EPFL

Analyse des Polluants dans l'Environnement

Part I

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&

Pascale Mulattieri - Biol'Eau Sàrl

2024

Program - Part I

Day 1: Introduction (chemical analysis + sampling of macrozoobenthos) (GC D0386)

Day 2: Field work (water and sediment sampling) (**Sept. 18**)

Analysis of macro-pollutants in water (Lab GR C0 528)

Day 3: Analysis of organic and inorganic micro-polluants in

sediments (Lab GR C0 528) (2 groups **Sept 25th or Oct. 2nd**)

Day 4: Marcozoobenthos identification (Lab GR C0 528) (2 groups **Oct. 9th or Oct. 16th**)

Day 5: Discussion and report (GC D0 386) (**Oct. 30th**)

13h15-19h

Objective of the course - Part I

The aims of this course:

- Learn how to collect and analyze environmental samples
- Learn how to interpret analytical data (critical thinking)
- Be able to assess the environmental quality of surface waters

Evaluation: **Scientific report (3.5 points part I and 2.5 points part II – total 6 points)**

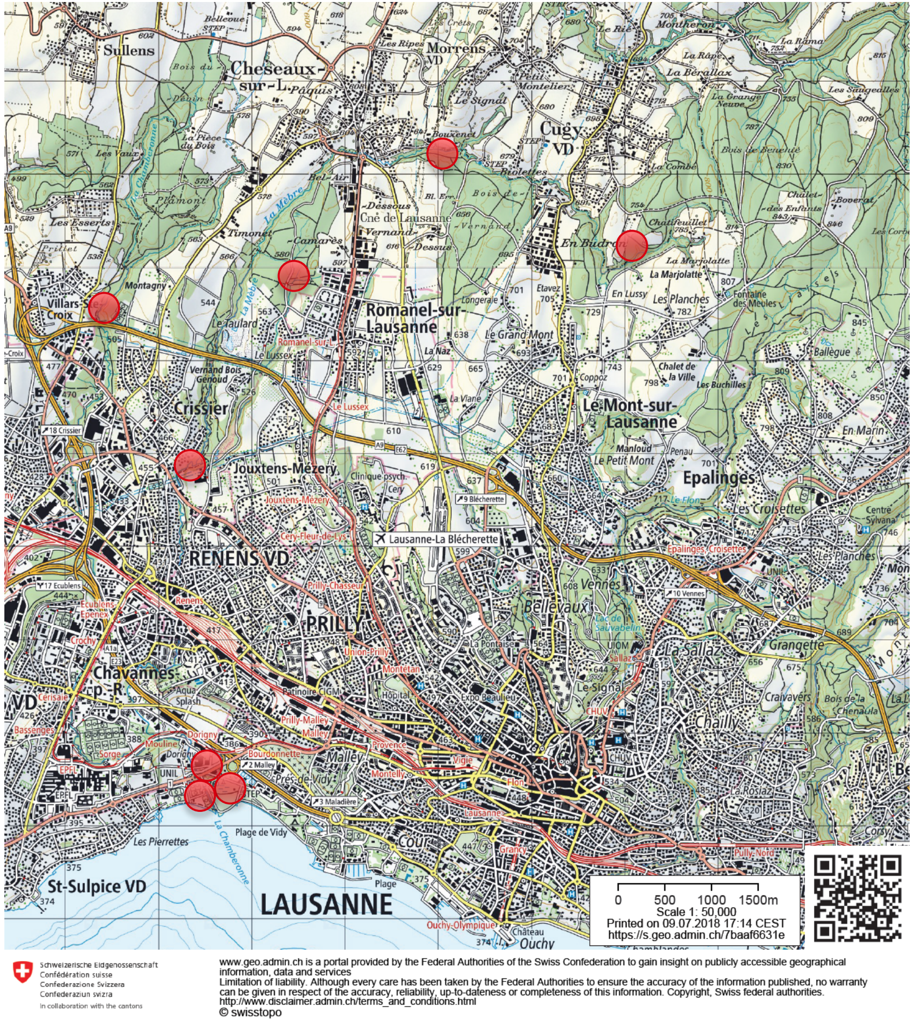
The final report should be submitted at the latest on December 13th (23:59) 2024

Lab and field work

14-15 groups of 3 students / 13 sites

Septembre 18th (Field work)

meeting at the north entrance (C) of the GR building with your boots or hiking shoes…



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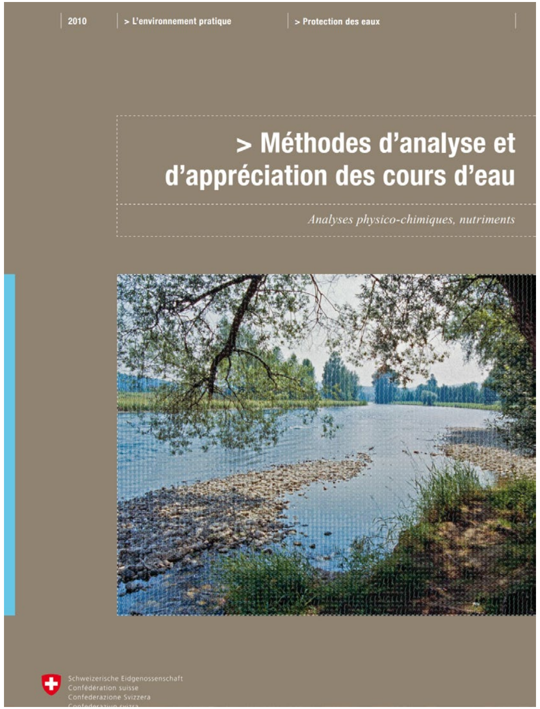
Modular Stepwise Procedure (Système Modulaire Gradué)



https://www.bafu.admin.ch/bafu/fr/home/themes/eaux/etat/eaux--methodes/systeme-modulaire-gradue.html

Methods to asses the environmental quality of streams

- General aspect
- Ecomorphology
- Physico-chemical analysis and nurtiments
- Marcozoobenthos

Appréciation	Ortho-P [mg/L P]	P total non filtré[6] [mg/L P]	P total filtré [mg/L P]
très bon	jusqu'à < 0,02	jusqu'à < 0,04	jusqu'à < 0,025
bon	0,02 à < 0,04	0,04 à < 0,07	0,025 à < 0,05
moyen	0,04 à < 0,06	0,07 à < 0,10	0,05 à < 0,075
médiocre	0,06 à < 0,08	0,10 à < 0,14	0,075 à < 0,10
mauvais	0,08 et plus	0,14 et plus	0,10 et plus

Appréciation	Nitrites [mg/L N][7] (<10 mg/L Cl-)	Nitrites [mg/L N] (10 à 20 mg/L Cl-)	Nitrites [mg/L N] (>20 mg/L Cl-)
très bon	jusqu'à < 0,01	jusqu'à < 0,02	jusqu'à < 0,05
bon	0,01 à < 0,02	0,02 à < 0,05	0,05 à <0,10
moyen	0,02 à < 0,03	0,05 à < 0,075	0,10 à < 0,15
médiocre	0,03 à < 0,04	0,075 à < 0,10	0,15 à < 0,20
mauvais	0,04 et plus	0,10 et plus	0,20 et plus

Report

- One report per group (in french or english)

1. Introduction (goals, context)
2. Material and methods (sampling, sample preparation, analytical methods, etc.)
3. Macropollutants analysis (methods, results and discussion)
4. Micropollutants analysis (methods, results and discussion)
5. Macrozoobenthos (results, dicusssion and link with the concentration of pollutants)
6. Discussion (comparison with previous reports and the littérature)
7. Conclusion
8. References
9. Supporting information (chromatograms, tables, etc.)

Summary

1. General introduction
2. Macro-pollutants
3. Organic micro-pollutants
4. Inorganic micro-pollutants
5. Analytical methods in environmental chemistry
6. Biological quality of streams and rivers (P. Mulattieri)

Définitions (1)

- Paracelsus (16e siècle): « Toutes les substances sont des poisons. La dose juste différencie un poison d'un médicament ».

- Selon Chapman* (2007), on parle de **contamination** lorsque les substances chimiques trouvées dans les sédiments ne sont pas habituelles ou lorsque les concentrations ne sont pas habituellement si élevées, alors qu'on parle de **pollution** lorsque la contamination provoque des effets biologiques délétères.

* Chapman, P. M., 2007. Determining when contamination is pollution - Weight of evidence determinations for sediments and effluents. Environment International 33(4): 492-501

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Définitions (2)

- **Macropolluants**: On appelle macropolluants des molécules naturelles qui sont présentes localement et/ou temporairement dans l'environnement à une concentration ne s'inscrivant pas dans l'intervalle habituel. Les réactions biochimiques impliquant ces molécules n'en seront pas changées, mais leurs cinétiques seront différentes.

- **Micropolluants**: On appelle micropolluants les molécules anthropiques présentes dans l'environnement à des concentrations <1mg/L. Les micropolluants peuvent être toxiques à de très faibles concentrations et peuvent causer des effets chroniques. Ils nécessitent en général des moyens sophistiqués et couteux pour leur analyse.

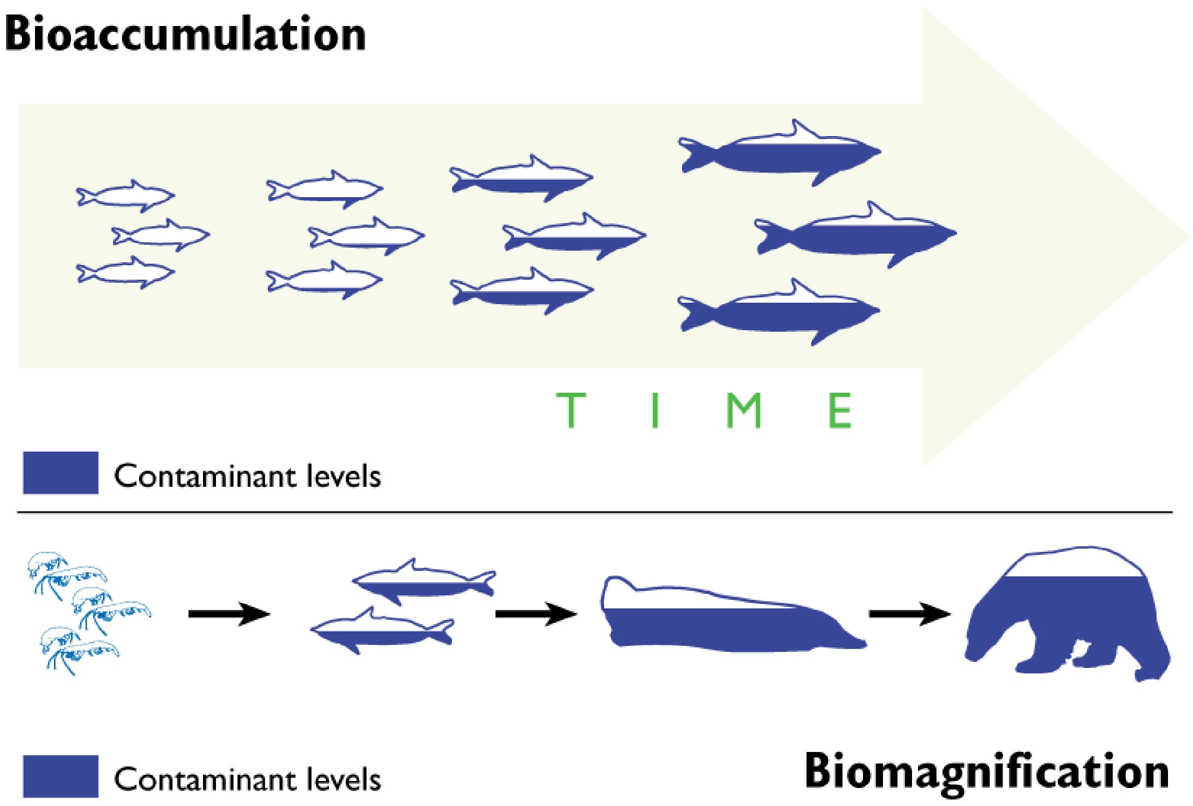
Définitions (3)

- **Bioaccumulation**: phénomène par lequel une substance présente dans un biotope pénètre dans un organisme.

