E-kursuse
"Keskkond ja mõõtmine"
("Environment and Measurement")
materjalid

Aine maht 3 EAP

Siiri Velling

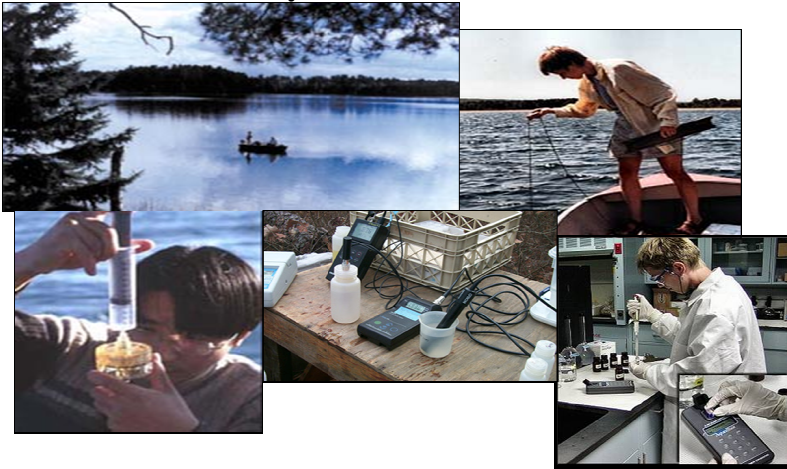**Tartu Ülikool 2011**

Sisukord
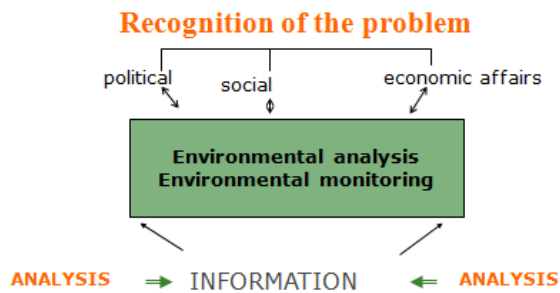
1 Introduction. Need for environmental analysis

Versatile field of study:

1.1 Need for environmental analysis?

Concerning warm up☺:
Describe a conflict situation between political, social and economic affairs where results of environmental analysis are cruisial for the settlement!

Basics of environmental analysis
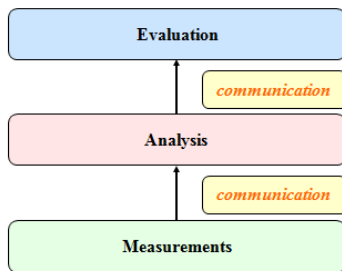
 Keyword here is COMMUNICATION.
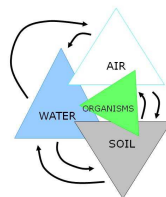
Taking into consideration that   one should have in mind that environmental analysis provides to
- control the present
- predict the future
- study the past.

Basic goals of environmental analysis are
1) determination of problems
2) diminishing the problems
3) affirm, that the probleem has diminished and
4) improvenemt of control means.

The field of environmental analysis involves both natural and artifical environment, lobal environmental problems and issues associated with human environment, the analysis of soil, water and air as well all the interesting topics connected with living organisms and appropriate environments.
The link between the compounds under study, as well methods of analysis and theres connection points has been described below:

Legislation
Environmental laws and control requirements
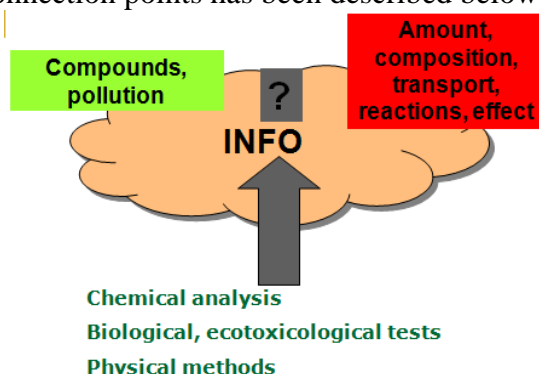- Natural waters
- Wastewater
- Drinking water
  - Microbiological parameters
  - Chemical parameters
  - Indicators – radiological parameters

Rules and requirements for analytical processes

Standards for environ. measurement
-standards ensure quality, reliability and interchangeability
-provide governments with a technical base for environmental legislation.

For example: ISO (International Organization for Standardization) standards
- ISO/TC 146 Air quality
- ISO/TC 147 Water quality
- ISO/TC 190 Soil quality

APHA (American Public Health Association)
Standard methods For the Examination of Water and Wastewater

OECD (Organisation for Economic Co-operation and Development)
The OECD is the main reference for the certification and standardisation of certain agricultural commodities and inputs.

Main drinking water constituents

Major constituents, >5 mg/L
- sodium
- calcium
- magnesium
- chloride
- sulfate
- bicarbonate
- silica

Minor constituents, 0,01-10 mg/L
- potassium
- strontium
- iron
- carbonate
- fluoride
- nitrate

Trace constituents, <0,1 mg/L
- aluminium
- arsenic
- barium
- bromide
- cadmium
- chromium
- cobalt
- copper
- iodide
- lead
- lithium
- manganese
- molybdenum
- phosphate
- selenium
- uranium
- zinc

1.2 Wastewater quality indicators

Wastewater is any water that has been adversely affected in quality by **anthropogenic influence**.
It comprises:
- liquid waste discharged by domestic residences,
- commercial properties,
- industry, and/or agriculture
- and can encompass a wide range of potential contaminants and concentrations.

In the most common usage, it refers to the municipal wastewater that contains a broad spectrum of contaminants resulting from the mixing of wastewaters from different sources.

Wastewater constituents
- ✓ Water 95%
- ✓ Pathogens such as bacteria, viruses, prions and parasitic worms.
- ✓ Non-pathogenic bacteria (> 100,000 / ml for sewage)
- ✓ Organic particles such as faeces, hairs, food, vomit, paper fibers, plant material, humus, etc.
- ✓ Soluble organic material such as urea, fruit sugars, soluble proteins, drugs, pharmaceuticals, etc.
- ✓ Inorganic particles such as sand, grit, metal particles, ceramics, etc.
- ✓ Soluble inorganic material such as ammonia, road-salt, sea-salt, cyanide, hydrogen sulfide, thiocyanates, thiosulfates, etc.
- ✓ Animals such as protozoa, insects, arthropods, small fish, etc.
- ✓ Macro-solids such as sanitary napkins, nappies/diapers, condoms, needles, children's toys, dead pets, body parts, etc.

✓ Gases such as hydrogen sulfide, carbon dioxide, methane, etc.
✓ Emulsions such as paints, adhesives, mayonnaise, hair colorants, emulsified oils, etc.
✓ Toxins such as pesticides, poisons, herbicides, etc.

BOD and **COD**

Biochemical oxygen demand and chemical oxygen demand

Any oxidizable material present in a natural waterway or in an industrial wastewater will be oxidized both by biochemical (bacterial) or chemical processes. As a result the oxygen content of the water will be decreased. Basic reaction for biochemical oxidation may be written as:

Oxidizable material + bacteria + nutrient + $O_2 \rightarrow$

$CO_2 + H_2O$ + oxidized inorganics such as NO_3 or SO_4

N – compunds - NH_4^+, NO_2^-, NO_3^- and others
P - compounds - PO_4^{3-}, total phosphorus
TOC – total organic carbon
DOC - dissolved organic carbon
VOC – volatile organic compounds
AOX - adsorbable organohalogens eg. organically bound halogens
EOX - extractable organic halogens.

Which parameters should be analyzed?
Legislation *(monitoring)*
- European Union Council Directive 91/271/EEC on Urban Waste Water Treatment
- National level, for example „Heitvee veekogusse või pinnasesse juhtimise kord" (http://www.riigiteataja.ee/ert/act.jsp?id=13136367)
- Local authorities
- Lab experience *(research)*.

2 Sampling
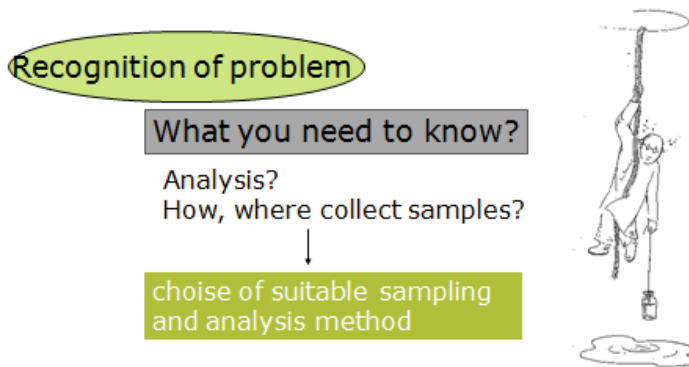
Sampling goals:
- Description
- Monitoring
- Control
- Special samples

Sampling is an essential part of **analytical process** and a sample must be a representative part of analyzing object.

Sample collection should fulfill the goals of study as well the sample must correspond to the requirements of analysis.

