1. Introduction

Intercalibration of biological methods for assessing the stability of the aquatic ecosystems development is of particular relevance on the territory of Eastern Europe. The area chosen for the approbation of the method of short-term bioindication is an elevated southwestern part of the foundation of the Eastern European platform and includes the Ukrainian crystal shield. This led to significant variations in the diversity of the parent rocks, soil conditions, and, accordingly, the diversity of the vegetation cover. A sharp and frequent change of the natural and territorial complexes also caused a sharp change in the areas that were not used in economic activities, with the areas of intensive anthropogenic influence. This could not but affect the quality of aquatic ecosystems.

In addition, the growth of the anthropogenic influence, the levels of natural water consumption, the extensive type of management in the middle and large river basins led to a deterioration of the ecological state not only of surface waters, but also led to a significant modification of the composition and state of living organisms, including aquatic
macrophytes. Modern methods for assessing water quality used by monitoring services are reduced to ascertaining the presence of certain levels of toxic substances in the water, while their influence on a number of inhabitants of reservoirs is leveled. In recent years, the necessity and importance of operations of strategic level aimed at providing an integrated assessment of the ecological sustainability of aquatic ecosystems are increasing. This integrated assessment is also envisaged by the EU Water Framework Directive 2000/60/EU, but in Eastern Europe it requires significant adjustments that involve increasing the role of the biological component, expanding the list of biological indicators which can be included into the system of the integrated assessment of surface water quality.

Equally important is the necessity to analyze the state not only at the time of the analysis, but also the negative impact in retrospect. All these conditions are provided by the method of morphogenetic analysis of the development of living organisms in the studied area, which allows assessing the homeostasis and sustainable development of ecosystems.

2. Literature review and problem statement

Stress in the development of aquatic ecosystems, including under the influence of anthropogenic factors, causes morphological and physiological deviations in living organisms. Environmental conditions affect the size of leaves and the features of their structure [1]. Of a particular importance for biomonitoring are deviations in the development of bilaterally symmetrical organisms [2].

The most interesting for the implementation of biomonitoring is the fluctuating asymmetry. The influence of external conditions on the magnitude of fluctuating asymmetry has been convincingly proved [3]. Fluctuating asymmetry is slight non-directional differences in the manifestation of features on the symmetrical sides of a biological object [4]. Therewith, from the point of view of the statistical characteristic, fluctuating asymmetry is the distribution of differences of sides with zero mean value and obeys the normal law [5].

But one of the actual problems of practical use of fluctuating asymmetry indices is the choice of a necessary object and parameters of its evaluation, with a view to further interpreting the results obtained [6]. This method is widely used for the analysis of the ecological situation on the basis of the reactions of some representatives of dendroflora, with the most commonly used species Betula pubescens, Betula alnifolia, and others [7]. In other studies, species with a bright color of leaves were used as indicators [8]. Besides, the use of fluctuating asymmetry indices allows analyzing not only the existing environmental impact, but the impact that has occurred in the past [9]. However, probably the most informative is this method for analyzing the quality of the aquatic environment using macrophytes, since it is these phytocenes that are most closely in contact with the environment. This is confirmed by a number of scientific studies carried out using higher and lower aquatic organisms [10]. Aquatic organisms are distinguished by a variety of reactions to stress in the environment and many macrophytes and algae can be used as an indicator of its condition [11].

3. The aim and objectives of the research

The aim is to substantiate the theoretical and methodological aspects of the application of fluctuating asymmetry of macrophytes for the implementation of the bioindicative evaluation of the sustainable development of aquatic ecosystems.

To achieve this aim, it is necessary to accomplish the following objectives:

- to establish the parameters for the application of a short-term (instantaneous) biomonitoring system based on morphogenetic changes in some plant species growing on the territory of Eastern Europe;
- to determine the regularity of the distribution of integral indices of the fluctuating asymmetry of water mac-
rophytes depending on the presence of toxic compounds in bottom sediments and coastal soils.

4. Methodology of the experiment on short-term bioindication of aquatic ecosystems

The study of the possibilities of using the morphogenetic approach in determining the ecological tolerance and the stability of the development of aquatic ecosystems was determined in the conditions of the Ukrainian Polissya zone. Among various types of reservoirs, rivers belonging to the class of medium and large watercourses – Teterev, Gnilopyat, Irsha, Guyva and Zdvizh – flowing through the territory of Zhytomyr and Kyiv regions were chosen, as well as fairly large reservoirs – Chudnove, Otsechnoye, Zhytomyr, Malyn and Irsansk (Fig. 1).

