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BIOINDICATION AND BIOTESTING METHODS APPLICATION FOR WATER QUALITY ASSESSMENT

Досліджено екологічний стан природних водних об'єктів на основі аналізу якісного та кількісного складу біоіндикаторних організмів. Апробовано методики відбору проб та проведено лабораторні дослідження біологічного матеріалу, відібраного з природних екосистем. Досліджено такі біологічні об'єкти: перифітон, що розвивається на скелястій та кам'яній поверхні узбережжя водойм, водорості та організми, що належать до мейофауни *(Nematoda, Harpacticoida, Cladocera, Rotifera)*, а також макробентосні прісноводні річкові організми. Визначено головні впливи та залежності поширення організмів.

Ключові слова: моніторинг довкілля, водні об'єкти, біоіндикація, мейофауна, перифітон.

In this article the environmental monitoring of the natural water bodies based on qualitative and quantitative analysis of bioindicative organisms were conducted. Approbation of the sampling methods, techniques and laboratory analysis of biological objects from natural ecosystems were carried out. The next biological objects have been analyzed: periphyton from rocky and stony coasts, algae and meiofaunal organisms *(Nematoda, Harpacticoida, Cladocera, Rotifera)*, and macrobenthos freshwater river-living organisms. Main influences and distribution dependences were found.

Key words: environmental monitoring, water bodies, bioindication, meiofauna, periphython.

Research and publications analysis. Environmental monitoring of the area near wastewater treatment plants is very important and necessary issue. Implementation of environmental monitoring by bioindication and biotesting, including the use of meiofauna, is widely used to carry out the express control. Biological objects could be not only objects of environmental monitoring, but also a tool for its implementation.

Most of the classic biological tests with microorganisms and algae provide systematic observations and visual estimation, and that is subjective estimation. The use of the software combined with the classical method could provide precise and continuous indication of water ecosystem quality assessment. This could prevent morphological and physiological changes of people's health, which could be induced by environmental pollution. Implementation of automated biological analysis for example could enable determination of correlation between the pollutants concentrations and the kinetic parameters of algae biomass growth.

Besides the use of biotests with microorganisms and algae, highly efficient is estimation of water bodies pollution by using chemo- and phytoindication. Analysis of ecosystem's biocenosis and computational modeling enable performance of environmental monitoring of different water bodies and environmental prediction [1-2].

It's known that after bringing of the new bacterial species (as biological preparation) or genetically modified microorganisms, or random microorganisms into the environment it is needed specific ecological niche (specific conditions, source of nutrients) for them to establish. If the massive release of microbial

biomass into the soil or water takes place, the change of the energy and matter flows in trophic chains, the change of the structure and function of natural biocenosis, the decrease of ecosystems self-treatment activity and the global changes in the functions of living organisms could occur. Also all biocenoses, as a complex of phytocenosis, zoocenosis and microbiocenosis (microbial associations), are formed according to the competitive exclusion principle, and that need to be considered when using biological treatment, remediation or application of biological preparations [3].

Materials and Methods Purpose of the study – the implementation of the express methods of water quality assessment for environmental monitoring. Objects – meiobenthos, macrobenthos, algae, periphyton.

Sampling. For the sampling of periphyton and meiofauna from rocky surface the Brush Sampler was used as described in [4]. Brash Sampler consists of a glassy cylinder made from Plexiglas®, to the one end of which a ball valve and rubber gasket (inner diameter 2 cm; inner area 3,14cm2) are attached. Inside the cylinder is a stainless steel plunger with strip brush on the top, a guide rail with the knob for pulling it out. When the plunger is moved a vacuum is created, that ensures sucking in the biomass with water inside the cylinder. When sampling under the water level, the water, needed for transporting biomas from the stone into the cylinder, comes through the 20 µm filter. When sampling above the water surface, an additional source of water is needed. Field work was based on taking samples from two different types of coast. The surface of the stones with the biomass that is choosen has to be quite flat to ensure good sealing between sampling equipment – the "brush sampler" and the substrate. Also the stone should be fairly slope, so

Fig. 1. Macrobenthos sampling

different layers of periphyton quantity can easily be detected.For sampling of macrobenthos the 0,5 mm net was used (Fig. 1).

Laboratory analysis. After sampling we got 44 samples of periphyton at total from two types of coasts, which were analysed in the laboratory for measuring biomass parameters (chlorophyll a, ash-free dry mass) and meiofaunal distribution. After all samples were taken to the laboratory, they immediately were puted into the climate room in the dark, with temperature 4 ºC for storage. The next day laboratory work started. All samples had different volume, so first step was to adjust all solutions to the same volume - 100 ml. Then 10 ml of each solution was taken for chlorophyll a analysis, 10 ml - for organic matter analysis and at last 80 ml - for measure the quantity of meiofauna.

