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The sex System of the Population of *Sagittaria Saittifolia* as an Indicator of the Ecological Status of a River

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Abstract. The ecological state of small rivers that define the water regime of the territory, as well as the water supply of larger rivers, the quality of their waters is a matter of a serious concern. The ecosystems of watercourses in urban areas exposed to pollution by industrial and domestic wastes are under the maximum anthropogenic load. Bioindicative methods, which let to assess directly the status of aquatic ecosystems and their individual components, occupy an important place in the system of ecological monitoring of water bodies. Plants respond to toxicants in an aquatic habitat by the abnormality of biosynthesis of chlorophyll, carotenoids and other pigments, change of mineral balance, phytohormones, which leads to a change in their reproductive differentiation.

In this regard, water quality assessment was carried out on the basis of chemical analysis and bioindication concerning Sagittaria sagittifolia populations in the upper and middle stream ways of the river Tyosha flowing through the Nizhny Novgorod region.

The article presents the results of some organoleptic and chemical parameters such as pH level of overall hardness, concentration of nitrates and nitrites, ions of ammonium, phosphates, mercury, concentration of oxygen and carbon dioxide in the water. It was detected that there were exceeding quantities of ammonium ions, nitrite ions, phosphates in the water of all ranges of the river Tyosha, and also high content of iron in the downstreams of the river. The findings suggest that the reproductive structure of arrowhead inflorescences can serve as a bio-indicator of the degree of water pollution. The harder the environmental conditions of the plant habitat in the water body, the more intensive is a shift in the ratio of flowers to the feminine side. It has been shown that due to the deterioration of environmental conditions, the number of compound inflorescences of the arrowhead and the numbers of whorls in them decrease and, on the other hand, there is an increase in the percentage of plants with simple typical inflorescences. A credible shift in the reproductive ratio of arrowhead inflorescences to the female side is spotted on the downstream river station near Lukoyanov and industrial community Shatky, where the complex of environmental factors for its growth is the least favorable. It was established that anthropogenic pollution of the river has a negative effect on the reproductive sphere of Sagittaria sagittifolia, causing a reduction in pollen fertility, which is confirmed by the chemical analysis of water.

On the basis of the studies the assessment of the ecological status of the water in the Tyosha river was given.

Keywords: anthropogenic load, bio-indication, bio-indicator, pollen fertility, sex differentiation, reproductive structure.

I. INTRODUCTION

The ecological state of small rivers in Russia is alarming. Small rivers define the water regime of the territory, as well as the water supply of larger rivers, the quality of their water [6]. A particularly acute situation is connected with watercourses within the city limits, where their ecosystems are polluted by industrial, municipal and rainfall wastes [15]. In the Nizhny Novgorod region, there are more than 9 thousand water bodies, among which small rivers are very important. Many of them are degraded today: they have become shallow; their banks are demined, their water quality is deteriorating. Among the most dangerous pollutants of freshwater reservoirs are oil products, heavy metals, pesticides. The sources of biogens are fertilizers coming from the fields, and wastes from livestock farms [3]. Thus, the relevance of the study of the ecological state of small rivers is obvious.

An important role in assessing the state of the rivers is played by biological control methods that allow a direct estimation of the state of aquatic ecosystems and their individual components. The presence of bio-indicators and their condition help to reveal the changes in the environment. In plants pollutants often transgress the biosynthesis of chlorophyll, carotenoids and other pigments, impact the mineral balance, phytohormones, causing changes in sex differentiation.

The issue of the day is to find adequate test-objects reflecting actual ecological state of the environment. One of these bioindicators is *Sagittaria sagittifolia*. As one of the main environmental

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indicators determining the status of the environment, we have chosen the reproductive ratio. According to modern scientific views gender is not only a means of reproduction, but also an effective tool of the evolution, responsive to directional change in the environment [4]. According to A.G. Sidorsky [13], the reproductive structure of inflorescences of the arrowhead can serve as a bio-indicator of the degree of water pollution. Petroleum products, surfactants in high concentrations lead to a shift in the sex differentiation of plants to the female side and the increase in the percentage of populations of plants with simple typical inflorescences. The greater the ratios of flowers of female type, the harder are the environmental conditions of plant habitats [11].