- **Bioconcentration**: phénomène par lequel des êtres vivants absorbent des substances naturellement présentes dans leur biotope ou polluantes et les accumulent dans leur organisme à des concentrations supérieures à celles se rencontrant dans le milieu naturel.

- **Bioamplification**: phénomène par lequel une substance naturelle ou un contaminant présent dans un biotope connaît un accroissement de sa concentration au fur et à mesure qu'il circule vers les maillons supérieurs d'un réseau trophique.

Définitions (3)



Macro/Micro-pollutants

Macro-pollutants

Inorganic: PO_4^-, NO_3^-, NO_2^-, SO_4^{2-}, Cl^- …

Organic: plastics, surfactants, polymers, organic matter (feces, industries…)

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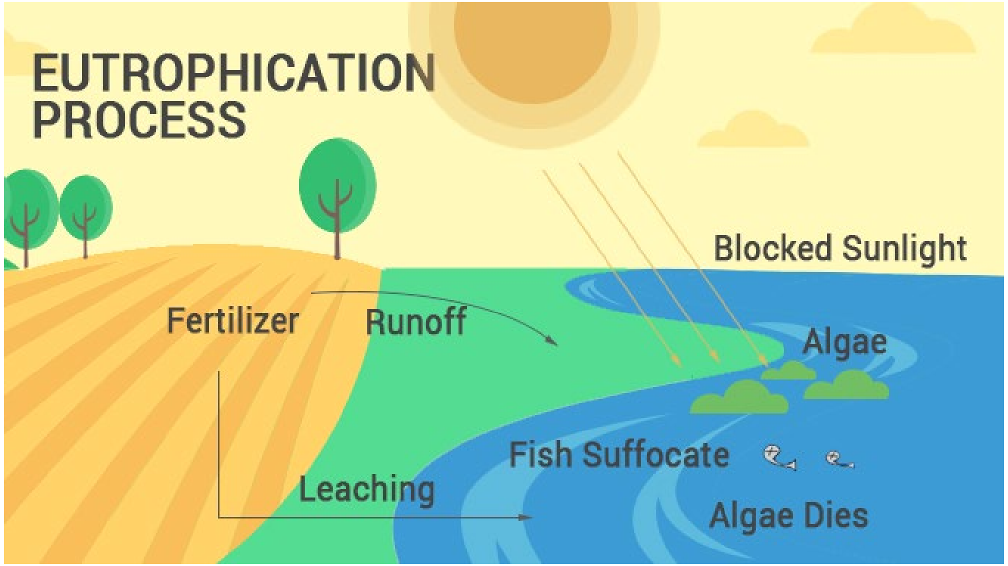
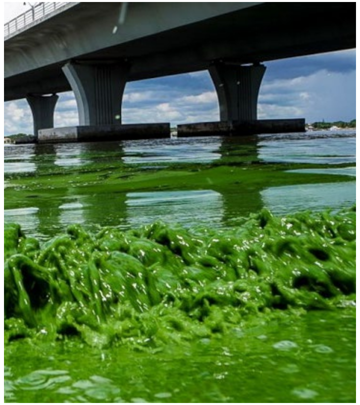
Macro-pollutants

PO_4^-

The orthophosphate concentration is an indicator of the level of pollution of rivers by human activities.





Macro-pollutants

PO_4^-

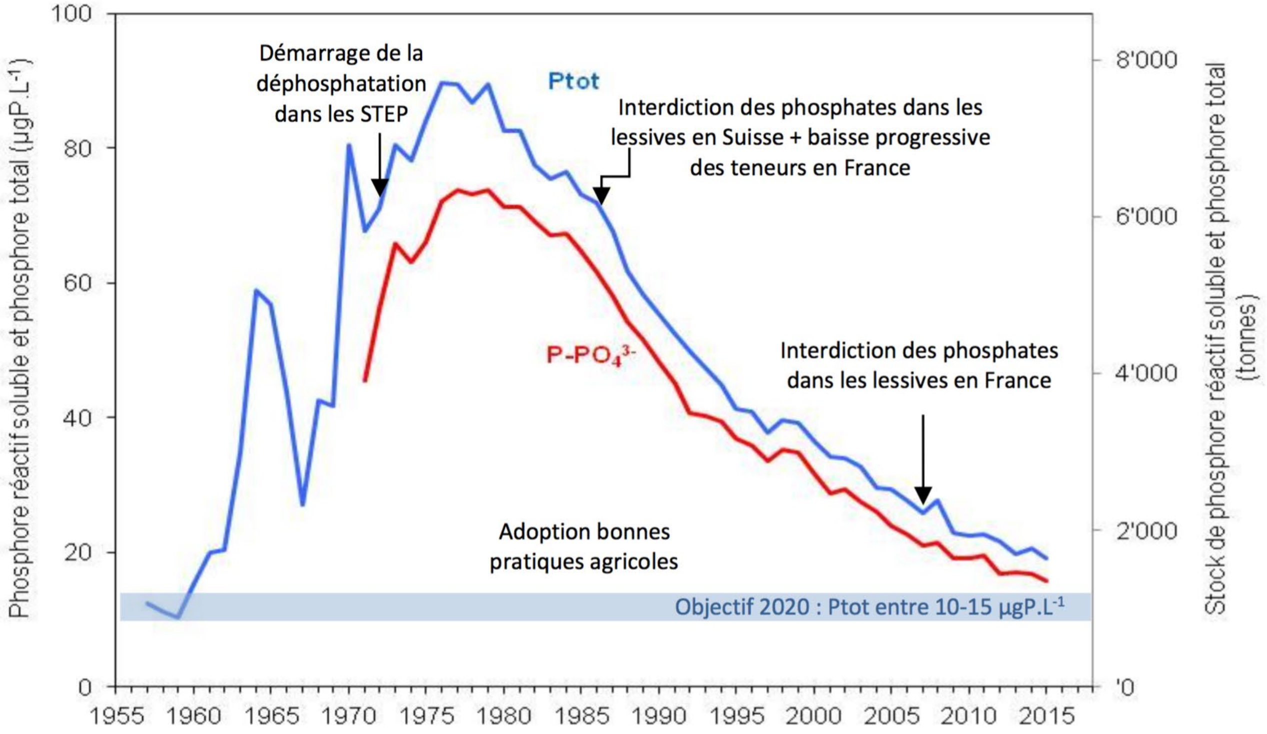


Figure 31 : Evolution de la concentration moyenne annuelle pondérée et du stock de phosphore réactif soluble et de phosphore total pour l'ensemble de la masse d'eau du lac de 1957 à 2015, Léman - Grand Lac (SHL2)

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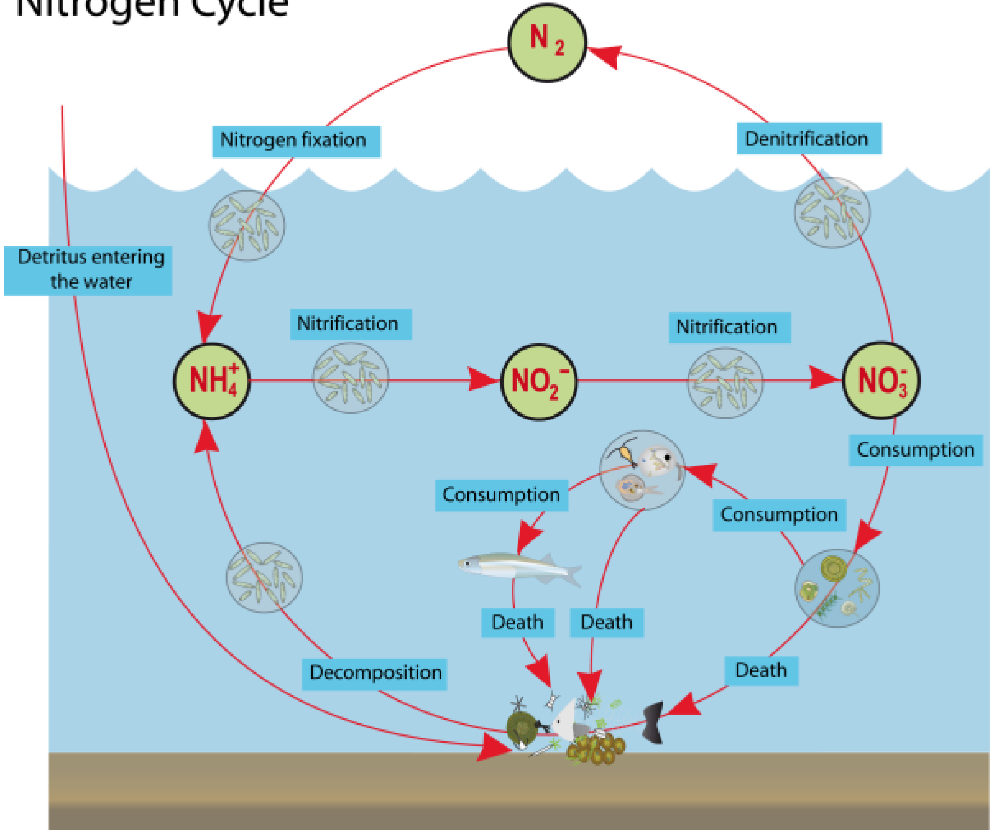
Macro-pollutants

NO_2^-/NO_3^-

Nitrogen Cycle

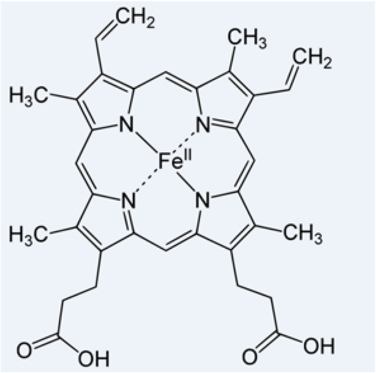


Macro-pollutants

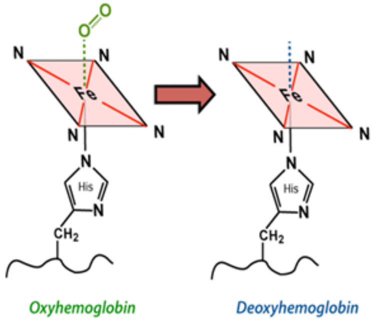
NO_2^-/NO_3^-

High nitrite concentrations in freshwater ecosystems may cause toxicity to aquatic animals. These living organisms can take nitrite up from water through their chloride cells, subsequently suffering oxidation of their respiratory pigments (hemoglobin, hemocyanin). Because NO_2^- and Cl^- ions compete for the same active transport site, elevated chloride concentrations in the aquatic environment have the potential of reducing nitrite toxicity.