A filtration of 10 ml periphyton solution on glass fibre filters (Whatman® GF6, Ø 25 mm) on vacuum was made for chlorophyll a analysis. Pigment extracting with ethanol (90%) in the dark for 20h was processed directly after filtration. For samples, which were taken from the depths 0 cm, +10 cm and +20 cm was used 2,5 ml of ethanol and for samples took from under the water - 5 ml of it (volume depends on the concentration chlorophyll in the sample). The last step was to measure concentration of chlorophyll a by spectrophotometer.

Organic and inorganic matter were determined by measuring ash-free dry mass analysis (AFDM). The first step was to weight the pre-combusted (Whatman® GF6, Ø 25 mm) filters, then filtration of 10 ml periphyton solution was made. Filters were dried in the oven at 105 ºC for 24h then weighted. Then filters were combusted at 550 ºC for 5h in the oven and had been weighted again.

To do the meiofaunal analysis the remainder (80 ml) of each sample was sieved through a mesh net (20µm) for the separation of meiofauna. Then material was removed into a small bowl where Rose Bengal (1 drop/10ml) was added for colouring and afterwards fixed with formaldehyde (4%, 1ml/10ml). The meiofaunal organisms were counted and classified into taxonomic groups by using stereomicroscope (Olympus® TL 2 – 1) with 30x magnification.
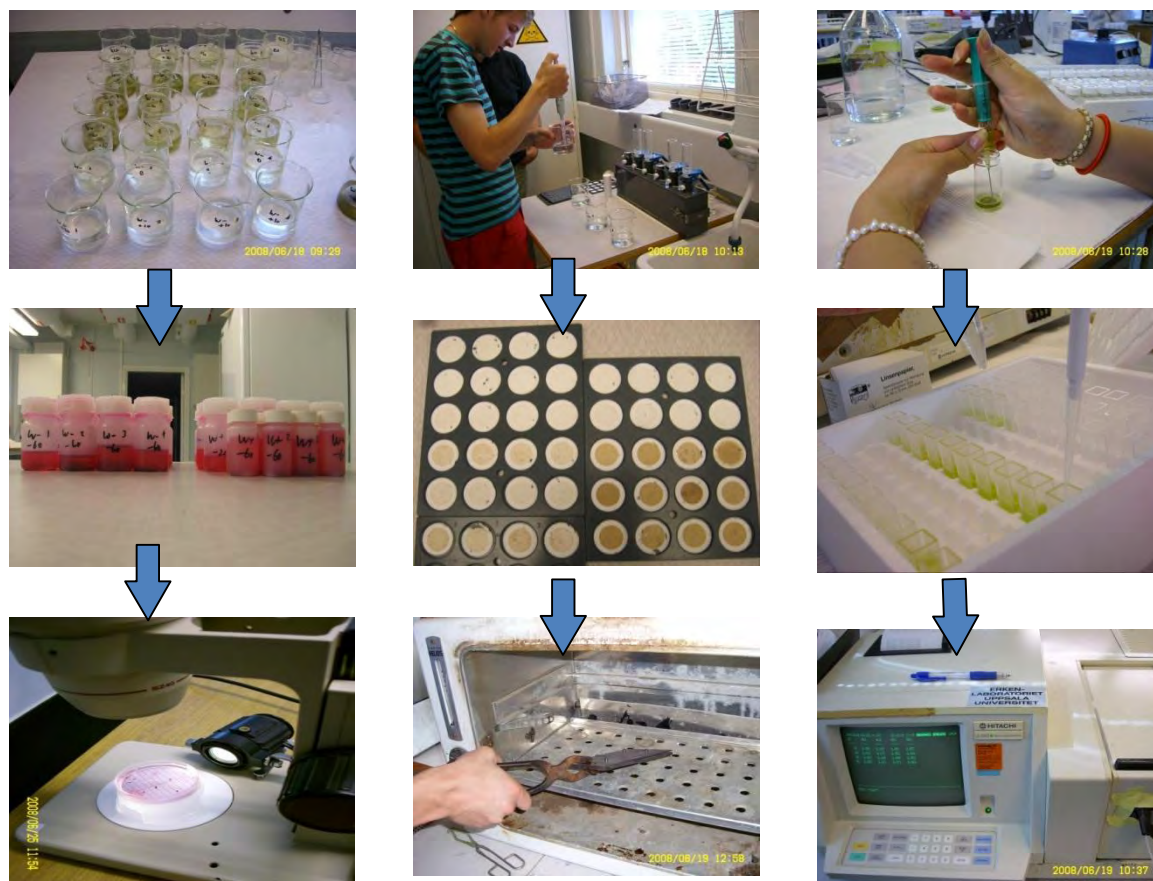
Fig. 2. Laboratory analysis

Results and discussion. To estimate the water treatment efficiency at Lviv wastewater treatment plant and, also, to asses the water quality of the natural water bodies of north western part of Lviv region the investigations of the implementation of different methods, including bioindication, biotesting and microbiological analysis, has been started. An advisability and accuracy of the methods by analyzing of amount and taxonomic composition of meiofaunal and benthos organisms has been investigated [5–7].

