BIOACCUMATION

Many persistent pollutants such as potentially toxic elements, fluorides, chlorinated hydrocarbons, polycyclic aromatic ones, radionuclides, are difficult to detect in the air in appreciable concentrations using analysis instruments. Therefore it is very difficult to study their diffusion in a territory, and verify potential emission sources.

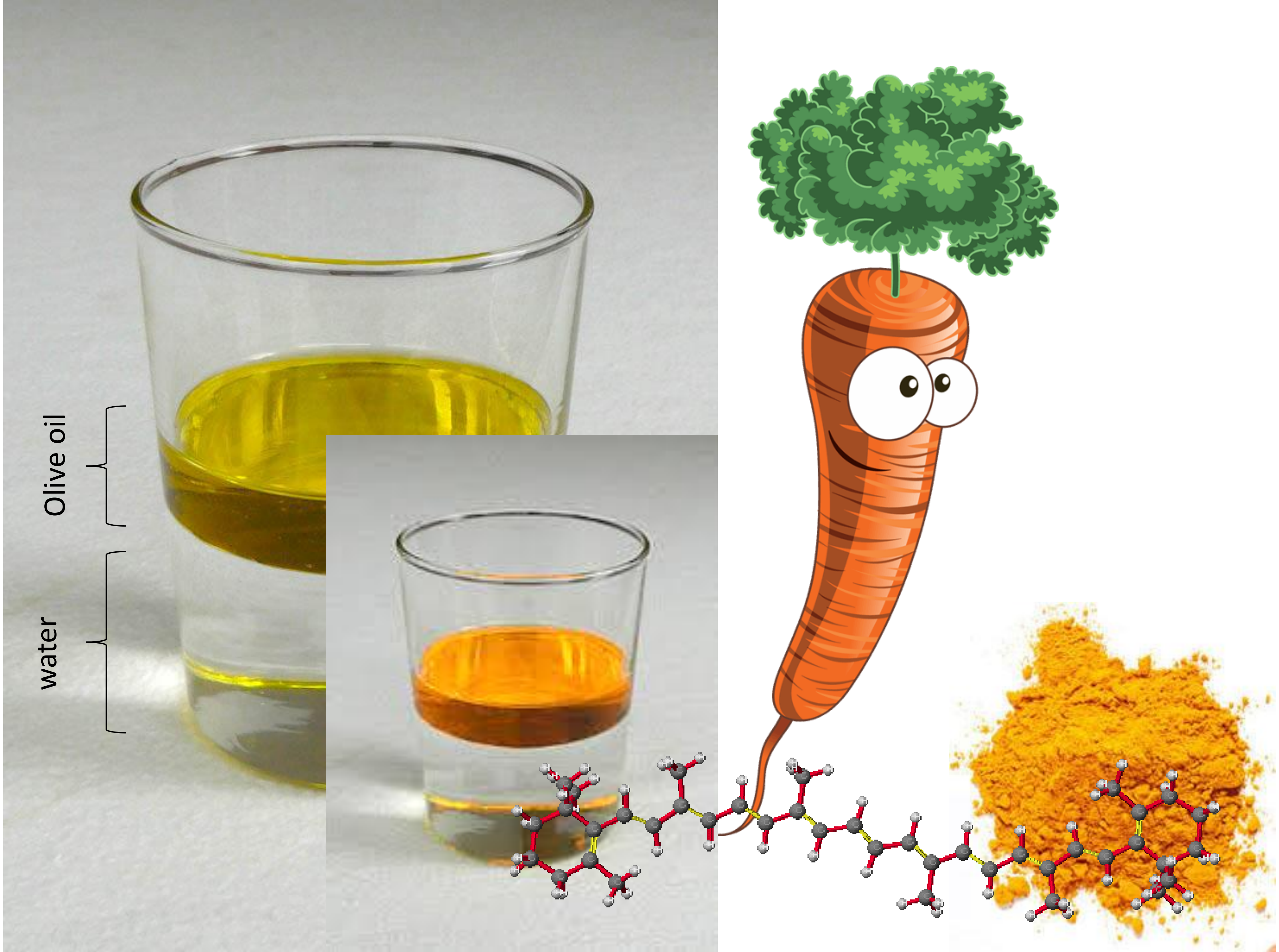
Some biological matrices allow us to more easily measure the relative abundance of these potentially dangerous pollutants, identifying the areas of greatest deposit on the ground, the source etc.

Many substances present in the environment can have - based on their chemical-physical properties - specific affinities for certain organic matrices.

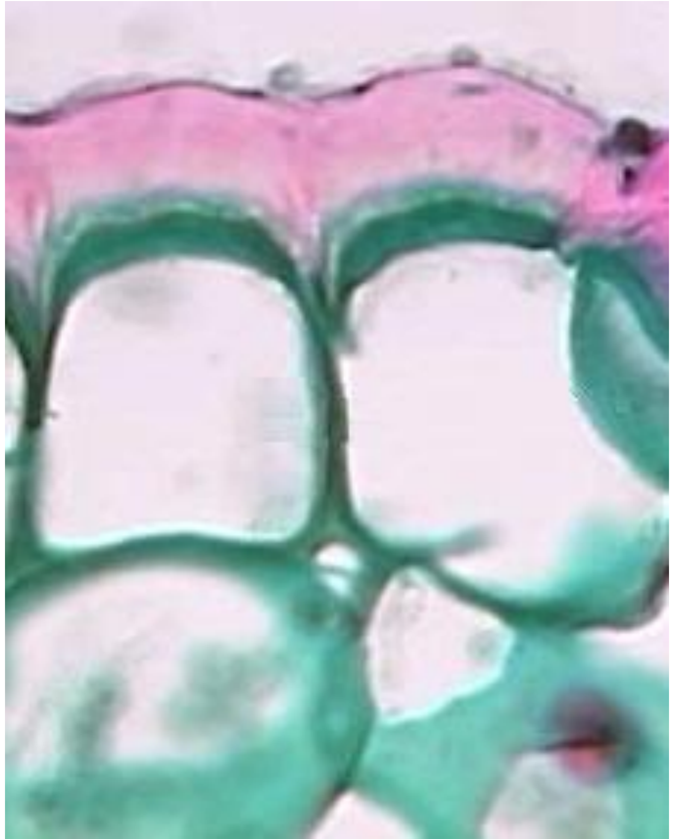
In the physical sciences, a **partition coefficient** (P) is the ratio of concentrations of a compound in a mixture of two immiscible solvents at equilibrium. This ratio is therefore a comparison of the solubilities of the solute in these two liquids. The partition coefficient generally refers to the concentration ratio of un-ionized species of compound, whereas the distribution coefficient refers to the concentration ratio of all species of the compound (ionized plus un-ionized).

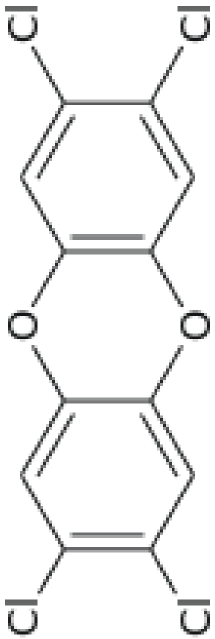
Both phases usually are solvents. Most commonly, one of the solvents is **water**, while the second is hydrophobic, such as **1-octanol**. Hence the partition coefficient measures how hydrophilic ("water-loving") or hydrophobic ("water-fearing") a chemical substance is.

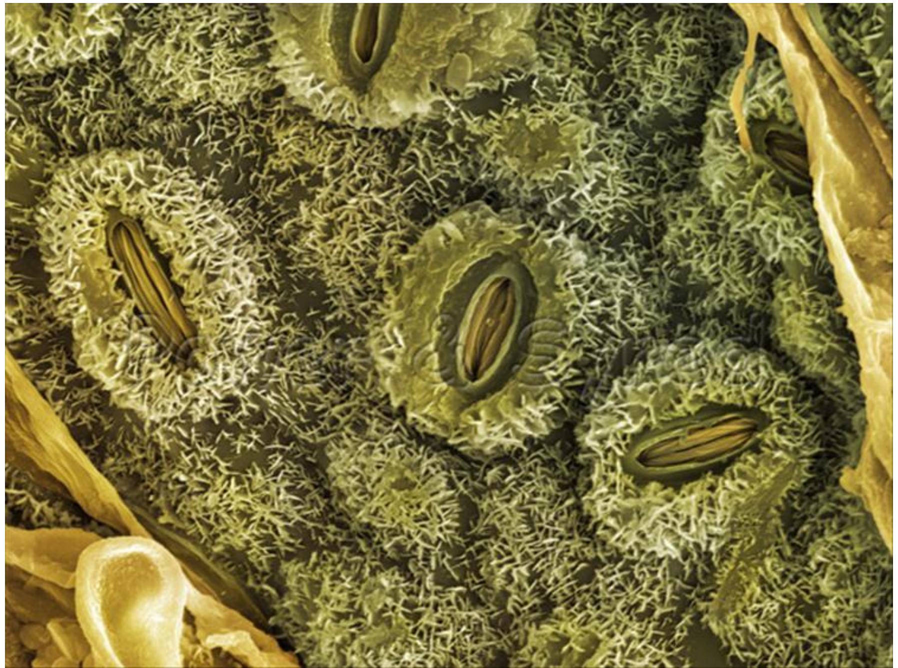
Partition coefficients are useful in estimating the distribution of drugs within the body or of polluting substances in environmental matrices.


Olive oil
water

In general, a highly apolar substance is unlikely to reach high concentrations in a tissue very rich in water, on the contrary it is absorbed by adipose tissue or by structures rich in lipids, including cuticles and epicuticular waxes.



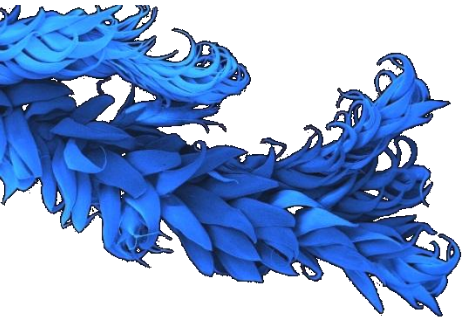




In the case of biomonitoring on *Homo sapiens*, for example, a lipophilic compound will be measurable in milk or blood, while a hydrophilic compound will be measurable in urine.

By choosing suitable biological matrices, the presence of a certain substance in the environment can be highlighted, following its dispersion kinetics, or on the contrary, a presumed absence can be substantiated.

In this case, only a well-trained person can understand whether the choice of the analyzed matrix is correct or not.



BIOACCUMULATION

Bioaccumulation techniques are applied to study the diffusion of pollutants emitted from the most varied sources, from point sources (incinerators, cement factories, foundries, nuclear power plants) to diffuse ones.

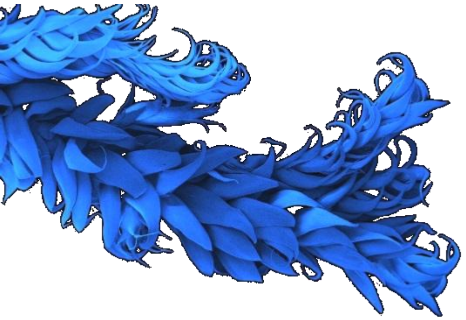
No particular taxonomic knowledge is required, unlike the use of bioindication techniques (e.g. those based on floristic-vegetation approaches), as one operates with only one or in any case with a few target species, generally well characterized morphologically (therefore easily identifiable).

However, sophisticated laboratory equipment and instruments necessary for the analysis of contaminants are required.

It is highly desirable that the operator knows the phenomena involved well, including the physiological ones.

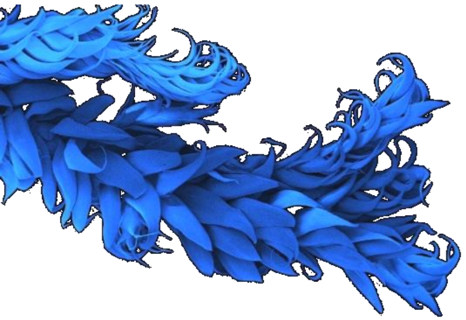
The properties that make an organism a good bioaccumulator are the following:

- high tolerance to the test substance(s);
- ability to accumulate the substance(s) examined to a (relatively) indefinite extent;
- possibility of defining the age of the organism (older parts will tend to have higher concentrations of pollutant(s) than younger parts);
- presence of many specimens in the study area (for passive biomonitoring) or in any case in natural environments (for active monitoring).



The choice of bioaccumulator to use depends on a series of factors:

- by the work tradition of the laboratory or research group;
- by the availability of consolidated and possibly shared protocols, if not even regulated at a national or international level;
- the season in which the investigation is to be carried out;
- if a passive biomonitoring study is carried out, by the presence of the organism in the area.



The possible phenomenon of **biomagnification** or **bioamplification** should be carefully considered, which is the process of bioaccumulation of substances (including toxic and harmful ones) in living beings with a progressive increase in concentration going towards the highest levels of a trophic chain, due to the inability to part of the various organisms to eliminate the substance in question, which is not degraded by normal cellular metabolism, nor can it be excreted.