The aim of this work was to evaluate the water quality using a number of chemical analyzes and bio-indication of *Sagittaria sagittifolia* populations in the upper and middle stream of the river Tyosha, flowing through the southern part of the Nizhny Novgorod region.

II. MATERIALS AND METHODS OF RESEARCH

The object of the research of river water pollution is Old-World arrowhead (*Sagittaria sagittifolia*) - a perennial herbaceous plant. Its underwater leaves are of simple elongated shape, floating ones – elliptical and above-water leaves are shaped like an arrow. The flowers are diclinous, three pieces form an acervulus, have a green trimerous bell and three white with rose base petals. The material was collected at 4 stations of the river Tyosha with a different anthropogenic load (Fig. 1).

Fig. 1. Sketch-map of sample drawing on the river Tyosha (✦ - points of sample drawing)

The first point – is the Tyosha River, downstream of Lukoyanov. The main pollutants of the river in this area are municipal sewage of the city and the pig-breeding complex near the head of the river; the 2nd Point – is the Tyosha River below the industrial community Shatky. Here, the pollutants come from factories, situated upstream: Shatkovsky plant, JSC "Shatkovsky butter factory", Shatkovsky instrument-making factory, a district hospital.

The second point is the Tyosha River near the village Kozhino, located on its left bank. At this station of the river a watermill had been working for a long time. Currently, there is only a construction made of timber, boulders and concrete, which creates rifts of swirling water. The fourth point is the Tyosha River below Arzamas - quite a large industrial city of Nizhny Novgorod region, with the developed sectors of mechanical engineering and instrument making.

The research was carried out in July 2016. The temperature ranged from 18°C to + 25°C, which corresponds to average long-term observations.

The length of the route along the river was 67 km from Lukoyanov (headstream) to the city of Arzamas, including the monitored river range below the city. In each of 4 points we collected 150 plants that were analyzed in respect to the reproductive structure of their inflorescences. Depending on the depth of the water in which the arrowhead grows, it forms different ecological forms: 1) *Sagittaria sagittifolia Forma typical* – a typical form, with roots at a depth of 15 – 30 cm, with three generations of leaves. 2) *Sagittaria sagittifolia Forma natans* – a form with floating leaves, rooted at a depth of 70 – 85 cm 3) *Sagittaria sagittifolia Forma vallisnerifolia* – a form rooted at a depth of 150 - 500 cm, and having only a long ribbon-like tender leaves. 4) *Sagittaria sagittifolia Forma terrestris* – a terraneous form, usually found on the banks of reservoirs. In this environment they develop a large number of aerial leaves, and the first underwater ones are very few. The botanical literature often presents the notion that the Old-World arrowhead inflorescence resembles a simple whorl, in which one or two lower whorls have a pistillate type, and all the rest - the staminate type of flowers. 200 reproductive forms of the arrowhead were described; they have the following features: some plants have one or two bottom whorls with 2 side clusters forming the complex whorl cluster; the number of whorls varies from 1 to 10. There are rare forms of inflorescence, forming androdioecious flowers instead of pistillate flowers. We used the method of counting of the whorls with male and female flowers, described by A.G. Sidorsky [13]. To determine the pollen fertility we used the acetone-carmine method [9]. Hydro-chemical composition of water has been studied at the research points. To control the thermal pollution we measured water temperature. When there is a difference in the measured temperatures in a few degrees (temperature gradients) we can talk about thermal pollution of water bodies [1].

The validity of differences was assessed by Student's criteria.

III. DISCUSSION AND RESULTS

It is known that plants can utilize and involve in their metabolism some amount of toxic compounds accumulated on the water surface. Some of them can be inactivated in plant cells, and a portion - accumulated in certain organs [12]. In the Old-World arrowhead the reproductive structure of inflorescences changes depending on the environmental conditions [14].