The techniques were overtaken during the investigation of meiofauna distribution in littoral periphyton communities of Lake Erken under supervision of Lars Peters (Norr Malma, Sweden). Determination of quantity and taxonomic composition of meiofaunal organisms, which were the object of the biomonitoring, and analysis of chemical composition of periphyton enable water quality assessment and anthropogenic contraventions evaluation. It's known that meiobenthos communities have reasonable influence on physicochemical characteristics and composition of sediments, take part in mineralization of organic matter, could stimulate bacterial growth, therefore significantly influence biological capacity of ecosystem.

The task of investigations was qualitative and quantitative analysis of meiofauna and taxonomic distribution according to the depth of sampling and spatial location of the coast according to the systematic wind influence. Thereby by this approach it was tried to determine the wave action influence on these two parameters. Besides qualitative and quantitative analysis of meiofauna, composition of organic and inorganic matter of periphyton and algal quantity have been analyzed.

The wind action creates waves, which has a strong influence on meiofaunal distribution. When the wave splashes against the stone, some part of the organisms loses their grip and being washed off into the water. And in this case, the quantity of meiofauna is diminished. There are two main affects which are caused by wave action: the vertical and horizontal gradients of meiofauna distribution. The horizontal gradient is displayed by division all coasts into two types: wind exposed and non-wind exposed. Which means that the wind exposed coast gives way to the influence by the waves and the non-wind exposed coast doesn't. The vertical gradient means that the wave action is bigger on the surface than in the water, which caused vertical depending of the meiofauna biomass variability.

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The first part of sampling was taken place on the non-wind exposed coast , which is located on the north side of Lake Erken. The samples were taken from 5 different depths: 20, 40, 60 cm under the water zero line, from zero line and from 10 cm above the water. Four replications from each depth was made. The total amount was 20 samples, for the further investigation in the laboratory. The second half of sampling was made on the same day on the wind exposed coast . Samples were taken from 6 different depths: 20, 40, 60 cm under the water zero line, from zero line and also from 10 and 20 cm above the water, also four replications each. Quantity of samples was 24.

Algal biomass distribution. Investigation of algal biomass distribution was made by measuring Chlorophyll a concentration within samples. The results show, that algal biomass value depends considerably on sampling location. Two basic dependences can be distinguished : horizontal dependence (on coast) and vertical dependence (on depth) (Fig. 3).

The dependences are expressed in following: There is bigger quantity of algae on the stone surface above the water on wind exposed coast then on wind sheltered. And bigger quantity of algae under the water on non-wind exposed coast comparatively to wind exposed. Quantity of algae is increasing with growth of depth especially on non-wind exposed coast. On the non-wind exposed coast the biggest jump befalls from depth 0 to 20 cm under the water. On the wind exposed coast changes of algal biomass are quite smooth from depth to depth.
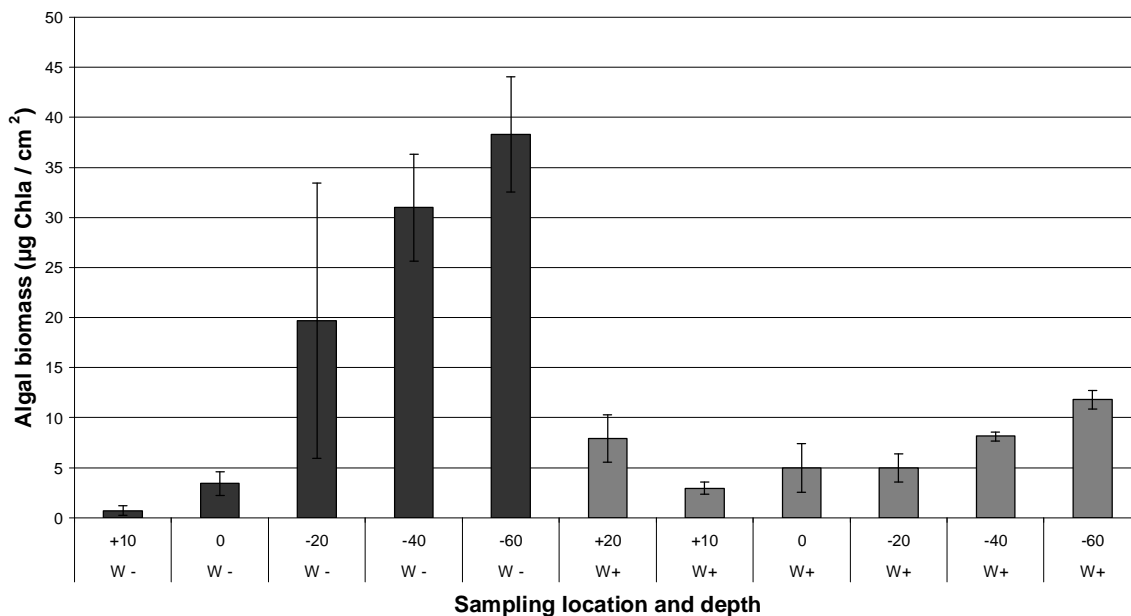