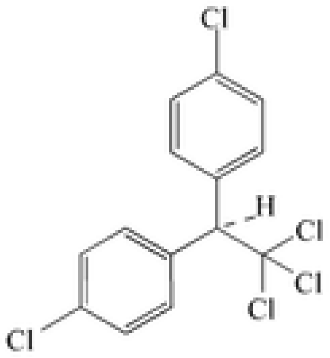
“Bioaccumulation occurs within an organism, and biomagnification occurs across trophic (food chain) levels”

In the presence of biomagnification phenomena, by analyzing animals (in particular certain tissues chosen *ad hoc*) at the top of the trophic web, substances present in the environment at very low concentrations can be highlighted, well below the limits of instrumental analysis, and it is therefore possible obtain a much less optimistic picture of the real environmental compromise.

A frequent objection (to be rejected) is that matrices are analyzed which "notoriously" have high concentrations of that specific substance, and therefore the presence in the environment would be overestimated.
The interesting aspect is that if in the samples the concentrations are low, you can be sure that the bioavailability of that specific substance in the environment is very low and certainly does not cause concern for human health.

The most famous cases of biomagnification, very well documented, are those of DDT and methylmercury.

DDT is a synthetic product, a chlorinated organic compound used as an insecticide.



Methyl mercury, an organometallic cation, is linked to the action of anaerobic microorganisms that live in aquatic ecosystems and that modify inorganic mercury released by industrial processes (where it is used as a catalyst) or natural sources (e.g. volcanoes). At each step of the food chain the concentration of methylmercury in the organism increases, and its concentration in aquatic predators at the top of the food chain can be a million times greater than the concentration in water.

CH_3Hg^+

In the 1950s, inhabitants of the seaside town of Minamata, on Kyushu island in Japan, noticed strange behavior in animals. Cats would exhibit nervous tremors, and dance and scream (“*neko odori byo*”). Within a few years this was observed in other animals; birds would drop out of the sky.

Symptoms were also observed in fish, an important component of the diet, especially for the fishermen. When human symptoms started to be noticed around 1956 an investigation began.

It was found that the Chisso Corporation, the first-class petrochemical company and maker of plastics such as vinyl chloride, had been discharging heavy metal waste into the sea for decades.

From 1951, they used ferric sulphide instead of manganese dioxide as co-catalyst, causing the formation of a 5% of methylmercury, which was discharged in the wastes. After some years, 2 kg of mercury per ton of sediment was measured at the mouth of the bay: a level that would be economically viable to mine…

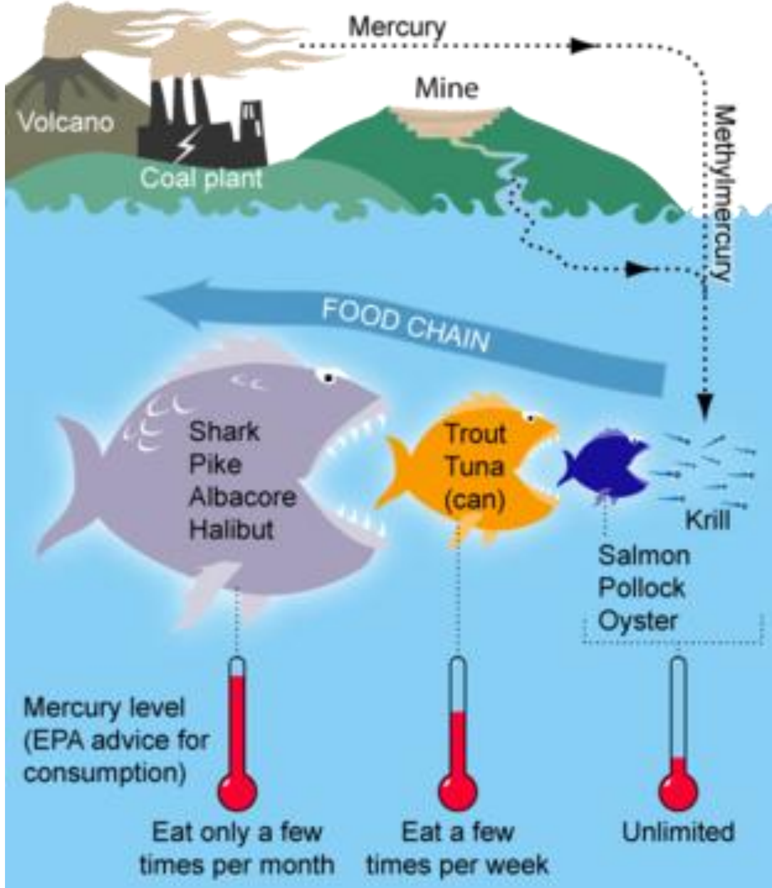
It is believed that about **5,000 people were killed** and perhaps 50,000 have been to some extent poisoned by mercury.

Mercury poisoning in Minamata, Japan, is now known as *Minamata disease*.

Hair samples were taken from the victims of the disease and also from the Minamata population in general.

The maximum mercury level recorded was:

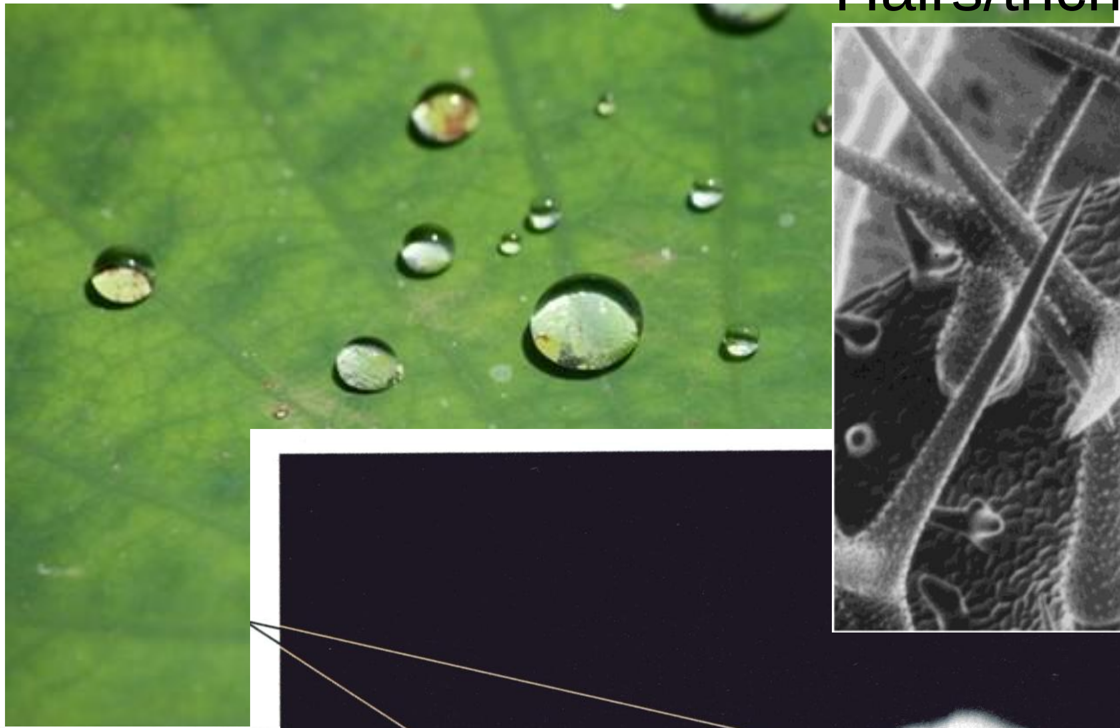
- 705 parts per million (ppm) in patients
- 191 ppm in non symptomatic Minamata residents
- 4 ppm for people living outside the Minamata area

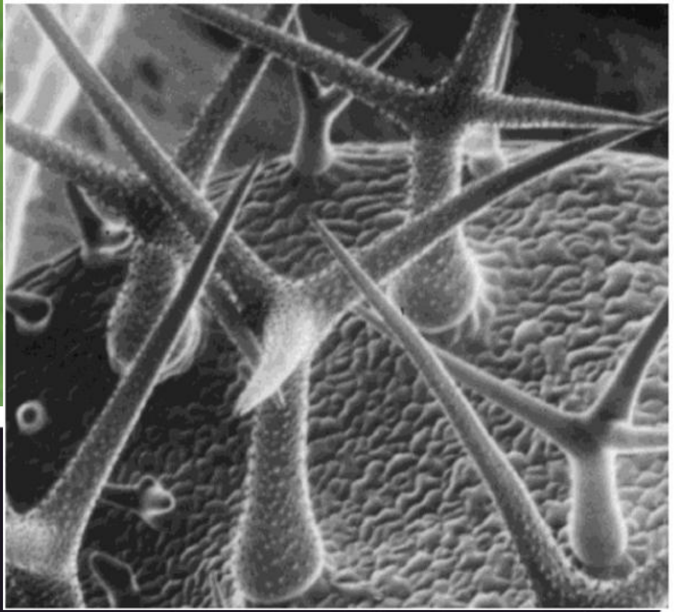


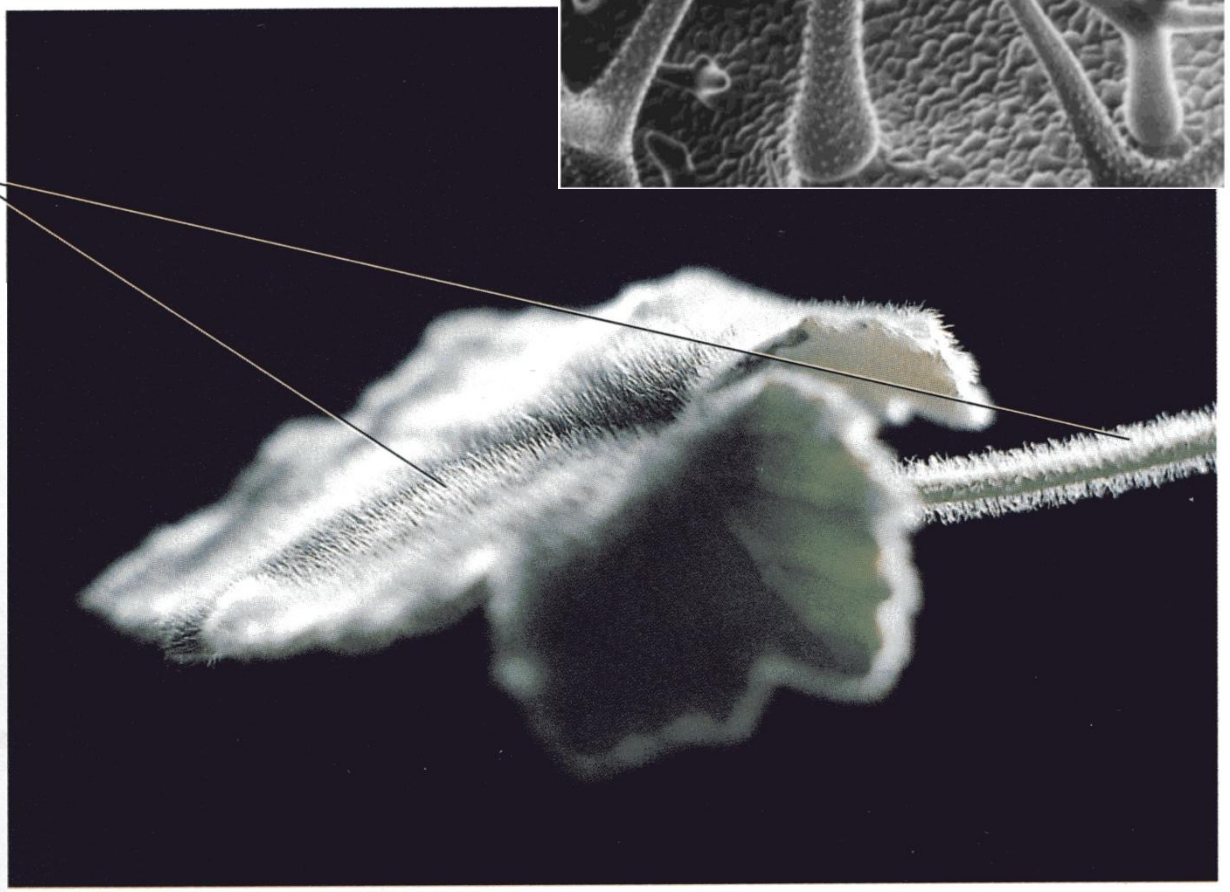
Materials derived by seed plants are frequently used as matrices for the detection of persistent pollutants, such as potentially toxic elements (PTEs), organic compounds, radionuclides (remember: the water internal translocation may represent a problem).