From the above-described environmental forms of the arrowhead we found three forms in the areas of the Tyosha river: *Forma typical* about 25.3%, *Forma natans* (70%), *Forma vallisnerifolia* (5.7%). All arrowhead population consisted of specimen of different ages, had inflorescences at different stages of budding, flowering and fruiting. Analysis of the reproductive structure of inflorescences of the Old-World arrowhead revealed a high variability of the plants on this basis. All plants can be divided into groups of specimen with simple and complex whorled cluster (Fig. 2; 3), with staminate, pistillate and mixed whorls (Fig 4; 5).

Fig. 2. simple (а) и compound (б) inflorescences. Fig. 3 Compound inflorescences.

Fig. 4. Stamina whorls.

Fig. 5. Pistillate whorls.

In the course of the research it was found that the number of compound inflorescences of the arrowhead is minimal (1%) at the first point, which is below the source of domestic sewage in Lukoyanov, and wash-outs from the fields (Fig. 6).

There are much more compound inflorescences of this plant (31%) in the area of Shatky – point II. The number of plants with a compound type of inflorescences at the site near Kozhino at point III (which is far from Shatky and especially from Lukoyanov) is 50 times bigger in comparison with Lukoyanov river station. Downstream, the river takes wastes of Arzamas, and environmental conditions again become less favorable for the growth of *Sagittaria sagittifolia*, which manifested itself in a sharp decrease in the number of compound inflorescences – 11% (Fig. 6).

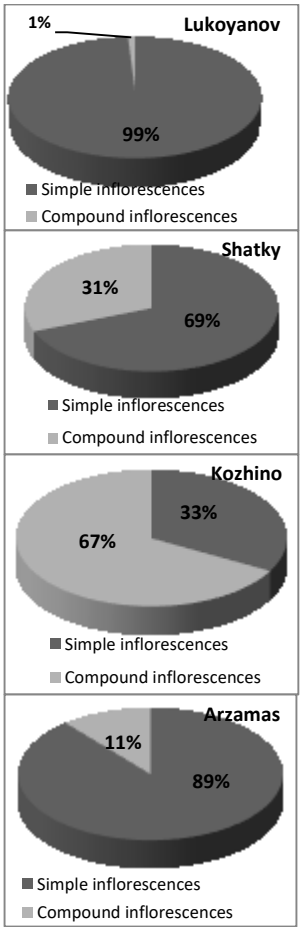

Fig. 6. Dependence of the number of inflorescences of different type on the region of the research

It is likely that the initiation and then bloom of simple inflorescences are faster than that of the compound, which is, apparently, an adaptive property of plants with this type of generative shoot in ecologically unfavorable growing conditions.

In the area of the river near Kozhino – at point III – we recorded the largest number of mixed whorls (with male and female flowers, mainly in the side cluster) - 1.2 specimen, compared to other river stations (Fig. 7).

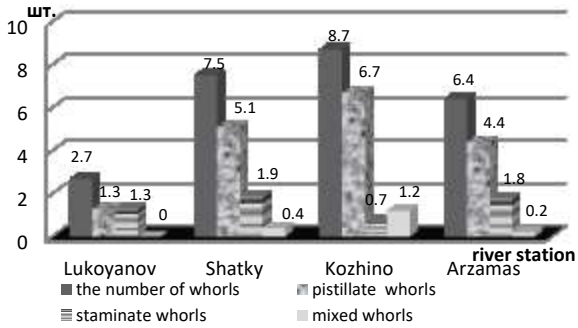

Fig. 7. Dependence of the average number and type of whorls in the arrowhead inflorescences on environmental conditions

Here we saw the maximum number of whorls in inflorescences of the arrowhead (8.7 pcs.), among which we noticed the smallest number of pistillate whorls (0.7 pcs.). A smaller, compared with point III, number of whorls was found at the river station near Shatky (7.5 pcs.), among which there were only 0.4 pc. of the mixed type due to water pollution by local enterprises. The river station below the city of Lukoyanov was characterized by the smallest of all the points of the study number of whorls in the inflorescence (2.7 pcs.), among which there were no mixed, and the number of staminate and pistillate was equal. In point IV, below the city of Arzamas, only 0.2 pc. mixed whorls were noted among inflorescences of the arrowhead.