General sample collection problems:
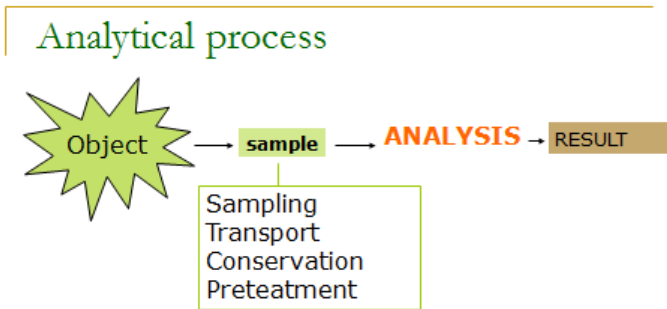- change in the properties of an object in time or space
- heterogenous, complicated systems
- low concentrations, lots of parameters to study

Sample collection "musts":
- The representative part of object remains unchainged
- Correspondence of sample properties to the requirements of analysis method
- No changes in sample properties during sample collection, transport and/or conservation

What has to be considered?
- ✓ specifics of analysis
- ✓ sampling frequency
- ✓ sampling place
- ✓ volume of a sample
- ✓ suitable containers
- ✓ handling and preservation

2.1 Water samples

Individual and joint samples
- o Individual – one time sample or a point sample
- o Individual samples unified into **joint averaged samples**
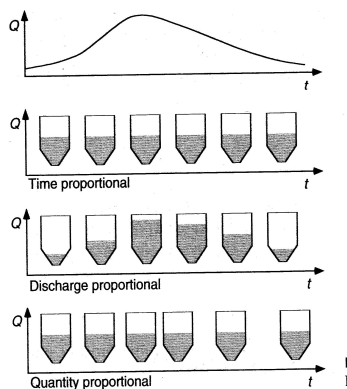
- **Time proportional** - collecting individual samples of a certain volume after known (assigned) time interval
- **Discharge proportional -** the time intervals are constant, but the volume of each sample is proportional to the volume of discharge during the specific time interval
- **Quantity proportional -** the volume of each sample is constant, but the temporal resolution of sampling is proportional to the discharge
- **Event-controlled sampling -** depends on the trigger signal.

General guidelines
1. Location
   - according to the pupose and characteristics of object
    - easy of approach
    - allows to collect samples at the same place always
    - below 30 cm form the surface of water bodies
    - from the point of strong stream in rivers
      (well-mixed area)

    ! Places that are not typical to the water body, should be avoided!
2. How often?

    - thermal stratification, which leads to variations of quality in depth
    - effects of influent streams
    - lake morphology
    - wind
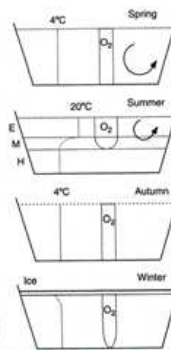
    E-epilimnion, M-metalimnion, H-hypolimnion
3. Sample volume
    - amount of components to be analyzed
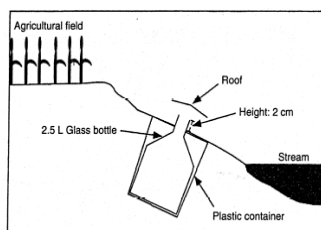   - methods of analysis or study
4. Sample containers
    - up to the components to be analyzed
    - washed and labelled beforehand (in laboratory)
    - should be rinsed with sample before final collecting of the sample
      (exept for the analysis of oil products, oils, fats etc)
    - usually are filled to the brim.
5. Water samplers
    Automatic, portable, specific

General Considerations
- ✓ Always fill sample containers - no air is left above the sample.
- ✓ Use an appropriate container. For example polyethylene bottles should not be used for hydrocarbons, since adsorption on to the bottle's surface is likely to occur.
- ✓ Glass containers are suitable for most determinations. Brown bottles should be used since this will reduce photosensitive reactions to a considerable extent.
- ✓ Containers must be clean.
- ✓ Samples should be kept at a temperature below that at the time of filling. Cooling between 2 degrees and 5 degrees (ie. in melting ice, refrigerator or cool bag with ice packs) is adequate. It is not suitable for long-term storage.
- ✓ Suspended matter, sediment, algae and other micro-organisms should be removed at the time of sampling by filtration or centrifuging or immediately on receipt at the laboratory. Filtration should not be carried out if the filter is likely to retain one or more of the constituents to be analysed.

Changes in sample composition may occur due to:
- consumption of certain constituents by bacteria, algae etc.,
- certain compounds being oxidised by the dissolved oxygen in the sample,
- precipitation from the liquid, eg. calcium carbonate, aluminium hydroxide,
- loss into the vapour phase,
- absorption of carbon dioxide from the air, changing the pH value,
- adsorption of metals and certain organic compounds on to the container's surface,
- depolymerisation of polymerised products and vice versa.

Sampling mistakes:
- Not enough partial samples
- By the sample collection procedure caused
     precipitation of particles,
     evaporation of substances
- Changes of sample properties before analysis
   biodegradation, adsorption

Proper **handling and preservation** are very impotent to keep the sample content unchanged. The goal of specificd handling and preservation requirements are to remain the representative part of object unchainged for as long as possible or as needes.

Conservation and maintenance
- Samples should be as fresh as possible
- Preferably avoid the conservation of samples
- Non-conserved samples should be analyzed guring 24 hours from sample collection
- Conservation – to maintain the (specific) properties of samples and concentrations of ingredients for as long as possible (needed)

SPECIAL REQUIREMENTS
- Depending on the components to be analyzed in the sample or properties of sample

Sample transport
- Pick a suitable container
- Keep samples cool, no warming or frozing of samples is accepted
- Keep samples in dark.

2.2 Soil samples

By the collection of soil sample, one should keep in mind, that
- every sample shoud characterize a certain type of region or land
- from a certain layer appropriate to a certain depth (topsoil)
- different layers of depth separately
- joint averaged sample
  point samples shoud be collected over the whole region
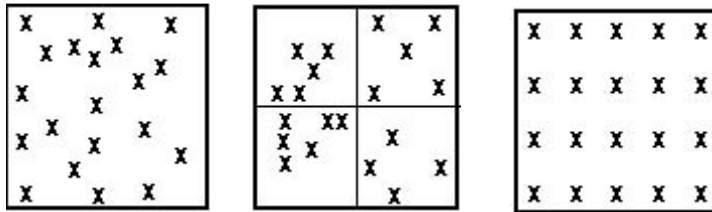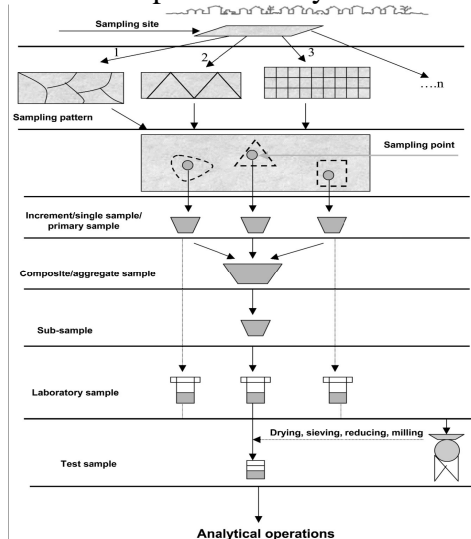

Pick the best one!

Sampling site: A well delimited area, where sampling operations take place
Sampling point: The place where sampling occurs within the sampling site

To ensure the representativity of soil sample:

Terminology in soil sampling (IUPAC Recommendations 2005)

Transport of soil samples:
- ✓ no warming or frozing of samples is accepted
- ✓ keep in dark.

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Soil sample pretreatment
Washing, drying
Grinding/homogenisation
      Biologically active samples should not be exposed to prolonged warming !
Extraction of analyte
- o   solvent extraction – neutral organic compounds
- o   ashing and subsequent dissolution – elemental composition
- o   extraction in aqueous solutions – "available" ions

Problems:
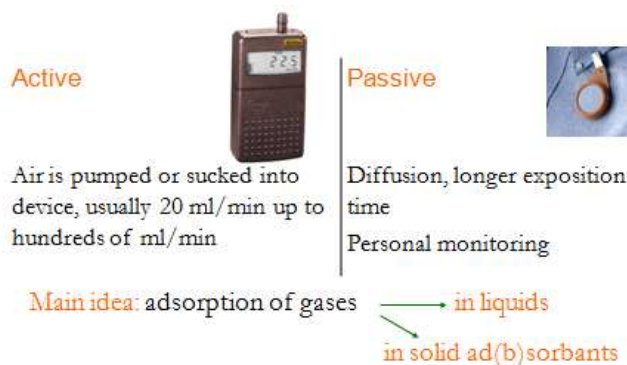General contamination with pesticides
Internal standard
Reference material

Reference materials
A material or substance one or more of whose property values are sufficiently homogeneous and well established to be used for the calibration of an apparatus, the assessment of a measurement method or for assigning values to materials
- Pure standards
- Solutions   **-** one analyte
  - several analytes
  - analyte and unwanted constituent
- Synthetic mixtures
- Matrix reference materials   – natural
                                          – fortified

2.3 Atmospheric analysis and air sampling

- Collection of one specific substance
- Collection of several substances
- Solid sample analysis
- Absorption of gases in liquids (special reagents)
- Adsorption of substances on solid sorbents
         Passive or active sampling devices
- Filtration of particles

Active   Passive

Air is pumped or sucked into device, usually 20 ml/min up to hundreds of ml/min

Diffusion, longer exposition time

Personal monitoring

Main idea: adsorption of gases   in liquids
                                in solid ad(b)sorbants

One specific compound or mixture of compounds, filtration of solid particles

3. Titrimetric ja gravimetric methods

Environmental analysis

analysis

qualitative
Different components
(compounds)
in sample ?