Hemocyanin	Hemoglobin
Preforms better in cold environments with low oxygen pressure	Preforms better in oxygen rich environments
Contains copper linking other parts of the molecule	Contains iron surrounded by atoms of carbon, nitrogen, and hydrogen
Binds with oxygen non co-operatively most of the time (when non co-operatively, is one quarter as efficient as hemoglobin)	Binds with oxygen co-operatively all of the time
Free floating in blood	Connected to red blood cells



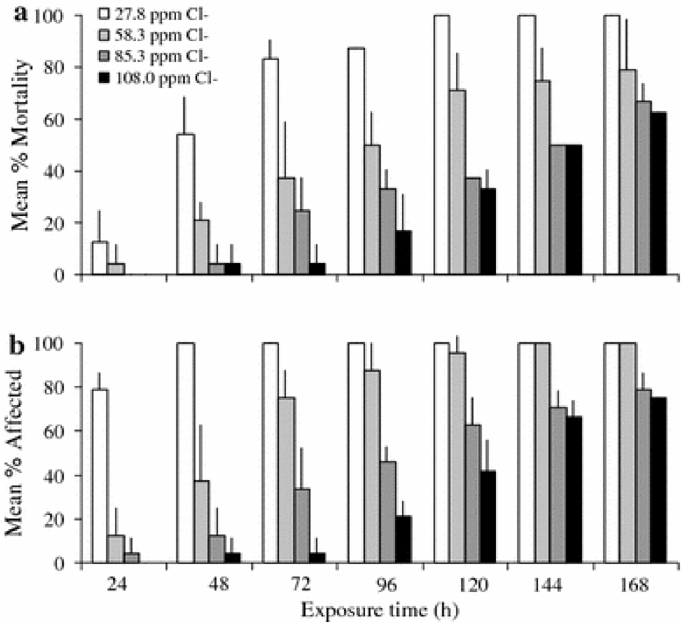
Heamoglobin



Macro-pollutants

NO_2^-/NO_3^-

Antagonist effect of chloride on nitrite toxicity



Mean percentages (+SD) of mortality (a) and affected individuals (b) for Eulimnogammarus toletanus exposed to 5.1 ppm NO_2-N through seven different exposure times (hours) and at four different chloride concentrations (ppm Cl^-)

Eulimnogammarus toletanus

Appréciation	Nitrites [mg/L N][7] (<10 mg/L Cl-)	Nitrites [mg/L N] (10 à 20 mg/L Cl-)	Nitrites [mg/L N] (>20 mg/L Cl-)
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mauvais	0,04 et plus	0,10 et plus	0,20 et plus

Système Modulaire Gradué

Macro-pollutants

NO_2^-/NO_3^-

Blue baby syndrome (methemoglobinemia) is caused by exposure to elevated levels of nitrite in infants <6 years.

Nitrite and nitrate can lead to the formation of *N*-nitrosamines (potent carcinogenic compounds) under certain conditions (e.g. gastric fluid, oxidative water treatment).

Blue baby syndrome Nitrates in water are ingested by an infant and converted to nitrite by the digestive system. The nitrite then reacts with oxyhemoglobin to form metheglobin, which cannot carry oxygen

Macro-pollutants

SO_4^-

Corrosion of water distribution systems.

Sulfate in drinking-water can also result in a noticeable taste.

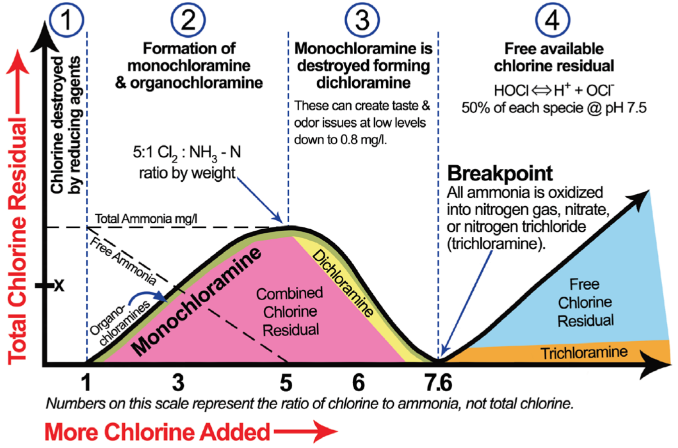
Cl^-

Salinization

NH_4^+

Indicates the level of pollution caused by municipal wastewater or leaching of crop lands.

Formation of chloramines during water chlorination.



Macro-pollutants

Alkaline earth metals and (post)transition metals

Level	Concentration range	Exemples
Major	1-100%	Al 1-30% Ca 0.01-10% Fe 0.7-15%
Minor	0.01-1%	Mg 0.06-0.6% Mn 0.03-0.3%
Traces	0.1-100 ppm	Cu 2-100 ppm Zn 10-300 ppm Pb 2-200 ppm
Utra-traces	<0.1 ppm (<100 ppb)	Hg 0.01-0.8 ppm Ag 0.01-0.1 ppm

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Macro-pollutants

Alkaline earth metals and (post)transition metals

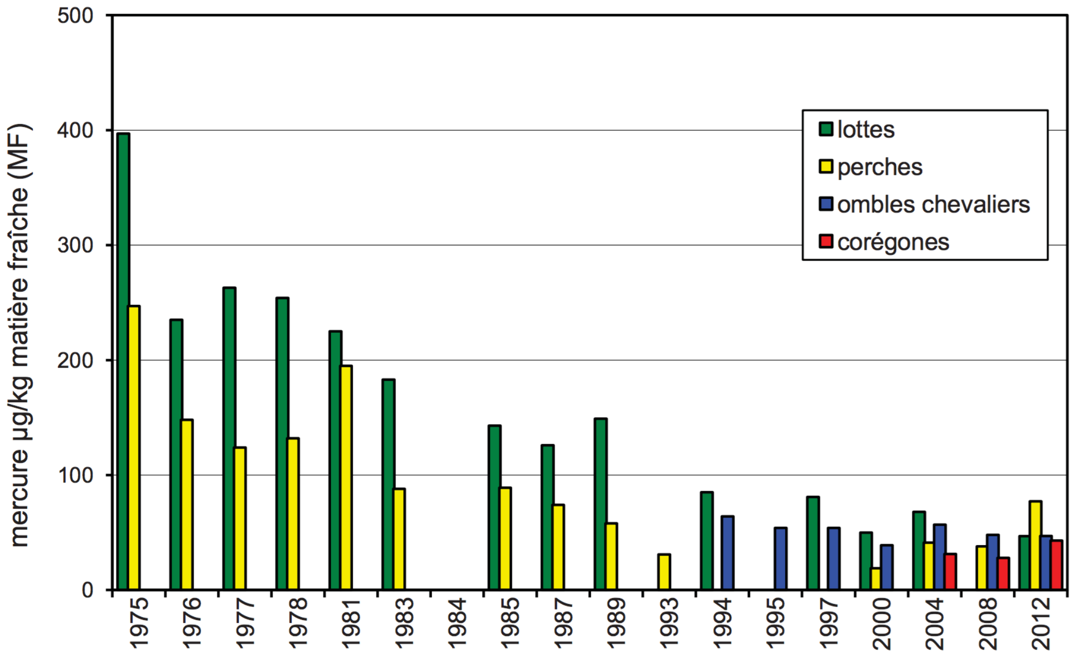

Figure 9 : Evolution de la teneur en mercure dans les poissons du Léman de 1975 à 2012.

Micro-pollutants

Micropollutants refer to residue from organic and inorganic substances, used everyday in modern society, including for example pharmaceuticals and personal care products (PPCPs), hormones, pesticides and industrial chemicals.

Concentration range

mg/L to ng/L or ppm to ppt

Effects at 10 ng/L

Olympic sized swimming pool
2,500,000 L

= 25,000,000 ng

= 25000 µg

= 25 mg

= 0.025 g

109 grains
of sugar

= 4.2 g
= 18,312 grains

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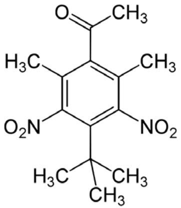
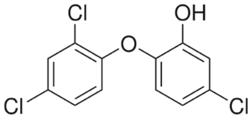
Micro-pollutants

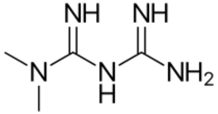
PPCP – Pharmaceutical and Personal Care Products



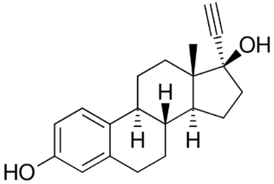
Musk ketone



Triclosan



Metformin



Ethinylestradiol

More than 85,000 chemicals are commercially available, and seven new substances are introduced on the market every day.

