ENVIRONMENTAL TOXICOLOGY PRACTICE

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Wrocław, 2019

Laboratory 1

AIM: Evaluating toxicity of selected toxicants on water organisms: the performance of lethal tests with the use of indicator species and assessment the dependence on type, concentration and time of disinfectant activity.
The aim of this exercise is to assess the sensitivity of tested organisms to studied/ selected toxicants.

Exercise 1.

Materials:
Four/ five different types of toxicants, few species of tested organisms: *Tubifex tubifex*, *Chironomus* sp. larvas, *Chaoborus* sp larvas and *Daphnia magna, Artemia salina*.
The influence of selected toxicant on the mortality of tested organisms will be studied.

Procedure:

- Prepare half- solutions of toxicants which as we assume the initial concentration as 100%. It is necessary to prepare at least 5 solutions from each of the selected toxicants (at least 100 ml per sample).

Remarks: 100-120 ml per 1 set of experiment (with 3 types of organisms without replicates).

- Chose 10 specimens from each tested organism and introduce them **at the same time** into 6 plates.
- Remove excess water from the plates with a Pasteur pipette and add prepared dilutions of disinfectants (5 ml) (3 replicates for each dilution)
- Prepare the control (water) (3 replicates)
- Count the number of dead and alive specimens after 10, 20, 30, 40 minutes in all samples.

IF the 10% of the studied specimens in control samples will die you have to repeat the experiment

Results:
Place obtained results in table form. Draw a chart of dependence of mortality on concentration and time exposure for each disinfectant. Calculate LC_{50} with REED method and logit method. Calculate LOEC, NOEC, MATC (**maximum acceptable toxicant concentration)** MATC=$\sqrt{(NOEC)(LOEC)}$.

Toxicants:

$CuSO_4$

Initial concentration: 10 g/l

$(CH3COO)_2Pb$

Initial concentration: 10g/l

Deltrametrin (Decis)

Initial concentration 10%

Glyfosat (Roundup)

Initial concentration 40%

Ecotoxicology - laboratory 2

The application of bioindicators for the assessment of water quality

Exercise 1
Growth test assessing the influence of various chemicals on the producer *Lemna minor*

Lemna minor is a plant. Because of its small size and easy growth it is commonly used in toxicology. The influence of a toxicant on *Lemna* sp. is assessed on the basis of its physiological condition (biomass, chlorophyll amount), its morphology, size, leaf shape, their color (chlorosis), root length.

Materials:
Water for dilution, chemicals, *Lemna minor*, nutrient for *Lemna*, pipettes, loops, measuring cylinder, jars.

Prepare half-concentrations of the study compounds using water for dilution in order to obtain final concentrations after introducing culture medium.

The test should be done in 5 concentrations and in 3 replicates (5 ml) for each concentration (+ control sample). Introduce ten plants (two fronds and least) from the maternal culture into the nutrient, weigh them. Then samples should be kept under the same conditions as the maternal culture. Incubate for 7 days; mix the culture once a day.

Results:
After 7 days: observe the morphological changes of plants, determine their weight. Calculate EC50. The results should be put in a table with conclusions drawn.

Exercise 2
Growth test assessing the influence of various chemicals on algae representatives: *Chlorella* sp. or *Scenedesmus*.

Materials:
Liquid maternal culture of algae: *Chlorella* or *Scenedesmus*, chemicals, nutrient for algae, pipettes, measuring cylinders, slides, filter paper, Fuchs-Rosenthal counting chamber.

Procedure:
Prepare the half-concentrations of studied compounds using water for dilution in order to obtain final concentrations after introducing the culture medium.

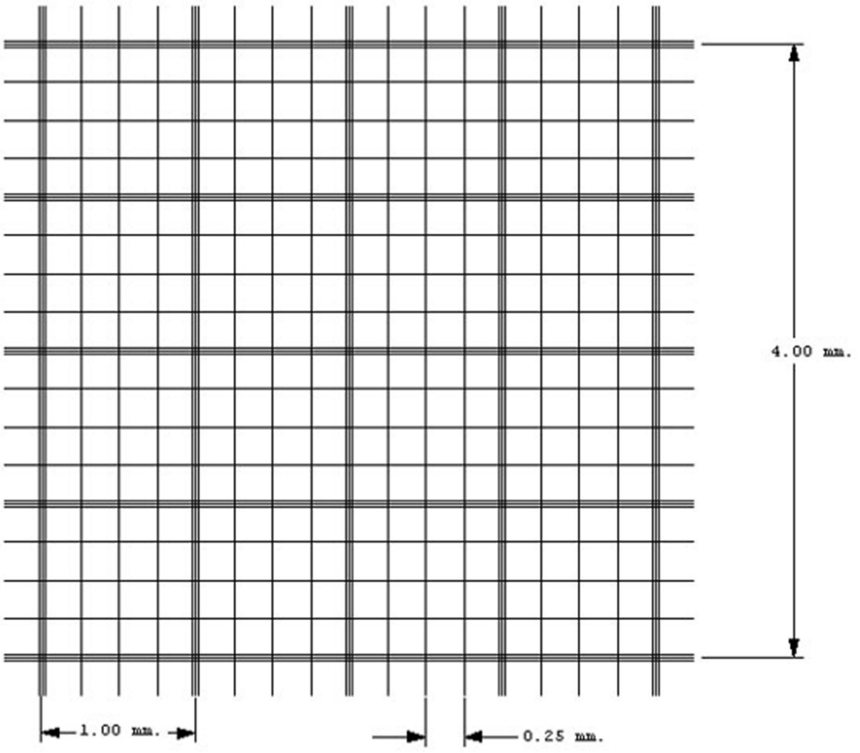
The test should be done in 5 concentrations (2ml) and with 3 replicates for each concentration (+control sample). Add 0,5 ml of algal suspension to each concentration.
The prepared samples should be kept under the lighting of 2500 lux and a temperature of 20°C. Incubate them for 7 days. After 7 days, determine the number of algae in cultures. The results should be put in a table with conclusions. Calculate LC50