quantitative
Amount or concentration
of the compound
in sample

Classical" methods
Gravimetry
Titrimetry

Instrumental analysis
Spectrophotometry
Chromatography

Chemical analysis

DETERMINATION OF
INDIVIDUAL COMPOUNDS

PARAMETERS INDICATING
TOTAL AMOUNT OF SPECIFIC
COMPOUNDS
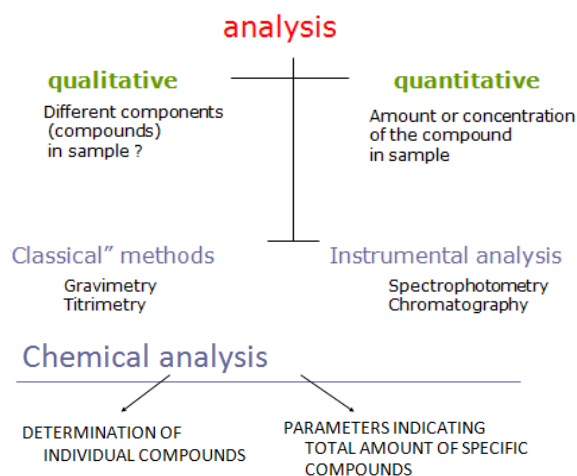
2.1 Titrimetric method

Titrimetry is a method of volumetric analysis.
Titration is a quick, accurate and widely used way of measuring the amount of a substance in solution and is performed by adding an exact volume of a standard solution needed to react with the analyte in the sample.

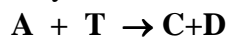Thereby a **titration reaction** between **titrant T** and **analyte A** occurs.

References:
Concentration C_T, volume V_T of titrant
volume of sample V, concentration of analyte $\mathbf{C_A}$

Equivalence point
The equivalence point or stoichiometric point responds to the stoichimetry of chemical reaction:

$$\mathbf{A + T \rightarrow C+D}$$

the amount of added titrant is chemically equal to the amount of analyte, per moles.
The **end point** is the point in a titration when a physical change occuring immediate after the equivalence point

Calculation of results
if **1 mole** of analyte reacts with **1 mole** of titrant

$$c_T V_T = c_A V$$

c_T - molar concentration of titrant
c_A – molar concentration of the analyte in the sample
V_T – volume of titrant used during titration up to equivalence point
V - sample volume

The equivalence point can be determined by or with
- precipitation
- pH
- potentiometry
- conductance
- isothermal titration calorimeter
- thermometric analysis.

Requirements for successful titration:
- the reaction should be stoichiometric, there should be a definite ratio between the number of moles in reaction
- rapid rate of reaction
- end point and equivalence point should coincide
- other components of a sample should not alter the stoichiometry of the reaction.

2.2 Methods of titrimetry

Direct titration
Determination of equivalence point or nd point by
physical changes:
occurence or disappearing of precipitate
occurence or disappearing of colour
change of colour.
What kind of titration terror can not be avoided?

$$A + T \rightarrow C+D \qquad \textit{Indicators!}$$

$$\underset{colour1}{Ind} + T \rightarrow \underset{colour\ 2}{Ind\ after\ reaction}$$

Backtitration - if the reaction is slow or determination of end point is complicated, then
- measured volume if titrant is added in excess (compared to theoretically needed amount)
- excess titrant is back-titrated with a regent

Substitution titration
- sample should contains stronger chelating agent
- sample is titrated with the reaction produkt of titrant and weaker chelating agent
- the volume of weaker chelating agent that separates is determined
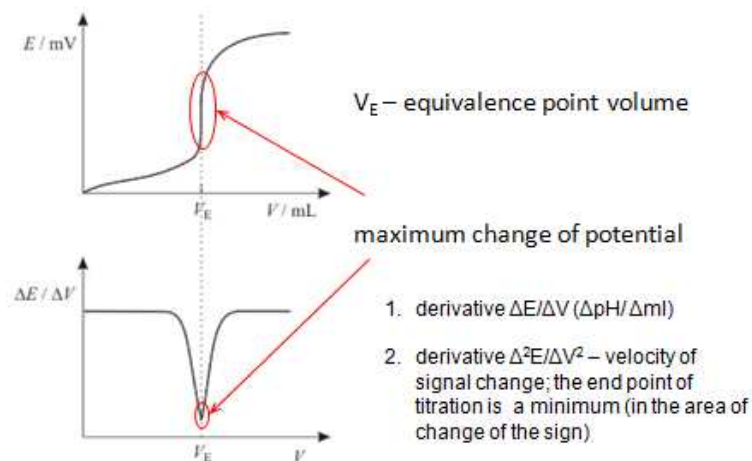
Potentiometric titration

http://www.postech.ac.kr/class/chem241/0129-1.jpg
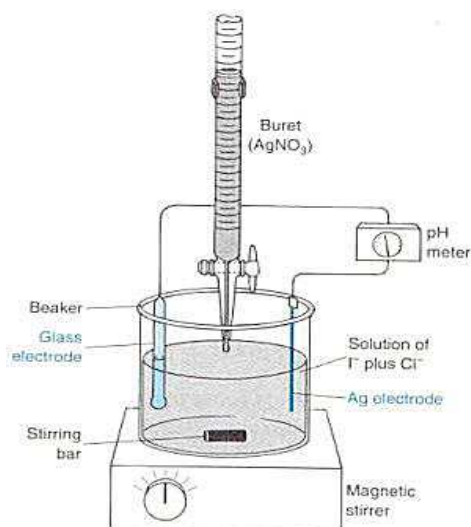
✓ potential between strandard electrode and working electrode changes suddenly as the equivalence point is reached

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✓ exact method
✓ suitable for turbid solutions
✓ relatively low selectivity (determined by the selectivity of the reaction)
✓ labor-consuming
✓ slow

Calculation of results

V_E – equivalence point volume

maximum change of potential

1. derivative $\Delta E/\Delta V$ ($\Delta pH/\Delta ml$)

2. derivative $\Delta^2E/\Delta V^2$ – velocity of signal change; the end point of titration is a minimum (in the area of change of the sign)

Conductivity of a solution depends on the ions that are present in it. During many titrations, the conductivity changes significantly.

Isothermal titration calorimeter uses the heat produced or consumed by the reaction to determine the endpoint.

Thermometric - heat of the reaction is measured and the end point is determined by the rate of temperature change.
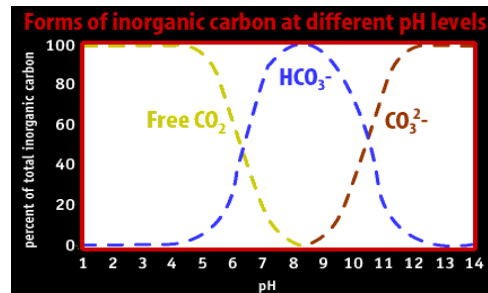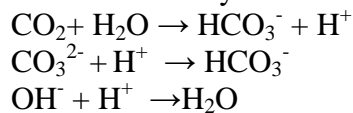
2.2.1   Applications for environmental analysis

o Acid-base titration - is based on the neutralization reaction between the analyte and an acidic or basic titrant.
   Applicable for the determination of alkallinity, that is the capacity of water sample to accept **H^+** ions

Alkalinity of water is its acid-neutralizing capacity. It is the sum of all the titratable bases. The measured value may vary significantly with the end-point pH used. Alkalinity is a measure of an aggregate property of water and can be interpreted in terms of specific substances only when the chemical composition of the sample is known.

Alkalinity is significant in many uses and treatments of natural waters and wastewaters. Because the alkalinity of many surface waters is primarily a function of carbonate, bicarbonate, and hydroxide content, it is taken as an indication of the concentration of these constituents. The measured values also may include contributions from borates, phosphates, silicates or other bases if these are present. Alkalinity in excess alkaline earth metal concentrations is significant in determining the suitability of a water for irrigation. Alkalinity measurements are used in the interpretation and control of water and wastewater treatment processes. Raw domestic wastewater has an alkalinity less than, or only slightly greater that, that of the water supply!

Alkalinity serves as a pH buffer and reservoir for inorganic carbon. Basic species responsible for alkalinity :

$$CO_2 + H_2O \rightarrow HCO_3^- + H^+$$
$$CO_3^{2-} + H^+ \rightarrow HCO_3^-$$
$$OH^- + H^+ \rightarrow H_2O$$

Forms of inorganic carbon at different pH levels

Alkalinity is titrated with different indicators:
- phenolphthalein – up to pH 8,3
- general – up to pH 4,3 (methylorange).

Alkalinity units

mol/L, meqv/L, eqv/L , mg/L $CaCO_3$, mg/L $Ca(HCO_3)_2$

If 0,005 mol H^+ is aded to change 1 L of sample to lower pH to 4,3 then alkalinity is 0,005 mol/L (0,005 eqv/L)

HCO_3^- solution: 1 mmol/L corresponds to 1 meqvL
                1M   1 eqv/L

CO_3^{2-} solution: 1 mmol/L corresponds to 2 meqv/L
                2 M   2 eqv/L

How many grams of $CaCO_3$ should be dissolved in water to get appropriate alkalinity?

