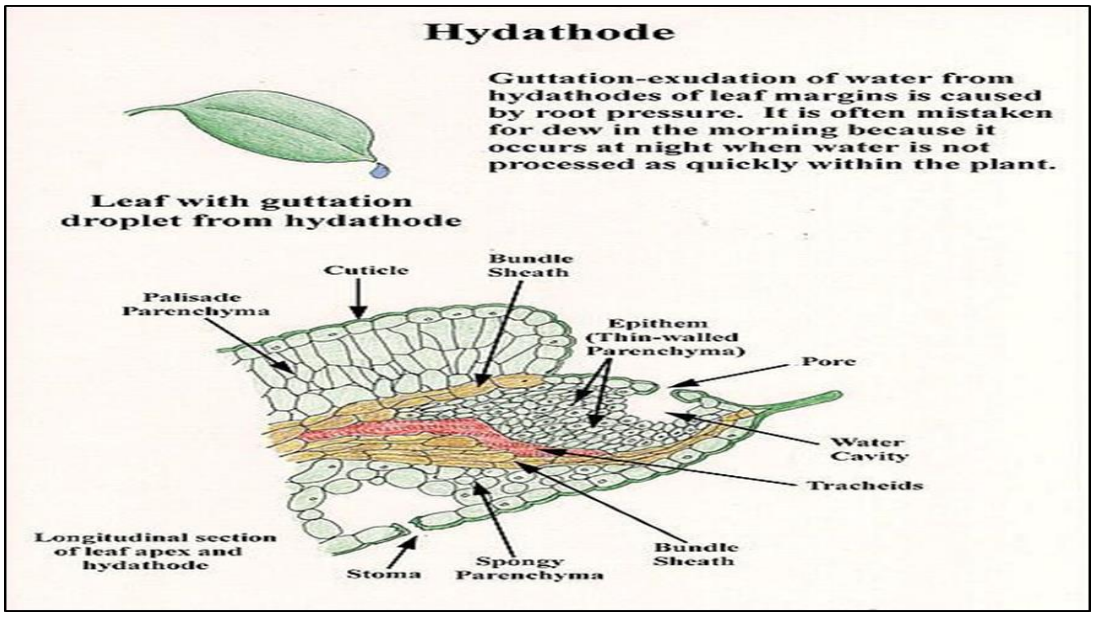
   The next day examine the leaves. (Record the result)

Guttation

In small, herbaceous plant it has been observed that in cool, humid conditions in early morning hours, there is a high water pressure in the root it forces water into the leaf and it is actually released from the leaf through small opening called ***hydathodes***

In a process called ***guttation.***

Transpiration	Guttation
1-In transpiration, water is lost from aerial parts of plants in the form of invisible water vapors	1-In guttation ,water solution oozes out from uninjured margins of aerial leaves only
2-It occurs in all vascular plants	2-It occurs only in some Angiosperms Such as strawberry, garden nasturtium (Tropaeolum), Colocasia, tomato etc
3-Transpiration occurs mostly through stomata. It may also take place through cuticle and lenticels	3-occurs only through hydathodes (water stomata)
4-Usually it takes place throughout the day, its rate being maximum	Usually it takes place only early in the morning when root pressure and rate of water absorption are higher.



Lab 8

Seeds Dormancy

Dormancy (True dormancy) is a mechanism to prevent germination of certain seeds under environmental factors that are normally suitable for the germination of the non-dormant seed. While, **quiescence**, is different than true seed dormancy and occurs when a seed fails to germinate because the external environmental conditions are too dry or warm or cold for germination, but the seed will germinate if suitable environmental conditions happen.

Important functions of seeds dormancy are:

1- Allows seeds for disperse and prevents germination of all the seeds at same time.
2- 2- The delaying of germination safeguards some seeds and seedlings from suffering damage or death from periods of bad weather or transient herbivores.
3- Allows some seeds to germinate when competition from other plants for light and water might be less intense.
Many species of plants have seeds that delay germination for many months or years, and some seeds can remain in the soil seed bank for more than 50 years before germination. Some seeds have a very long viability period, and the oldest documented germinating seed was nearly 2000 years old.

