Development of biotesting method for water quality control

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Abstract. The article presents a method for biotesting natural waters based on the rate of growth of nasturtium pollen tubes on a nutrient medium prepared using tested water samples; pollen tubes of flowering plants are used as an indicator; the waters of a large water-transport artery Moscow Canal and the Klazhmskoye Reservoir are subject to biotesting. For the research, pollen of nasturtium plants, which has convenient for observation sizes of pollen grains and a high initial growth rate of pollen tubes, was selected. Within the process of research, the factors influencing the development of pollen tubes were identified and optimal conditions for germination were proposed. As well, the article attempts to indicate the state of water in the Moscow Canal during the season using the proposed method.

1. Introduction.
The water quality in reservoirs and streams can be assessed using physicochemical and biological methods. Biological assessment methods denote a characteristic of aquatic ecosystem state with regards to the plant and animal population of the reservoir.

Any aquatic ecosystem being in equilibrium with environmental factors has a complex system of mobile biocommunication disrupted under the influence of anthropogenic factors. First of all, the influence of anthropogenic factors, particularly pollution, is reflected in the species composition of aquatic communities and the abundance ratio of their constituent species.

The biological method for assessing the state of a reservoir defines the qualitative state of natural waters based on the biota state analysis without involving instrumental studies using hydrophysical and hydrochemical methods. A reconnaissance assessment of the degree of reservoir pollution according to the composition of aquatic organisms quickly establishes the reservoir sanitary state, determines the degree and nature of pollution and the ways of its spread in the reservoir, and gives a quantitative characteristic of natural self-purification processes. Plankton is a collection of aquatic organisms that are not able to move actively or slowly but do not resist water currents. [1–3]

Phytoplankton is the most important component of aquatic systems. It actively participates in water quality formation and is a sensitive indicator of the state of aquatic ecosystems and the reservoir as a whole.

Emphasizing the importance of bioindication research methods, it should be noted that bioindication identifies the existing or ongoing environmental pollution by the functional characteristics of individuals and the ecological characteristics of organisms’ communities. Gradual changes in the species composition are due to long-term intake of pollutants into the reservoir, and they become obvious in the case of sufficiently high levels of pollution. Thus, the species composition
of aquatic organisms living in a polluted reservoir serves as a final characteristic of the toxicological properties of the aquatic environment for a certain period of time and does not provide its assessment at the time of the study. During the year, there is a regular change in the number and species composition of plankton. This makes the assessment of the aquatic system state using the method of biological indication by quantitative and qualitative indicators of planktonic organisms’ development extremely unstable. Bioindication is a method for assessing anthropogenic load based on the response of living organisms and their communities. Biotesting is the use of biological objects (test objects) under controlled conditions to identify and assess the effect of environmental factors (including toxic ones) on an organism, its individual function or a system of organisms.

The most complete biotesting methods are developed for aquatic organisms and can be used to assess the toxicity of natural waters pollution, wastewater toxicity control, apply express-analysis for sanitary and hygienic purposes, conduct chemical analyzes for laboratory purposes and solve a number of other problems.

Depending on the goals and objectives of toxicological biotesting, various organisms are used as test objects, specifically, higher and lower plants, bacteria, algae, aquatic and terrestrial invertebrates, and others.
3. Results and Discussion.
Physical factors leave a noticeable imprint on the behavior of pollen grains during germination. Specifically, they significantly affect the nature of growth. As well they influence the percentage of pollen grains germination. Before conducting the main experiments to define the role of phytohormones in the fertilization process, we set up a series of experiments to optimize the conditions for growing pollen in vitro.

Factors such as the nutrient medium composition, temperature and acidity of the medium have especially substantial effect on the viability of pollen grains during in vitro germination.

The study of the temperature impact on the growth rate of nasturtium’s pollen tubes showed that pollen did not germinate at +5°C, +40°C and +50°C, the growth rate of pollen tubes slowed down at +10°C, and the growth of pollen tubes was accompanied by the destruction of the apical parts at +30-37°C. The highest growth rates were noted in the options +20-21°C and +26°C. There were no significant differences between these options (Fig. 1).

Information on the influence of the medium’s pH on the viability of pollen varied greatly. The optimum of the medium’s pH was in the range of 4-5. Petunia pollen germinated better in an acidic environment (pH 5), and maize pollen required pH 7. A number of authors confirm that different types of pollen have a pH optimum in a fairly wide range (from pH 1.3 to 7.8). Based on the aforesaid, we studied the effect of acidity on the growth of nasturtium’s pollen tubes (Fig. 2). At pH 2, pollen did not germinate, which was probably due to the complete disorganization of enzymatic processes and the death of pollen grains. Optimal growth was observed at pH values of 4 and 5. Thus, it was confirmed that the effect of acidic growth also occurs during germination of the nasturtium’s pollen tube.

When germinating pollen on nutrient media, it is necessary to introduce sucrose being a nutrient substrate into the composition of the medium. The maximum percentage of germination and the greatest length of the pollen tube are observed at a strictly defined concentration of sucrose.

Nasturtium pollen appeared to be very sensitive to the concentration of sucrose in the nutrient medium. The highest growth rates of pollen tubes were observed in the variant with a 20% sucrose concentration (Fig. 3). With a further increase in the sucrose content to 30-40%, the growth rate of pollen tubes slowed down sharply. In a nutrient medium that did not contain sucrose, not a single case of germination of pollen grains was observed.