$M(CaCO_3)$=100 g/mol, therefore we have 100 g of substance in 1 L of 1M solution and alkalinity is 1 mol/L or 2 eqv/L
What about mg/l $Ca(HCO_3)_2$? $M(Ca(HCO_3)_2)$=61 g/mol
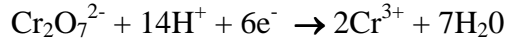
- o Precipitation titration
  For example for the determination of chlorides: $Cl^- + Ag^+ \rightarrow AgCl$ (s)
 (titrant $AgNO_3$)

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o Redox titration

Determination of COD, the indirect measurements of the amount of organic compounds in water. During analysis the amount of oxygen needed to reduce the organic matter present in a water sample is determined with chemical methods.

COD is expressed in milligrams per liter (mg/L), which indicates the mass of oxygen consumed per liter of solution
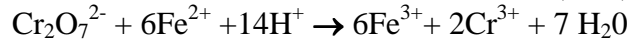
organic substances + oxidant $\rightarrow CO_2 + H_2O$

$$Cr_2O_7^{2-} + 14H^+ + 6e^- \rightarrow 2Cr^{3+} + 7H_2O$$

$HgSO_4$ is used for the precipitation of chlorides, to avoid overevaluation of COD

$$Cr_2O_7^{2-} + 6Cl^- + 14H^+ \rightarrow 3Cl_2 + 2Cr^{3+} + 7H_2O$$
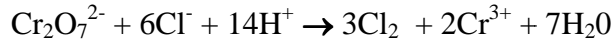
The rest of $K_2Cr_2O_7$ is back-titrated with Mohr's salt $(NH_4)_2Fe(SO_4)_2$

$$Cr_2O_7^{2-} + 6Fe^{2+} + 14H^+ \rightarrow 6Fe^{3+} + 2Cr^{3+} + 7 H_2O$$
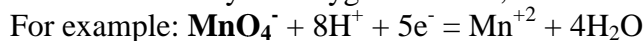　　　　light- green　　　　　　　　　brown

and the amount of used $K_2Cr_2O_7$, is calculated as a amount of oxygen (mg/L).

o Permanganometric titration

The content of $C_2O_4^{2-}$, NO_2^-, H_2O_2 and other reducing agents in natural waters are determined by the oxygen demand,

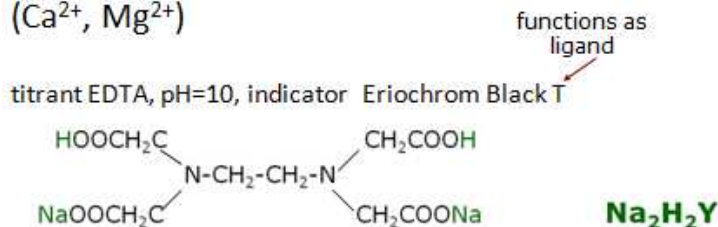For example: $\mathbf{MnO_4^-} + 8H^+ + 5e^- = Mn^{+2} + 4H_2O$

o Complexometric titration
  • In theory, any **complexation reaction** can be used as a volumetric technique provided that:
  • the reaction reaches equilibrium rapidly after each portion of titrant is added.
  • interfering situations do not arise. For instance, the stepwise formation of several different complexes of the metal ion with the titrant, resulting in the presence of more than one complex in solution during the titration process.
  • a complexometric indicator capable of locating equivalence point with fair accuracy is available.
  • In practice, the use of **EDTA** as a titrant is well established.

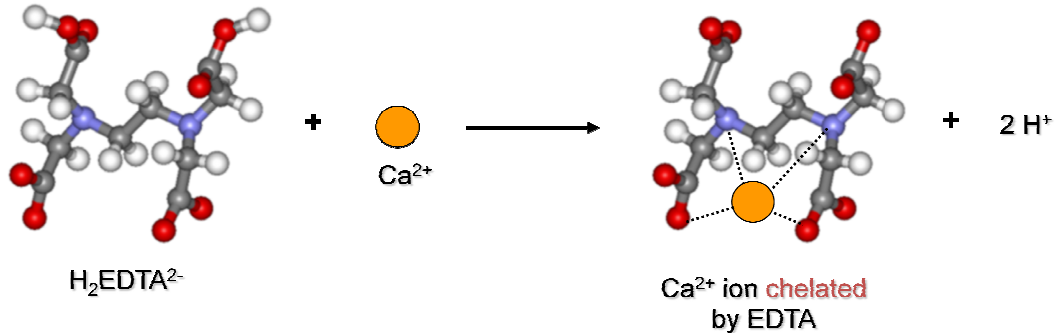**Determination of water hardness**

(Ca²⁺, Mg²⁺)

functions as ligand

titrant EDTA, pH=10, indicator Eriochrom Black T

HOOCH₂C　　　　　　CH₂COOH
　　　＼N-CH₂-CH₂-N／
NaOOCH₂C　　　　　　CH₂COONa　　　**Na₂H₂Y**

$Ca^{2+} + H_2Y^{2-} \rightarrow CaY^{2-} + 2H^+$

Mg²⁺　　　　　　MgY²⁻

metal complex with indicator should be weaker than metal complex with EDTA

Indicator Eriochrom Black T:

$$Me^{2+} + HInd^{2-} \leftrightarrow MeInd^- + H^+$$

$$MeInd^- + H_2Y^{2-} \leftrightarrow MeY^{2-} + HInd^{2-} + H^+$$

H_2EDTA^{2-}   +   Ca^{2+}   →   Ca^{2+} ion chelated by EDTA   +   2 H^+
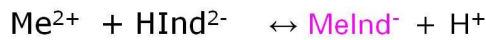
Color of indicator changes as soon as all of the metal (Me) is involved in a stronger, EDTA complex and we can determine the concentration of metal (Ca^{2+}, Mg^{2+}).

Also, water hardness is determined with complexometric titration.

2.3 Gravimetric analysis

Gravimetric analysis is suitable for relatively larger concentrations of analyte in the sample. Method has low requirements on apparatus and proovides relatively quick response.

Procedure: **WEIGHING**

Precipitation
Precipitation is the formation of a solid in a solution during a chemical reaction.

PRECIPITATE   filtering
   washing
                        drying
                        weighing

Properties of the precipitate: relatively insoluble
             readily filterable
             stable
             "pure" and known chemical composition
             nonhygroscopic.

Requirements for precipitate and precipitation reagent

Solution   Supernate
Suspension   Precipitate

- estimated, certain chemical composition
- selectivity of precipitation reagent
- precipitate washable and filterable
   big pure cristals
- insolubility of precipitate
   total precipitation – there are 1/1000 of analyte
   in solution
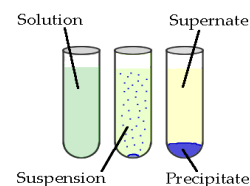
- stable precipitate
  for example, reaction with O_2 may unstabilize
  precipitate

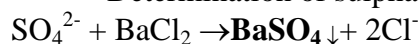Two processes for should occur the formation of a stable precipitate:
  -formation of precipitation centers, e.g. nucleation
  -growth of precipitate particles
To increase the size of precipitate particles:
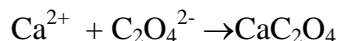 - high concentration of precipitate
  - low concentration of precipitating reagent, slow addition
  - pH adjusting.
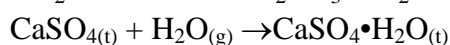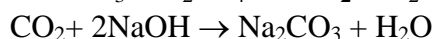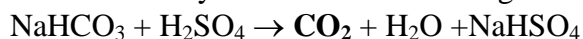
Applications
  ✓ Determination of sulphate
$SO_4^{2-} + BaCl_2 \rightarrow \mathbf{BaSO_4} \downarrow + 2Cl^-$
  ✓ Determination of calcium
$Ca^{2+} + C_2O_4^{2-} \rightarrow CaC_2O_4$

Product is filtered, dryied and weighed:
$CaC_2O_4 \rightarrow \mathbf{CaO} + CO + CO_2$

  ✓ Gravimetric vapour diffusion/evaporation method
 Direct - volatile analyte is collected and weighed
  $NaHCO_3 + H_2SO_4 \rightarrow \mathbf{CO_2} + H_2O + NaHSO_4$
  $CO_2 + 2NaOH \rightarrow Na_2CO_3 + H_2O$
  $CaSO_{4(t)} + H_2O_{(g)} \rightarrow CaSO_4 \bullet H_2O_{(t)}$
 Indirect –volatile part is calculated by the weighed mass of solid sample

Co-precipitation is mostly undesirable phenomenon, when soluble substances
precipitate with analyte.
There are three main mechanisms of co-precipitation:
  - inclusion - formation of impure crystals, e.g crystallographic efect
  - adsorbtion - adsorbtion on the surface
  - occlusion - adsorbed impurity gets physically trapped inside the crystal
    during growing process.

3 Instrumental analysis. Spectroscopy

Spectroscopy can be calssified vased on for example **interaction**:
  ✓ Radiation spectroscopy – interaction between radiation and matter as a
    function of wavelength
  ✓ Mass-spectroscopy – interaction of charged species with magnetic and/or
    electric fields, giving rise to a mass spectrum
And according to **object**:
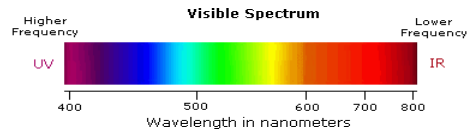 **Molecule spectroscopy**
  Atomic spectroscopy

Methods and appropriate wavelengths of radiation spectroscopy:
- ✓ X-ray spectroscopy   0,01-10 nm
- ✓ UV-Vis spectroscopy (10-) 180-800 nm
- ✓ Near-Infrared (NIR)   800-2500 nm
- ✓ Infrared (IR)   800 nm- 300 μm
- ✓ Radiospectroscopy   startin a few cm.