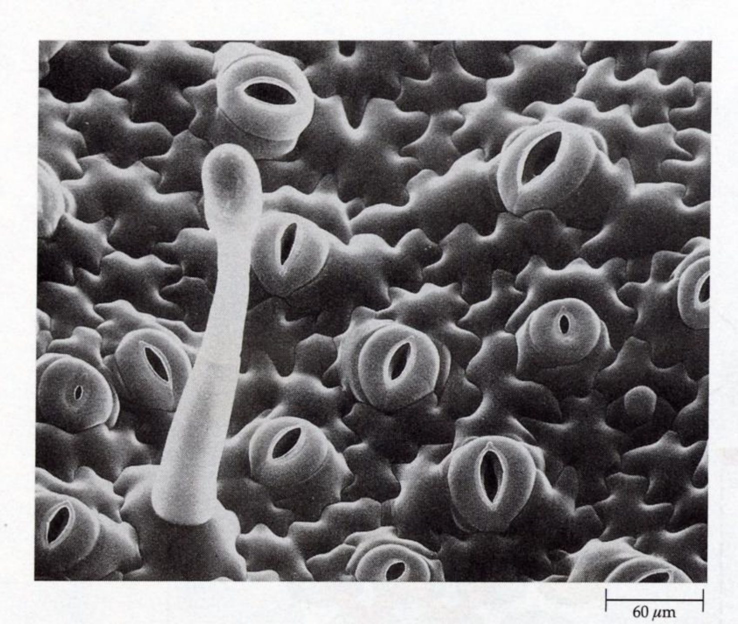
Hairs/trichomes

At leaf level, the surfaces are covered by a water-repellent cuticle, have adjustable openings (stomata), and possibly a covering of trichomes, which trap particulate matter.



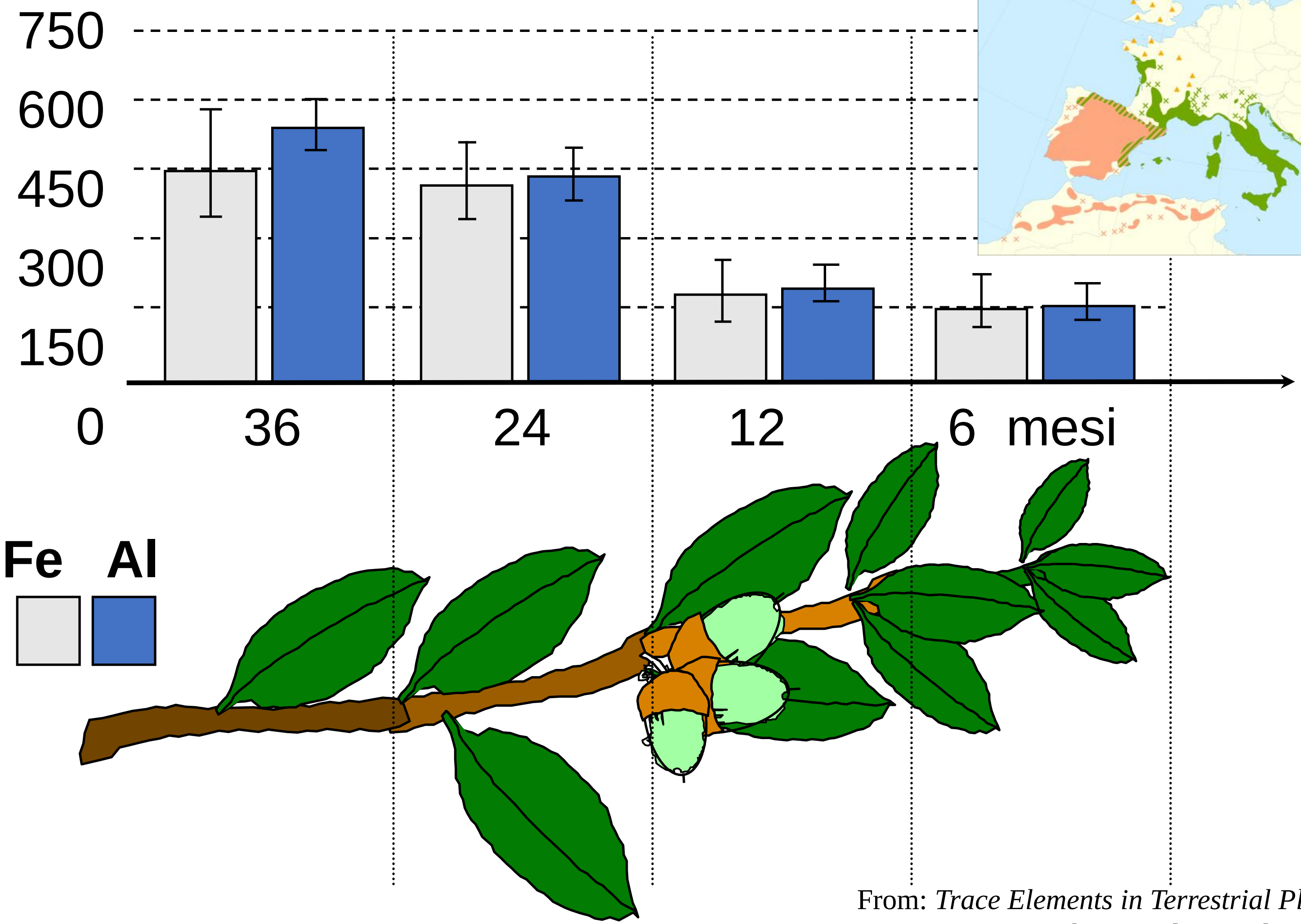






Leaves have a specific life cycle; their life span is an important factor to consider, since bioaccumulation phenomena are time-dependent.

Iron and Aluminium mean concentration in Quercus ilex *leaves of different age along a road with intense traffic.*



From: *Trace Elements in Terrestrial Plant*, R. Bargagli, Springer, New York, 1998 disegni di Dario Gasparo

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Environmental Pollution 153 (2008) 376–383

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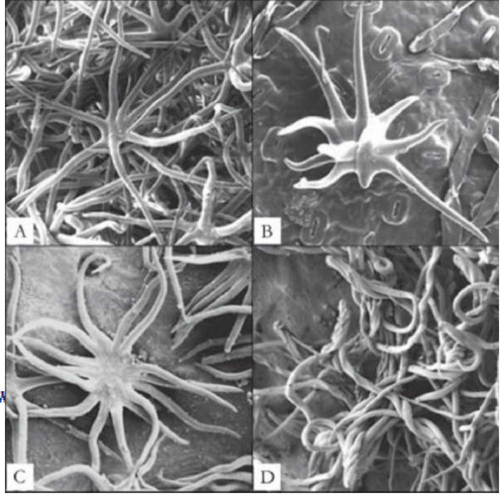


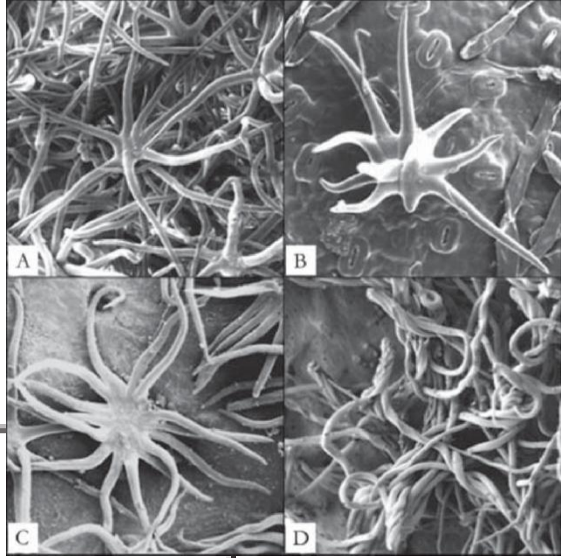
Environmental Pollution 153 (2008): 376-383

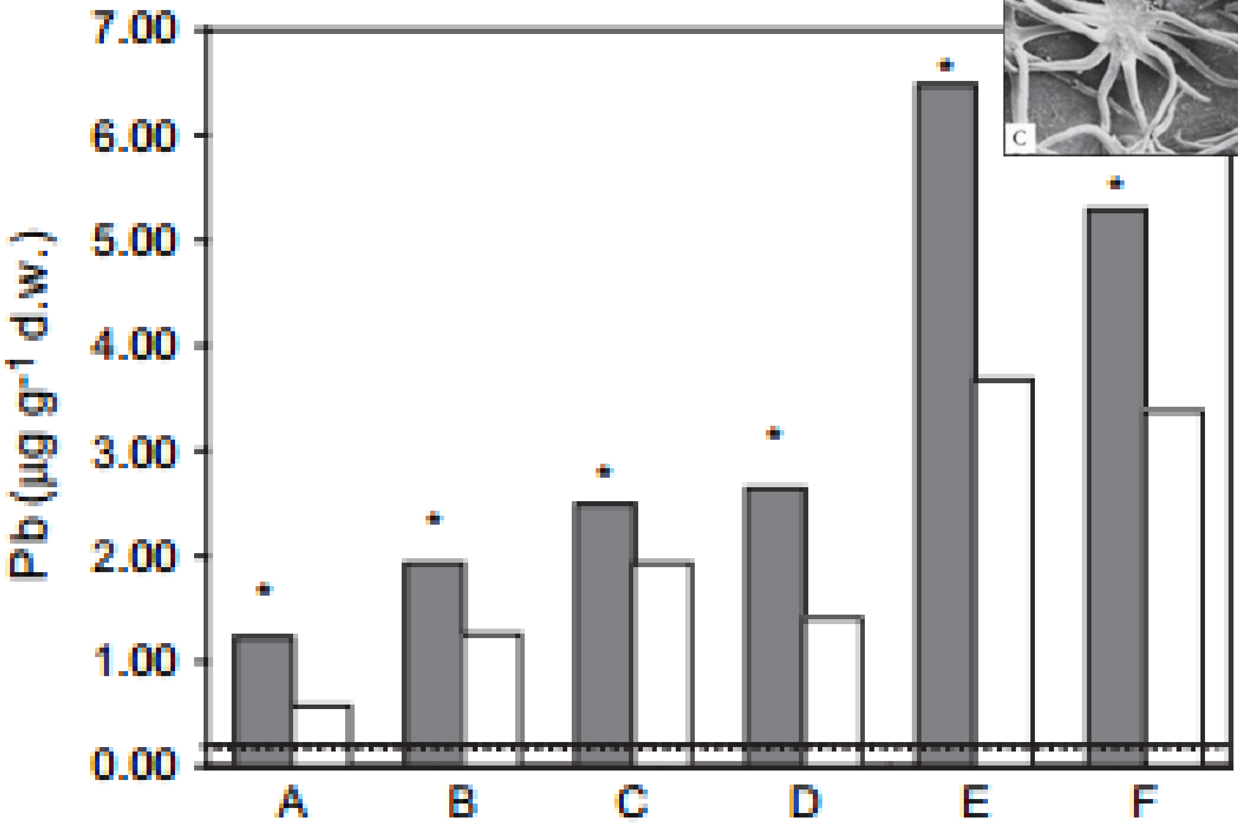
Leaf accumulation of trace elements and polycyclic aromatic hydrocarbons (PAHs) in *Quercus ilex* L.

F. De Nicola [a,*], G. Maisto [b], M.V. Prati [c], A. Alfani [d]

Table 1
Mean concentrations over time (± s.e.) of each trace element in unwashed and washed *Quercus ilex* leaves at the urban and remote sites

	Urban		Remote	
	Unwashed	Washed	Unwashed	Washed
Cd	0.15 (0.02)	0.13 (0.01)	0.11 (0.03)	0.10 (0.03)
Cr	1.88 (0.20)	0.94 (0.15)	0.37 (0.10)	0.30 (0.10)
Cu	13.75 (1.00)	9.05 (0.81)	3.90 (0.20)	3.82 (0.17)
Fe	0.77 (0.08)	0.39 (0.05)	0.17 (0.04)	0.09 (0.03)
Pb	3.34 (0.61)	2.04 (0.44)	0.20 (0.07)	0.18 (0.08)
V	1.97 (0.29)	1.21 (0.19)	0.47 (0.08)	0.26 (0.08)
Zn	32.13 (2.73)	23.89 (3.14)	23.50 (6.80)	22.5 (6.63)


A
B
C
D


7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Pb (μg g^{-1} d.w.)
A
B
C
D
E
F


Pontedera
Bagno a Ripoli
Montevarchi
AREZZO
Poggibonsi
Città di Castello
Gubbio
SIENA
Cortona
PERUGIA
Assisi
Follonica
INGV
Foligno
GROSSETO
Orvieto
Spoleto
TERNI
Narni
VITERBO