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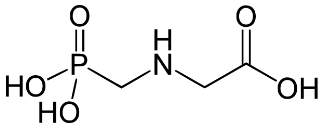
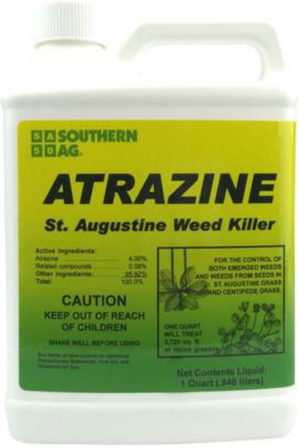
Micro-pollutants

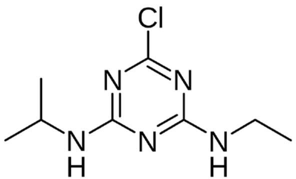
Agrochemical substances

Glyphosate





Atrazine



Micro-pollutants

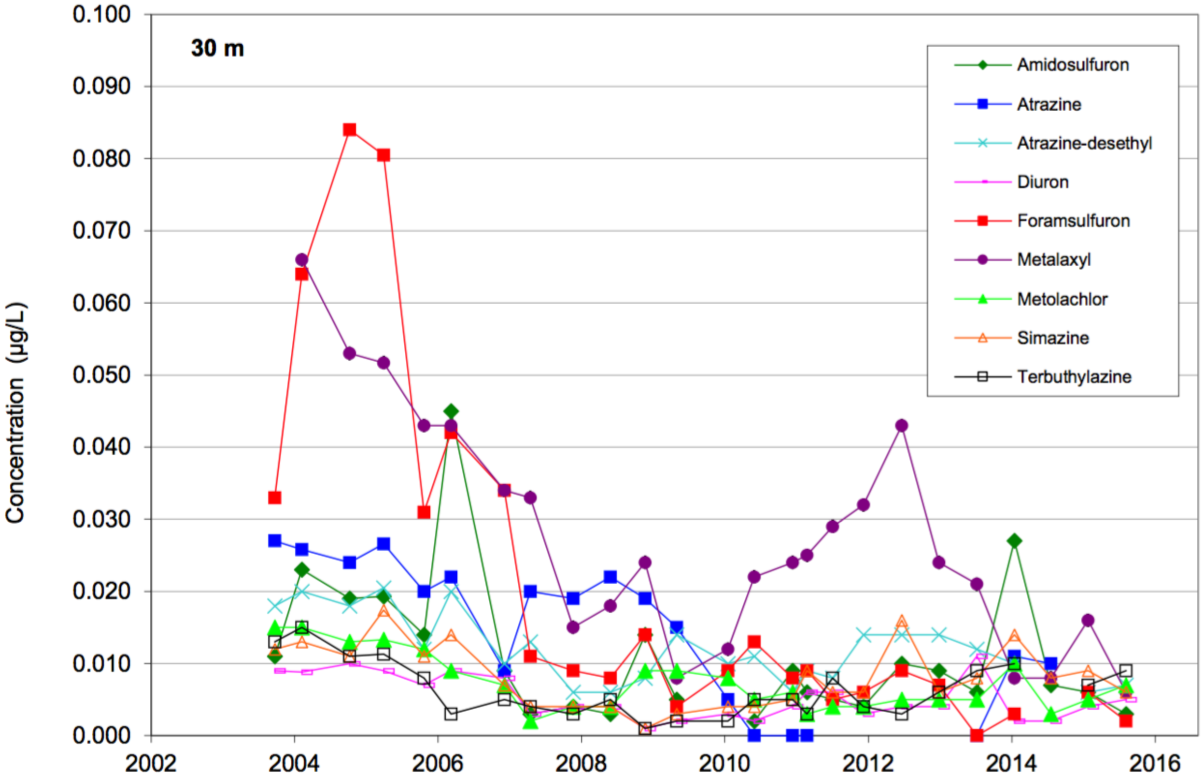


Figure 3 : Evolution des concentrations en divers pesticides au centre du Léman à 30 m (station SHL2) de 2004 à 2015.

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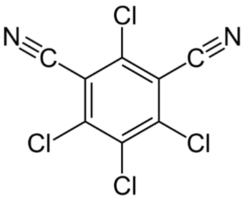
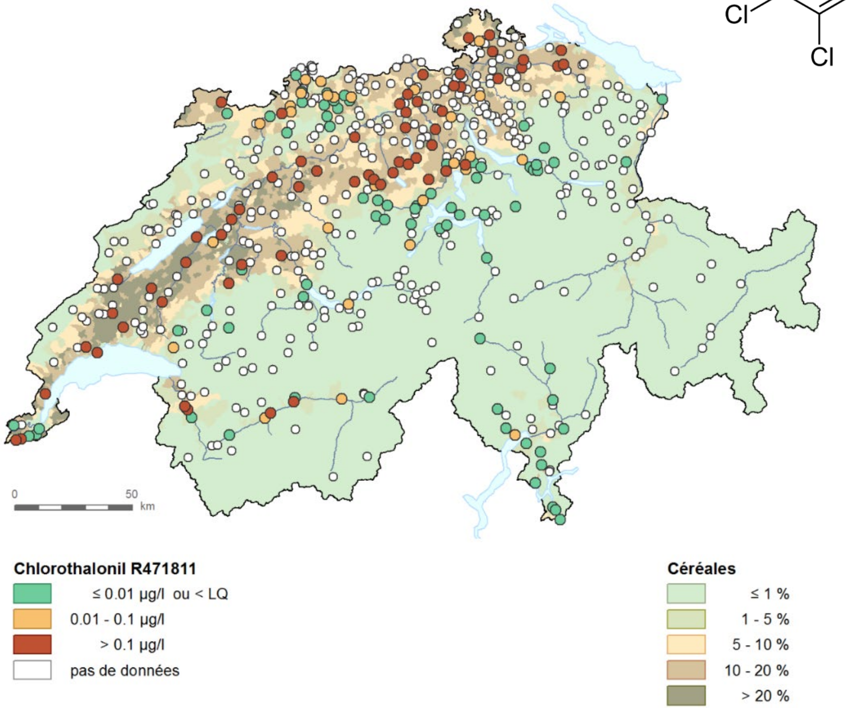
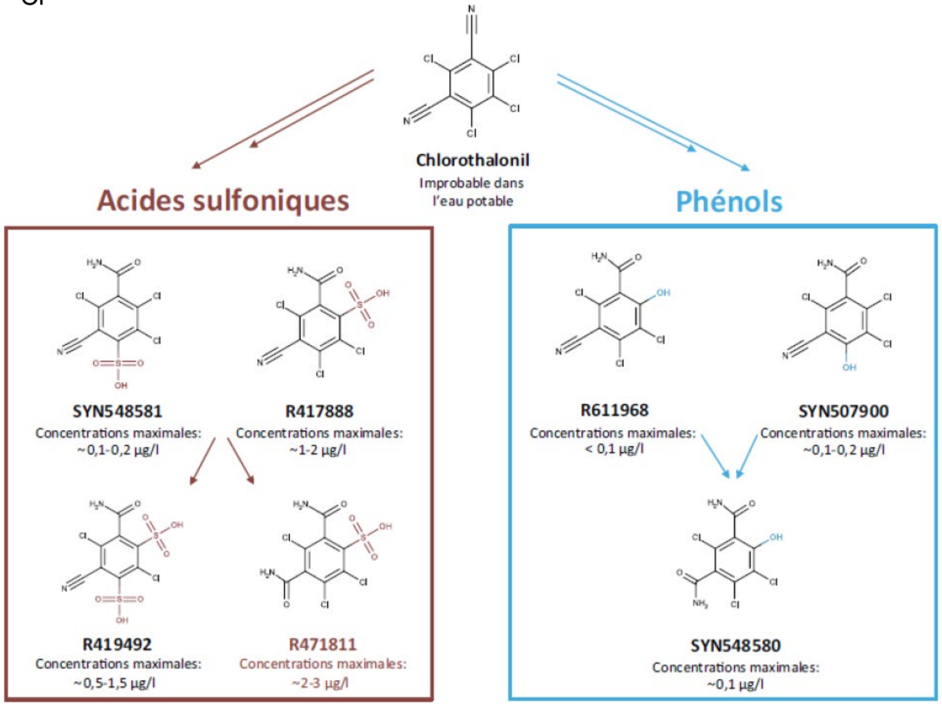
Micro-pollutants

Agrochemical substances



Chlorothalonil

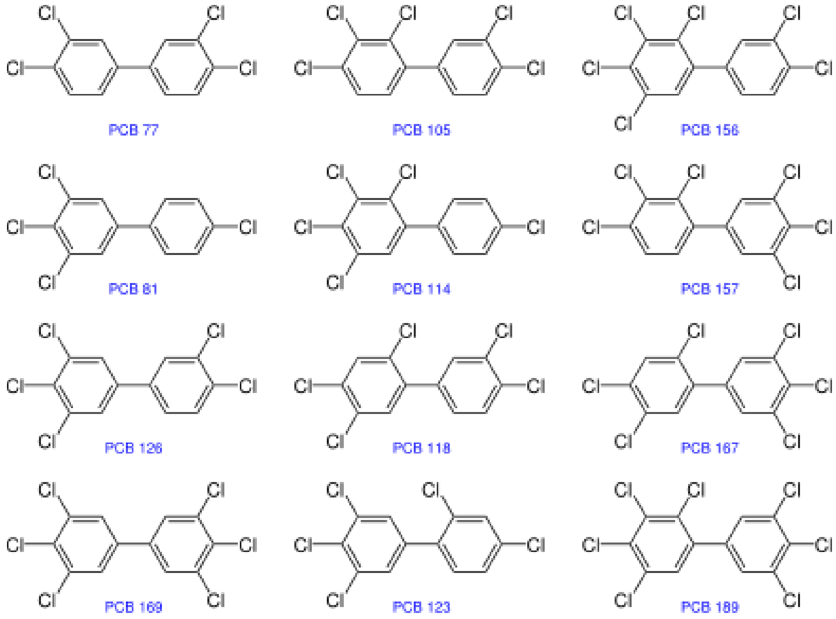




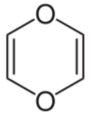
Max conc. of the metabolite R471811 groundwater

Micro-pollutants

PCBs – Polychlorinated Biphenyls



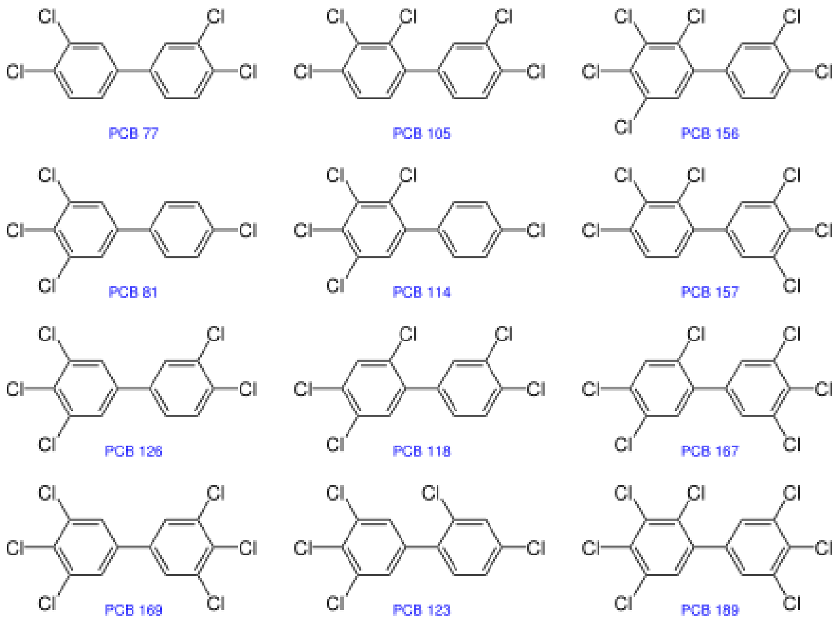
- Persistent organic pollutant
- 209 PCB congeners
- Fat soluble
- Classified dioxin-like PCBs as human carcinogens
- Endocrine disruptors
- Bioaccumulation