Calculating the number of algae in the Fuschs-Rosenthal counting chamber
The chambers are ruled with the Fuchs-Rosenthal pattern. This consists of 16 one square millimeter areas orientated by triple lines and each area is sub-divided into 16 squares. It is generally recommended to count randomly 16 one square millimeter areas preferably 8 in each chamber. Each sample should be diluted and transferred with the use of a pipette into a chamber which has a 3.2 mm^3 volume.

Results:
The number of algae in 1ml of suspension should be calculated using the following formula:

$$X = (a * 2)* 1000 /3.2*R$$

where:
x – number of cells/ ml,
a – the number of cells counted in 8 squares,
500 – conversion factor which enables a value into the volume of
0.5 cm^3 (0.5 ml),
3.2 mm^3 – internal volume of the counting chamber,
R – dilution.



Toxicants:

$CuSO_4$

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Deltrametrin (Decis)

Initial concentration 10%

Glyfosat (Roundup)

Initial concentration 40%

Ecotoxicology - laboratory 3 and 4

The application of bioindicators for the assessment of soil and waste quality

Exercise 1
Physiological test assessing the influence of toxicants on the enzymatic activity of soil bacteria. The aim is to become familiar with the influence of toxicants on the dehydrogenase activity of bacteria.

Materials:
- Control soil – 50 g per section
- Contaminated soil – 50 g per section
- Plastic containers
- Glass test tubes (20 ml)- 6 per section
- Buffer Tris, c = 0,1 mole/l (dilute 12,11 g of Tris in 600 ml of water and lower pH into 7,6 (HCl) then fill in water (up to 1000 ml))
- 1 % TTC in buffer TRIS).
- Triphenyloformazane (TF) for standard curve: final concentration 5 mg in 50 ml of acetone.
- Standard curve: prepare 5 dilutions of TF in acetone in volume 10 ml: 6.3 µg/ml.
- 12.5 µg/ml , 25 µg/ml, 50 µg/ml, 100 µg/ml,
- Methanol or acetone – *60 ml per section*
- sterile filter paper, funnels, test tubes

Procedure:
Weigh 2 grams of control and contaminated soil (2 replicates) and introduce them into glass test tubes, then add 2 ml of TTC into each sample close them and incubate during 1 week in the dark.

The determination of dehydrogenase activity
Triphenyltetrazolium chloride (TTC), as an acceptor of hydrogen and electrons, changes into water-insoluble red triphenyloformazane (TF) as a result of its reduction. The colour intensity relates to the dehydrogenase activity of the studied material.

After incubation add to samples 5 ml of acetone and mix it very often during 1hour. Correct the volume of acetone and rotate 6 000 rotation per minute /or mix samples on Vortex and filtrate them. Read the extinction of acetone fraction for the wavelength 490 nm. Prepare the standard curve as it was recommended above (concentrations of TF in acetone). On the basis of extinction read the concentration of TF in mg from the prepared standard curve .

Dehydrogenase activity should be calculated on the basis of formula below:

$$a = \frac{TF \cdot V \cdot 100}{m \cdot t}$$

Where: a – dehydrogenase activity, µg/g d.m.,h
TF – concentration of TF, µg/l
V – solution volume (exrahent + solution of TTC)/ concentration of TTC
m – sample mass, g d.m.
t – incubation time, h

Moreover, calculate inhibition of dehydrogenase activity

$$I = \frac{A_k - A_b}{A_k} \cdot 100$$

Where : I – inhibition of dehydrogenase activity , %
A_k – absorbance of control sample (avarage)
A_b – absorbance of studied sample (average)

Exercise 2
Influence of toxicants on soil respiration

Materials

- Bottles 100 ml
- Glass vials 7 ml
- Pipettes, spoons
- NAOH 1.0 M per group
- HCL 0,5 M
- Flasks
- Phenolophtalein

Procedure:
Introduce 20 grams of soil contaminated with selected toxicants and garden soil (add 2ml H_20 to make it wet) into 2 separate bottles (every 20 grams of soil to one bottle). Prepare 3 glass vials and put 10 ml of 1, 0 M NaOH inside. Then introduce each vial into bottles with soil (one vial with NAOH to each bottle). The third vial should be put into empty bottle (blind sample). Close the bottles. Incubate two of them 25ºC during 7 days. Blind sample should be titrated at the end of laboratory!