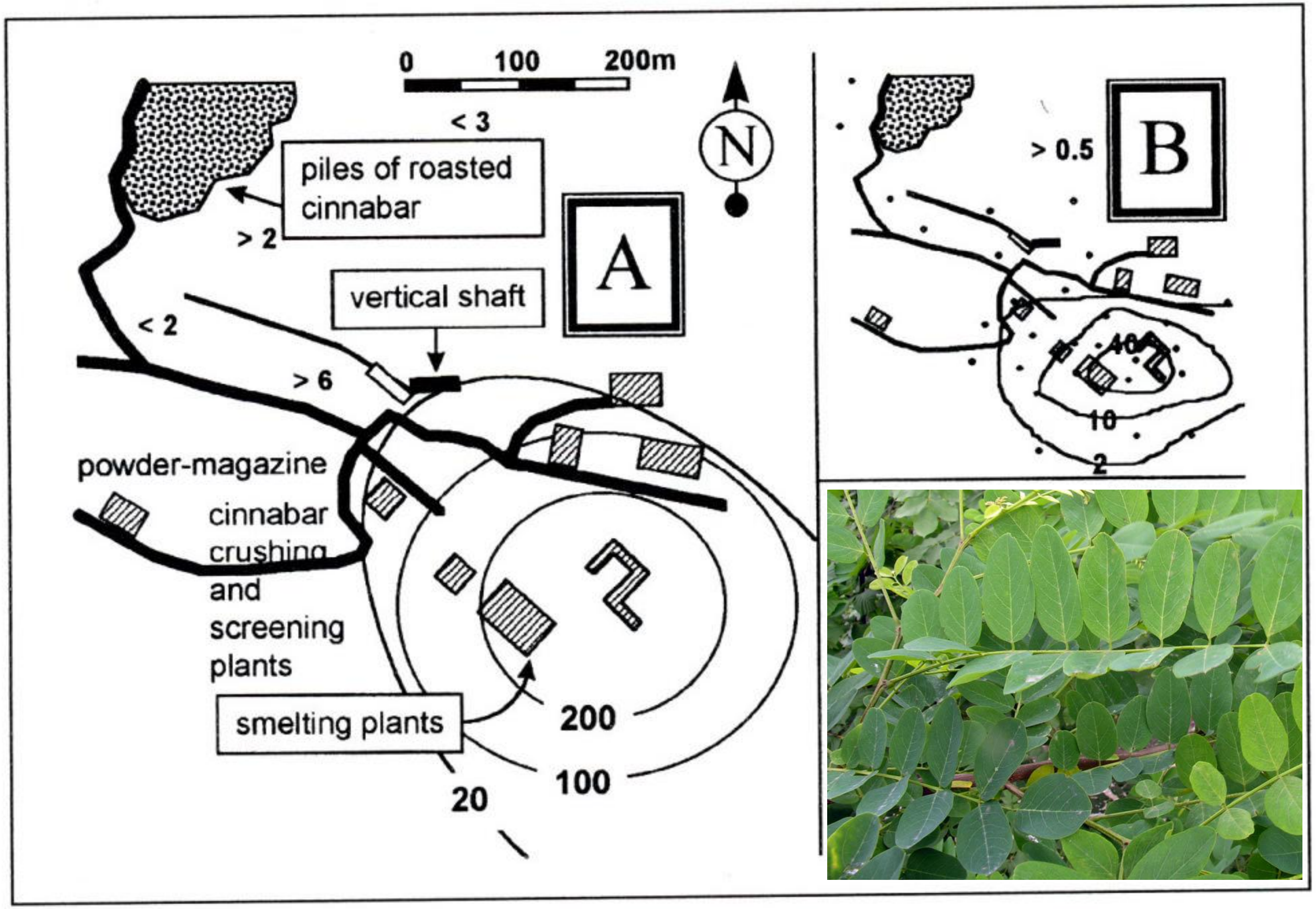
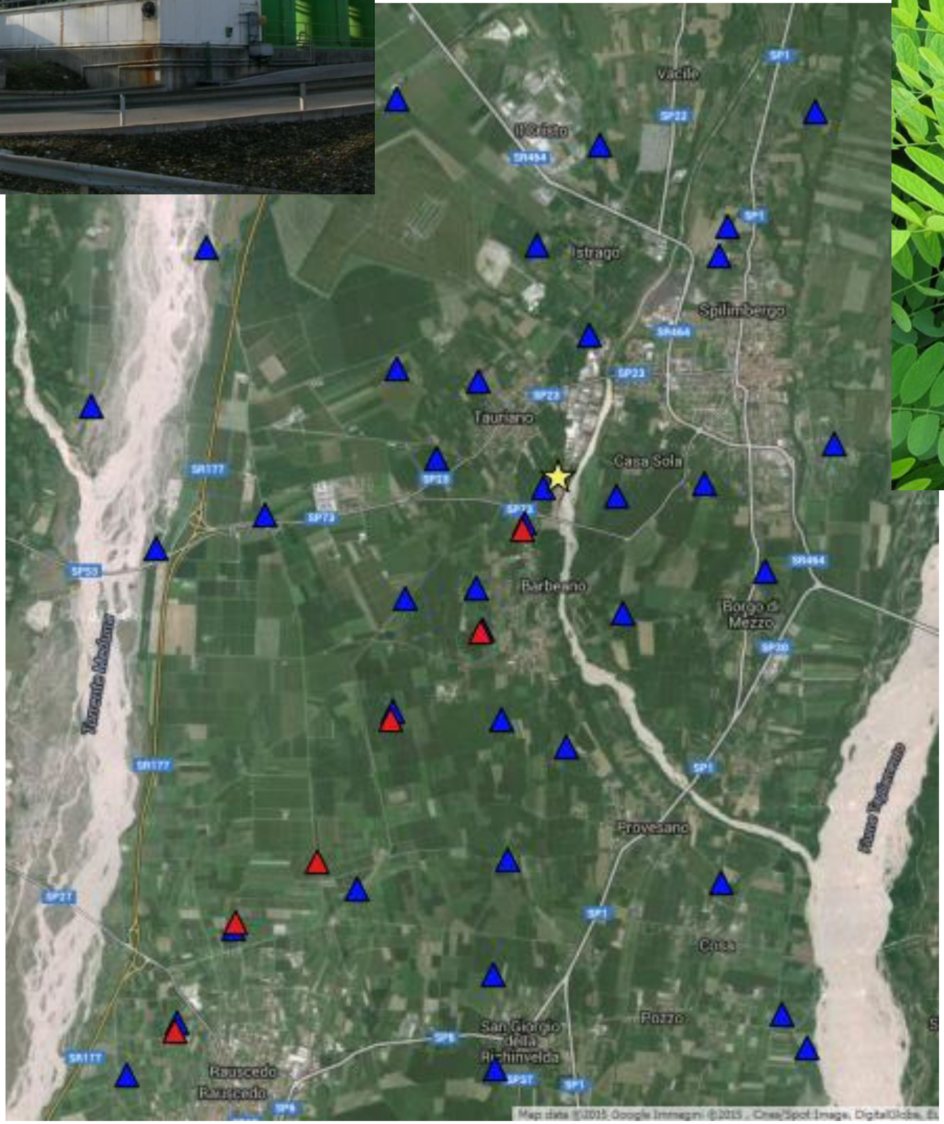
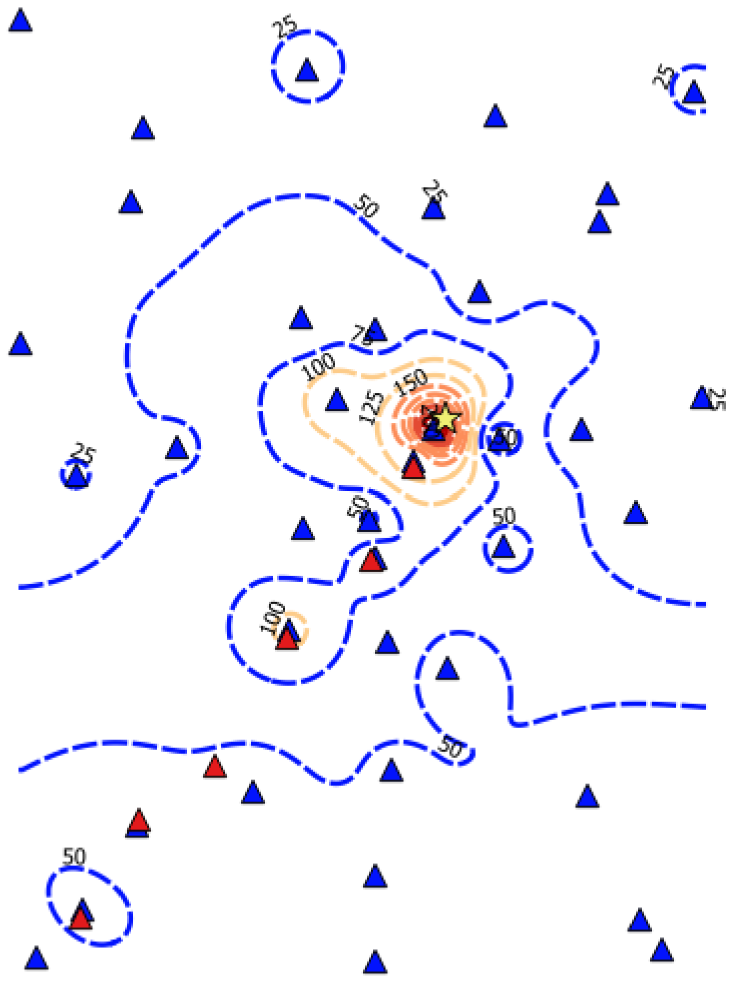
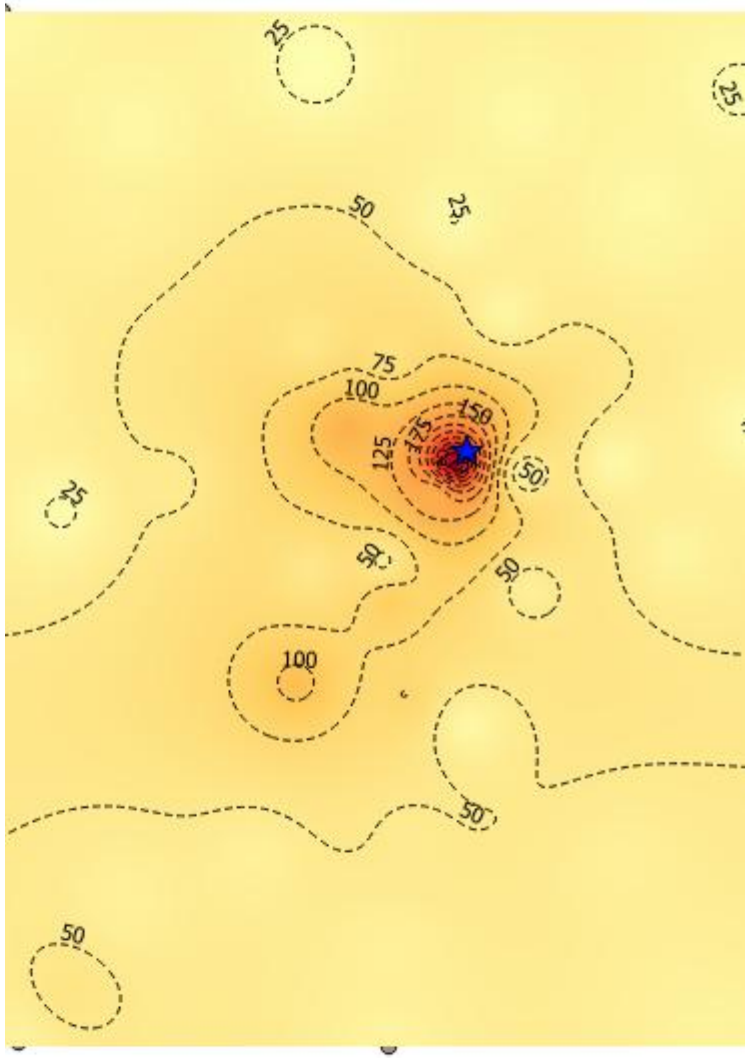


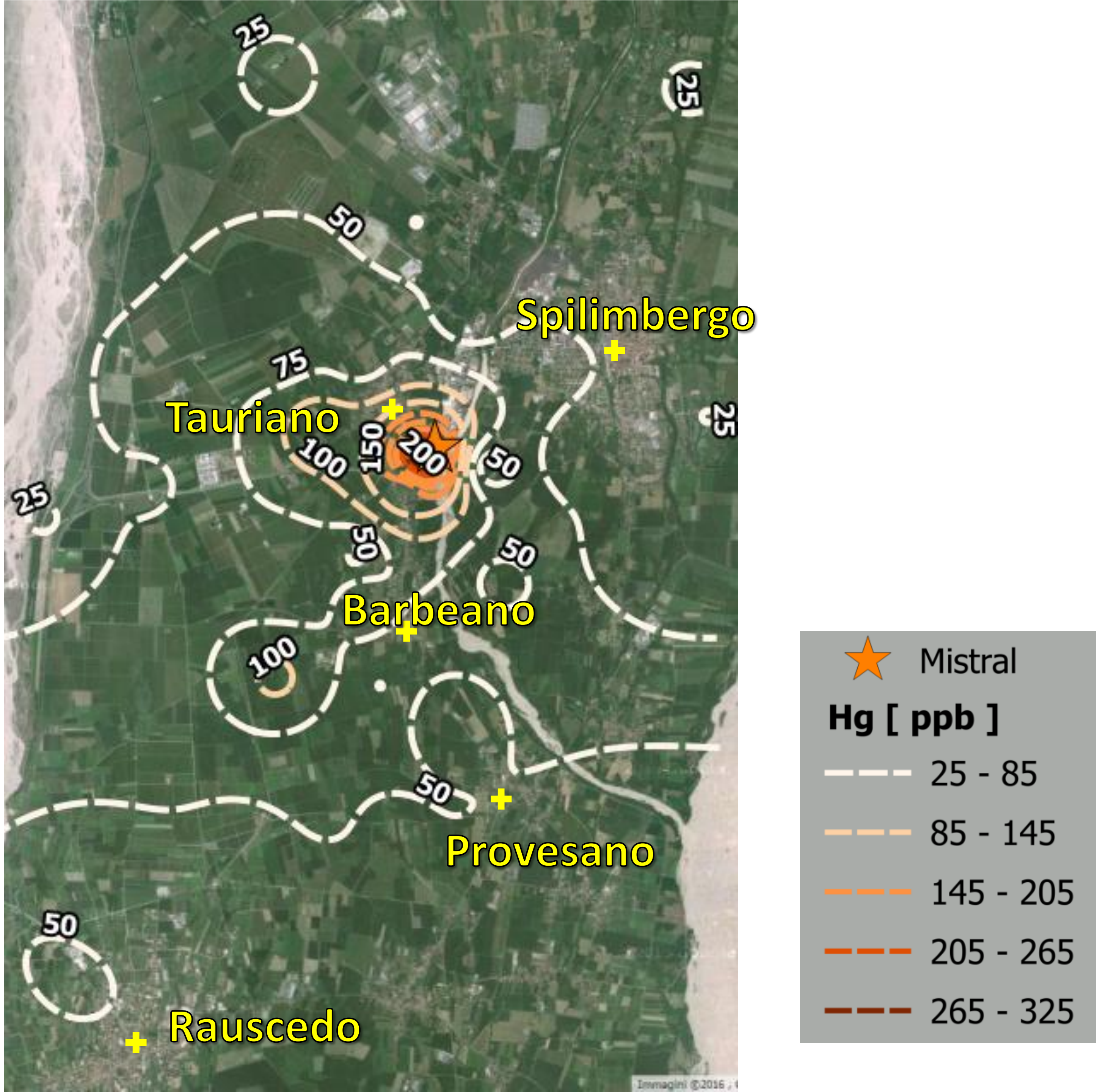
Fig. 5.1. Isopleth maps of gaseous mercury distribution at an abandoned mercury mining and smelting site, obtained by direct long-term instrumental measurements (A; ng/m^3), and biomonitoring using *Robinia pseudoacacia* leaves (B; µg/g dw)