3.1 UV-Vis spectroscopy
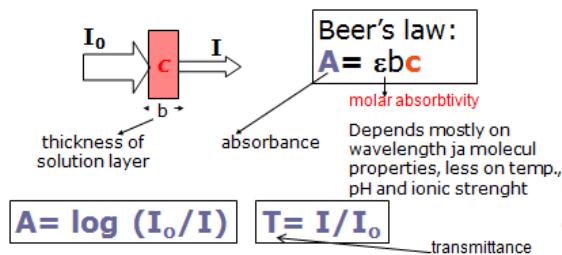
Molecular Absorptsion Spectroscopy

- • We measure the amount of ultraviolet or visible radiation absorbed by molecules
- • By the peak, e.g the magnitude of molar absortivity concentration is determined, shape of spectrum can be used for identification, in principle

UV radiation   100...400 nm
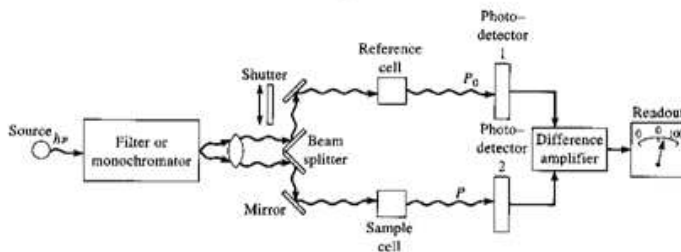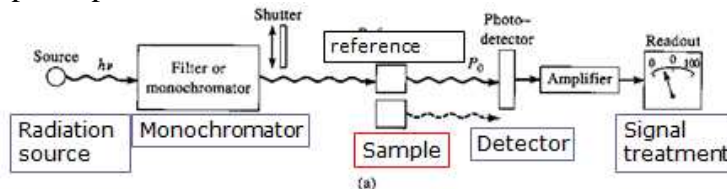Visible light   400...800 nm
Near-Infrared 800...2500 nm

□ absorbance of a solution/sample on a certain wavelength is proportional to concentration of analyte

Beer's law:
$$A = \varepsilon bc$$

molar absorbtivity

Depends mostly on wavelength ja molecul properties, less on temp., pH and ionic strenght

thickness of solution layer

absorbance

$$A = \log (I_0/I) \qquad T = I/I_0$$

transmittance

Spectrophotometers:

Applications
- ✓ Quantitative analysis
- ✓ Broad area of applications
  - o Analysis of absorbing substances
  - o Analytes/substances that absorb radiation after a certain reaction
  - o Calibration with standard solutions

✓ Suitable filter, wavelength
✓ Choise of cuvette: material, size
✓ Adsorption may depend on: solvent choise, temperature, reaction time, other ingredients of sample
✓ Limit of detection 10^{-3} .. 10^{-5} M

o **Determination the content of orthophosphates (PO_4^{3-})**

Phosphorus has been identified as a prime nutrient needed for algae growth in inland environments. Eutrophication caused by the overabundance of nutrients in water can result in a variety of water-quality problems, including fish kills, noxious tastes and odors, clogged pipelines, and restricted recreation. In freshwater, phosphorus is often the nutrient responsible for accelerated eutrophication. Many algae blooms in rivers and lakes are attributed to elevated phosphorus concentrations resulting from human activities. Phosphorus enters surface waters from agricultural and urban runoff as well as from industrial and municipal wastewater treatment plant effluent.

Quantification of phosphorous requires the conversion of the phosphorus to dissolved orthophosphate followed by colorimetric determination of dissolved orthophosphate. The analysis of different phosphorous forms (e.g. particulate or organic-P) is obtained by various pretreatment steps. Pretreatment may consist of filtering to remove suspended matter or various digestion techniques designed to oxidize organic matter.

Phosphorus can be present in surface waters as organic phosphorus, orthophosphate (an inorganic form of PO_4), or as condensed (solid) phosphates. The phosphorus may be in solution or as a component of suspended particulates. The wet chemical colorimetric analysis of phosphorus only works for orthophosphates and thus other forms of phosphorus must be converted to this form if they are to be analyzed. Organic phosphorus can be oxidized (digested) using perchloric acid, nitric acid-sulfuric acid, or persulfate with the persulfate technique being the safest and least time consuming. The digestion methods are detailed in APHA method 4500-P B.[1]

Three techniques for colorimetric analysis of phosphorus are available. The technique most commonly used is the ascorbic acid method, which can determine concentrations of orthophosphate in most waters and wastewater in the range from 2-200 μg P/L. Ammonium molybdate and antimony potassium tartrate react in an acid medium with dilute solutions of orthophosphate-phosphorus to form an intensely colored antimony-phospho-molybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid measured colorimetrically at 850 nm. The color is proportional to the phosphorus concentration. The complex is not stable and thus analysis must be performed within 30 minutes of adding the ammonium molybdate and antimony potassium tartrate.

Experimental set-up

Add to sample: solution of ammonium molybdate $(NH_4)_6Mo_7O_{24} \times 4H_2O$

potassium antimonyl tartrate $K(SbO)C_4H_6O_6$
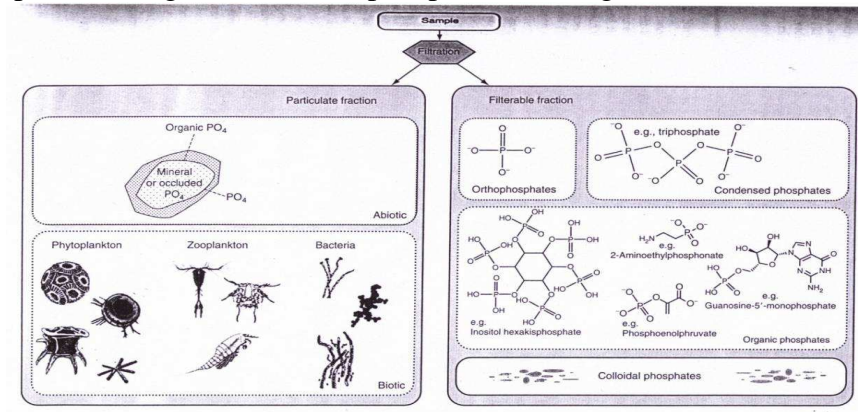
ascorbic acid

PO_4^{3-} → **$H_3[P(Mo_3O_{13})_4]$**

+ ascorbic acid → **molybdenum blue**
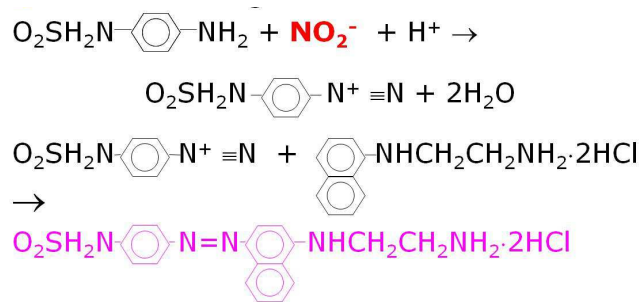
The measurements are performed at wavelenght 880 nm, standard solutions prepared from KH_2PO_4

Possible disturbing factors
In acidic environments may dissolved phosphor organic compounds as well colloidal phosphorous degrade into orthophosphates causing the overestimation of the result.


- o **Determination of total phosphorus**
  $K_2S_2O_8$ acidic solution is added, boiled and PO_4^{3-} ions are determined.

- o **Determination of ammonium nitrogen**
  - Nesslerization (20μg/l-5mg/l NH_4-N)
    $2HgI_4^{2-} + 2NH_3 \rightarrow NH_2Hg_2I_3 + NH_4I + 4I^-$
    in basic environment , λ=425 nm
  - Phenate method (10μg/l - 5mg/l NH_4-N)
    $C_6H_5OH + NH_3 + ClO^- + 2OH^- \rightarrow$ benzoquinone chloramine $\rightarrow$
    indophenol (intensely blue compound)
    λ=640 nm

- o **Nitrites**
  - Griss reaction for organic nitrite

    O_2SH_2N—◯—NH_2 + **NO_2^-** + H^+ $\rightarrow$

    O_2SH_2N—◯—$N^+ \equiv N$ + $2H_2O$

    O_2SH_2N—◯—$N^+ \equiv N$ + ◯◯—$NHCH_2CH_2NH_2 \cdot 2HCl$
    $\rightarrow$
    O_2SH_2N—◯—$N=N$—◯◯—$NHCH_2CH_2NH_2 \cdot 2HCl$

    λ=540 nm

- o **Nitrates**
  - Salicylate method, λ=415 nm
    Reaction product between sodium salicylate and sulfuric acid is sulfosalicylic acid, that in alkaline environment after reaction with nitrate ions gives a yellow product. The intensity of the yellow color is measured spectrophotometrically in 415 nm. To avoid the precipitation of Ca and Mg solution of EDTA-Na_2 is added to system. If sample contains a lot of nitrite (NO_2^-) ions, sulfamic acid is added before analysis.

