

## Practice No. 9

### Diagnosis of fish poisoning

Every time you must do! :

- examination of the place of poisoning
- examination of water
- biological test of water toxicity
- examination of dead fish

Also you can do, but it is not obligatory:

- Hydrobiological examination
- Sediment examination (in pesticide, PAH, metal contamination)
- Examination of food, if it is a fishing pond and we feed the fish

### Examination of the place of poisoning

- Important!!!! – clinical signs in damaged fish – especially colour of gills and mucus layer structure (it changes quickly on the air) and changes of behaviour; possibly but not obligatory also dissection of fish right on the place
- Temperature and concentration of oxygen in water – must be measured immediately and in the place – we cannot do it lately in lab!
- Draw a plan of place – factories nearby, estuaries, tributaries, houses or villages (towns), locations of sample withdrawal

### Water examination

- colour
- turbidity (opacity, transparency for light)
- odour
- temperature (important in ammonia intoxication)
- content of nitrates and nitrites
- pH - optimum lies between 6,5 and 8,5  
Salmonidae - death - lower than 4,2 and higher than 9,2  
Cyprinidae - death - lower than 5,0 and higher than 10,8
- Alcalinity = acid neutralisation capacity (ANC) – how much of strong acid you need to change pH to certain pH value (usually to 4,5). It depends on an ability of  $\text{H}_2\text{CO}_3$  -  $\text{HCO}_3^-$  buffer system to work. Optimum is up to 3,0 mmol/l
- Oxygen – in mg/l or % - we measure it with oximeter IN THE PLACE of poisoning!  
Salmonids - need between 8-12 mg/l of oxygen in water  
Cyprinids – need between 6 - 8 mg/l of oxygen in water

- Ammonia
  - It is an assessment of whole ammonia in water including both  $\text{NH}_3$  and  $\text{NH}_4^+$
  - According to a table in which temperature and pH are involved, we count the amount of toxic form  $\text{NH}_3$
  - Lethal dose is 0,5 mg/l for Salmonids and 1,0 mg/l for Cyprinids
- Chemical oxygen demand
  - Assessed by  $\text{MnO}_4^-$  or  $\text{Cr}_2\text{O}_7^-$
  - Non-direct method of assessing oxygen in water
  - We detect the value of organic compounds in water - these are reduced by above mentioned ions and for this reduction they need oxygen which they take/spend from water
  - Measures both substances biologically degradable and non-degradable
  - Optimum is less than 10 mg/l in Salmonids and less than 20 – 30 mg/l in Cyprinids
- Biochemical demand of oxygen after 5 days
  - Only biologically degradable organic substances are measured
  - Oxygen amount is assessed in time 0, then we put the water sample into thermoregulator for five days keeping it at dark in  $20^\circ\text{C}$
  - After that we measure oxygen concentration again
  - The concentration of used/spent oxygen shouldn't be more than 5 mg/l in Salmonids and more than 8-15 mg/l in Cyprinids
  - Biochemical demand is always lower than chemical demand

### **Biological test of toxicity**

- On *Daphnia magna* and on aquarium fish – *Poecilia reticulata*, *Danio rerio*
- Ten pieces of *Daphnia* and fish into examined water for 24 hours – if they survive, there is not a toxic agent in harmful concentration
- Warning! - *Daphnias* are quite insensitive to increased amounts of ammonia and increased pH and to oxygen depletion
- Also when manipulating with a sample, we change the amount of oxygen in it, so it tells us nothing about the lack of oxygen as a source of poisoning

### **Fish examination**

- we make a section and look for specific signs to exclude parasitic or infectious origin of the death, and to check gastric content and spleen size

### **Protocol about fish poisoning**

- Date, when it was found out
- All present people, including their addresses and phones
- Locality
- Owner if it is a pond
- Length or area of affected place
- Kinds, age and numbers of dead fishes
- Behaviour and clinical signs in living fish
- Possible sources of contamination

- Samples – what was taken, how much, where it was sent
- Measurements in place – temperature and oxygen !!!
- Conclusion of present people what kind of toxin could be a source of poisoning
- Plan/map of the place
- Signatures of all present people

More info: <http://www.epa.qld.gov.au/publications?id=366>

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Practical work: Complete analysis of different water samples  
Biological test of toxicity on *Daphnia magna*