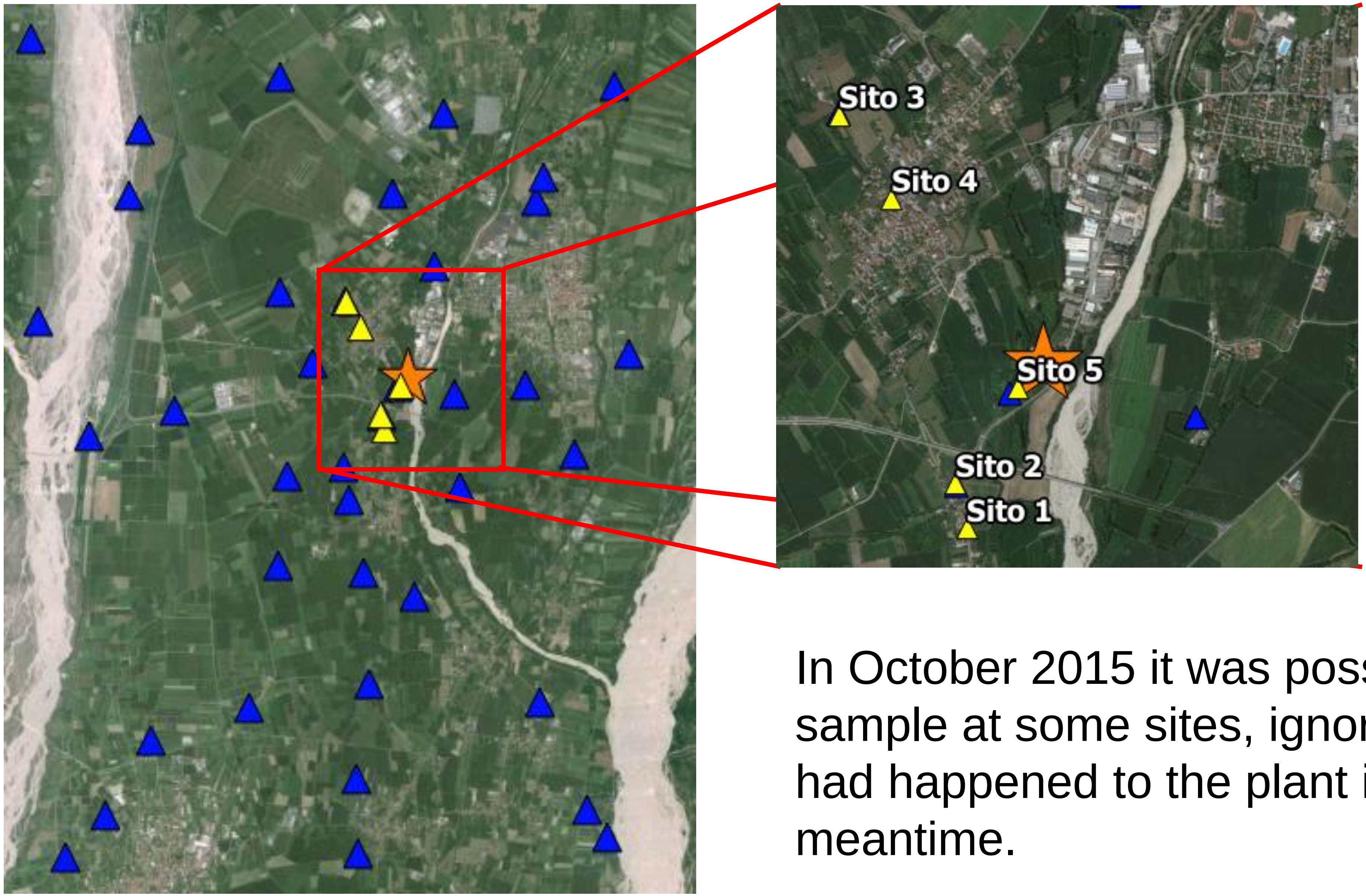


In mid-November 2014, black locust leaves (above) were taken from 3 trees (left), forming a single sample, in each station around an incinerator near Spilimbergo (PN, NE Italy). The ground samples were analyzed using DMA (Direct Mercury Analysis) at the laboratories of the University of Santiago de Compostela (Spain).


25
50
75
100
125
150
100
50


25
50
75
100
125
150
175
100
50


Spilimbergo
Tauriano
Barbeano
Provesano
Rauscedo
25
50
75
100
150
200
Mistral
Hg [ppb]
25 - 85
85 - 145
145 - 205
205 - 265
265 - 325

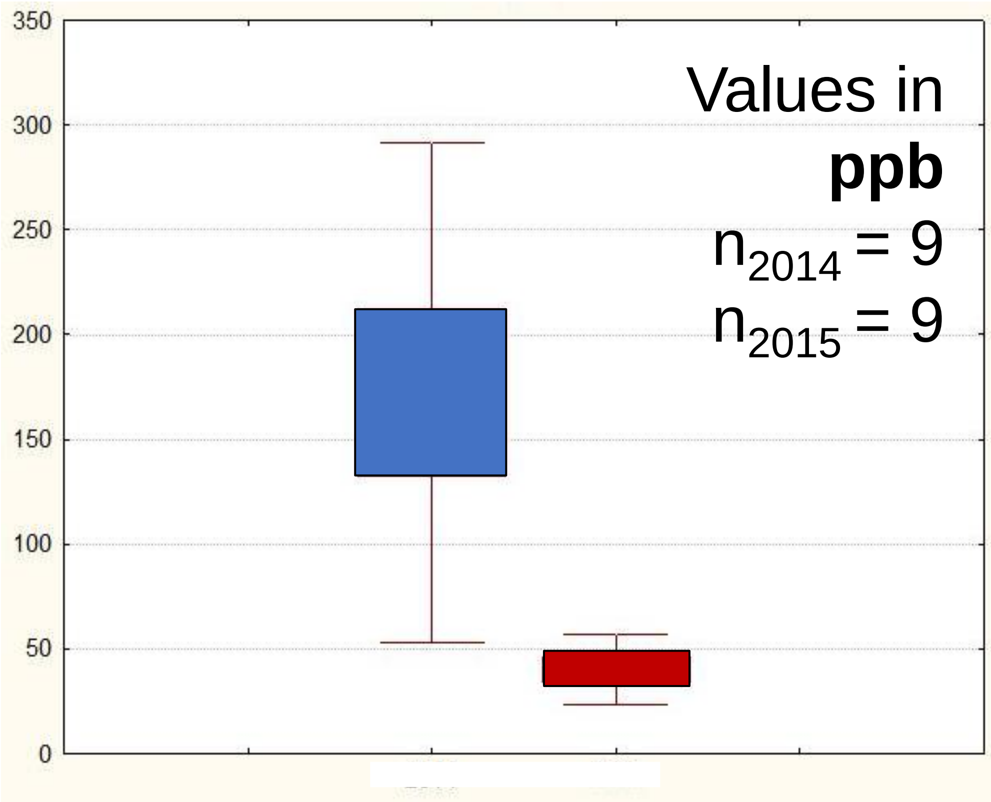


2015

In October 2015 it was possible to sample at some sites, ignoring what had happened to the plant in the meantime.

2014

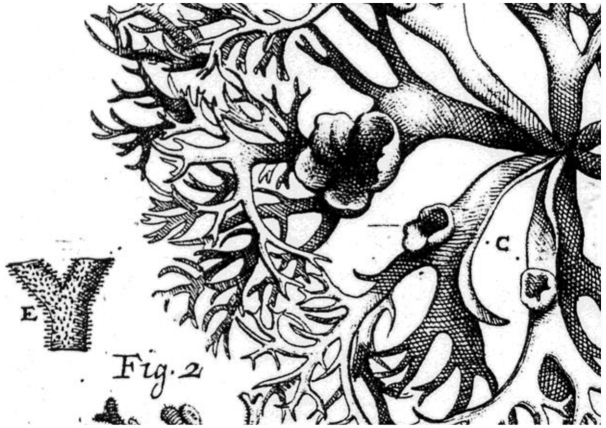
The statistical comparison was possible for sites 2, 3 and 5, for which *Robinia* samples were available for both years.



2014
normally working plant

2015
plant shut down since July

Fortuna L., Candotto Carniel F., Capozzi F., Tretiach M., **2019**. *Atmosphere* 10 (4): 183



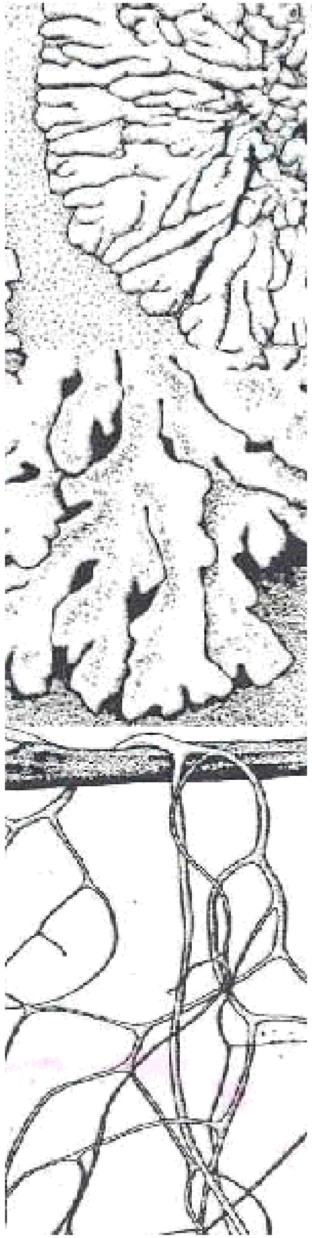
Lichens and **mosses** are among the organisms most frequently used in bioaccumulation studies.

Hundreds of studies carried out over the last 30 years confirm that the chemical composition of lichens and mosses largely reflects the availability of potentially toxic elements (PTEs) and persistent organic pollutants (POPs) in the environment, because these organisms behave as long-living collectors.

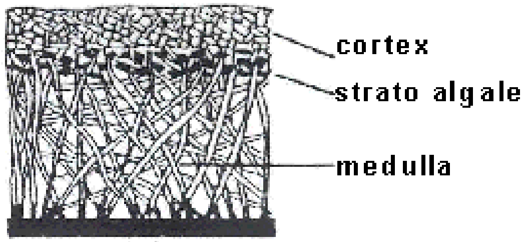
- Lichens and mosses are perennial, and are active for most of the year, and particularly in winter, when pollution is higher

Interception is increased by the thallus growth form and by the nature of the surfaces.

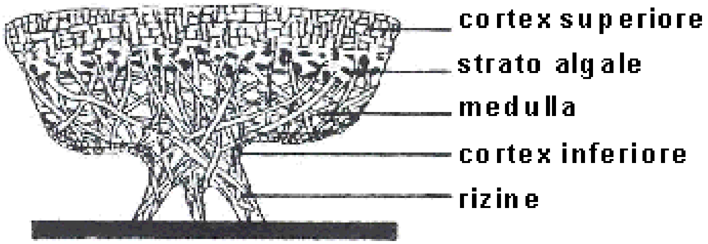
In lichens, in the series crustose$\rightarrow$ foliose $\rightarrow$ fruticose there is a strong increase in the external surface in contact with the atmosphere.