Often seed dormancy is divided into two major categories based on what part of the seed produces dormancy: exogenous and endogenous.

1- Exogenous dormancy

Exogenous dormancy is caused by conditions outside the embryo and is often broken down into three subgroups:

a- Physical dormancy

Physical dormancy is the result of impermeable layer(s) for water. These layers are lignified and impregnated with water-repellent.

Physical dormancy is broken by several factors including high temperatures, fire, freezing/thawing.

b- Mechanical dormancy

Mechanical dormancy occurs when seed coats permeable for water but too hard to allow the embryo to expand during germination. This type of dormancy is broken by puncture the seed coat with hot needle.

c- Chemical dormancy

Includes chemical inhibitors present in the coverings around the embryo. They may be leached out of the tissues by washing or soaking the seed in hot water.

2- Endogenous dormancy

Endogenous dormancy is caused by conditions within the embryo itself, and it is also often broken down into three sub group:

a- Physiological dormancy

Physiological dormancy prevents embryo growth and seed germination this happen in presence of **inhibitors** that often retard embryo to the point where it is not strong enough to break through the seed coat. Physiological dormancy is broken often by a period of cool moist conditions, or by light that effect on **Abscisic acid** is usually the growth inhibitor within seeds.

b- Morphological dormancy

Embryo undifferentiated. Immature embryos – some plants release their seeds before the tissues of the embryos have fully differentiated tissues, germination can be delayed from a few weeks to a few months.

c- Combined dormancy

Seeds have both morphological and physiological dormancy.

Morphophysiological dormancy occurs when seeds with undifferentiated embryos, also contain inhibitors both responsible for

such type of dormancy. Therefore, these seeds require dormancy-breaking treatments as well as a period of time to develop a fully grown embryo.

Experimental procedure:

Breaking the seed coat dormancy

Hot water treatment:

A. Make groups of 10 uniform and healthy looking seeds of the test plant species (10 groups for each group of students)
B. Immerse 3 groups of seeds in boiling water for 3 minutes and other 3 groups for 6 minutes .Leave the remaining 4 groups untreated as a control.
C. Place the seeds in petri dishes (10seeds/petri dish) in between filter paper and add appropriate amount of water.
D. Count the germination per cent after one week from sowing.

Acid treatments:

A. Make groups of 10uniform and healthy looking seeds of the test plant species (10groups for each group of students).
B. Immerse 3 groups of seeds in H_2SO_4 placed in glass beaker for 3 minutes and other 3 groups for 6 minutes. Leave the remaining 4 groups untreated as control.
C. Place the seeds in petri dishes (10 seeds /petri dish) in between filter paper and add appropriate amount of water.
D. Count the germination per cent after one week from sowing.

Hot needle treatments:

A. Sting 3 groups of seeds by hot needle
B. Follow the same steps in (C and D) .

Lab 9

Plant growth regulators

- **Plant growth regulators** can be defined as chemical compounds synthesized in plants or outside in plants (in factory) and have the ability to regulate plant growth and development. Examples: auxins, 2, 4 D and vitamins.
- **Plant hormones:** It is organic compounds synthesized in specific regions in plants and moved to other regions to express their effects. Example: Auxins and Gibberellins.

1. Auxins:

- Darwin was the first scientist who found that the oat coleoptiles bend towards the source of light in process called phototropism.
- Additional work was conducted on this compounds and led to the following facts:
  1. The compound responsible for phototropism is synthesized in **apical region** of the coleoptiles.
  2. The compound is accumulated in the region away from light, increased the growth of this region and caused phototropism.
  3. The compound accumulated in the dark part moved downward the coleoptiles.
  4. It was found that the extract of coleoptiles contained the compound.