- By Cd reduction method where NO_3^- is reduced to NO_2^- (see photo) and NO_2^- ion is determined spectrophotometrically
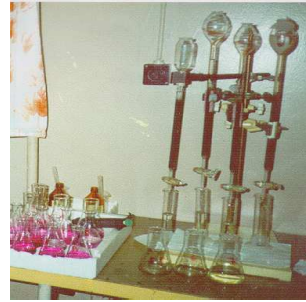
o **Kjeldahl nitrogen**

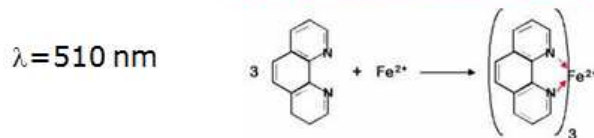- Boiling in the presence of H_2SO_4
  Org. substance (C, H, N) $\rightarrow$ NH_4^+ + CO_2 + H_2O
- NH_4^+ + OH^- $\rightarrow$ NH_3 + H_2O
- NH_3 distillation into HCl standardsolution
  NH_3 + H^+ $\rightarrow$ NH_4^+
- Back-titration of HCl
  H^+ + OH^- $\rightarrow$ H_2O

Fullautomatic Kjeldahl
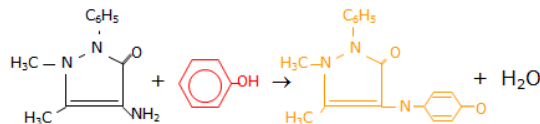
o **Spectrophotometric determination of iron**

- Phenanthroline method (0,02-2 mg/L)
  Sample + HCl, boiling $\rightarrow$ Fe^{3+}
  Add HONH$_3$Cl $\rightarrow$ Fe^{2+}
  Add o- phenanthroline (pH=3) $\rightarrow$
  iron- phenanthroline complex

λ=510 nm

o **Determination of phenols**

- Phenol, phenolic compounds
  - Distillation
  - pH=10 (pH=8)
    4-aminoantipyrin
    $K_4Fe(CN)_6$
    λ=510 nm

3.2 IR spectroscopy

Identification and quantification of organic compounds
✓ Infrared radiation, absorbed by compound is measured
         Absorption of molecules
✓ Intensity of absorbance
IR spectrum 4000...400 cm^{-1}

3.3 Atomic spectroscopy

Metal analysis

Atomic spectroscopy:
- gives analytical data about the identification and concentration of atoms
- All the methods below determine elements.
  Compounds can be identified according to the determined atoms.

✓ Atomic absorptionspectroscopy **AAS**
  is a technique for determining the concentration of a particular metal element in a sample by absorption intensity.

✓ Atomic emission spectroscopy **AES**
  is a method of chemical analysis that uses the intensity of light emitted from a flame, plasma, arc, or spark at a particular wavelength to determine the quantity of an element in a sample.

3.4 Atomic absorptionspectroscopy (AAS)

The atoms of each element absorb radiation at a specific wavelength - atomic absorption spectroscopy
Befroe AAS, sample must be atomized in the flame, graphite cuvette or with cold vapor.
Atomic absorptionspectrometer:
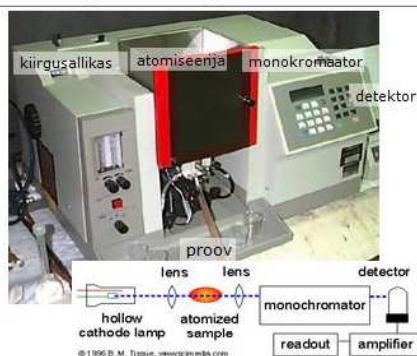
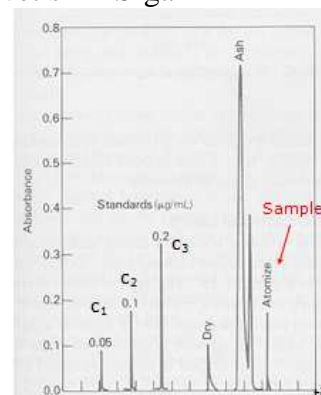

Pb analüüs grafiitküvetis AAS-ga

Analysis of Pb
graphite cuvette, AAS

Quantitative analysis
A = f (c)
   Beer's law!

2µl orange juice as a sample,
conc. of Pb was 0,1 µg/ml

Beers law can be applied for quantitative analysis although the non-linearity is relatively high and linear relationship between the response and analyte content occurs in narrow concentration range. Additionally other parameters are not always stable and it is recommended to compile the calibration plot regularly.

Disturbing effects to AAS
- ✓ Spectral disturbancies – other particles in flame absorb radiation
    Spectral lines may overlap or the absorption lines are too wide.
- ✓ Chemical – different equilibriums may occur in the flame that produces ionization of atoms or other transformations (for exapmple oxide)

Means:
variation of process parameters, spectrochemical buffers: releasing agents
protecting agents
suppressors of ionisation

AAS is used for
- ✓ analysis of metals
- ✓ minerals
- ✓ biological samples
- ✓ analysis of traces.

3.5 Atomic emission spectroscopy (AES)

Aatomid ergastatakse kõrgel temperatuuril. registreeritakse aatomite poolt emiteeritud kiirgust, lainepikkused on UV-Vis spektrialas. The atoms are excited at high temperatures and the atomic radiation is registered. The wavelengths are in UV-Vis spectrum band.

For the atomisation:

flame (1700-3200K) stable
electric arc (4000-5000 K) unstable
electrical spark (40 000 K) unstable
plasma (6000-8000 K)   stable.

4 Methods based on oxygen demand
Characterization the environmental impact of contaminants

The impact of potential contaminants can't be evaluated solely by chemical analysis. The influences of contaminants, their effects and environmental impact can't be evaluated only by their concentration data. To assess if a contaminant is a threat or toxic, we should consider at least some of the following aspects
- concentration in water
- biological availability
- hydrophobic
- stability of substances in environment.

For the analysis of environmental impact ecotoxicological studies can be implemented, as well bioaccumulation and biodegradation properties of the substance can be analysed. Also, there are to approaches:
- ✓ Study of environmental contaminants;
- ✓ Study of chemical substances.

4.1 Degradation

……. e.g stable *vs* degradable.

Biodegradation can be primary or total and compounds are classified accordingly:
- easily biodegradable
- biodegradable substances
- slowly biodegradable or stable substances.

Biodegradability is dependent on
- ✓ structure of molecules
  - ▪ Concentration
- ✓ Environment
- ✓ Microorganisms
- ✓ Time.

Evaluation of biodegradation can be implemented generally:
- ✓ by the biodegradability study of a compound,
- ✓ the prognostics of biodegradation extent of the material (mixture, sample)under study.

International standardized tests (OECD; ISO are used.

Differencies in methods:
- • aerobic or anaerobic environment
- • time period
- • amount and type of microorganisms
- • batch-type or continuous systems.

You can measure:
- ✓ Biodegradation extent, %
- ✓ Biodegradation rate.

Determination of biodegradation extent:
- - First you need to know the initial concentration of organic substances in sample
- - In the end of experiments: how much organics is degraded

How is degradation calculated?
- ✓ Through the change of oxygen concentration
- ✓ By the change of total carbon composition (TOC)

ORG. substance + $O_2 \rightarrow CO_2$ + H_2O + biomass
TOC, DOC
COD
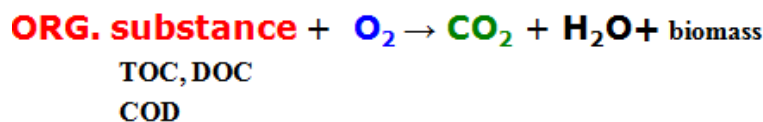
Estimation of biodegradability: DEGRADATION INDEX: $\dfrac{BHT_7}{KHT}$
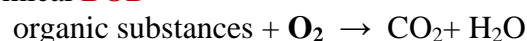
OECD criteria: $\dfrac{BHT_7}{KHT} > 0{,}43$

easily biodegradable substances
easily biodegradable wastewater

4.2 Determination of oxygen demand

Oxygen demand (OD):
- o theoretical TOD
- o chemical COD
- o biochemical BOD

organic substances + $O_2 \rightarrow CO_2 + H_2O$
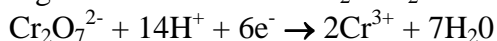
Theoretical oxygen demand

…is calculated by the chemical reaction of a compound with O_2 in the case of total degradation to CO_2 and H_2O.

Chemical oxygen demand

The determination of chemical oxygen demand (COD) is widely used in municipal and industrial laboratories to measure the overall level of organic contamination in wastewater. The contamination level is determined by measuring the equivalent amount of oxygen required to oxidize organic matter in the sample.

Most types of organic matter are oxidized by a boiling mixture of chromic and sulfuric acids. A sample is refluxed in strongly acid solution with a known excess of potassium dichromate ($K_2Cr_2O_7$). After digestion, the remaining unreduced $K_2Cr_2O_7$ is titrated with ferrous ammonium sulfate to determine the amount of $K_2Cr_2O_7$ consumed and the oxidizable matter is calculated in terms of oxygen equivalent. Keep ratios of reagent weights, volumes, and strengths constant when sample volumes other than 50 mL are used. The standard 2-h reflux time may be reduced if it has been shown that a shorter period yields the same results. Some samples with very low COD or with highly heterogeneous solids content may need to be analyzed in replicate to yield the most reliable data. Results are further enhanced by reacting a maximum quantity of dichromate, provided that some residual dichromate remains.

organics + oxidant $\rightarrow CO_2 + H_2O$

$Cr_2O_7^{2-} + 14H^+ + 6e^- \rightarrow 2Cr^{3+} + 7H_2O$

Biochemical oxygen demand (BOD$_7$)

The biochemical oxygen demand (BOD) determination is an empirical test in which standardized laboratory procedures are used to determine the relative oxygen requirements of wastewaters, effluents, and polluted waters. The test has its widest application in measuring waste loadings to treatment plants and in evaluating the BOD-removal efficiency of such treatment systems. The test measures the molecular oxygen utilized during a specified incubation period for the biochemical degradation of organic material (carbonaceous demand) and the oxygen used to oxidize inorganic material such as sulfides and ferrous iron. It also may measure the amount of oxygen used to oxidize reduced forms of nitrogen (nitrogenous demand) unless their oxidation is prevented by an inhibitor. The seeding and dilution procedures provide an estimate of the BOD at pH 6.5 to 7.5.