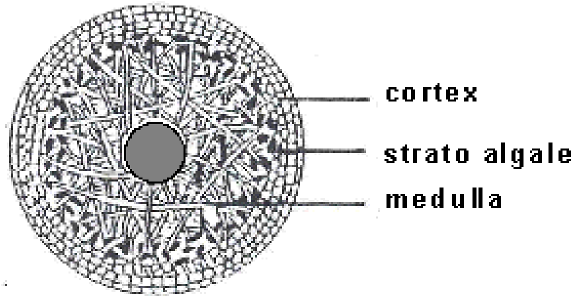
crustose



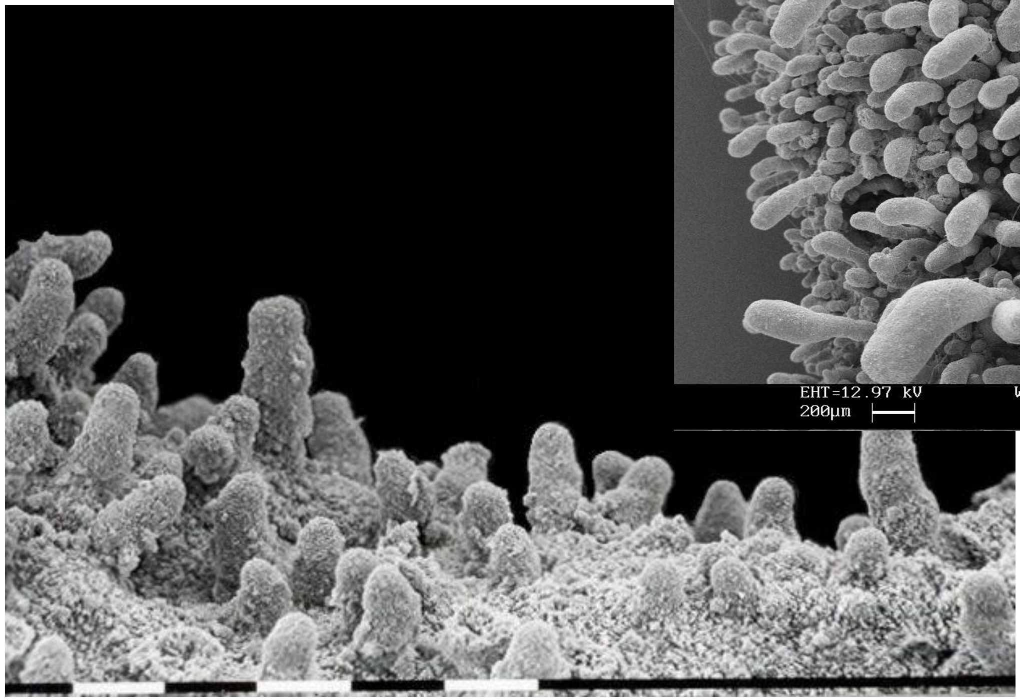
foliose

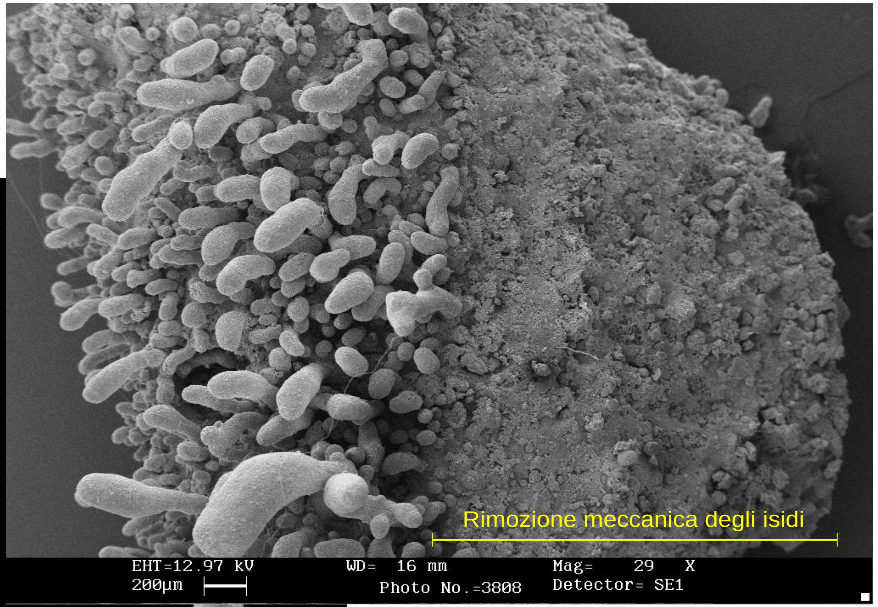


fruticose



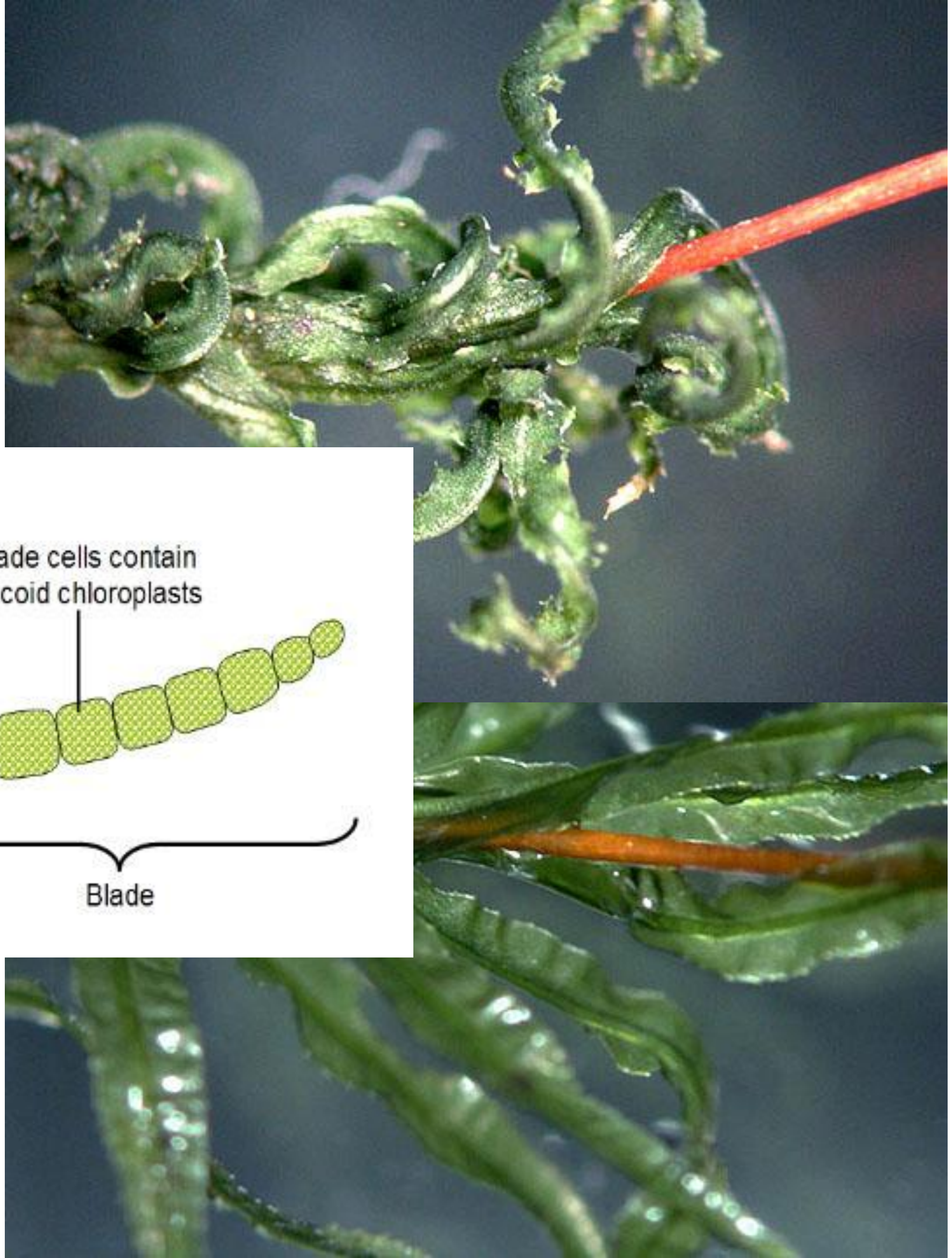
The interception of particulate matter is increased by rough, irregular surfaces: in lichens, interception is intuitively higher when the thallus produces vegetative or reproductive structures, or when it is covered by a layer of small crystals, produced by the metabolic activity of the mycobiont.

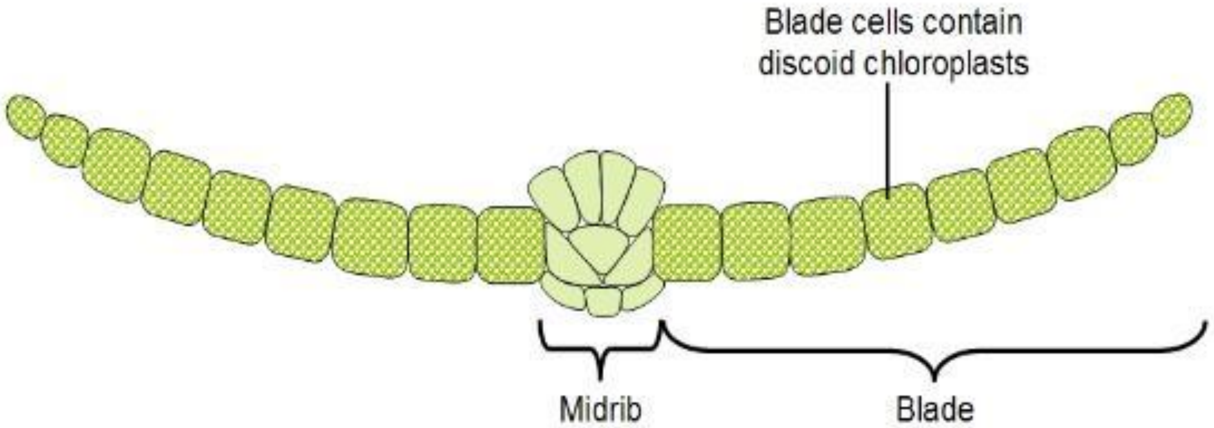




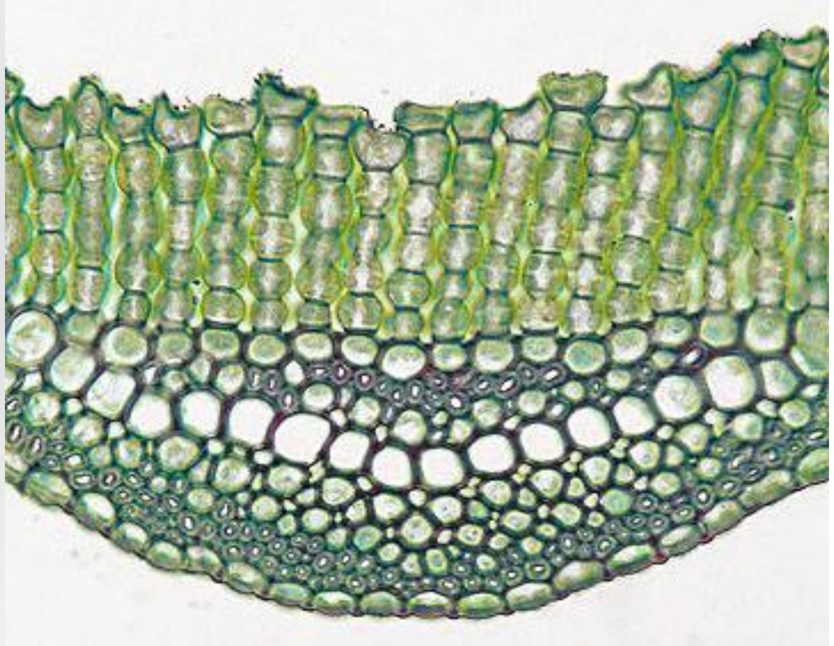
A good example of structures increasing interception are the isidia of *Pseudevernia furfuracea*.

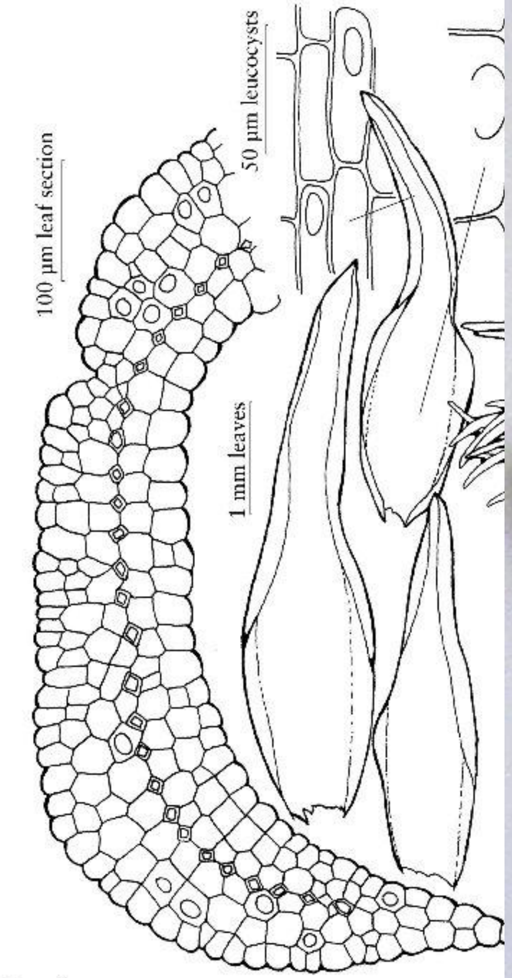

200 µm




Blade cells contain
discoid chloroplasts
Midrib
Blade







L. glaucum

Leucobryum glaucum


BETTER-GRO
Sphagnum Moss
Long Fibered
All Natural
Easy to Use Potting Medium Excellent for: Lining Hanging Baskets, Growing Seedlings & Decorative Soil Covering
Helps Retain Soil Moisture & Nutrients
Excellent for Lining Wire Baskets and Topiary Forms
240


20 µm
© Dr. R. Wagner

The basic of (bio)-accumulation processes in lichens and mosses

Particles, normally present in the atmosphere or incremented by human activities, arrive to lichens and mosses in two not mutually exclusive forms: **dry and wet depositions**. The latter are particularly important for lichens and mosses, because they are highly hygroscopic.