![Fig. 1. Distribution of registration points of higher aquatic vegetation on the territory of Ukrainian Polissya](image)

The water quality at the observation points was assessed in accordance with the "Methodology for the environmental assessment of surface water quality according to relevant categories" [25]. Therewith, 43 indicators of the physical and chemical composition of water were determined. According to the obtained data, the overall water quality index WQI was calculated as the average of three block indices: SI – the Salt Index; TSI – index of trophy-saprobological indicators; TI – index of the content of specific toxic and radiation substances.

During the research, the application of the method of short-term bioindication was tested, according to which geobotanical descriptions of groups of higher aquatic plants and evaluation of the data obtained using a morphogenetic approach were carried out. The primary materials for the bioindication analysis were independent studies of the authors, which included descriptions of aquatic ecosystems of certain parts of the river ecosystems of Ukrainian Polissya. The author has collected the materials during 2011–2015. The research was carried out at 57 points simultaneously with water sampling. For a more detailed analysis of the anthropogenic impact on the species composition of macrophytes, the samples were also taken along the river before and after the territories of settlements.

To assess the ecological state of landscapes within the location of observation points, the coefficient of environmental sustainability of the landscape (KESL1) was calculated [24]. To carry out the research, we selected species of plants that freely grew in all the selected hygroteops in quantities sufficient to obtain reliable data: Potamogeton perfoliatus L., Salvinia natans and Nuphar luteum (L.) Smith. The sample size was 500 – 600 leaf plates. The leaf plates were scanned with Epson Perfection V33 scanner and measured by means of the electronic beam compass BCE-1-125-0.01 with an accuracy of 0.01 mm. Measurements of the length of the second rib were carried out by the mechanical curvimeter CU-A.

The coefficient of the fluctuating asymmetry was estimated by determining the integral index calculated by the average difference between the parties for each feature [25] using the formula

\[ K_{fa} = \frac{\sum (d_{lr})}{nk}, \]

where

\[ d_{lr} = \frac{d_l - d_r}{d_l + d_r}, \]

\[ k \] is the number of features, \( d_l \), \( d_r \) are the values of the feature measurements on the left and right of the leaf plate, and \( n \) is the sample size.

5. Results of studies on the application of the method of short-term bioindication of aquatic ecosystems

As a result of the comparative analysis, a correlation was defined with a high degree of reliability between the values of the water quality indicators and the fluctuating asymmetry indices (\( r = 0.5841 \)), as well as between the values of KESL1 and fluctuating asymmetry parameters (\( r = -0.2639 \)). Thus, according to the research, the growth of fluctuating asymmetry coefficients was found in the places of deterioration of the ecological state of water (Fig. 2).

Here we constructed a three-dimensional model of the formation of the fluctuating asymmetry in the leaves of Potamogeton perfoliatus L. under conditions of changing the ecological stability of the landscape and water quality, which is described by the equation:

\[ K_{fa} = 0.008 + 0.014x - 0.011y - 0.0011x^2 - 0.0019xy + 0.0067y, \]

where \( K_{fa} \) – the coefficient of the fluctuating asymmetry of the leaves of Potamogeton perfoliatus L.; \( x \) – the integral ecological index of water quality, Ie; \( y \) – the coefficient of ecological stabilization of the landscape, KESL1.

The obtained equation makes it possible to determine the extremums of the development of the indices of the fluctuating asymmetry of Potamogeton perfoliatus L. under deterioration of integral ecological indices and KESL1. Thus, the asymmetry shift of more than 0.039 was observed with deterioration in water quality below Category 2 (Class II), and
extreme values (below 0.044) were observed at points where the quality of water was at the transition category 3.5 from Class II to Class III. Thus, the coefficient of the fluctuating asymmetry of the leaves of Potamogeton perfoliatus L. is a clear indicator of the formation of Class III of water quality in which the state of aquatic ecosystems is defined as “satisfactory” – by status and “slightly contaminated” – by purity.

The distribution of the fluctuating asymmetry indices in relation to the indicators of landscape destabilization showed the extremes of the symmetry shift of the leaves of the Potamogeton in landscapes with KESL1 below 0.4, that is, starting with the transition category from “slight stable” landscapes to “unstable” landscapes.

Symmetry shifts of leaf plates were observed in slight stable landscapes with KESL1 below 1.0, where the values were recorded at the level of Kf = 0.039.