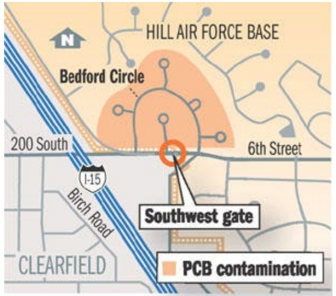


dioxin

Micro-pollutants

PCBs – Polychlorinated Biphenyls





PCB facts (Polychlorinated biphenyls)

- **Health effects:** may cause cancer, suppressed immune system, low birth weight and learning deficits
- **Environmental sources:** transformers, hydraulic equipment, paints, plastics, dyes, rubber products, carbonless copy paper
- **How much:** 1.5 billion pounds manufactured in the United States prior to cessation in 1977

SOURCE: U.S. Environmental Protection Agency

Saône : la pêche de certains poissons bientôt interdite

La consommation des poissons de fond va être interdite entre les confluents du Doubs et du Rhône. Des pêcheurs et des écologistes critiquent une gestion minimaliste de la pollution aux PCB

ux fois supérieur à
èmes et une silure

Micro-pollutants

PCBs – Polychlorinated Biphenyls

Décharge de la Pila (Fribourg)

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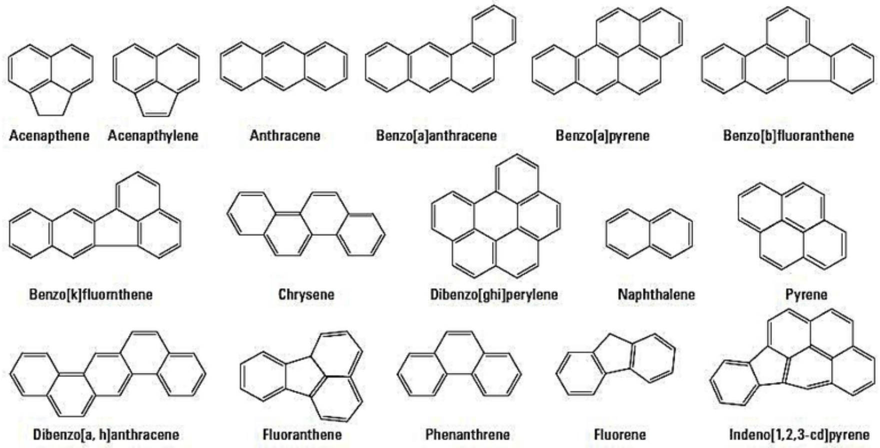
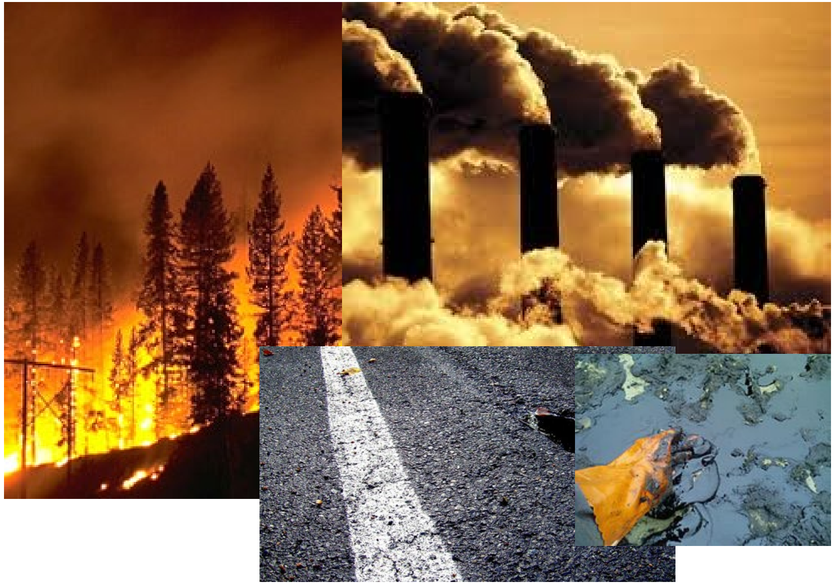
Micro-pollutants

PAHs – PolyAromatic Hydrocarbons



- Persistent organic pollutant
- linked to skin, lung, bladder, liver, and stomach cancers
- Cardiovascular disease
- Developmental impacts



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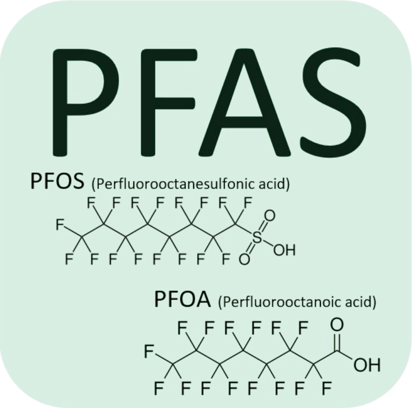
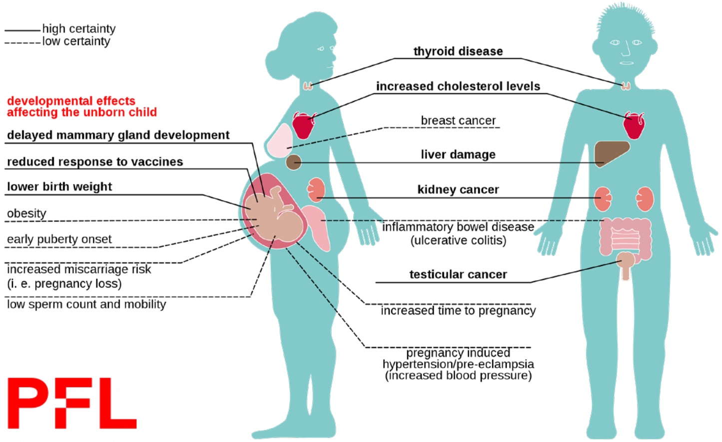
Micro-pollutants

Per- and polyfluoroalkyl substances (PFAS)





Mico/Macro-pollutants

Plastic pollution

Macro/meso-plastics >5mm
Microplastics <5mm – 1μm
Nanoplastics <1μm – 1 nm

Primary microplastics
Primary microplastics are small pieces of plastic that are purposefully manufactured.

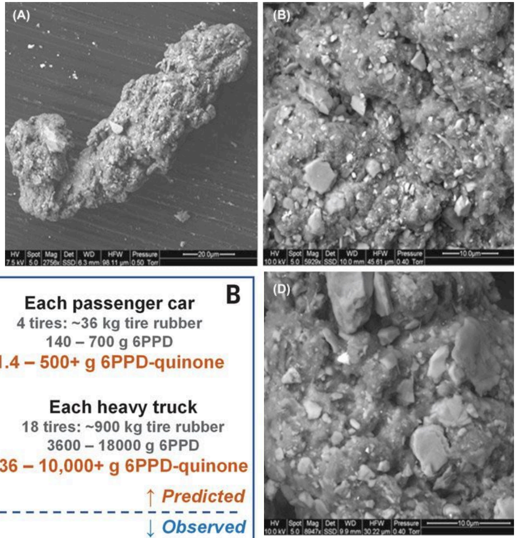
Secondary microplastics
Secondary plastics are small pieces of plastic derived from the breakdown of larger plastic debris, both at sea and on land.

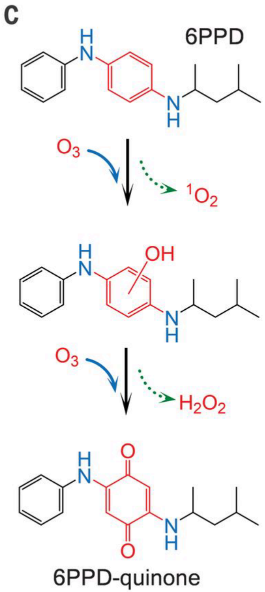
Mico/Macro-pollutants

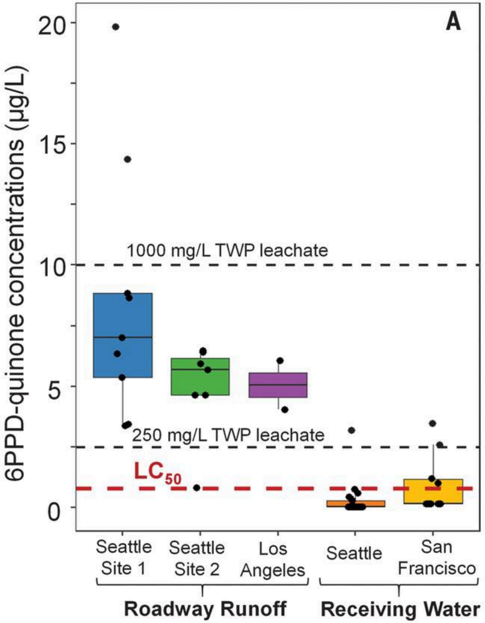
Plastic pollution

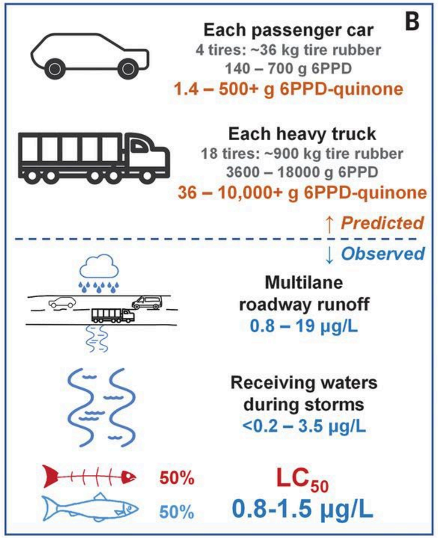
Tire and Road Wear Particles (TRWP)

- PAHs, heavy metals, antioxidants,…









Micro-pollutants (physico-chemical properties)

Partitioning (Kow)

Solubility

Photoreactivity

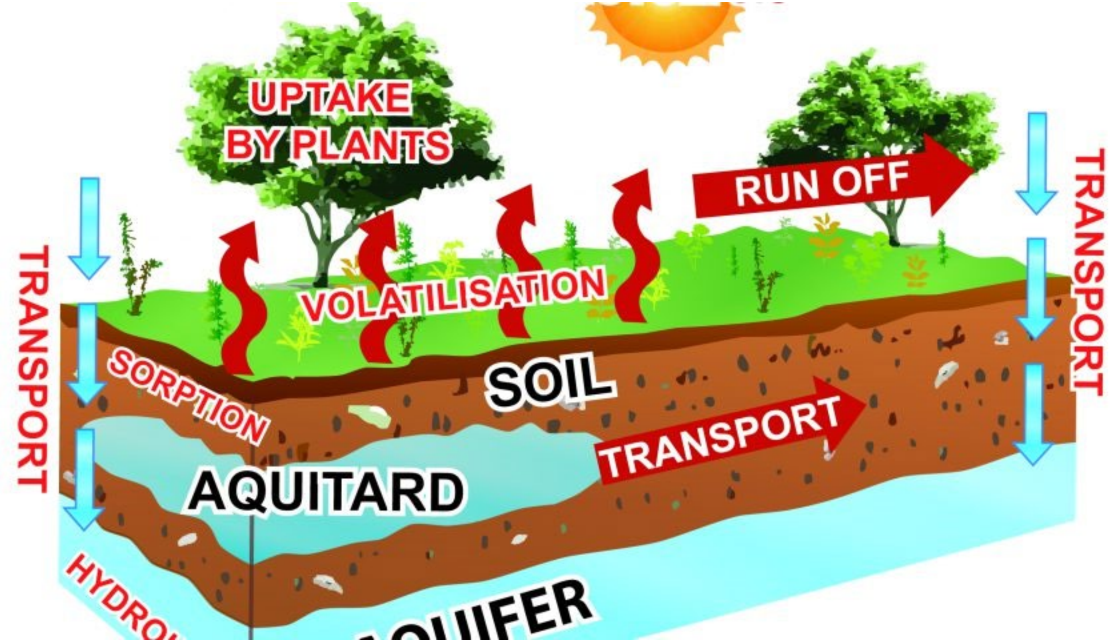
Vapor pressure

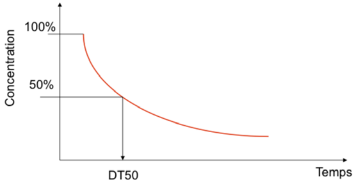
Acid-base properties (pKa)

Chemical reactivity

Sorption precesses

Biodegradability
Half-life time
Etc…





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Measure of pH, conductivity and dissolved oxygen

How pH is measured?

