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Impact of Biogenic Amines on the Growth and Biochemical Characteristics of Microalgae

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TABLE OF CONTENTS

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INTRODUCTION

Background. The present thesis is concerned with the effect of neuroactive compounds such as biogenic amines (BAs) on the growth-related and biochemical characteristics of green microalgae (Chlorophyta) and cyanobacteria. This work is directly related to one of the most rapidly developing subfields of modern ecology, *environmental toxicology* that focuses on the detrimental effects of physical, chemical, and biological factors on living organisms as well as their populations and ecosystems (Матвиенко, 2006). Among various toxic factors, the environment including water bodies is increasingly affected by *active pharmaceutical ingredients (APIs).* Some types of APIs are present in the environment in amounts that exceed safe levels (maximum acceptable concentrations, MACs). These dangerous APIs include neuroactive substances such as antidepressants, antihistamine preparations, β-adrenoreceptor antagonists, anesthetics, calcium channel blockers, etc. (Bouzas-Monroy et al., 2022). Aquatic ecosystems are affected by very low concentrations of some APIs because they are identical with or homologous to the signal molecules of diverse hydrobionts. «Presumably, a wide spectrum of aquatic organisms including bacteria, algae, invertebrates, and fishes have receptors for such neuroactive pollutants, and this accounts for their responses to these compounds. Specifically, widely used psychopharmacological preparations such as Prozac (an antidepressant) and amphetamines (cognitive capacity stimulants) cause major disruptions in the functioning of bacterial and algal communities within aquatic ecosystems" (Олескин, Постнов, 2022).

Evolutionarily conserved compounds that perform signaling and regulatory functions in various components of aquatic ecosystems include the neurotransmitters (reviewed: Олескин, 2024) that were tested in the present work for their toxic effects on model organisms. These were acetylcholine (ACh) and the biogenic amines serotonin (5-HT), histamine, norepinephrine (NE), and dopamine (DA). These compounds are not included in the official lists of globally dangerous

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APIs, and they relatively rapidly decompose in the aquatic environment. However, they are likely to be constantly released into the environment with the wastewater of pharmaceutical factories, healthcare institutions, and food-producing plants. Dopamine, norepinephrine, and serotonin are known to be produced by pharmaceutical enterprises in Russia and other countries. For instance, dopamine as a cardiotonic and hypertensive preparation is supplied by the Promomed company in Russia and Admed Arzneimittel in Germany. Acetylcholine (Brain Food™) is utilized as a food additive. Food industry wastewater inevitably contains neurotransmitters that are present in the blood and other biological liquids of animals (notably, 0.5 to 1.5 μM serotonin is contained in the human blood, McPherson and Pincus 2011). Histamine accumulates in food items during their storage, due to the bacterial conversion of histidine to histamine (see: Oleskin, Shenderov, 2020). In addition, neurotransmitters are secondarily produced and accumulated in natural ecosystems under the influence of other humankind-produced ecotoxicants including APIs. For instance, the release of cocaine and other neuroactive pollutants into rivers, e.g., the River Thames in London (Learn, 2018) results in increasing the dopamine concentrations in the organism of eels and other fishes that release dopamine into the environment under stress or after an injury.

In the thesis, humankind-produced neurotransmitters in the environment are considered in connection with their endogenous production by the components of natural ecosystems including unicellular organisms that use them as signals and regulatory agents.

An important specific type of intercellular communication is referred to as quorum sensing (QS) that subsumes various mechanisms of density-dependent regulation of physiological, biochemical, genetic, and behavioral characteristics of microorganisms in a population. Over the course of the last decades, the main classes of quorum sensing signals were characterized, including aromatic signal molecules (exemplified by AI-3) that operate in enterobacteria and other

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microorganisms and are structurally similar (homologous) to catecholamines, a group of amines functioning as neurotransmitters and hormones in animals and humans (Lyte, 2016; Олескин, Шендеров, 2020). Therefore, research on the effects of biogenic amines (BAs) in microalgae is not only of ecotoxicological importance. It also is of much interest in terms of communication between microalgae and other organisms in aquatic or soil ecosystems in which microalgae occur in nature. The dependence of microbial culture growth on the availability of BAs in the medium was predominantly investigated with respect to bacteria, and less attention was paid, in terms of BA effects, to such eukaryotic microorganisms as microalgae as well as to an important subgroup of prokaryotes, *cyanobacteria.* There is only fragmentary data exemplified by the effects of acetylcholine on the microalgae of the genus *Chlorella* (Czerpak et al., 2003; Parsaiemehr et al., 2015) and of dopamine on *Haematococcus lacustris* (Zhao et al., 2025a).

It should be noted that the concept of neurotransmitters as potentially significant ecotoxicants encompasses both their inhibitory effects on the growth and biochemical (fatty acid content in cellular lipids and concentrations of photosynthetic pigments) characteristics of microalgae, which threaten to disrupt the food chains in ecosystems that depend on the phytoplankton, and their possible stimulatory effects, which can lead to uncontrolled proliferation (blooming) of microalgae.

The model organisms belonging to microalgae that were used in this work are of significant biotechnological importance as producers of valuable substances in aquacultural systems where they risk contacting the wastewater of drug and food industry.