Fig. 3. Mean algal biomass. [Sampling location: Non-wind exposed coast (“W-”) and Wind exposed coast (“W+”), depths from 20 cm above (“+20”) to 60 cm under(“-60”) the water].

The periphyton biomass distribution. The periphyton biomass distribution was assessed by ash-free and dry-mass analysis (AFDM). This analysis gives data about concentration of organic and inorganic matter in samples. There is considerable dependence of periphyton dry mass and organic-inorganic composition on sampling place and depth (Fig. 4).

The most common dependences are: there is more material above the water on the wind exposed coast and under the water on the non-wind exposed coast comparatively to each other. Increasing of periphyton dry weight with growth of depth on the non-wind exposed coast befalls.

The biggest jump happens from depth 0 to 20 cm and from 20 to 40 cm under the water. Also there are nearly identical values of periphyton dry weight on depths 20, 40 and 60 cm under the water on the wind exposed coast. And there are no big changes of periphyton dry weight value during moving from levels above to levels under the water. There is less quantity of inorganic matter above the water surface

and it increases with growth of depth. The most common phenomenon is the dominating of organic material almost at all depths.
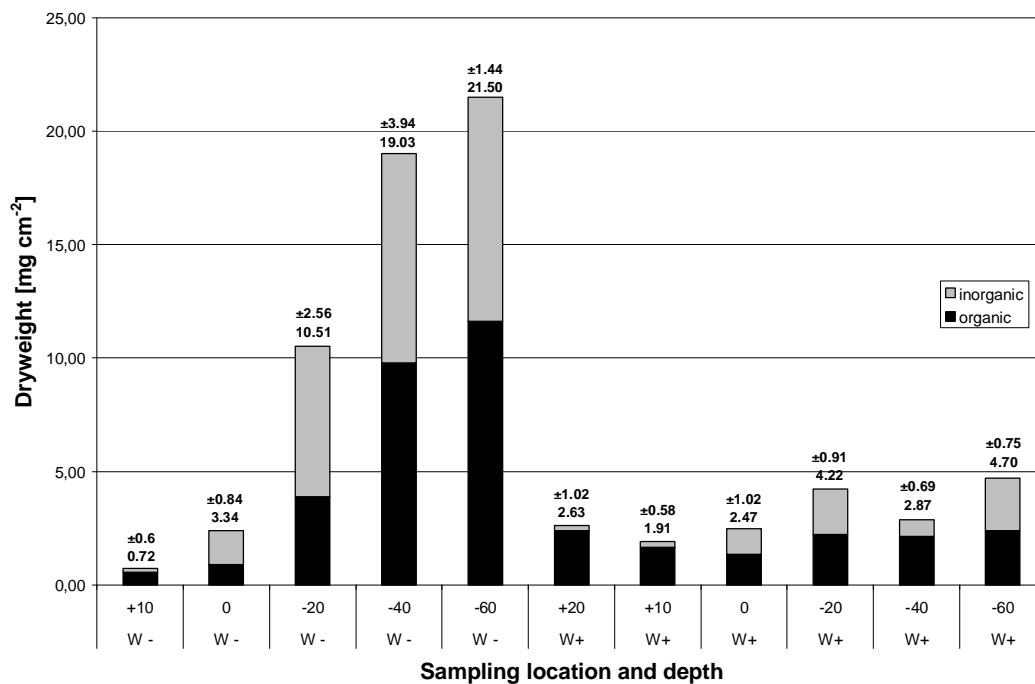

Fig. 4. Mean periphyton biomass separated into values of organic and inorganic matter. [Sampling location: Non-wind exposed coast ("W-") and Wind exposed coast ("W+"), depths from 20 cm above ("+20") to 60 cm under("-60") the water. The number above the bars give the mean value (located lower in the form [mean]) and standard deviation (located higher in the form [±SD]) of the dry weight.]