Pure water dissociates into hydronium ions (H3O+) and hydroxide ions (OH-). This dissociation is called autoprotolysis of water:

water is an acid: H2O = H+(aq) + OH-(aq)

water is a base: H2O + H+(aq) = H3O+(aq)

hence the reaction: 2 H2O = H3O+(aq) + OH-(aq)T

he dissociation constant of water (Kw) is 10^{-14} and corresponds to the sum of the activity of the hydronium and hydroxide ions.

Kw = aH+ + aOH-= 1 x 10^{-14}

For a neutral solution aH+ = aOH- = 10^{-7} at 25°C. Because the concentration of hydronium ions is 1 x 10-7 M, the pH of pure water is 7.00 at 25°C.This forms the basis of the pH scale.

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How pH is measured?

The source of the potential is measured by the electrode. The measuring system consists of a glass measuring electrode whose voltage varies proportionally to the activity of the hydronium ions contained in the solution and a reference electrode which produces a constant and stable voltage. The measuring electrode and the reference electrode can be housed in the same body (referred to as combined electrodes) or mounted separately (separate electrodes). The pH meter measures the potential difference between the two electrodes and displays the results either in mV or, after conversion, in pH units.

Conversion is based on the following equations

$E_{obs} = E_c + N_f \text{ Log } aH+$

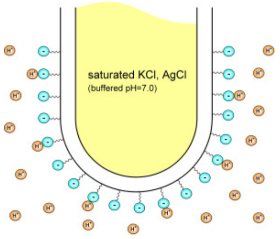
Where

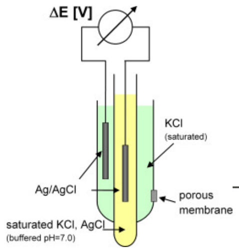
E_{obs} is the observed potential

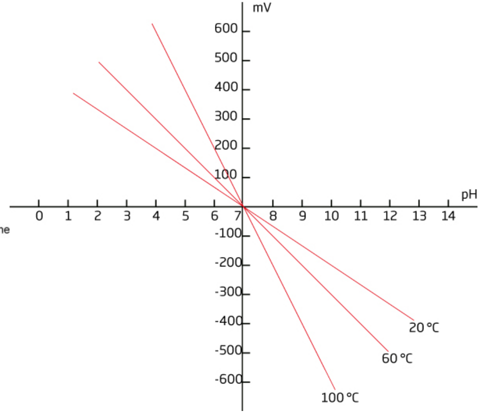
E_c is the reference potential including other stable and fixed potentials

N_f is the Nernstian factor of the slope

aH+ is the activity of hydronium ions







The degree of the slope Nf is the Nernstian factor, and is also a characteristic of the glass membrane, i.e. :$N_f = 2.3RT/ nF$

where:

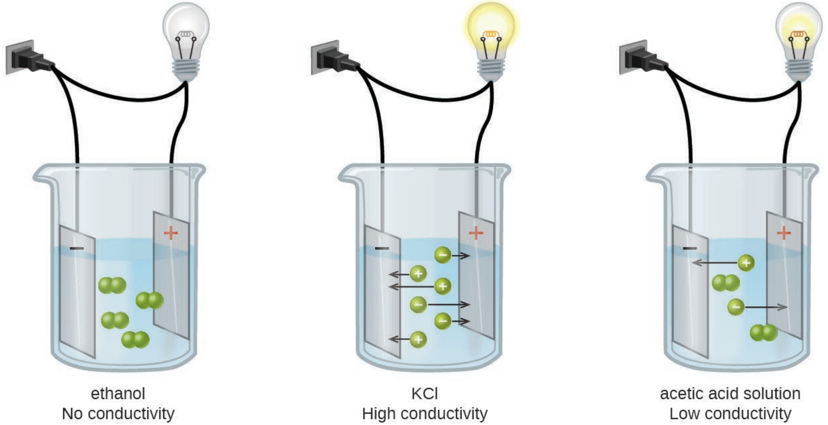
R=perfect gas constant = 8.313 J.K-1.mol-1,T=temperature in degrees Kelvin, F=Faraday constant = 9.65x104 C.mol-1, n=valency factor (n=1 for hydrogen)

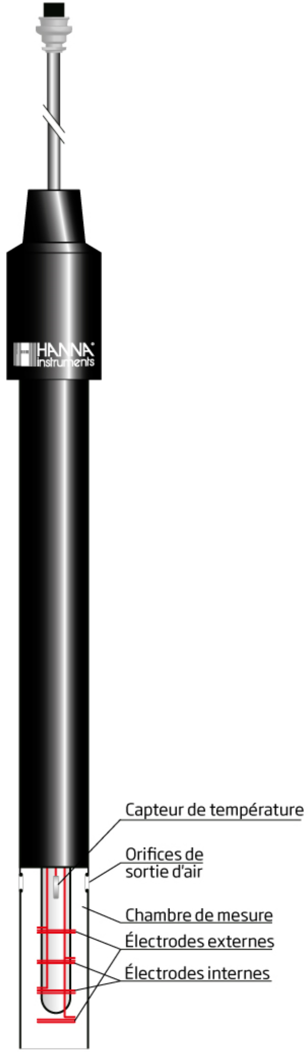
How water conductivity is measured?

An alternating current is applied to 2 electrodes (included in the conductivity cell) immersed in the sample and the resulting voltage is measured. The anions move towards the positive electrode and the cations towards the negative electrode. The sample behaves like an electrical conductor.

In practice, conductivity meters measure conductance. After performing the conversion operations, they display the conductivity.

Depending on the application, it may be preferable to measure resistivity (the inverse of conductivity), as in the case of pure water containing very few ions. The degree of conductivity of a solution depends on several factors:
- the ion concentration
- the valency of the ions
- their mobility
- the temperature of the solution.





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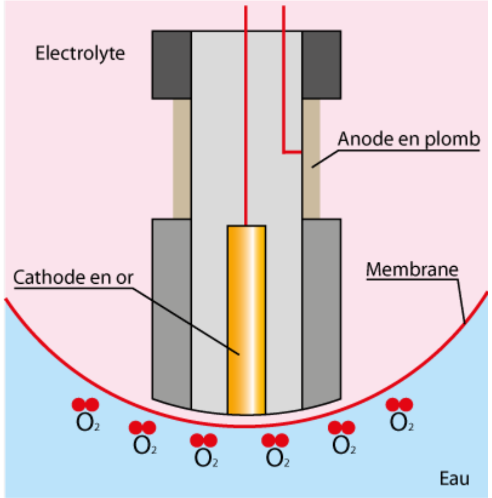
How dissolved oxygen (DO) is measured?



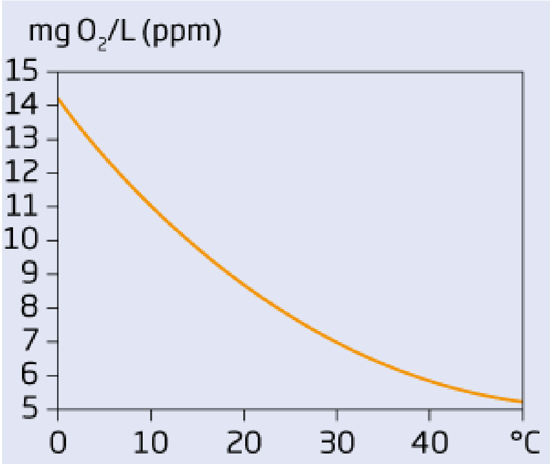
Probes for measuring dissolved oxygen consist of a cell containing two electrodes (anode and cathode), linked by an electrolyte providing the electrical bridge and insulated from the sample by a gas-permeable membrane.

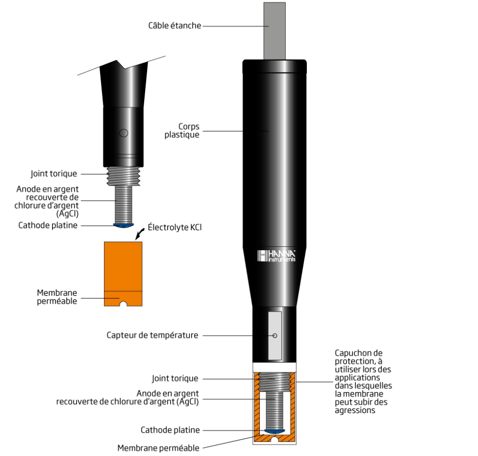
There are 2 types of cell:

- the polarographic cell, to which a polarisation voltage is applied to perform the measurement,

- the galvanic cell, where the electrode/electrolyte system is designed to generate its own potential, albeit with the help of an external power supply.

The 2 types of cell operate on the same electrochemical principle, the reduction of oxygen at the cathode.