Whatever the way of arrival, three different modalities of immobilization are known to occur in lichens and mosses, which can explain their bioaccumulation capacity:

1) **particles entrapping** (adsorbed onto the thallus surface or within intercellular spaces);

2) **extracellular ionic binding** (mainly at cell wall level, secondarily at cell membrane level);

3) **intracellular ionic binding**.

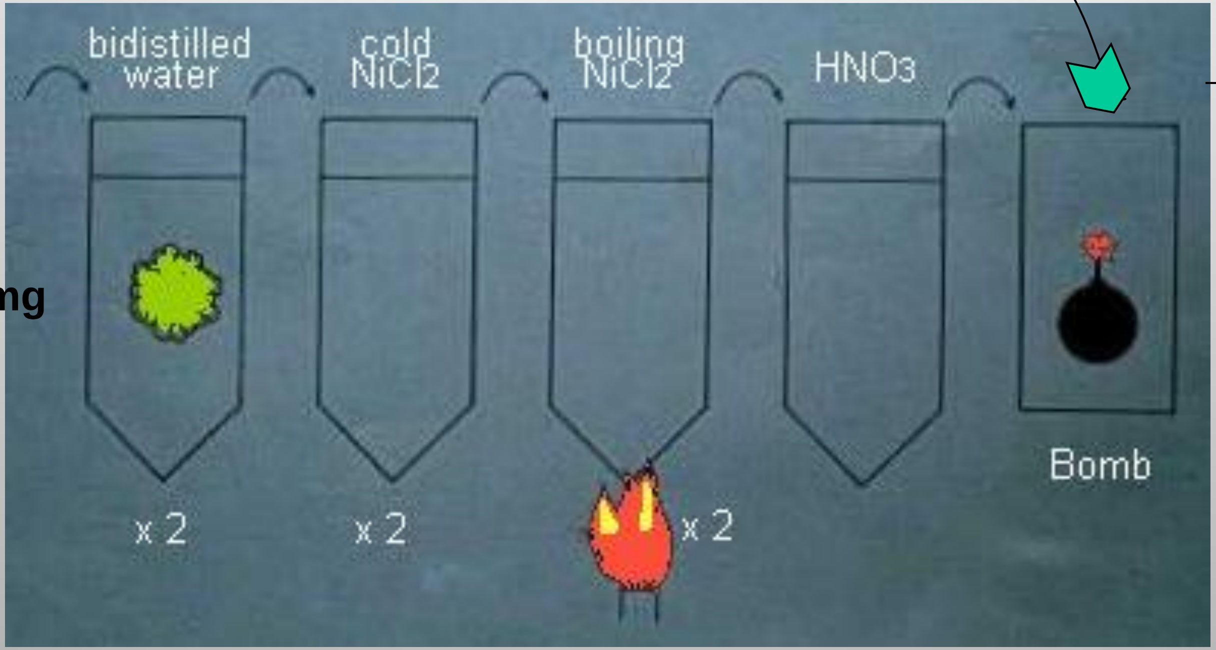
These modalities are qualitativavely and quantitatively different among species, and this can explain why some species are more tolerant than others to pollutants, and why some are stronger bioaccumulators than others.

The sequential elution technique (Brown and co-workers)

Sample 1

Sample 2

Vol.: 0.5 ml/ mg



TOTAL CONTENT

Ext. particulate

extracellular

intracellular

insol. particulate

FRACTIONS

Alternatives:

(EDTA)

(cold NHO_3)

What we understood using the sequential elution technique:

- The components made up of particulate matter and the extracellularly bound ionic fraction are prevalent.

- Some elements enter more easily than others at the cytoplasmic level, but they are of physiological, not ecotoxicological interest (e.g. K^+).

- The extracellular wall fraction can undergo significant turnover, i.e. it is not stable.

- There are important differences among species in the capability to entrap, adsorb and bioaccumulate PTEs: it is wrong to consider all the species as the same.

1999

2019

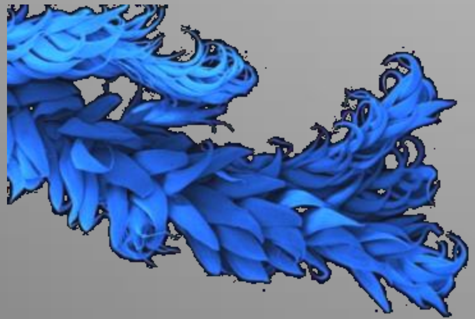
As for the use of lichens as bioindicators, also in this case a lot of work has done (at national level) to produce a standard protocol recognized by ISPRA, the Istituto Superiore per la protezione e Ricerca Ambientale. This time a clear way for data interpretation is offered, for both native and transplanted samples.

If we want to estimate the alteration entity, accumulation data can basically be used following three different approaches:

1) comparison with the minimum value measured in the survey area;

2) comparison with background values;

3) use of an “interpretative scale”.



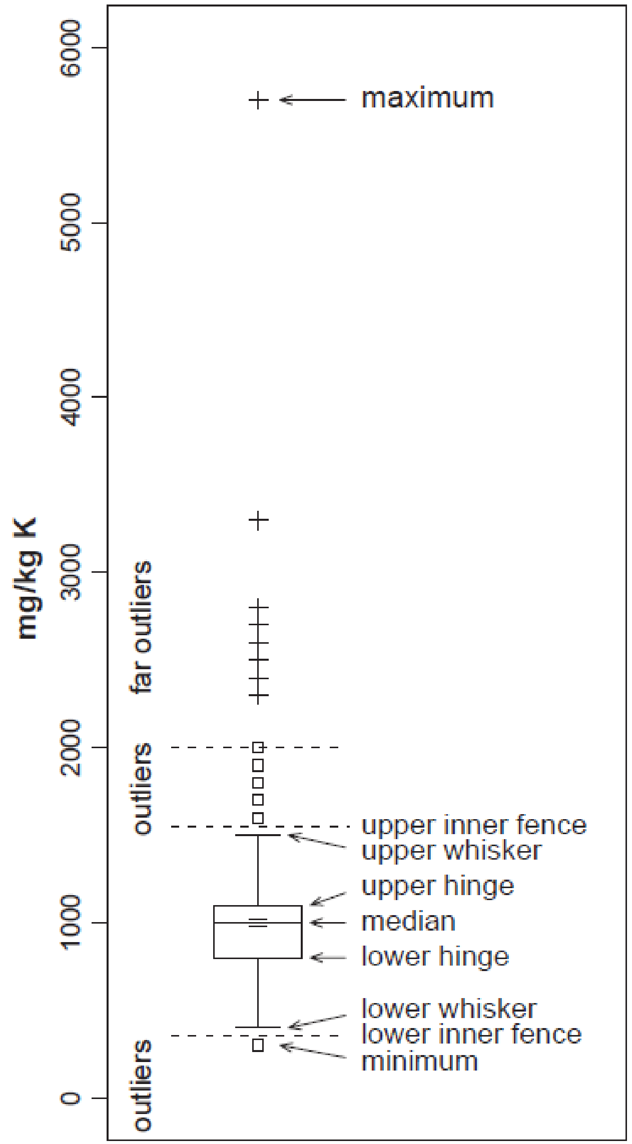


Fig. 1. Tukey boxplot for potassium (K%) concentrations in the O-horizon of podzols from the Kola area (Reimann et al., 1998). For definitions of the different boundaries displayed see text.

C. Reimann et al. / Science of the Total Environment 346 (2005) 1–16

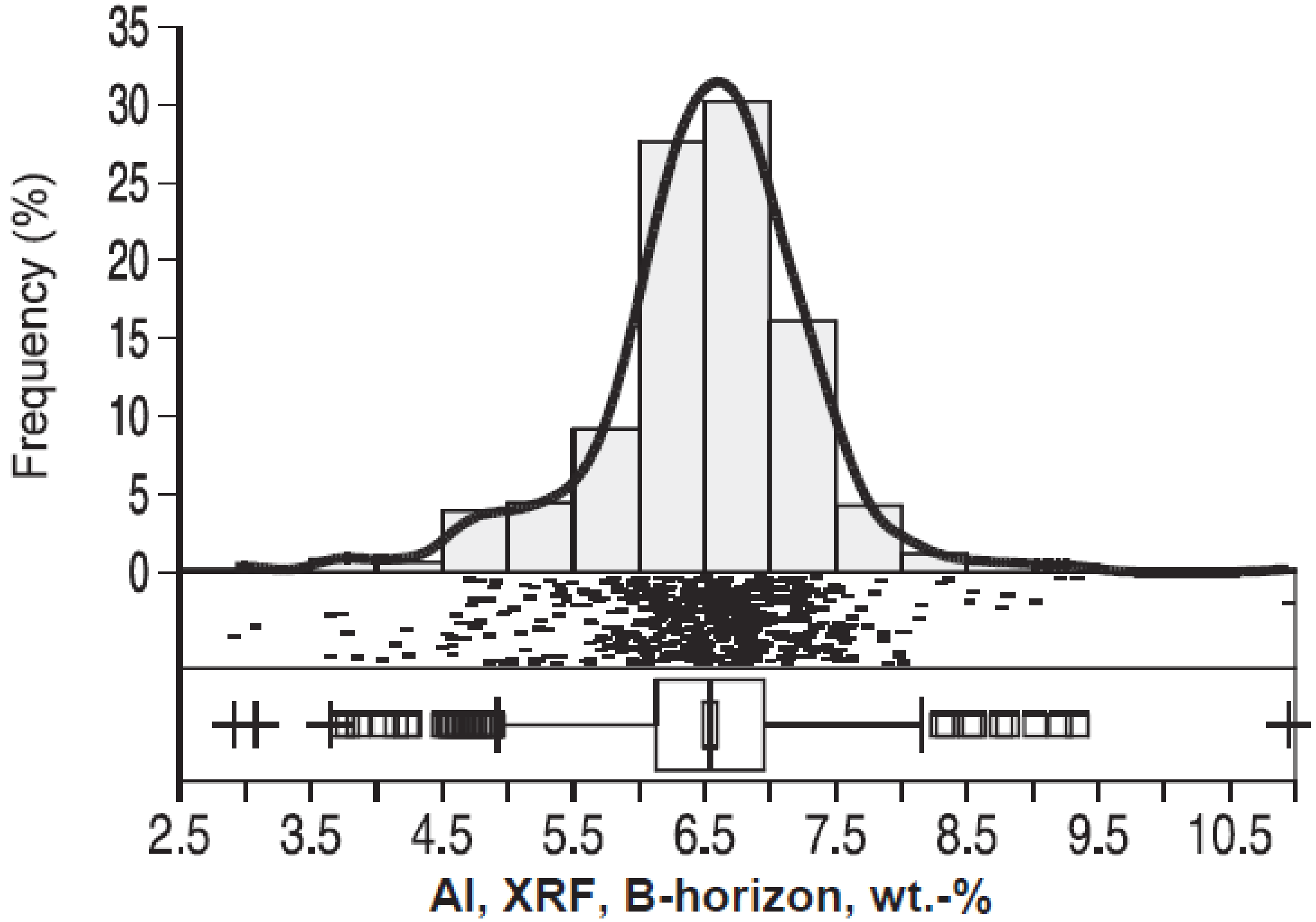


Fig. 5. Combination of histogram, density trace, one-dimensional scattergram and boxplot for the study of the empirical data distribution (data from Reimann et al., 1998).