Physiological effects of auxins:

1. Cell elongation of leaves, roots and stems:
2. Leaves and fruit drop:

   Tropism: It is defined as the movement of plant organ towards the environmental stimulus such as light, gravity, water, chemicals and mechanical stimulus. There are several types of tropism:

   A. **Phototropism:**
   B. **Geotropism:**
   C. **Chemotropism:**
   D. **Thigmotropism:**
   E. **Hydrotropism:**

3. Apical dominance:
4. Callus formation:
5. Roots initiation:
6. Fruit development:
7. Increase rate of respiration:
8. Induce the synthesis of ethylene:
9. Translocation of nutrients within plant:

2. Gibberellins (GAs)

Positions of GAs synthesis in plants:

1. Juvenile leaves
2. Roots
3. Buds
4. Embryos and fruits

Physiological effects of GA3:

1. Control genetic dwarfism:
2. Stimulation of flowering and growth of flower branches:
3. Inhibition of root formation in cuttings and callus.
4. Stimulate germination of seed which required dark condition.
5. Formation of parthenocarpic fruits.
6. Breaking the dormancy of seeds and buds.
7. Stimulate the synthesis of enzymes in endosperm of germinated seeds.
8. GA_3 stimulates male flowers in some plants.

3. Cytokinins

- Cytokinins were found to synthesize in roots and translocated to other part of the plant by xylem.
- **Rate** of movement of cytoinins is **slower** than the auxins and GAs.

The most important physiological effects are:

1. Cell division.
2. Cell enlargement.
3. Root initiation and growth.

4. Shoot initiation and growth.
5. Breaking dormancy.
6. Preventation of aging and senescence.
7. Enzymes formation.

4.Abscisic Acid (ABA)

It is considered as growth inhibitor hormone.

Physiological effects of ABA:

1-Regulation of stomatal opening and closing:
2- Bud dormancy.
3-Abscission and senescence of leaves and fruits

5. Ethylene

1. It is growth retardant hormone
2. Gas under natural condition
3. Simple chemical structure compared to other hormones
4. Found in a very low concentration in plants
5. It affects essential physiological process alone or in combination with auxins.

Physiological effects:

1. Fruit ripening. 2. Geotropism. 3. Apical dominance

Lab 10

Allelopathy

Allelopathy is the direct influence of chemicals released from one plant on the development and growth of another plant. Multiple physiological effects have commonly been observed from many allelochemicals released from allelopathic plants. These effects include decreases in plant growth, absorption of water and mineral nutrients, ion uptake, leaf water potential, shoot turgor pressure and rate of photosynthesis and respiration.

Allelopathy plays a major role in natural ecosystems by determining vegetational patterning, plant dominance, plant succession, plant biodiversity, preventing seed decay and causing seed dormancy. Also, allelopathy has a significant role in agricultural ecosystems such as weed - crop, crop - weed, crop - crop, forestry and nutrient cycling. During the last four decades, the results of allelopathic effects of crops on weeds revolutionized the scientists to put much effort on this aspect with the aims of using this phenomenon to reduce the dependence on chemical herbicides for weed control. Different strategies in which allelopathy is involved have been suggested such as using allelopathy in crop rotations, cover crops and mulches, smother crop, crop mixtures and intercropping and use of allelopathic crop residues or extracts. Allelopathic crop residues as mulch or incorporated into field soil have been found to be the most successful strategy in weed suppression. However, in most cases, the efficacy of allelopathic residues was generally below that of herbicides. Therefore, many researchers have discussed the possibility of integrating allelopathic residues with other managing options for weed control. suggested that a herbicide applied in combination with allelopathic conditions could enjoy a complementary interaction, and may help to

minimize herbicide usage for weed management in field crops. The combination of allelopathic crop extract with lower rate of herbicide was first explored by Cheema group in Pakistan during the last decade. These scientists postulated that herbicide use can be reduced by 50 – 70 % when herbicides are used in combination with aqueous sorghum extracts for weed control in field crops such as wheat, cotton, mung bean and maize. Although successful results have been obtained from allelopathic plants extract applied with low herbicide rates, additional work in other soil types and to employ this technology, large volumes of sprays are required for field application, and therefore appropriate concentrations for each crop should be determined for large scale field operations.

Due to these limitations, an alternative practical and feasible approach has been developed by Alsaadawi group where the residues of allelopathic crops including sorghum have been left to dry under field conditions and then promptly incorporated into production sites for weed management. Low herbicide doses were applied along with residue incorporation. By using this approach with faba bean, wheat, barley, cowpea and mung bean, it was found that application of half the labeled rates of the test herbicide in field soil amended with sorghum or sunflower residues suppress weeds and generated crop yield similar to that of the label (full) rate of herbicide.