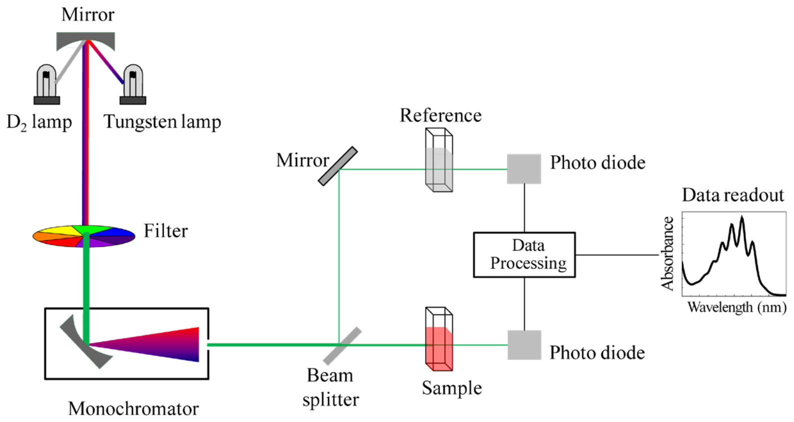


Samples preparation and Analytical methods

From sampling to the quantification of the analytes

Sampling	Water, soil/sediment, air, biological samples.
Samples preparation	Extraction, pre-contration, purification, derivatization…
Compounds separation	Chemical extraction,chromatography…
Compounds detection and quantification	Mass spectrometry, UV-vis spectrophotometrie, electrochemical detectors…

Compounds detection and quantification



UV-vis detector

For any particular wavelength,

$$A = \varepsilon b C$$

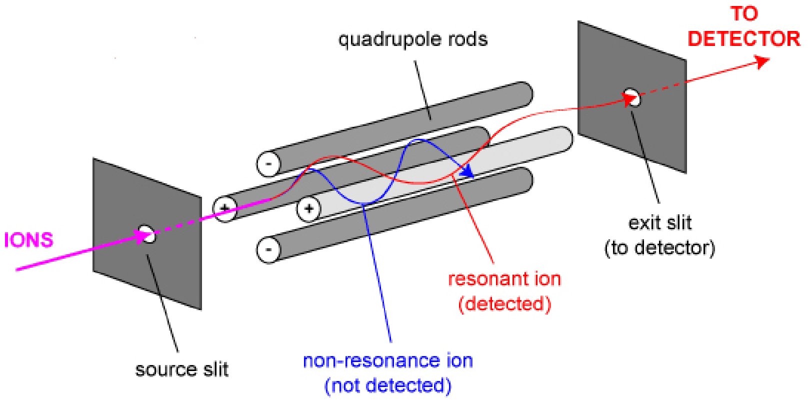
Absorbance

Molar absorptivity → L/(mol cm)

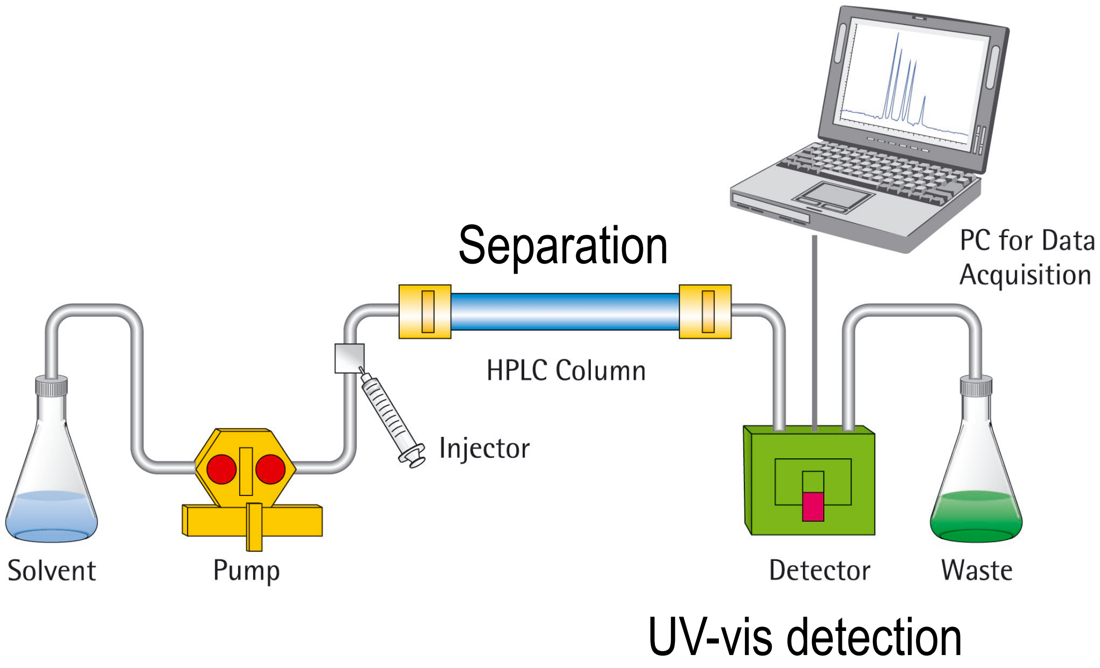
Path length → cm

Concentration Mol/L

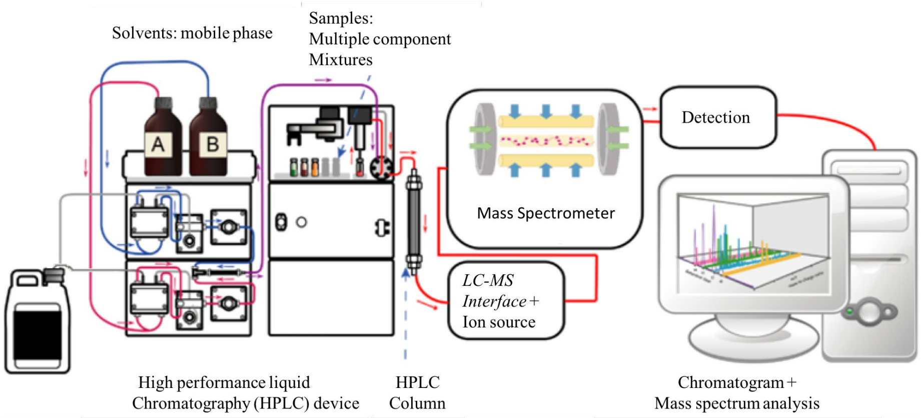
Mass detector

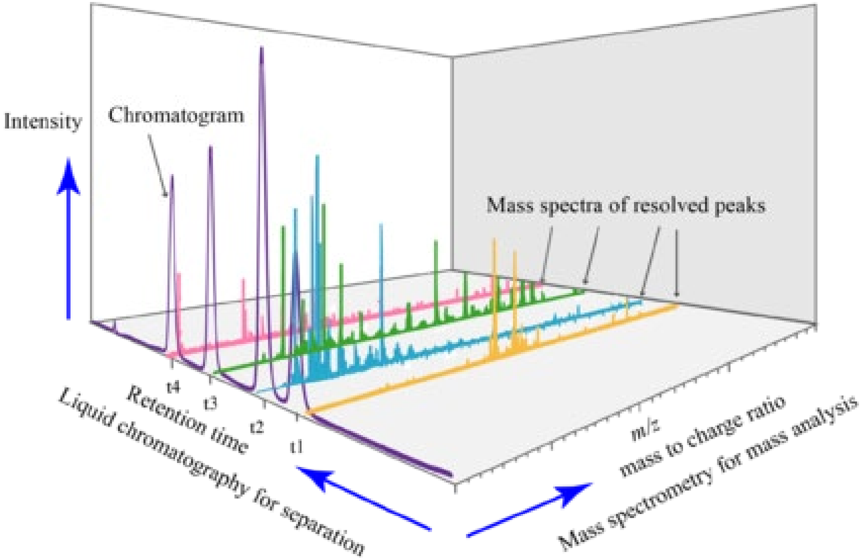


High Performance Liquid Chromatography (HPLC)



Liquid Chromatography – Mass Spectrometry (LC-MS)

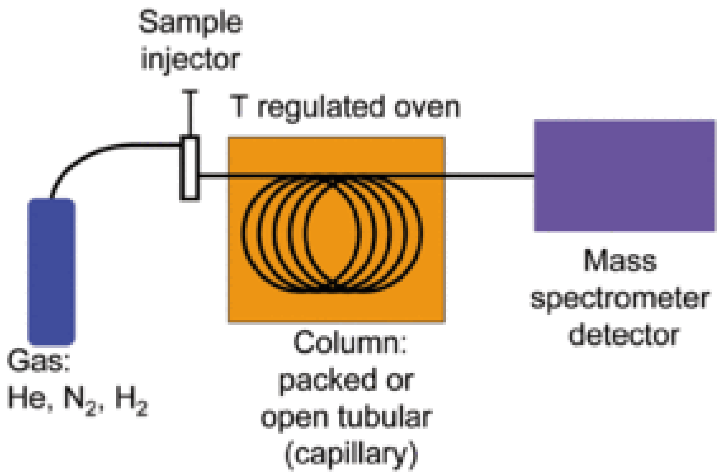




Liquid Chromatography – Mass Spectrometry (LC-MS)

- LC is a method for separating a complex mixture in to its components
- The high sensitivity of mass spectrometry provides the information for the identification of compounds or structural elucidation of compounds
- Combination of these two techniques is LC-MS
- In the most of the cases the interface used in LC-MS are ionization sources (electron, photon or chemical ionization)

Gas Chromatography – Mass Spectrometry (GC-MS)



For low molecular weight and volatile/semi-volatile compounds

LC-MS vs. GC-MS

GC/MS	GC/MS or LC/MS	LC/MS
MW < 700	100 < MW < 800	50 < MW < 50k
Highly volatile	Moderately volatile	Non-volatile
Non-polar	Moderately polar	Thermally labile
Moderately polar		Highly polar
Hydrocarbons	Most pesticides	Proteins/peptides
Flavors	Many drugs	DNA
Fragrances	Many industrial cpds	Oligosaccharides
Pesticides	Some vitamins	Surfactants
Some drugs		Dyes
	With derivatization	Drug glucuronides
	Many more!	Glyphosate
		Chlormequat

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LC-MS vs. GC-MS

GC/MS	EI 70eV	Hard	Large number of fragments (best finger print); M+ varies, maybe zero
GC/MS	PCI	Soft - varied	M+H dominant; fragments with "harder" reagents
GC/MS	NCI	Very soft	M- dominant; may fragment to yield electroneg groups
LC/MS	ESI	Very soft	Mol ion and adducts (+ or -) Few fragments
LC/MS	APCI	Soft - varied	Mol ion and adducts (+ or -) Few fragments

