BIOLOGY LABORATORY
A METHOD FOR DETERMINING AIR QUALITY

Determination of the air quality using a bio-indicator: the pink yeasts on tree leaves.

CONTEXT:

– Some living organisms are particularly sensitive to the presence of polluting agents in their living environment. Their presence in more or less large quantity can thus give us an indication of the air quality. This is why these living organisms are called bioindicators. This is for example the case of a microscopic fungus (called pink yeast) which lives on the underside of the leaves of some very common trees such as ash or maple. These yeasts are sensitive to nitrogen dioxide (NO_2)*, which is why, their number on the leaves decreases sharply in case of pollution.* Mainly due to road traffic)

1. BACKGROUND:

– Installation of air quality sensors in schools in Brussels (link to actions carried out by the non-profit organization "Chercheurs d'air").

2. MOBILIZATION:

Become aware of the problem and the possible consequences of too much air pollution. Reading of a press article and reflection on the question: "What are the possible consequences of an increase in air pollution?"
Article: "Children breathe poor air in nearly two schools out of three" Le vif—March 14, 2018

3. MANIPULATIONS TO BE CARRIED OUT:

3.1. Preparing a culture medium
– For 40 Petri dishes (sterile plastic dishes used for culture of yeast), prepare a culture medium with 15g Agar-agar, 5g glucose and one liter of water.
– Boil the mixture then distribute it in the dishes.
– Close the dishes and let the mixture cool down and gel.
Note: Make your work as sterile as possible by disinfecting the tables and hands with alcohol.

3.2. Sampling collection
Before the experiment:
– Define a 500m radius around the school.
– Locate 6 trees (ash or maple) in this area and name them A to F.
– Prepare six plastic bags (freezer bags).
—In each bag, insert a card with the following information:

See dichotomous tree recognition key
—Students' Names
—Reference letter given to the tree: A, B, C, D, E, F
—Date and time of sampling
—Commune, sampling village
—GPS data of the sampling location
—Tree species
– Description of the environment (woods, garden, downtown, main road, busy crossroads, quiet street, ...)

The sycamore maple is a tree with single, lobed, opposite leaves. Its fruits (samaras) are grouped in pairs. It is very common in our country.
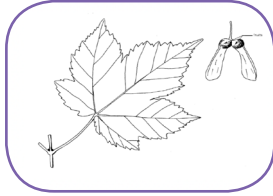
The maple should not be confused with the plane tree whose leaves are alternate, with more spaced teeth and whose fruits are in the shape of balls.

The ash tree has compound leaves that are pinnate, opposite and with 7 to 13 leaflets. The presence of black buds is typical of the species.
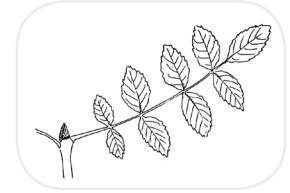
3.2. Sampling collection

The day of the experiment:
— Take 2 or 3 leaves from each tree and place them in the corresponding plastic bags.
Remarks: Do not choose the fallen leaves instead, take the leaves still present on the branches. If it is not possible to perform the sampling on the day of the experiment, this can be done the day before by storing the plastic bags in the fridge.

3.3. Carrying out the experiment

— Thoroughly clean the work table with denatured alcohol.
– Take the first three leaves of a tree out of their bag, holding them by the petiole and place them on the table with the bottom side up.
— Cut out 6 disks from these 3 sheets, using a cookie cutter (apple corer knife).
– Without touching them with your finger, using a pair of pliers, place the disks on the table (still with the underside facing upwards).
– Using a marker, draw 3 lines on the bottom of the dish in order to delimit 6 zones.

– Open the dish and place the lid with the inner side up and the other part with the culture medium down to prevent it from becoming infected when in contact with the air.

Rondelles de feuilles

couvercle

Milieu de culture
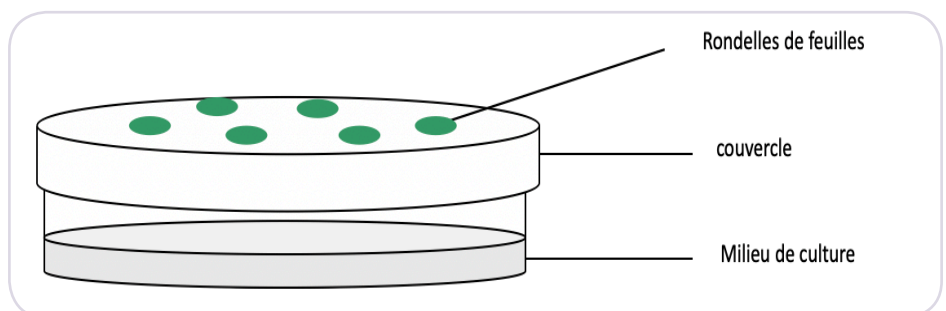
– Using a spatula, place a point of petroleum jelly in each area on the inside of the lid.
— Attach the leaf disks (always with the underside upwards) to the jelly dots.
— Close the box and fix the lid with sticky paper.
— Paste a label with your name and the tree reference (A, B, C, D, E or F).
— Repeat the operation for the other trees.
— Group all the boxes of the classroom on the same table.
- The next day, 24 hours after the beginning of the experiment, turn the boxes over (lid down) to stop the spores from falling out.
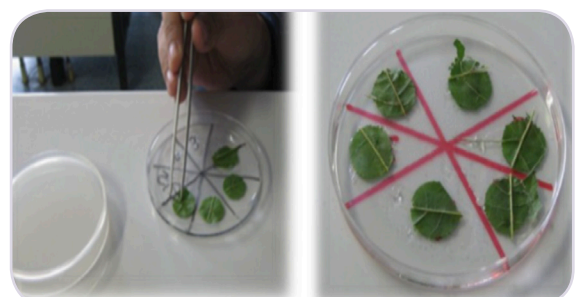
WHAT HAPPENS DURING THIS EXPERIMENT?

The pink yeasts on the underside of the leaves will release spores.
These will fall on the medium of culture and will develop in colonies. Each spore giving in principle birth to a colony, the number of these colonies gives us a rather reliable indication of the quality of the air at the place where the sampling was carried out.

4. RESULTS

– One week after the beginning of the experiment, observe and count with a magnifying glass or binocular the number of colonies per zone

Note: Colonies are recognizable by their pale pink color. Sometimes colonies may merge. Therefore, try to estimate the number of colonies that have merged.

– Indicate the results in the chart in the appendix.
– Calculate the averages per tree and then for the region studied.

	Nombre de colonies par rondelle							Moyenne
Arbre A								

5. CONCLUSION:

Based on the calculated averages, draw a conclusion about the air quality.

Classes (cultures/cm²)	Signification (degré de pollution)
De 0 à 5	Qualité de l'air mauvaise
De 6 à 20	Qualité de l'air médiocre
De 21 à 50	Qualité de l'air modérément bonne
De 51 à 90	Qualité de l'air bonne
91 et plus	Qualité de l'air excellente

Communicate the results as soon as possible and before November 13, 2020 at the following address: g.ferenc@ hypothese.be.

If you participated in the "Chercheurs d'air" campaign, it is interesting to compare the results of the two experiments. Compare the results obtained in this experiment, using bioindicators with the nitrogen oxide levels measured by chemical method.

You can also consult the daily nitrogen dioxide measurements available on the irCELine platform (Cellule Interrégionale de l'Environnement): https://www.irceline.be/fr/qualite-de-lair/mesures/dioxyde-dazote

Thank you for your participation