As already stated, the research concerned river ecosystems with features of both natural and anthropogenic nature. This caused the difference in the anthropogenic development of the left-bank and right-bank parts of the ecological corridor studied. On the left bank of the rivers Teterev and Irsh, the main settlements and industrial enterprises are located, and therefore there is more water pollution in the coastal zone. Since the water flows in the river in the city do not completely mix, there is a difference in their contamination at the opposite banks.

It was found out (Fig. 3) that the value of the asymmetry coefficient of the leaves of Potamogeton perfoliatus L. on the left bank exceeds these values for plants of the right bank. A sequential increase in the values from a point located above the mouth of the river Kaminka (No. 1–15 and No. 1–16), to the point near the settlement of Sloboda-Selets (points No. 1–21 and 1–22) was revealed, where the indicators of the asymmetry coefficients reach their maximum. Violation of the symmetry of leaf plates of Potamogeton perfoliatus L. and Salvinia was also observed in points (No. 1–33 and No. 1–32 – 1 km below the city of Radomyshl, and correspondingly in points of intense anthropogenic load.

The lowest indicators of leaf asymmetry are confined to the areas where the records were taken at points No. 1–01…1–06, 1–29…1–32 and others, completely confirm the data obtained with other hydrochemical and bioindicational criteria.

The distribution of the values of the asymmetry coefficients calculated for plants on the right bank does not form such a clear dependence on the sources of pollution as on the left bank, however, an increase in the values within the city boundaries and their decrease outside the city is reliably revealed.

Besides, we divided all the data obtained into three categories according to the intensity of anthropogenic pressure on aquatic ecosystems. The prerequisites for the distribution of observation points into the categories of the intensity of anthropogenic pressure were water quality data of water bodies in accordance with the “Methodology for the Environmental Assessment of Surface Water Quality According to Corresponding Categories” and the indicators of destabilization of landscapes. So, objects with water quality categories 1 – 2.99 (from “excellent” to “good”), were classified as objects of low anthropogenic pressure, this included constant observation points No. 1–01…1–06, 1–09…1–10, 1–14, 1–23, 1–25…1–26, 1–29…1–32, 1–37. The objects with “the average level of anthropogenic pressure” included observations of water where the quality category was fixed at 3.0 – 3.99 (slightly contaminated). Thus, points from No. 1–07…1–08, 1–11…1–14, 1–16, 1–22, 1–24, 1–35, 1–38…1–42, 2–01, 2–03, 2–05…2–06, 5–01…5–02 were included into this category.

With the objects of strong anthropogenic pressure, we included all parts of the water courses, where the levels of the ecological state of water bodies are fixed in the range from 4.00 category and more. So, here were assigned the points No. 1–15, 1–17…1–21, 1–27…1–28, 1–33–1–34, 1–36, 2–02, 2–04, 4–01…4–04.

Thus, it was found that the intensity of the anthropogenic load significantly affects the indicators of the leaves asymmetry.

So, the increase in anthropogenic pressure on the state of the aquatic environment leads to a polynomial shift of the asymmetry coefficient distribution by 0.093 units for Potamogeton perfoliatus. 0.0074 for Salvinia natans (L.) All. and by 0.143 for Nuphar luteum (L.) Smith. And in case of a strong anthropogenic pressure, the basic values of the polynomial can be shifted by 0.0145 units for Potamogeton perfoliatus L. and Salvinia natans (L.) All. and by 0.0201 for Nuphar luteum (L.) Smith (Fig. 4–6).
For a more detailed analysis, the asymmetry coefficients were compared with the water quality indicators and the block of toxicity indicators obtained during the analysis of the species composition in some points of the territory of Ukrainian Polissya.

According to the results of the research (Table 1), the most vivid changes in morphogenetic indices, namely in the fluctuating asymmetry coefficients, were found at the points of intense anthropogenic pressure, confined to the areas of sewage impact (1–16, 1–22, 1–34, 2–04 and others), and variations in the fluctuating asymmetry coefficients were noted when a high concentration of heavy metals in the bottom soil was reached, but different plant species reacted differently to the variation of the heavy metal content in the bottom sediments and coastal soil.

For the species Nuphar luteum (L.) Smith, all the investigated groups of heavy metal cations in bottom sediments had an effect on morphogenetic indices. Thus, the noted direct close relationships are confirmed by the correlation coefficients obtained (r=0.4042...06579). Close ties are stated for Zn$^{2+}$ and Cu$^{2+}$.

At most observation points, the values of the fluctuating asymmetry coefficients were in the range from 0.02 to 0.06, and their upward shift occurred with an increase in the concentration of Cu$^{2+}$>2 mg/kg, Cr$^{2+}$>0.8 mg/kg, Zn$^{2+}$>15 mg/kg, Ni$^{2+}$>2.5 mg/kg, Co$^{2+}$>0.8 mg/kg.