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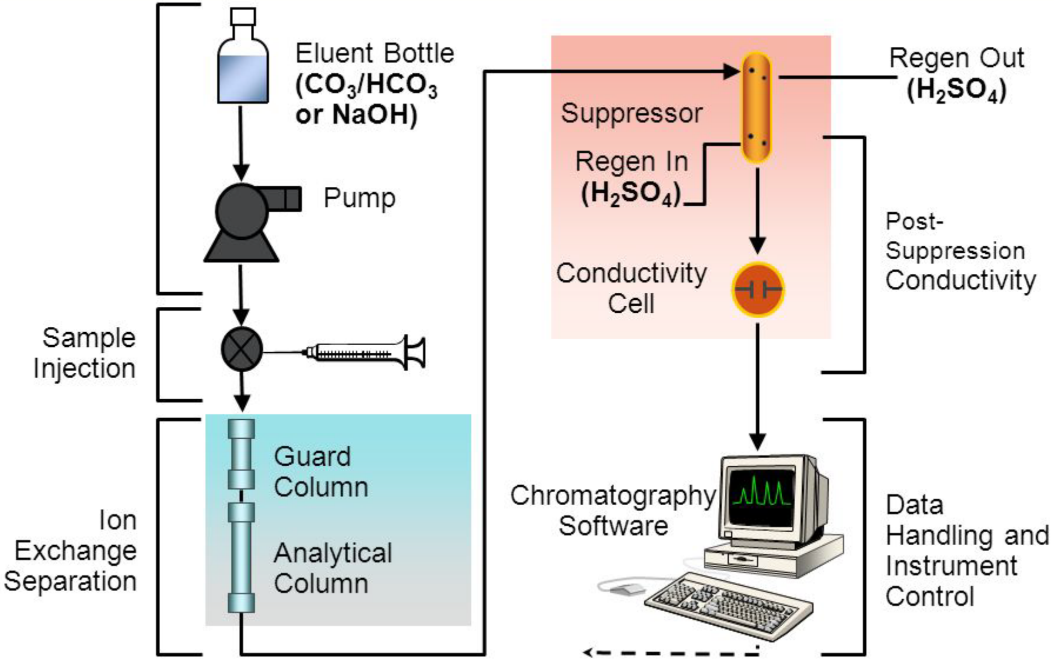
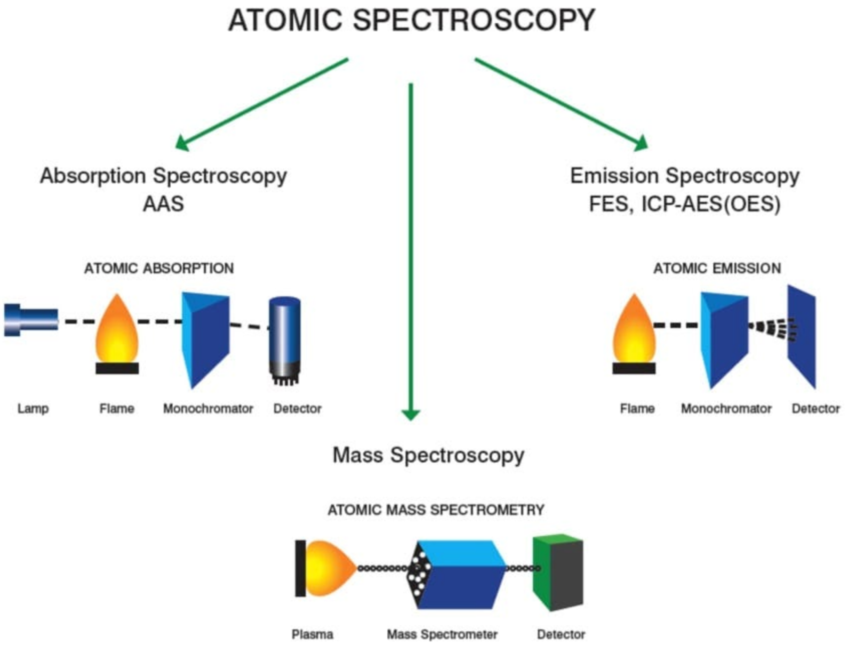
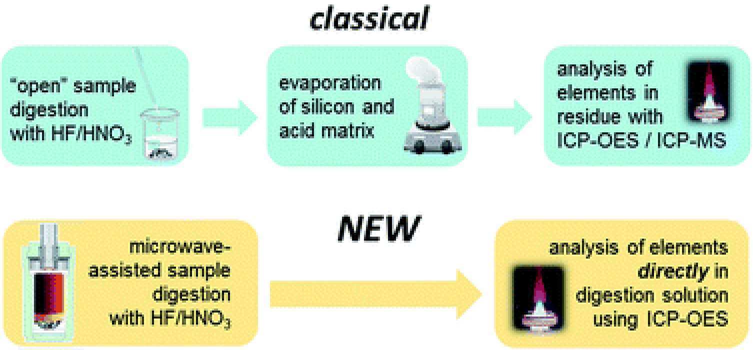
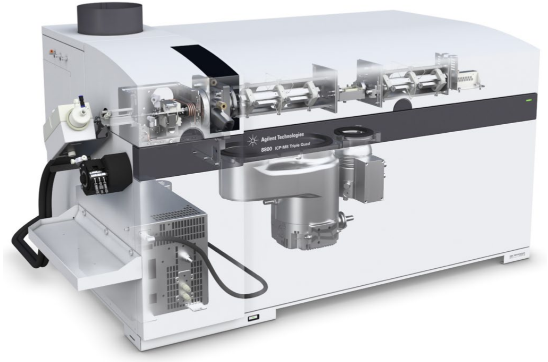
Typical ion chromatography system (anions)



Atomic spectroscopy (AAS, ICP-OES, ICP-MS…)







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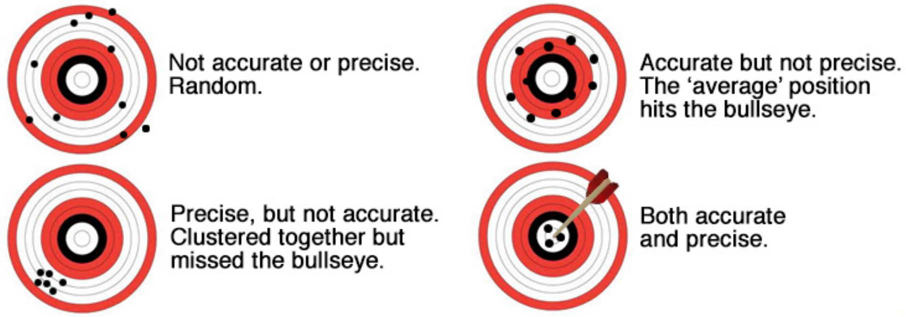
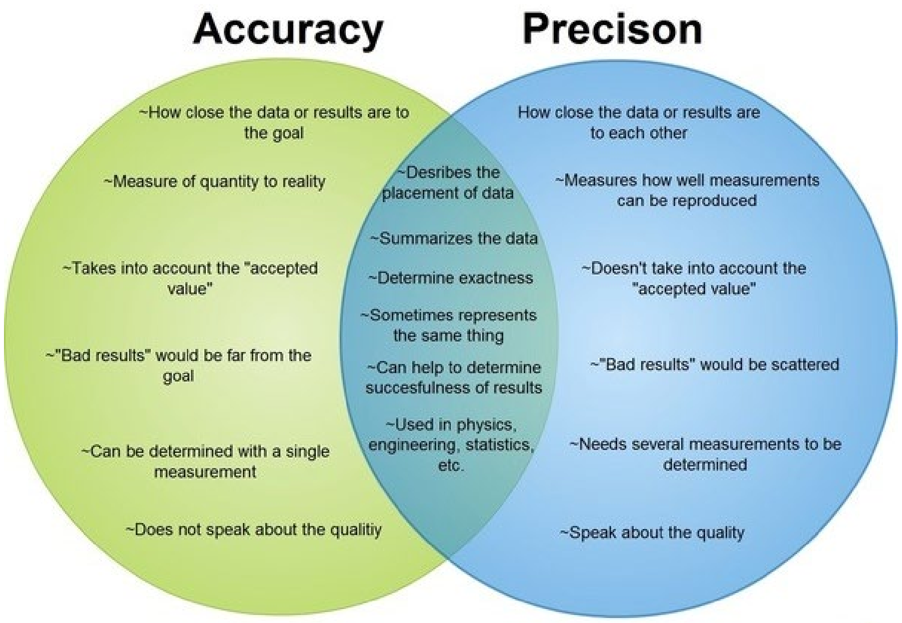
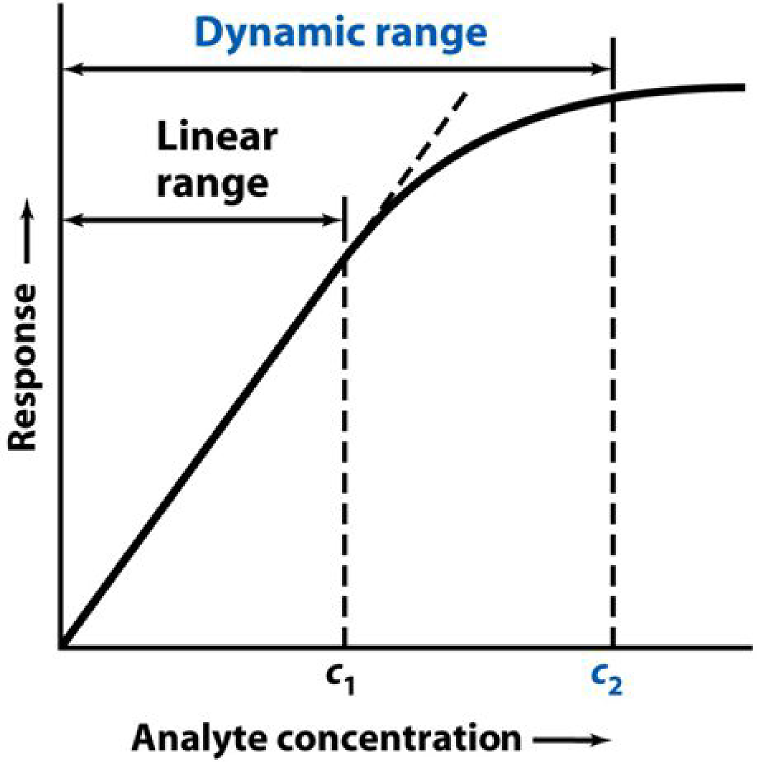
Accuracy vs. Precision





Calibration

It is not reliable to extrapolate any calibration curve, linear or non-linear. Measure standards in the entire concentration range of interests.



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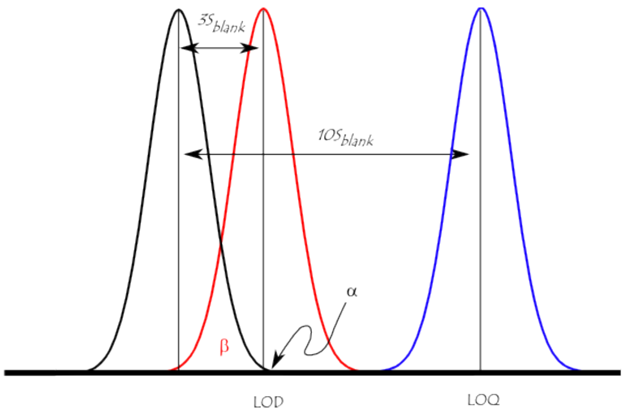
Limits of Detection and Quantification (LOD/LOQ)



LOD = 3.3 σ/Slope
LOQ = 10 σ/Slope
Where: σ = the standard deviation of the response at low concentrations
Slope = the slope of the calibration curve.

- The LOD is the lowest quantity of a substance that can be distinguished from the absence of that substance (a blank value) with a stated confidence level (generally 99%).

- The LOQ of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantified with suitable precision and accuracy.