Measurement of CO_2

Take each vial with NaOH and pour their content into small flasks (blind sample should be titrated at the laboratory 3 , the last two at laboratory 4) . Then add one drop of phenolophtalein and titrate left NaOH with solution of HCL (0,5 M). During the process the following reaction takes place. Write down the amount of acid used**.**

$2NaOH + CO_2 = Na_2CO_3 + H_2O$

The respiration pace should be calculated according to the following procedure:

$$R = 0,224 \frac{A - B}{2tm}, \quad ml\,CO_2 / g,$$

where: A – the amount of acid used for titration of blind sample
B – the amount of acid used for titration of studied sample
(2 types: control and contaminated soil)
T – incubation time, d
m – mass of soil sample, g d.m.

Exercise 3
Growth inhibition test for the following plants: barley (*Horodeum vulgare*), rye (*Secale cereale*), oats (*Avena sativa*), corn (*Zea mays L. V. saccharata*), white charlock (*Sinapsis alba*), black charlock (*Brassica nigra*), lupin (*Lupinus angustifolius*).

Materials: germinated plant seeds (36h - 48h at 20 ° C - incubated in the dark), vases, test soil (contaminated with heavy metals, etc.) and control soil.

Prepare half dilutions of contaminated soil using control soil. Fill the vases with the prepared samples (80 g / sample). Place 10 germinated seeds (of one selected species) in vases with the test soil at a depth of about 1.5 cm below the surface of the earth (2 replicates). Add 10 ml of water to each dilution. Incubate them in phytotrone for 7 days. Maintain a constant soil moisture level of 80% WHC (total water capacity). Apply artificial and natural lighting 16h/d, with night and day division at 20 ± 2ºC.
After appropriate time of growth, the plant should be removed from the vase, and then rinsed and the following parameters should be determined:

- **number of plants**
- **mass of plants**
- **shoot length**
- **root length**
- **the length of the longest root**

Compare the calculated mean values with those obtained from the control vases. The report should include following curves: the number of plants, the mass of plants, the shoot length, root length. Calculate EC 50 for each parameter.

Exercise 4 Germination test with *Lepidium sativum*

Materials: seeds of plants, Petri dishes, soil (contaminated with heavy metals, etc.) and control soil. For test, garden cress seeds (*Lepidium sativum*) are used.

For Petri dishes (9 cm diameter) enter 60 grams of the soil to be tested in different dilutions. Prepare the control plates with unpolluted soil. Introduce 25 seeds into each dilution. Place all samples in a thermostat at 25 ° C for 24 hours (in the dark). After incubation period: count the seeds that germinated (specify how many) to measure the root length.
Growth inhibition is assessed according to the formula:

$$I = \frac{L_k - L_t}{L_k} \cdot 100\%$$

Where L_k – the average length of roots in control samples, mm L_t – the average length of root in studied samples, mm

Determine the average for parameters separately for each soil type and compare with the results obtained for the control. The report should include charts illustrating the dependence of the percentage of germinated seeds N [%] and growth inhibition on the concentration of toxicant.
Calculate the IC_{50} for each parameter.

Toxicants:

$CuSO_4$

Initial concentration: 50g/kg d. s.

$(CH3COO)_2Pb$

Initial concentration: 50g/kg d. s.

Deltrametryna (Decis)

Initial concentration: 20g/kg d. s.

Glyfosat (Roundup)

Initial concentration: 20g/kg d. s.

Ecotoxicology - laboratory 5

REPORT

Each section prepares a presentation / report from the conducted research. The sensitivity of a chosen toxicant and its impact on aquatic organisms (invertebrates, plants and algae) as well as soil organisms (bacteria and higher plants) should be determined. As a part of the report, you have to calculate separately LC50 and EC50 (you have to use at least two methods: Reed and logit methods) and LOEC, NOEC, MATC for all organisms tested. Additional calculations with formulas given in the instruction are also required (respiration rate, calculations of dehydrogenase activity and plant growth inhibition). In addition, the presentation should include the following charts, for invertebrates: the dependence of dead invertebrates (different species) on the concentration of toxicant studied and dependence of number of dead specimens on exposure time (lab 1), for plants: the dependence of the concentration of toxicant on number of plants, plant mass, shoot and root length (lab 3 and 4) as well as the dependence of concentration of toxicant on the percentage of germinated seeds and the dependence of growth inhibition on the concentration of toxicant (lab 3 and 4). In addition, the report must contain the overall and detailed characteristics (scientific papers) of toxicant with complete information on its toxicity, studies done with other bioindicators on this toxicant in relation to your studies, the complete information about organisms used in the conducted tests together with the conclusions.