The response to anthropogenic pressure in the form of deformations of leaf plates of plants of the species Potamogeton perfoliatus L. had close linear dependencies in response to an increase in the content of mobile forms of heavy metals.

| Table 1 |

| Index | Nuphar luteum (L.) Smith | Potamogeton perfoliatus | Salvinia natans | Nuphar luteum (L.) Smith | Potamogeton perfoliatus | Salvinia natans |
|-------|--------------------------|------------------------|----------------|--------------------------|------------------------|----------------|
|       | r | t_{Stud.} | R | | r | t_{Stud.} | r | t_{Stud.} | r | t_{Stud.} | r | t_{Stud.} |
| Co$^{2+}$ | 0.404 | 3.247 | 0.343 | 2.680 | 0.335 | 2.615 | 0.397 | 3.176 | 0.369 | 2.913 | 0.203 | 1.526 |
| Cr$^{2+}$ | 0.522 | 4.495 | 0.302 | 2.325 | 0.161 | 1.201 | 0.530 | 4.594 | 0.419 | 3.388 | 0.674 | 6.710 |
| Cu$^{2+}$ | 0.637 | 6.069 | 0.504 | 4.288 | 0.414 | 3.344 | 0.596 | 5.456 | 0.505 | 4.296 | 0.445 | 3.651 |
| Ni$^{2+}$ | 0.486 | 4.081 | 0.411 | 3.314 | 0.429 | 3.487 | 0.306 | 2.358 | 0.371 | 2.939 | 0.395 | 3.163 |
| Zn$^{2+}$ | 0.658 | 6.419 | 0.572 | 5.124 | 0.480 | 4.016 | 0.608 | 5.620 | 0.448 | 3.686 | 0.484 | 4.061 |

Note: $t_{Stud.}$ is the actual value of the Student’s test, r – correlation coefficient. The significance of the connection for the performance of the feature is equated to the critical table value for n=20 ($t_{Student}$=2.10)
Thus, for cations of zinc, copper, cadmium and nickel they were $r=0.5720$, $r=0.5040$, $r=0.4147$ and $r=0.4111$, respectively. The dependence of the fluctuating asymmetry coefficients on the contents of the mobile forms of chromium and cobalt was weak ($r=0.3017$ and $0.3426$, respectively). At most observation points, the values of the fluctuating asymmetry coefficients were in the range from 0.03 to 0.05, and their upward shift occurred with an increase in the concentration of $\text{Cu}^{2+}>0.8 \, \text{mg/kg}$, $\text{Cr}^{2+}>0.8 \, \text{mg/kg}$, $\text{Zn}^{2+}>20 \, \text{mg/kg}$, $\text{Ni}^{2+}>2.5 \, \text{mg/kg}$, $\text{Co}^{2+}>1.0 \, \text{mg/kg}$.

In the form of the asymmetry shift of leaf blades of plants of the species *Salvinia natans* (L.) *All.*, adverse concentrations of mobile forms of cations of nickel, zinc, copper and cadmium were noted. Close linear links are established for these dependencies ($r=0.4287$, $r=0.4796$, $r=0.4142$ and $r=0.5449$, respectively). For other groups of heavy metals, there are no reliable dependencies. Most of the values of the coefficients varied in the range from 0.02 to 0.04, and their upward shift occurred with an increase in the concentration of $\text{Cu}^{2+}>4 \, \text{mg/kg}$, $\text{Cr}^{2+}>0.8 \, \text{mg/kg}$, $\text{Zn}^{2+}>20 \, \text{mg/kg}$, $\text{Ni}^{2+}>2.5 \, \text{mg/kg}$, $\text{Co}^{2+}>1.0 \, \text{mg/kg}$.

From the data presented, it can be seen that there is a clear tendency to a straight-linear correlation between the fluctuating asymmetry coefficient and the cadmium, copper, nickel and zinc content in bottom sediments, which show clear reactions in all the plant species studied. Thus, the most sensitive to the presence of $\text{Cu}^{2+}$ were the species *Potamogeton perfoliatus* L. and *Nuphar luteum* (L.) Smith, which showed the content of this pollutant at a concentration of 0.6 MPC or Category 3 (Class II) for this pollutant, the species *Salvinia natans* (L.) *All.* reacted to a high content of $\text{Cu}^{2+}>1.3 \, \text{MPC or Category 4 (Class III).}$

All these species were indicated for the content of mobile forms of chromium cations at a concentration of more than 0.8 mg/kg or 0.13 MPC, nickel – 0.62 MPC, cobalt – 0.1 MPC, zinc – 0.52 MPC.