The results of our research show that due to the worsening conditions of plants' habitat, there is a shift of sex differentiation (ratio of staminate and pistillate flowers) to the female side. This is consistent with the data of other authors [5; 7, 13, 14]. It was found that the maximum number of pistillate flowers is observed on the territory of the river near Lukoyanov – 50.6%, and the minimum – in the area near Kozhino – 14.4%

Table 1
Reproductive Differenciation of Sagittária Sagittifolia at Different Stations of the River. Tyosha

Features	River Station			
	Lukoyanov	Shatky	Kozhino	Arzamas
Overall number of staminate flowers	50.6%	28.6%	14.4%	30.2%
Overall number of pistillate flowers	49.4%	71.4%	85.6%	69.8%
Overall number of pistillate flowers in an inflorescence	4.1 ± 0.2	6.5 ± 0.9	3.7 ± 1.0	5.8 ± 1.6
Average number of pistillate flowers in an inflorescence	4.0 ± 0.3	16.1±3.8	22.2 ± 4.6	13.3 ± 2.8
Ratio (♂/♀) of flowers in an inflorescence	1.0 ± 0.2	2.5±0.3	6.0 ± 1.2	2.3 ± 0.4
Pollen fertility, %	59.0 ± 1.5	61.4±1.6	79.3 ± 1.8	62.0 ± 1.1

The maximum credible shift of the ratio of reproductive forms of arrowhead flowers to the female side is marked at the station of the river near Lukoyanov, where, apparently, the complex of environmental factors for its growth is the least favorable (Table. 1). Here, where there is a small river bed occupancy, a weak current, we found the 1st form of the arrowhead – *Sagittaria sagittifolia Forma typical*.

At the river stations II and IV of the Tyosha we found an approximately equal ratio of male and female flowers (♂/♀) in the inflorescences (2.5 – 2.3), which is almost 2.5 times less compared to station III. Thus, below Lukoyanov, industrial community Shatky and Arzamas there is the feminization of arrowhead populations under the influence of adverse factors of growth.

The most important indicator of functional reproductive differentiation of plants is the viability of pollen, because the conditions of cultivation of plants affect the condition of pollen grains, their viability and fertility [2, 8, 18, 19]. There is a credible difference of pollen grains quality of the arrowhead growing at the studied stations of the river (Table. 1, Fig. 8). The lowest pollen fertility in plants was marked in Lukoyanov downstream at point I, which is 20.3% lower than at point III. At stations II and IV arrowhead pollen also had low fertility, which indicates the adverse growing conditions at points I, II and IV.

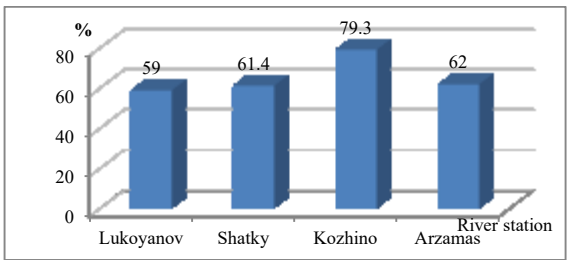

Fig. 8. Pollen fertility, % of *Sagittária sagittifolia* depending on the area of the research

Our bio-indication data are consistent with the hydrochemical analysis of water quality conducted in the laboratory of Arzamas weather station in July 2016. Studies were carried out on the surface water-level of four areas with the use of a variety of quantitative analysis methods: photometric, chromatographic, potentiometric, atomic absorption, titimetric and others. [10]. The data obtained are presented in Table 2.

Table 2
Hydrochemical Analysis of Water Quality of the Studied River Stations of the Tyosha

