

Review by the Official Opponent

of the dissertation submitted by Cao, Boyang for the academic degree
of Candidate of Biological Sciences on the topic: "Influence of biogenic
amines on the growth and biochemical characteristics of microalgae"

(specialty 1.5.15. Ecology)

Cao Boyang's dissertation and research lie at the intersection of ecology and microbial physiology—specifically, the area concerning microbial chemical communication mediated by specialized substances known as autoregulators. Microorganisms possess several such communication systems, the most thoroughly studied being quorum sensing (QS) systems. Some of these autoregulators bear a striking resemblance to neurotransmitters—substances that transmit signals between nerve cells in animals and humans. However, the role of these substances in the life of microorganisms such as phytoplanktonic microalgae—which can be either prokaryotic (cyanobacteria) or eukaryotic (e.g., green microalgae)—has received little research attention.

This gap defines **the relevance and objective of the dissertation**: to investigate the role of neurotransmitters—specifically biogenic amines—in regulating the physiological and biochemical parameters of microalgae. The study's ecological aspect is also highly relevant, given that biogenic amine neurotransmitters are emerging as significant ecotoxicants due to their increasing global production.

The key findings presented for defense are substantiated primarily by the author's own compelling experimental data, the **reliability** of which is beyond doubt. All data were obtained through four to five independent experiments, each with three replicates. The author employed appropriate standard statistical methods, including tests for data normality, Student's test for normally distributed data, and the non-

parametric Kruskal-Wallis H-test for data exhibiting significant skewness and/or kurtosis.

The findings presented for defense are consistent with existing literature.

The novelty of the dissertation lies in the newly established findings regarding the stimulation of growth and—in many instances—the synthesis of fatty acids (particularly valuable polyunsaturated ones) and photosynthetic pigments in microalgal cells (including cyanobacteria) under the influence of biogenic amines. Of particular interest are the data concerning neurotransmitter synthesis by *Daphnia* and river-bankst macrophytes; these findings reinforce the concept of neurotransmitters acting as ecosystem-level signaling agents (ecomones). The novel experimental data underpin the author's theoretical conclusions—specifically, the recognition of neurotransmitters as potential agents of "molecular mimicry" and their classification as a new generation of environmental pollutants.

A further strength of the work is the practical relevance of the study organisms—including the green microalgae *Chlorella vulgaris*, *Scenedesmus quadricauda* and *Haematococcus lacustris*, as well as the cyanobacterium *Limnospira platensis*—which enables the application of the results across various biotechnological fields, such as environmental management, algal biomass production, and the synthesis of target compounds.

The author's original hypothesis regarding the potential "cultivation" of prey (microalgae) by the predator *Daphnia magna* depicts Cao Boyang as a naturalist with broad perspective on the subject of study.

Structure of the reviewed work. The study spans 237 pages and follows a classic organizational structure. It comprises an introduction, a literature review, a methodology chapter, and four chapters dedicated to: 1) the growth-related effects of serotonin, dopamine, norepinephrine, histamine, and acetylcholine on the green microalgae *C. vulgaris*, *S. quadricauda*, and *H. lacustris*, as well as the cyanobacterium *L. platensis*; 2) the impact of these neurotransmitters on fatty acid content and composition within microalgal lipids; 3) the effect of neurotransmitters

on chlorophyll and carotenoid concentrations in microalgal photosystems; and 4) the levels of endogenous neurotransmitters detected chromatographically by the author in *Daphnia* and shoreline plants. Each experimental chapter includes a summary section, followed by a general conclusion and specific findings for the dissertation as a whole. The work concludes with a bibliography listing 184 sources (142 in English and 42 in Russian) and contains 37 figures and 55 tables.

The literature review is extensive; it begins with an overview of "new-generation" environmental pollutants—including active pharmaceutical ingredients, a category that encompasses anthropogenic neurotransmitters. The review then provides detailed information on neurotransmitters regarding both their "traditional" role in animal and human nervous systems and their function as signaling molecules across various components of aquatic ecosystems, including microbiota, phyto- and zooplankton, higher plants, and vertebrates (fish). It is regrettable that the extensive literature review contains no mention of other substances — specifically, the autoregulators of inter-organism communication such as acyl-homoserine lactones—which certainly worth inclusion.

The author also discusses the research subjects — microalgae — devoting a significant number of pages to their biotechnological applications.

