Sampling techniques

Microscope area computation. Examples of microscope slides analysis

The conversion factor counting
Sampler VPPS 2000 (Lanzoni)

cutting block

Weekly sampling drum
14 mm

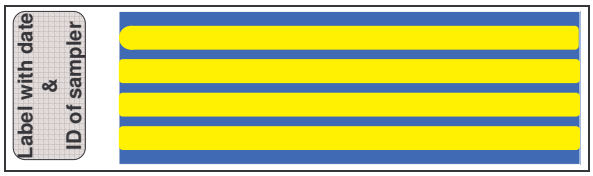
Tape's length = 336 mm

Speed 2mm/hour

24 hours x 2 mm = 48mm / day
48 mm x 7 days = 336 /week

Basic microscopy, calculating field of view.

The daily monitoring samplers are prepared to be examined under optical microscope to identify the different types of pollen grains with varing magnifications, the most diffuse of which are the 250x or the 400 x ones
The examined surface is a part of the entire figure of the daily sampler, because of the homogeneous deposition of the pollen grain on the plastic sampler
The total examined area corresponds to the sum of the rectangles which have the height as the diameter Ø of the microscopy field and the length of 48 mm which is length of the sampler



Pollen concentration computation

- diameter of microscopic field **Ø**
- number of horizontal lines of lecture
- number of grains counted on examined surface
- sampling area (48 x 14 = 672 mm^2)
- air sucked for a day (10l/minute x 60' x24 = 14,4 m^3)

The total daily sampler surface is :
14 x 48 mm = 672 mm^2
On this area bio- and abio- particles are deposited in 10 l/minute.
600l/h X 24 = 14400 l/day = 14.4 mc^3
total examined area :

field diameter x 48 mm x number lines
E.g. 0.72mm x 48mm x 4 = 138.2mm^2
P = pollen grain which are present on the total sampler
L= Pollen which have been counted

P:L= 672 mm^2 : 138mm^2
P= L x $\frac{672}{138.2}$ = L x 4.862
conversion factor :
ratio sampled area/examined area x daily volume
To calculate number of pollen grains which are concentrated in 1 mc
P = L x 4.862/ 14.4 mc = L x 0.338

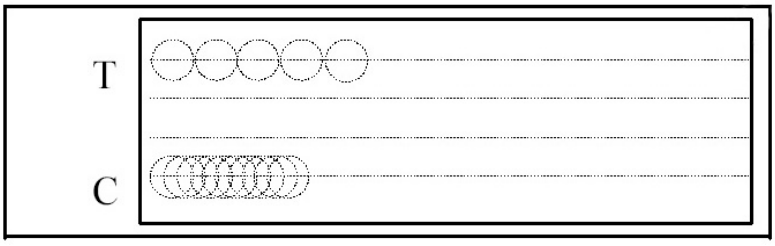
The examined surface is a part of the entire figure of daily sampler, because of the homogeneous deposition of the pollen grains on the plastic stream
At least the examined surface should not be less than 16% of entire sample and a study of more 20% does not provide a greater significance

Calculating the field of view

It is important to know the diameter of microscopic field.
This can be correctly measured by using a microscopic slide that has a printed micrometric scale
It is also possible to count the diameter dividing a known length by the number of microscopic fields necessary to scan the whole length

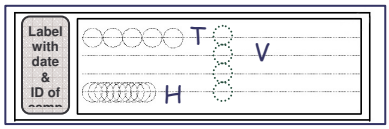
The microscopic field diameter is a characteristic of each microscope and of each magnification used and it can be modify by the adding of any apparatus (fluorescence, camera and so on)

Microscopy field



T = tangential fields
C = Horizontal sweeps

POSSIBLE LECTURE' S TYPE



HORIZONTAL SWEEPS

The sample is examined scanning 3, 4, or 5 horizontal lines separated by a space of 2 mm, to avoid overestimation or empty areas. The surface of each area of examined line is obtained multiplying 48 mm by microscopic field diameter.
Scannering of horizontal sweeps follows the direction of rotation of sampling tape and enables the recording the variation during 24 hour period.
Pollen caught in 1 hour is deposited on a surface of 2 x 14 mm.
So it is possible to know at specific hour which type of pollen was captured
For a correct examination begin by the upper left corner of tape,
Go down of 5 mm and start with the observation towards right.
At the end of line go down of 2 mm and start with the observation towards left.
And so on for all lines necessary to observe the area 16 and 20 %

TANGENTIAL FIELDS
With this method successive tangent fields positioned on 3,4 5, lines are examined.
After having counted the pollen in one field the slide is moved to the next tangential field.
Depending on the number of lines counted and the diameter of each field to calculate the pollen concentration per cubic meter of air, a factor is calculated which depends on the relation between the total examined surface and the total surface of the tape
VERTICAL FIELDS
The slides are examined in 24 transversal lines at interval of 2 mm one from the other; each one is 14 mm high and as wide as a microscopic field. In this way a line is read for every hour.

In this method the choice f the position of the lines could influence the final result expressed as average daily concentration because the concentration between the sweeps is unknown.
It is useful when it is necessary to know the pollen concentration at given moments of the day
In this case it is also necessary to know the diameter of the microscopic field and therefore the total examined surface to calculate a factor of correction which will enable the calculation of the number of pollen per cubic meter of air

Possible sources of error

For the pollens which are partially outside the counting sector , but identifiable, two methods may be adopted:

to count (or no to count) the pollens which are not completely inside the field

or

if more than half of the grain is visible, consider it inside the sector and not count those which are more than half outside

1. Broken pollen: if the are identifiable they are counted as they were whole otherwise they are counted with unidentifiable pollen.
2. Reading method: microscopic focus (continuously control in various focal plane the presence of pollen because pollen isn't perfectly flat), high number per field, peripheral pollens, eye position on the microscope, percentage of scanned surface
3. Sample interpretation: not very stained pollen, hidden pollens, broken pollen, monotony of the sample
4. Slide preparation: kind of adhesive and how it was applied, transparent polyester tape, application of cover glass over the sample