![Graph](image-url)  
**Figure 1.** Dynamics of growth of nasturtium’s pollen tube depending on temperature under experimental conditions
Figure 2. Influence of environment acidity on dynamics of pollen tubes growth

The pollen of most species cannot germinate in aqueous sucrose solutions. Therefore, agar-agar or gelatin was used to compact the medium. We chose agar-agar as a purer substance that practically does not contain mineral salts.

Figure 3. Influence of sucrose concentration on growth dynamics of nasturtium’s pollen tubes

The studied effect of this gelling agent concentration determined that the optimal content of agar-agar in the nutrient medium for nasturtium pollen was 1%. An increase in the concentration of agar-agar from 5 to 10% significantly reduced the growth rate of pollen tubes (Fig. 4). A further increase or
decrease in the concentration of agar-agar in the nutrient medium, almost completely inhibited the viability of pollen grains.

In all our further experiments, we germinated nasturtium pollen in vitro, taking into account the above results.

Inhibition of pollen germination and pollen tube growth at high sucrose concentrations in the nutrient medium was apparently due to an increase in the osmotic pressure of the medium and the resulting decrease of the water flow into the pollen grain. We made the assumption that the growth of the pollen tube at the first stage was primarily determined by the water flow, and not by enzymatic processes. To test this assumption, we carried out an experiment on the germination of pollen grains on a nutrient medium, where distilled water was replaced with heavy water (D$_2$O).

Heavy water, as is known, dramatically slows down biological processes and has a toxic effect on living organisms, inhibiting most of the enzymatic processes [9]. When replacing ordinary water with heavy water, the growth rate of the pollen tube decreased by about 1.8 times, which was slightly higher than the theoretical isotope effect for heavy water equal to 1.4 (Fig. 5).

![Figure 4. Influence of agar-agar concentration in a nutrient medium on the growth dynamics of nasturtium’s pollen tubes](image)

Probably, a slightly greater decrease in the growth rate of the pollen tube was associated with the negative effect of heavy water on enzymatic systems and (or) on the water flow into cells. However, the fact that growth by stretching did occur indicates that the control of pollen tube growth was mainly due to passive water transportation through the surface of the pollen grain, although enzymatic processes also took place but played a minor role.

To confirm this assumption, we conducted an experiment by increasing the permeability of the membranes of pollen grains by adding a membranotropic agent DMSO at a concentration of 2% to the medium (higher concentrations of the membranotropic agent led to the pollen grains destruction). If the process of pollen tube growth would mainly depend on the synthesis of the cell wall components, the use of DMSO would lead to a decrease in the growth rate of the pollen tube when using D$_2$O. However, in our experiments, DMSO supplements stimulated the growth of the pollen tube in both ordinary and heavy water by approximately the same amount (Fig. 5). The results of this experiment
confirmed the assumption that at the initial stages, the growth of the pollen tube was determined primarily by the rate of water absorption, and not by the growth of cell wall matter.

A series of experiments aimed to identify the influence of physical and chemical factors on the growth rate of the pollen tube defined that this indicator could be used as an express test of water pollution.

![Figure 5. Influence of heavy water content on growth dynamics of nasturtium’s pollen tubes](image)

We have carried out more than 200 laboratory experiments to study the impact of water on the preparation of a nutrient mixture using water taken from Moscow Canal in the sector where it flows into the Klyazminskoye reservoir. Bottled drinking water was taken as a control. The research data are presented in table 1.

**Table 1.** Results of biotesting water quality of Moscow Canal using proposed biotest based on nasturtium’s pollen tubes

| Month    | Change in the length of pollen tubes (c.u.) in time |
|----------|-----------------------------------------------------|
|          | 30 min   | 60 min   | 90 min   |
| Control  | 2.13±0.10| 3.29±0.09| 4.11±0.09|
| January  | 1.22±0.09| 2.97±0.10| 3.23±0.05|
| February | 1.25±0.02| 3.06±0.13| 3.28±0.03|
| March    | 1.32±0.08| 2.99±0.13| 3.45±0.13|
| April    | 1.08±0.07| 2.55±0.10| 3.13±0.08|
| May      | 1.12±0.07| 2.97±0.08| 3.13±0.05|
| June     | 1.22±0.08| 2.40±0.12| 3.23±0.13|
| July     | 1.29±0.17| 3.01±0.08| 3.42±0.06|
| August   | 2.16±0.10| 3.25±0.12| 4.08±0.08|
| September| 2.12±0.10| 3.01±0.12| 3.89±0.10|
| October  | 1.79±0.12| 2.87±0.11| 3.68±0.06|
| November | 1.28±0.09| 2.74±0.10| 3.18±0.05|
| December | 1.20±0.09| 2.97±0.10| 3.13±0.05|
4. Conclusion
As a result of the above experimental studies, a method for bitesting natural waters based on the growth rate of nasturtium’s pollen tubes on a nutrient medium prepared using the tested water samples was proposed. In the course of research, factors influencing the development of pollen tubes were identified and optimal conditions for germination were proposed.

An attempt was made to indicate the state of the water of Moscow Canal during the season using the proposed method. Data in Table 1 show that the differences in the growth rate of pollen tubes in water samples taken by the seasons exceeded the statistical error of determination within 30 minutes. Later, the results were leveled out and the difference did not exceed the measurement error. The proposed method enables to obtain a quantitative assessment of the water quality with minimal costs and within a short period. Further research aimed to identify the correlation of the proposed test with the real content of pollutants in the analyzed water samples is required.

This method can be used for screening and assessing water quality and identifying pollution periods and focuses.

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