Carbonaceous Versus Nitrogenous BOD

A number of factors, for example, soluble versus particulate organics, settleable and floatable solids, oxidation of reduced iron and sulfur compounds, or lack of mixing may affect the accuracy and precision of BOD measurements. Presently, there is no way to include adjustments or corrections to account for the effect of these factors.

Oxidation of reduced forms of nitrogen, such as ammonia and organic nitrogen, can be mediated by microorganisms and exert nitrogenous demand. Nitrogenous demand historically has been considered an interference in the determination of BOD, as clearly evidenced by the inclusion of ammonia in the dilution water. The interference from nitrogenous demand can now be prevented by an inhibitory chemical. If an inhibiting chemical is not used, the oxygen demand measured is the sum of carbonaceous and nitrogenous demands.

Measurements that include nitrogenous demand generally are not useful for assessing the oxygen demand associated with organic material. Nitrogenous demand can be

estimated directly from ammonia nitrogen (APHA Standard Methods, Section 4500-NH₃)[1]; and carbonaceous demand can be estimated by subtracting the theoretical equivalent of the reduced nitrogen oxidation from uninhibited test results. However, this method is cumbersome and is subject to considerable error. Chemical inhibition of nitrogenous demand provides a more direct and more reliable measure of carbonaceous demand.

The extent of oxidation of nitrogenous compounds during the 7-d incubation period depends on the concentration and type of microorganisms capable of carrying out this oxidation. Such organisms usually are not present in raw or settled primary sewage in sufficient numbers to oxidize sufficient quantities of reduced nitrogen forms in the 7-d BOD test. Many biological treatment plant effluents contain sufficient numbers of nitrifying organisms to cause nitrification in BOD tests. Because oxidation of nitrogenous compounds can occur in such samples, inhibition of nitrification is recommended for samples of secondary effluent, for samples seeded with secondary effluent, and for samples of polluted waters.

Report results as carbonaceous biochemical oxygen demand when inhibiting the nitrogenous oxygen demand. When nitrification is not inhibited, report results as BOD_7.

Typical values
- ✓ Most pristine rivers will have a 5-day carbonaceous BOD below **1 mg/L**.
- ✓ Moderately polluted rivers may have a BOD value in the range of **2 to 8 mg/L**.
- ✓ Municipal sewage that is efficiently treated by a three-stage process would have a value of about **20 mg/L** or less.
- ✓ Untreated sewage varies, but averages around **600 mg/L** in Europe and as low as **200 mg/L** in the U.S., or where there is severe groundwater or surface water infiltration.
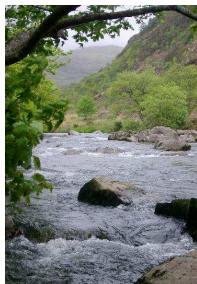
| <1 mg/L | 2-8 mg/L | <20 mg/L | 600 mg/L |

Experimental set-up

Biochemical oxygen demand analysis
Dilution method, 7 days

$$BOD_7 = C_{O_2, 1^{st} day} - C_{O_2, 7^{th} day}$$

- •Sample
- •Microorganisms
- •Dissolved oxygen
- •Minerals (N, P, Fe, Mg, Ca)
- •Nitrification indicator (ATU)

Dissolved O₂?

How fast biodegradation occurs?
- • depending on the composition of sample
- • adaption of microorganisms
- • other conditions: temperature, light, accessibility of air, oxygen, time?

Instrumental method:
- • Faster
- • Improved reproducibility due to automatization

Calculation

How much sample we need for BOD_7?

- BOD_7 analysis should be perfomed in a lab for a wastewater sample which COD is 1348 mgO_2/L. How many ml-s of sample should be initially measured into 1 L of inoculum (or dilution water) in the beginning of BOD_7 analysis considering that the OECD degradation index of the sample is 0,43?
- The result of BOD_7 analysis can be considered reliable when the oxygen concentration drops approx. 2-7 mg/l during the 7-days incubation period.
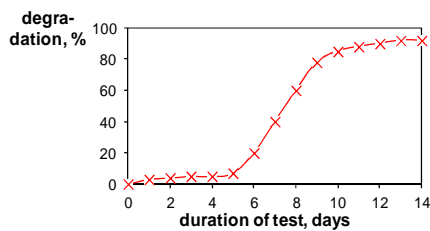
Content of organic carbon

Total carbon   **TC**
Inorganic carbon   **IC** (TIC)
Organic carbon   **TOC**
Dissolved organic carbon   **DOC**

$$\boxed{TOC = TC-IC}$$

Other methods for the determination of biodegradability

The biodegradability of different compounds and mixtures can be determined by various tests and analysis methods, for example OECD tests 301 A-E, 302 A-C and so forth.


Easily degradable:
 at least during 10 days of test beginning
   70% DOC removed
   60% degradation according to oxygen demand or CO_2 analysis

CO_2 "headspace" test – for the analysis of aerobic biodegradation of organic carbon by the measurement of inorganic carbon (ISO 14593).

The sample or chemical substance is a source of carbon and energy for microorganisms in the environment under study.
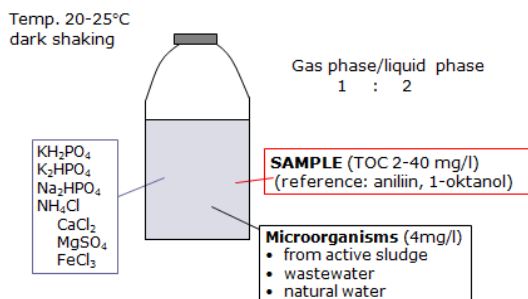
Calculation of results
If all of the substance would degrade into CO_2, then IC = TOC
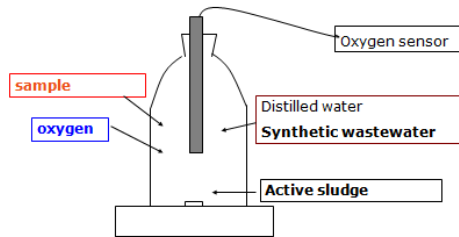**$D_t= (TIC_T-TIC_B)100/TOC$**
TIC_T mass of inorganic carbon (mg) in the sample at time t
TIC_B mass of inorganic carbon (mg) in reference sample (blind test) at time t
TOC initial organic carbon (mg)

Active sludge tests
Inhibition of oxygen demand of active sludge (ISO 8192)

the oxygen consumption of active sludge microorganisms is influenced by wastewater or other sample

Different amounts of sample
Change of oxygen concentration in time
Calculations of rate of oxygen demand

Similar measurement system is used for **inhibition of nitrification** (ISO 9509).

Anaerobic test for the evaluation of biodegradation(ISO 11734):
- anaerobic microorganisms
- sample
- duration of test 60 days.

5 Estimation of bioaccumulation

Bioaccumulation - concentration of a substance is higher in the organism compared to the concentration in environment**:**
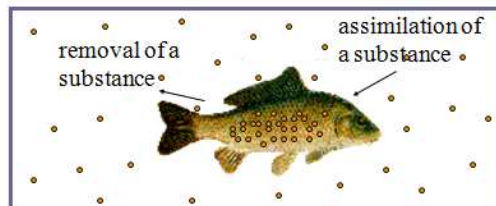
Which compounds bioaccumulate?
Bioaccumulation does not occur
- substance is biodegradable
- substance is not available to organisms
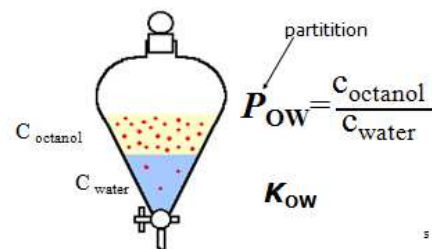- substance is easily removed from organism.

Estimation of hydrophobic compounds
...... with a two-phase system

In 1900 some scientists noticed, that the extent of bioaccumulation of organic compounds is proportional to the solubility in organic solvents

$$K_{water-solvent} = \frac{C_{solvent}}{C_{water}}$$

and the compound is divided in a two-phase system

$$P_{OW} = \frac{c_{octanol}}{c_{water}}$$

K_{OW}

5.1 Ecotoxicology

"the branch of toxicology concerned with the study of toxic effects, caused by natural or synthetic pollutants, to the constituents of ecosystems, animal (including human), vegetable and microbial, in an integral context" (Truhaut, 1977).

..... the science of poisons

Poison or toxicant is substance harmful to living organisms
- ✓ Detrimental effects on tissues, organs, biological processes
- ✓ Most are foreign to living systems (xenobiotics)
- ✓ Lipid affinity and ability to cross cell membranes
- ✓ Often metabolized to more or less toxic species
- ✓ Type of organism, amount of exposure, route of exposure.