C. Reimann et al. / Science of the Total Environment 346 (2005) 1–16

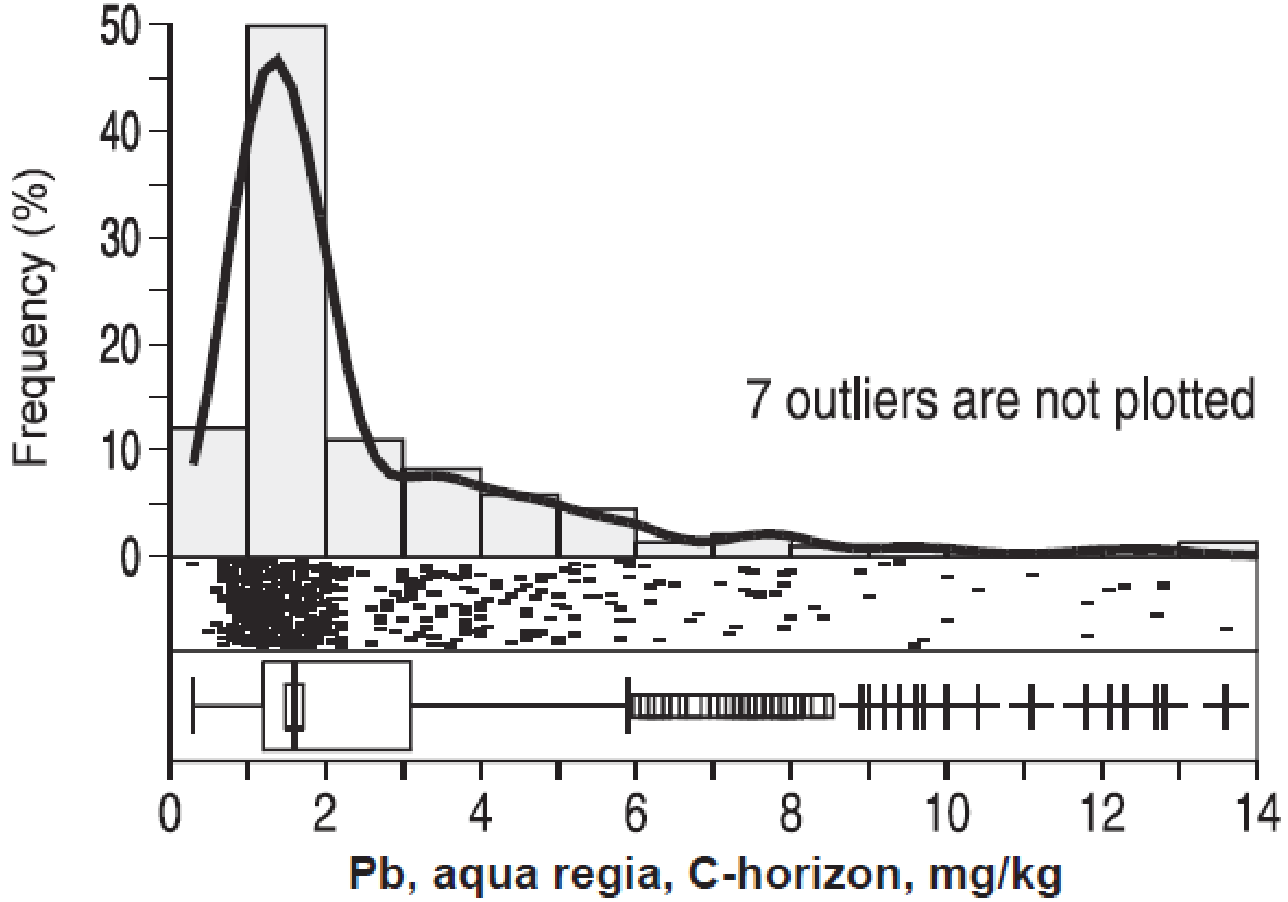


Fig. 5. Combination of histogram, density trace, one-dimensional scattergram and boxplot for the study of the empirical data distribution (data from Reimann et al., 1998).

C. Reimann et al. / Science of the Total Environment 346 (2005) 1–16

1998

Workshop "Biomonitoraggio della qualità dell'aria sul territorio nazionale"

Linee-guida per il bioaccumulo

Nimis & Bargagli (1999)

The “interpretative scale”

Nimis & Bargagli (1999) proposed an “**interpretative scale**” based on the analysis of hundreds of measurements carried out on in Italy using epiphytic foliose lichens,

- in survey areas with different climatic conditions,
- with different types of air pollution (intensity and sources)

Percentile distribution values were calculated for each element for which there were at least:

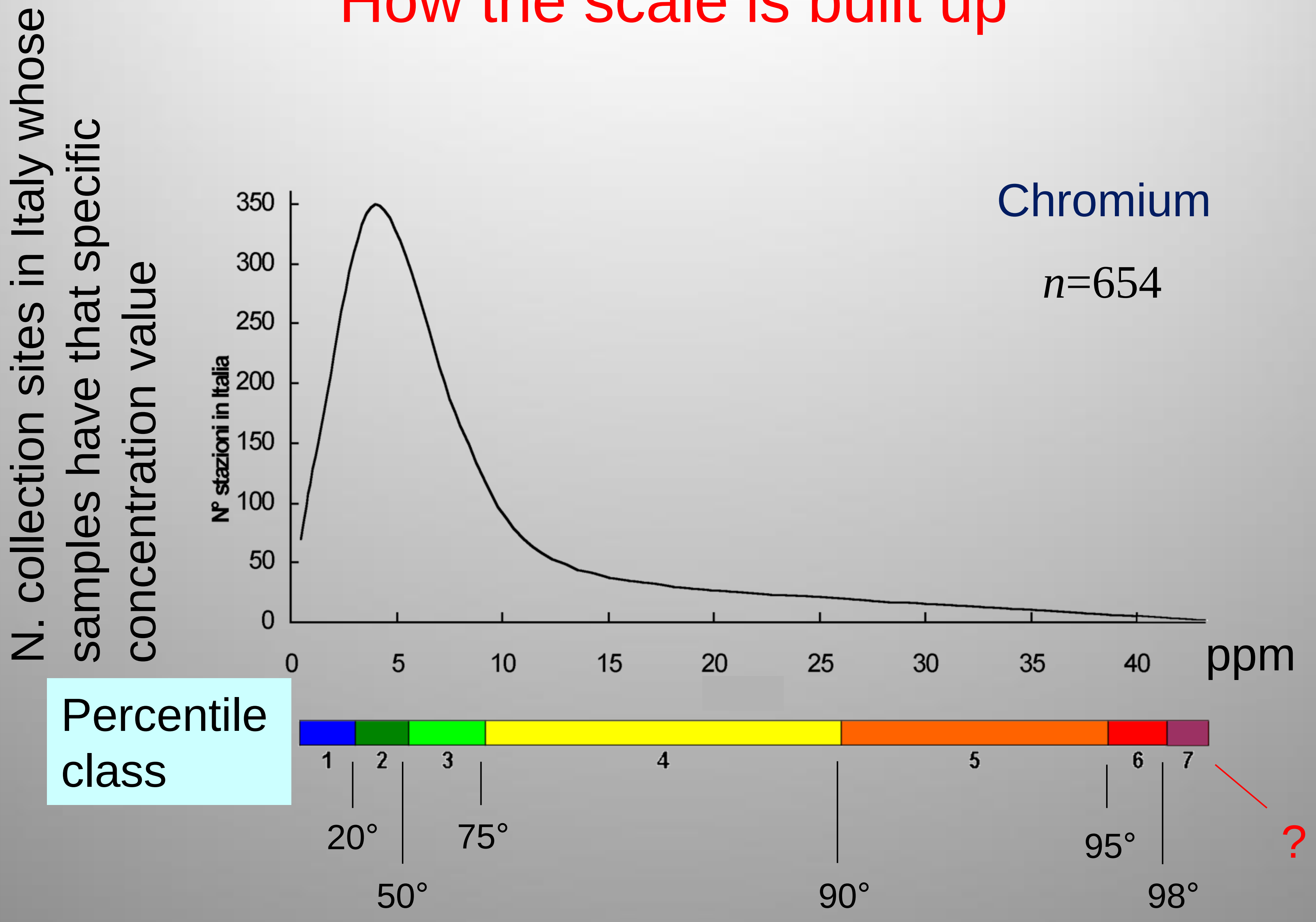
- 100 data collected in
- three “distant” areas of the country.

An ordinary scale of 7 grades was built up on the basis of **percentile classes** of different width.

The method suggests colours and definitions for each grade that can be used in graphic display and for the interpretation of the results.

I percentili sono dei valori che permettono di dividere un gruppo di dati ordinati in cento parti, ognuna delle quali comprende l’1% dei dati. Esempio: avendo un set di cento dati ordinati in ordine crescente, il 20° percentile è quel valore che divide il 20% dei dati dal restante, cioè i primi venti dagli ultimi ottanta. I comuni software di calcolo comprendono formule e opzioni che permettono di ricavarli automaticamente.

How the scale is built up



n° misure	definizioni		654
percentili	definizioni		Cr
20°	1 - Alterazione assente o trascurabile		<1,2
50°	2 - Alterazione molto bassa		2,2
75°	3 - Alterazione bassa		4,0
90°	4 - Alterazione media		6,0
95°	5 - Alterazione alta		9,0
98°	6 - Alterazione molto alta		16,0
	7 - Alterazione estrema		>16,0
max Italia			60,5

Interpretation scale according to Nimis & Bargagli (1999) (modif.). Values in $\mu g\ g^{-1}$ dry weight.

n° misure			626	435	350	223	626	654	656	647	606
percentili	**definizioni**		**Al**	**As**	**Ba**	**Be**	**Cd**	**Cr**	**Cu**	**Fe**	**Hg**
20°	1 - Alterazione assente o trascurabile		<350	<0,2	<3,3	<0,04	<0,2	<1,2	<7,0	<290	<0,07
50°	2 - Alterazione molto bassa		600	0,6	6,0	0,08	0,4	2,2	10,0	500	0,13
75°	3 - Alterazione bassa		1000	1,2	10,0	0,12	0,8	4,0	15,0	800	0,20
90°	4 - Alterazione media		1600	1,9	18,0	0,60	1,4	6,0	25,0	1200	0,29
95°	5 - Alterazione alta		2500	2,4	25,0	0,70	2,0	9,0	34,0	1500	0,42
98°	6 - Alterazione molto alta		3200	3,0	35,0	1,15	2,6	16,0	53,0	1800	0,74
	7 - Alterazione estrema		3200	>3,0	>35,0	>1,15	>2,6	>16,0	>53,0	>1800	>0,74
max Italia			8390	5,5	78,7	1,62	9,0	60,5	161,0	4276	1,84

n° misure			649	655	699	219	198	138	416	699
percentili	**definizioni**		**Mn**	**Ni**	**Pb**	**Se**	**Te**	**Ti**	**V**	**Zn**
20°	1 - Alterazione assente o trascurabile		<20	<1,0	<4,0	<0,04	<0,05	<13	<0,6	<30
50°	2 - Alterazione molto bassa		25	2,0	10,0	0,15	0,15	27	1,7	40
75°	3 - Alterazione bassa		35	3,0	25,0	0,30	0,35	70	3,1	65
90°	4 - Alterazione media		60	5,0	55,0	0,40	0,52	97	5,1	94
95°	5 - Alterazione alta		90	6,0	80,0	0,70	0,62	113	6,7	115
98°	6 - Alterazione molto alta		140	8,0	108,0	0,90	0,77	150	9,3	155
	7 - Alterazione estrema		>140	>8,0	>108,0	>0,90	>0,77	>150	>9,3	>155
max Italia			685	34,4	494,0	1,43	2,32	290	15,0	358

The idea was that the data set of each element had to be implemented year after year, as new studies were added, increasing the total number of data at disposal for the analysis

Brief summary on the interpretative scales of bioaccumulation data from native lichens

Olda scales N/A (Nimis & Bargagli 1999)

- Data from more species merged together;
- More than one digestion method;
- At least 100 data from three distant areas of the Country;
- no data-set available to the scientific community.

Data distribution frequency

7 percentile classes of variable range were determined, each one linked to a qualitative definition of naturality/alteration.

When this scale was applied, the single value of elemental content measured in the i-th sample could be directly compared with the ranges of the scale to verify in which class it belonged.

New bioaccumulation scales for native lichens (Cecconi et al. 2019; ISPRA 2019)

- Data-sets for single species (only two in the end);
- One standard digestion method (total);
- At least 40 data from at least 3 areas of the Country;
- data-set made available to scientific community, but not automatically modified when new data are published.

Data distribution frequency

5 classes of variable range were determined, with the highest threshold of the lowest class corresponding to the 25° percentile, as in the ecotoxicological disciplines, to define a background value (this is calculated as the mean of all values belonging to this class).

The ratio between each value available for the **x** species and the ***background (mean)*** value for the **x** species lead to the definition of a second distribution scale, which is not statistically different from the one calculated for the second species **y and similar for the elements tested**.
The values of this ratio are a-dimensional.

Because there is no difference between the two species, the new data (i.e. the ratios) are used in a cumulative way to build up a new reference distribution of frequency for all the elements. This scale is divided into the same 5 classes before mentioned, each one associated to a qualitative definition od bioaccumulation.

When we need to apply the new interpretative scale, the single value of element content measured in the i-th sample cannot be compared directly with the values of the new scale to determine in wich class it belongs.
It has to be calculated the ratio with the background value of the corresponding species.
This ratio has to be compared with the ranges of the new scale to determine in which clas it belongs, so to understand the bioaccumulation level as comparede to a background

The process is certainly more complex and less intuitive, but with the new interpretative scale ma con la nuova scala interpretativa it is possible to apply effectively the concept of deviation from a **normal** or **background** values.

N.B. – A critical point: the definitions applied to the 5 percentile classes of the new scale are less strong than the old ones: instead of speaking about «alteration» more or less important of the bioaccumulation values, the term «bioaccumulation» more or less high is used.