6. Discussion of the results of the study on the application of the method of short-term bioindications in aquatic ecosystems

The main load on the territory of Polissya of Ukraine is due to the receipt of organic substances and organogenic elements from the catchment area, which is largely plowed up and not adequately forested. Comparison of water purity classes of rivers determined with the help of laboratory methods with the results of the proposed method of instantaneous bioindication and indices of fluctuating asymmetry of macrophytes showed close correlation dependences for practically all indicators.

A sufficiently high degree of reliability of the obtained data allows us to state that the use of this method for water bodies of type 2 (large and medium rivers, as well as lakes and artificial reservoirs) will allow obtaining data on the quality of water bodies. At the same time, the study of the most common, within the limits of the accounting points, three plant species showed a high degree of response to anthropogenic pressure. This allows the implementation of a system of instantaneous biomonitoring of water quality at critical points.

Thus, within the areas of increased anthropogenic pressure, deformations as an asymmetry shift of leaf blades increased significantly. It is proved that under the conditions of anthropogenic impact on the ecosystems, the asymmetry index has higher values than points with the less anthropogenic load.

The obtained data on the distribution of the coefficients of asymmetry of leaf blades allow for a more accurate determination of the causes for phytocenosis.

Important data on the ecological assessment of water quality were obtained when estimating the fluctuating asymmetry of water macrophytes as a function of the concentration of heavy metals in bottom sediments and coastal soils. At the same time, the rectilinear correlation between...
the coefficient of the fluctuating asymmetry and the content of all groups of mobile forms of heavy metals in bottom sediments was clearly seen in the morphogenetic parameters of all the plant species studied.

Among the shortcomings of this study, it should be noted that it is impossible to determine exactly which of the factors provoked this or that deviation of the morphogenetic parameters of macrophytes. Identification of any deviation from the norm will require the use of additional methods for analyzing surface water quality. At the same time, variations in the development of higher aquatic plants may indicate the presence of anthropogenic influence and deterioration of environmental parameters. At the same time, the use of biomonitoring based on macrophyte reactions prevalent in Eastern Europe makes it possible to determine the possible negative influence exerted on individual species and their ecological communities. This greatly facilitates the monitoring of the quality of aquatic ecosystems by monitoring services and makes it possible to implement the recommendations of the EU Water Framework Directive 2000/60/EU in conducting an environmental assessment of water bodies in Eastern Europe. It may also be useful to implement the practice of developing calibration scales for evaluating the morphogenetic reactions of macrophytes in vivo, which can be a subject of further research.

Another shortcoming is the impossibility of implementing a biomonitoring system in places where the species studied have not been particularly widespread; therefore, further development of the list of indicator plants for revealing negative impacts on aquatic ecosystems should be noted among the prospects for further research.

### 7. Conclusions

1. When comparing chemical analysis data and fluctuating asymmetry coefficients, the following is revealed: an increase in anthropogenic pressure on the state of the aquatic medium leads to a shift of the polynomial of the asymmetry coefficient distribution by 0.093 units for *Potamogeton perfoliatus* L. 0.0074 – for *Salvinia natans* (L.) All. and by 0.143 – for *Nuphar luteum* (L.) Smith. And in the case of a strong anthropogenic pressure, the basic values of the polynomials can be shifted by 0.0145 units for *Potamogeton perfoliatus* L. and *Salvinia natans* (L.) All. and by 0.0201 – for *Nuphar luteum* (L.) Smith.

2. An assessment of the quality of the aquatic medium according to the indicator of developmental stability in the form of the fluctuating asymmetry coefficient of the leaves of the plant-indicators is justified, since this is confirmed by a number of estimates of other criteria. The indicator species reacted to elevated concentrations in bottom sediments:

   - **Cu**: 0.6–1.3 MPC or Category 3–4 (Class II–III);
   - **Cr**: 0.13 MPC, **Ni**: 0.62 MPC or Category 2 (Class II);
   - **Co**: 0.16–0.2 MPC;
   - **Zn**: 0.65–0.87 MPC or Category 3–4 (Class II–III), in coastal soils:
   - **Cu**: 0.6 MPC;
   - **Cr**: more than 0.13 MPC;
   - **Ni**: 0.62 MPC;
   - **Co**: 0.1 MPC;
   - **Zn**: 0.32 MPC.

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