Testorganisms: bacteria, protozoa, algae, crustazea, fish, angleworms, honeybees, active sludge

Dose - response relationships (exposure-response relationships) describes the change in effect on an organism caused by differing levels of exposure (or doses) to a stressor (usually a chemical)
- ✓ response of specific organisms to toxicant during fixed or known period of time under determined conditions
- ✓ determination of substance concentration that causes certain kind of response or has no harmful effect

Shortly, relevant aspects are:
- • a well determined result (death, growth, quantity of cells, inhibiton of enzymes)
- • well fixed time of the analyses.

Dose is the amount (per unit body mass) of toxicant to which the organism is exposed

Response is the resulting effect on an organism
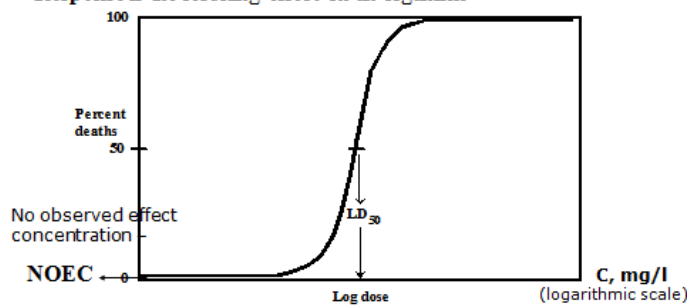
Acute toxicity

describes the adverse effects of a substance which result either from a single exposure or from multiple exposures in a short space of time (usually less than 24 hours). For example LC_{50}.

Different organisms have different sensibility on sample (contaminants, wastewater)

Toxicity rate of 100% wastewater

<20%	not toxic
20-49%	slightly toxic
50-99%	toxic
100% (1 test)	very toxic
100% (all tests)	highly toxic

Chronic toxicity
   organism is exposed to the substance continuously or repeatedly. Effects on growth, reproduction.
   NOEC – maximum concentration with no harmful effect

More experimental values
   ✓ No observed adverse effect level, NOAEL
   ✓ Lowest observed adverse effect level, LOAEL
   ✓ Maximum tolerable concentration, MTC, LC_0; Maximum tolerable dose, MTD, LD_0
   ✓ Minimum lethal concentration, LC_{max}; Minimum lethal dose, LD_{min}
   ✓ Median lethal concentration, LC_{50}; Median lethal dose, LD_{50}; Median lethal time, LT_{50}
   ✓ Absolute lethal concentration, LC_{100}; Absolute lethal dose, LD_{100}.

## 5.2 Ecotoxicological tests

Microtox^{TM} , Biotox^{TM}
…….change in luminescence of bacteria *Vibrio fischeri* is measured

Microtox test analysis the inhibition of bioluminescence of bacteria *Vibrio fischeri (formerly known as Photobacterium phosphoreum)*, thereby the intensity of bioluminescense decreases after exposure to toxic substances.
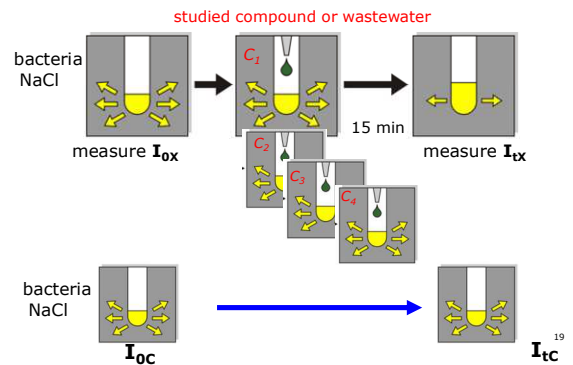Test is suitable for quick estimation of toxicity of chemicals, wastewater and sediments.

MICROTOX -time

Different chemicals affect the organisms different rates, producing distinctly different Light Level-Time Response curves
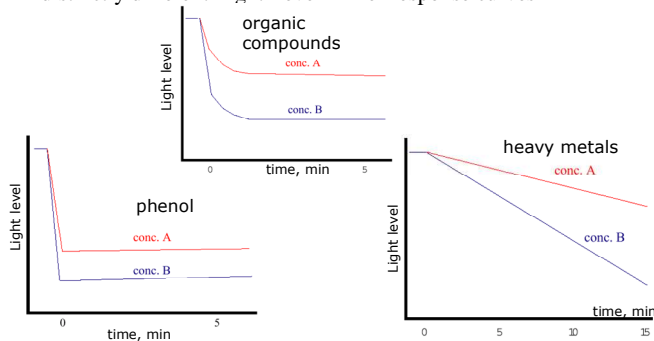
Microtox test – procedure

Calculation of results
Reference sample: $BR = I_{tc}/I_{0c}$
   • decrease of luminescence light level, %
   • decrease of residual luminescence
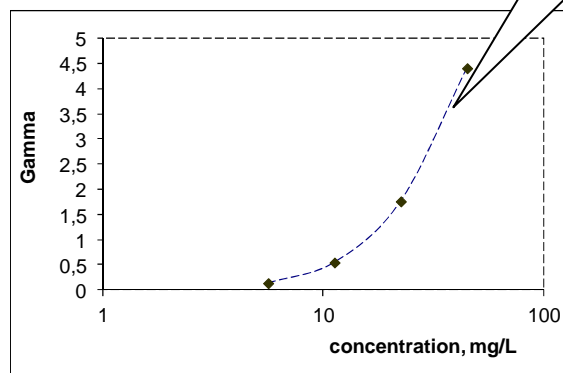
$$\Gamma = \frac{(I_{0X} \cdot BR) - I_{tX}}{I_{tX}},$$

EC$_{50}$ - half maximal effective concentartion, the concentration of a compound where 50% of its maximal effect is observed
IC$_{50}$ - measure of a compound's inhibition (50% inhibition)

Control of results

Testing a standard (reference toxicant)

- $ZnSO_4 \times 7H_2O$

EC_{50} (15 min) 6 ... 9 mg/l

- Phenol

EC_{50} (5 min) 13 ...26 mg/l

Nonlinear relationship

If the gammas are all larger than 1.0 for EC_{50}, retest the sample using a primary dilution testing lower concentrations.

Gammas less than 1.0 for EC_{50}, retest using a more concentrated sample.

DAPHTOKIT F[TM]

…..immobilizing chrustacea *Daphnia pulex*

Acute toxicity of *chrustacea*

Determination of mobility of freshwater microchrustacean *Daphnia Magna Straus* (ISO 6341)

Applicable for

- ✓ Water-soluble compounds
- ✓ wastewaters
- ✓ Soil leachate.

Experimental set-up

Principle: determination of sample (wastewater, contaminant) concentration that causes immobilisation of 50% of chrustacea during 24(48) hours.

- Every sample has equal number of
  Daphnia Magna Straus
- After 24 or 48 hours immobilised exemplars are counted
- Immobilisation in reference samples < 10%
- Reference toxicant $K_2Cr_2O_7$

$EC_{50} = 0{,}9 ... 2{,}0$ mg/l

EC_{50}

Lowest conc. that immobilises all
Highest conc. that immobilises none

Acute toxicity of *Pisches* (latin)

…determination of toxicity with freswater fishes *Brachydanio rerio* (zebra fish) (ISO 7346/2), the Zebra fish test.

Lethal dose of contaminant concentration during time/ testing periood (24, 48, 72 and 96 hours) is determined.

Testorganisms:
Brachydanio rerio
- 30± 5 mm
- cultivated under special conditions
- Conditions: 23 ±1°C, light, length of day 12-16 h
- Every 24 hours dead fishes are counted and living fished are moved to new solutions
- Any changes in the behavior of fishes are determined
- Reference toxicant : $K_2Cr_2O_7$
    24 h LC_{50}= 200 ... 400 mg/l

Lemna minor test
.........measures the ihibition of growth rate of plants.

**Toxicity tests with *Lemna*, Common Duckweed or Lesser Duckweed**
is a species of Lemna (duckweed) with a subcosmopolitan distribution, native throughout most of Africa, Asia, Europe and North America, occurring everywhere that freshwater ponds and slow-moving streams occur.
Lemna test
The growth of certain number of duckweeds in culture medium and research sample is observed

How to measure growth?
- by counting the number of leaves
- with the determination of biomass
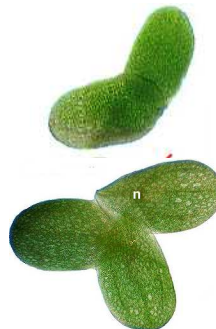
Calculation of results

$$I(\%) = \frac{(N_0 - N_t) \cdot 100}{N_0}$$

Chronic toxicity tests with microchrustacea
Keywords:
- ✓ *Dapnia magna Straus*
- ✓ 21 days
- ✓ Change of water-environment 3 times a week
- ✓ Feeding with green alga
- ✓ day/night   16:8 h, t= 18-22°C

Mortality, reproduction (No of descendants)
- ✓ LC50, EC50, NOEC.

6 Videos

Short study-videos to be discussed on the e-course and optionally during lectures:
1) collection of soil sample   http://uttv.ee/naita?id=6847
2) determination of BOD_7 http://uttv.ee/naita?id=6846
3) COD analysis of wastewater http://uttv.ee/naita?id=6844.

7 Literature

1. Standard Methods for the Examination of Water and Wastewater, APHA, AWWA, WPCF, 1985
2. R. N. Reeve, Environmental Analysis. John Wiley & Sons, England, 1994.