Meiofauna distribution. Meiofauna distribution was investigated by counting amount of organisms with the stereomicroscope. The results showed dependence of Meiofauna distribution on sampling location (Fig. 5).
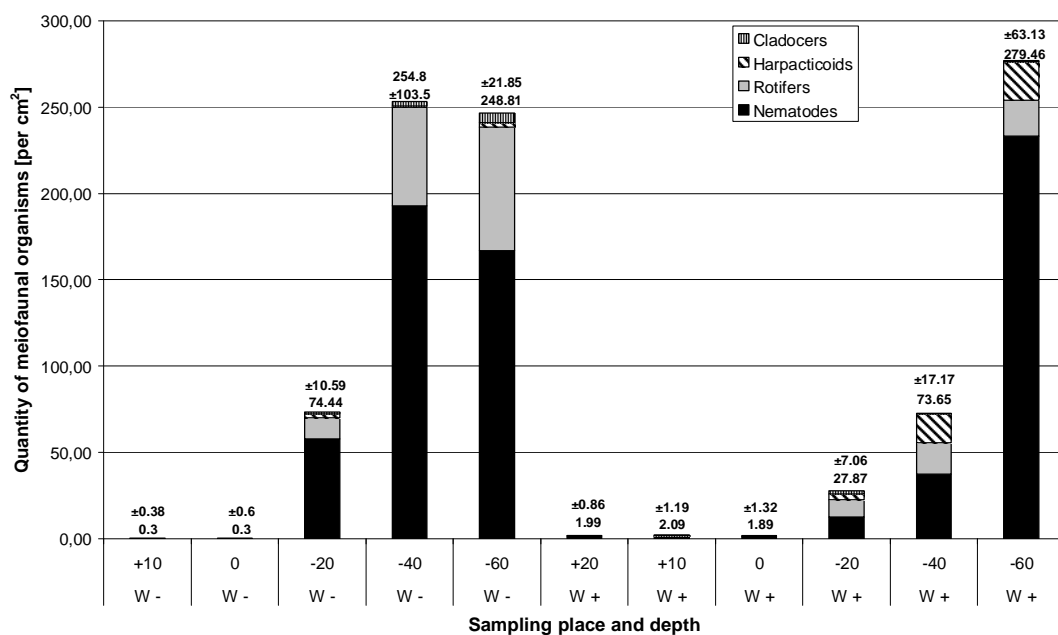

Fig. 5. Quantity of meiofauna. [Sampling location: Non-wind exposed coast ("W-") and Wind exposed coast ("W+"), depths from 20 cm above ("+20") to 60 cm under the water ("-60"). The number above the bars give the mean value and standard deviation]

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The dependences are: there is very low quantity of meiofaunal organisms above the water and at the zero depth. Increasing quantity of meiofauna with growth of depth befalls. Meiofaunal abundance on wind exposed coast at depths 20 an 40 cm is bigger than on opposite coast, but at 60 cm it almost the same. On non-wind exposed coast the biggest jump within amount of meiofauna befalls from 20 to 40 cm under the water. Amount of organisms at depths 40 and 60 cm under the water is equal. On the wind exposed coast the biggest jump within amount of meiofauna befalls from depth 40 to 60 cm under the water. During moving from depth 20 to 40 cm under the water there are no big changes. The dominating specie at all depths is *Nematoda*. Big amount of *Rotifera* was found at non-wind exposed coast. *Harpacticoida* were founded at the wind exposed coast, especially big amount of them at depths 40 and 60 cm. Also *Cladocera* were founded.

Conclusions. The investigation of littoral periphyton communities and meiofaunal organisms contributed important information about meiofaunal distribution on littoral hard substrates and influence of wave action on it. The wave action has the biggest influence on meiofaunal distribution, its taxonomical composition and the periphyton biomass parameters in lake. Wave action influence is increasing with grows of depth and depends on coast location. Nematode is the dominant species within the meiofaunal composition. However, in the future this research could be prolonged in order to get more detailed information about this phenomenon. Future research can be connected with more detailed study of taxonomic composition and taking samples from bigger depth. Obtained results could be used for environmental prediction and investigations of changes in meiofaunal taxonomic and quantitative parameters. Methods of bioindication and biotesting could be used for express environmental monitoring of surface water bodies or technological units.

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