Exponent	Norm	Research rezults			
		№1 Lukoyanov	№2 Shatky	№3 Kozhino	№4 Arzamas
Temperature difference Δ t°		2 ° C	1.5 ° C	0 ° C	0.5 ° C
Smell in points	2	3	2	1	1
pH	6.5 – 8.5	7.5	7.5	8	8
Total hardness (GH), mg-equ/l	7	5.5	10	24.5	25
Carbonate hardness (KH), mg-equ/l	1.43 – 5.35	5.3	6.5	5.9	6
ammonium (NH_4^+), мг/л	0.5	1.6	1.05	0.65	0.78
Nitrite (NO2-), mg / l	0.2	0.5	0.4	0.2	0.28
dissolved oxygen (O2), mg / l	Not less than 6	8	10	10	10
nitrates (NO3-), mg / l	40	10	1	0.5	0.5
phosphate (PO43-), mg / l	0.05	1	0.6	0.1	0.4
Sulfates mg / l	100.0	723.5	815.6	924.6	954.7
iron (Fe), mg / l	0.1	0.4	0.4	0.1	0.5
carbon dioxide (CO2), mg / l	2 – 10	13	14	10	13
active chlorine (Cl), mg / l	0.00001	0	0	0	0
chromates (Cr6 +), mg / l	0.02	0	0	0	0
Copper mg / l	0.001	0.002	0.004	0.0042	0.0048
Zinc mg / l	0.01	0.01	0.020	0.015	0.019
Mercury mg / l	0.00001	<000001	0.00002	0.00001	0.00008

The most significant change in the organoleptic characteristics of water (the temperature difference (2°C), changes in odor and color of water) are observed at the station of the river near Lukoyanov, indicating that processes caused by eutrophication.

The chemical analysis data indicate a rather high degree of contamination of the river. Hydrogen index of samples was recorded in the normal range (7.5 – 8.0), i.e. water is slightly alkaline which is probably due to the discharge of mine water. Total hardness of water in the Tyosha in the middle stream is high and ranges from 24 – 25 mg / eq. dm3 which is probably due to the natural content of sulfates. In the upstream water hardness is within the norm limits (5.5). Biogenic elements are nitrogen and phosphorus compounds.

At the same time, there are ammonium ions, nitrite ions and phosphates in concentrations exceeding the norm. The concentration of ammonium ions ranged from 0.65 mg / l (1.3 MAC) to 1.5 mg / l (3 MAC) in the area of Lukoyanov. There is a high level of water pollution in the river due to nitrite ions, from 0.28 mg / l (MAC) in Arzamas to 0.5 mg / l (2.5 MAC). Exceeding of the maximum allowable norms of content of nitrate ions in the water was not found. Along with nitrogen, the most characteristic pollutants of the river Tyosha are phosphates. The highest concentration of phosphate (20 MPC) is detected at point number 1. The lowest concentration of phosphate (2 MAC) is noted at point number 3. Phosphate sources are phosphate fertilizers and detergents.

The iron content in the upstream of the river exceeded up to 4 MPC, probably due to iron-bearing clays, and agricultural run-off; in the middle stream the marked level is the MPC. Chromate and chlorine concentration at all areas of the study were below detection limits. The content of heavy metals (copper, zinc and mercury) in water exceeded the maximum allowable parameters: copper 4.2 times, 1.5 – 2 zinc, mercury 2 – 8 times. This may be due to the discharge of waste water from instrument-making plants, electroplating shops, fertilizers washouts from the fields.

IV. CONCLUSION

The study revealed that the number of arrowhead compound inflorescences and the number of whorls in them is reduced due to the deterioration of environmental conditions. The maximum number of this type of generative shoots and the largest number of whorls were found in the area of the Tyosha near Kozhino, and the minimum - in the Lukoyanov area. The maximum credible shift of the ratio of reproductive forms of arrowhead flowers to the female side is marked at the station of the river below Lukoyanov, industrial community Shatky and Arzamas where the complex of environmental factors for its growth is the least favorable.

In the water of the river Tyosha below Lukoyanov there are processes caused by eutrophication. In the water of all the river stations of the Tyosha we detected excess of standards for ammonium ions, nitrite ions, phosphates, sulfates, copper, zinc, mercury, especially in the area of Lukoyanov, Shatky and Arzamas. Iron content exceeds the norm 4 times at the river stations near Lukoyanov and Shatky and 5 times near Arzamas.

On the basis of the research it was found out that in the upstream of the river water is polluted to a greater extent than in its middle stream. To solve the environmental problems of the Tyosha certain activities must be initiated:

1) the discharge of untreated and insufficiently treated wastewater of sewages must be prohibited;

2) control over the observance of the regime of water protection zone of the river and its tributaries;
3) the landscaping of the coastal zone, coastal zone for recreation;
4) monitoring of the ecological state of the river.

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