The "Materials and Methods" chapter details the characteristics of the specific subjects studied: green microalgae and cyanobacteria, as well as *Daphnia* and shoreline plants in which neurotransmitters were identified. Experimental procedures regarding the effects of biogenic amines on microalgal growth characteristics are described in detail, including the ratios of vegetative, palmelloid, and encysted cells (the latter being rich in biotechnologically valuable carotenoids) in the case of *H. lacustris*. Separate sections cover gas chromatography-mass spectrometry (used to determine the fatty acid composition of lipids and photosynthetic pigments) and high-performance liquid chromatography (used to identify neurotransmitters in the test subjects), as well as statistical procedures. It is somewhat regrettable that the author includes photographs of *Daphnia* and higher

plants but not of the microalgae (with the sole exception of *H. lacustris*, shown in experimental Chapter 3).

Experimental Chapters 3 through 5 follow a consistent structure: data on green microalgae are presented first, followed by data on the cyanobacterium *L. platensis*, and concluding with a summary. The experimental chapters are well-illustrated. The generalizations presented therein relate to the putative mechanisms of action of neurotransmitters: primarily as stimulators of growth and the synthesis of fatty acids and photosynthetic pigments at concentrations of 0.1–10 μM , and conversely, as inhibitors at higher concentrations. The author hypothesizes a potential receptor-mediated interaction; however, further research employing modern molecular-biological approaches is required to substantiate this claim. It is also advisable to consult existing databases on 1) neurotransmitter receptors and 2) microalgal proteomes in order to compare the data.

All results are summarized in the General Conclusion section, which posits that neurotransmitters pose a risk as ecotoxins that "mimic" signals produced by ecosystem components. A calculation is also provided demonstrating the cost-effectiveness of using low (micromolar) concentrations of biogenic amines to increase microalgal biomass yield and enhance the synthesis of target products.

The conclusions are fully consistent with the content of the thesis.

The abstract, available in both English and Russian versions, accurately reflects the main content of the dissertation.

Comments on the dissertation. Certain remarks were made in the review text above; the following shortcomings should also be noted.

Point 4 of the research objectives appears unconventional: "To compare one's own results with literature data to assess the role of neurotransmitters as ecotoxins..." After all, any scientific study inherently involves analyzing one's own data and comparing them with the findings of other researchers. It would have been more logical to include "analysis of the obtained data regarding their potential

use in ecological biotechnologies" as a separate objective. Furthermore, the fulfillment of objective No. 4 is not reflected in the "Conclusions."

The method employed by the candidate to assess microalgal growth (microscopy) is quite imprecise and could be improved. It would be more accurate and easier to assess growth based on one or several biochemical markers (protein, pigments), and using multiple markers would have provided more informative results. The fact that growth was determined solely by biomass accumulation for a single test organism means this criticism remains valid.

The text on page 10 of the abstract is somewhat unclear: "The experimental methodology did not involve setting up positive control experiments — which are typical when working with complex biological materials — because pure substances were added."

It is also regrettable that the candidate did not fully notice all the novelty of his primary experimental data. Another important biological effect of neurotransmitters went unnoticed: their influence on the cytodifferentiation of *H. lacustris* microalgae cells. This effect is evident from the way acetylcholine and noradrenaline increased the proportion of palmelloid cells while the number of resting cells remained constant or decreased.

Some stylistic and linguistic errors, as well as typos, were identified in the text, though these do not detract from the overall positive impression. The formatting and font styles of the figures and tables are not always consistent.

Conclusion

Despite the above minor criticisms, the work meets the requirements of M.V. Lomonosov Moscow State University regarding Ph.D. degree theses. The content of the work is in full conformity with specialty 1.5.15. Ecology (Biological Sciences) and the criteria of Articles. 2.1-2.5 of the regulations regarding scientific degrees awarded in M.V. Lomonosov Moscow State University. The design of the thesis

complies with the requirements laid down in the regulations of the degree-awarding council of the M.V. Lomonosov Moscow State University.

To re-iterate, Cao Boyang's thesis is a complete independent work dealing with an important subject and characterized by scientific validity, data credibility, and novelty. Therefore, it complies with the criteria to be met by scientific degree theses, and Cao Boyang deserves being awarded the Ph.D. degree in specialty 1.5.15. Ecology

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