Research subjects: the green microalgae *Chlorella vulgaris* Beijer*, Scenedesmus quadricauda* (Turp.) Breb. K-1149*, Haematococcus lacustris* (strains IPPAS H-239 and BM-1), the cyanobacterium *Limnospira platensis* IPPAS B-256, the water flea *Daphnia magna,* and the flowering plants *Plumeria rubra* L. cv. *acutifolia, Syzigium jambos* (L.) Alston, *Buxus megistophylla (Euonymus japonicas* cv.

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aureoma), and *Cinnamomum bodinieri* Levl.

Main research topic: Influence of neurotransmitters on the growth-related and biochemical characteristics of the test subjects

Goal of the present work: Conducting detailed studies on the impact of biogenic amines on the growth-related and biochemical characteristics of such phytoplankton components as green microalgae and cyanobacteria within the context of the regulatory and signaling functions of these substances with respect to various constituents of aquatic ecosystems.

Main Objectives:

1. Estimating the effects of BAs (5-HT, histamine, DA, NE, and ACh) on the growth-related characteristics of the green microalgae *Chlorella vulgaris, Scenedesmus quadricauda,* and *Haematococcus lacustris* as well as of the cyanobacterium *Limnospira platensis.*

2. Performing the analysis of the impact of these neurotransmitters on the fatty-acid composition of the lipids in the aforementioned green microalgae and cyanobacteria during the growth of their cultures.

3. Evaluating the influence of the tested neurotransmitters on the content of photosynthetic pigments including biotechnologically important products during the development of microalgal cultures.

4. Juxtaposing the data obtained with those available in the literature for assessing the role of neurotransmitters as ecotoxicants whose specific effects are due to the fact that they also represent endogenous signals or regulators produced by the components of natural ecosystems.

Novelty of the data obtained. The work demonstrated for the first time the stimulatory effects of the biogenic amines (BAs) dopamine, norepinephrine, serotonin, histamine, and histamine at low (micromolar) concentrations on the growth and the contents of fatty acids and photosynthetic pigments the green microalgae *Chlorella vulgaris, Scenedesmus quadricauda,* and *Haematococcus*

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lacustris, as well as in the cyanobacterium *Limnospira platensis,* which may result in uncontrollable growth of these phytoplankton components in the presence of neurotransmitters as ecotoxicants.

Theoretical and practical importance of the work. The present work demonstrates the effects of low concentrations of neuroactive compounds on the growth characteristics of green microalgae and cyanobacteria, the fatty-acid composition of their lipids, and the photosynthetic pigment content; from the ecotoxicological viewpoint, this poses the threat of uncontrollable proliferation of these phytoplankton components even in the presence of trace amounts of neurotransmitters in the wastewater of drug and food industry. From the viewpoint of aquatic ecology and hydrobiology, this testifies to a plausible contribution of the tested neurotransmitters to the chemical dialogue between these representatives of the phytoplankton and various neurotransmitter-producing ecosystem components. In addition, the results of the present work are usable for educational purposes in terms of teaching microbiology, algology, ecology, or hydrobiology.

Methodology: the present work is based on a complex of cultivation techniques, spectrophotometric and chromatographic methods, genomic analysis tools, and statistical procedures (see the Materials and Methods section for more details).

Main points of the present work:

1. Micromolar concentrations of the neurotransmitters DA, NE, 5-HT, histamine, and ACh promote biomass accumulation in the green microalgae *Chlorella vulgaris* Beijer, *Scenedesmus quadricauda* (Turp.) Breb. K-1149, and *Haematococcus lacustris* IPPAS H-239 and BM-1 (only 5-HT and ACh stimulate this strain). 5-HT, ACh, and histamine also facilitate biomass accumulation in the cyanobacterium *Limnospira platensis.* These results point to a possible role of BAs as ecotoxins that pose the threat of water blooming and eutrophication.
2. At least some of the tested neurotransmitters increase the content of photosynthetic pigments (chlorophylls and/or carotenoids) in microalgal cells.

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In *H. lacustris*, neurotransmitters augment the percentage of palmelloid cells containing both chlorophylls and carotenoids and/or that of resting cells that accumulate only carotenoids including the important antioxidant astaxanthin.

3. ACh and some of the other neurotransmitters, in a species-specific fashion, increased the fatty acid content of the lipids of the microalgae/cyanobacteria.
4. The neurotransmitters influenced the ratio between saturated and unsaturated fatty acids in the microalgae under study. For instance, NE in *H. lacustris* and *L. platensis* elevated the percentage of unsaturated fatty acids; conversely, it increased the saturated fatty acid share in *S. quadricauda*.

Credibility of the results of this work. Their presentation. At least 4-5 independent repeats of all experiments contained in this work were conducted. The mean values and standard deviations were calculated, and statistical tools such as the Student's *t*-test and the non-parametric Kruskal-Wallis test were applied.

The main ideas and data of the qualifying scientific work were presented at the following pan-Russian and international conferences: Lomonosov Readings (Moscow, 2021, 2024), 3rd Pan-Russian Microbiology Congress (Pskov, 2021); Seventh Puschino Conference on the Biochemistry, Physiology, and the Biospheric Role of Microorganisms (Puschino, 2021, Puschino); the International Roundtable Conference on Human Development and Human-centered Health Protection (Moscow, 2022); Tenth Sabinin Readings on the Ecological Physiology of Aquatic Phototrophs (Moscow, 2023), and the 5th Edition of International Nutrition Research Conference (Valencia, 2023).

The author's contribution. The author personally planned and carried out all studies on the neurotransmitter impact on the growth characteristics of microalgae and cyanobacteria, spectrophotometric measurements, statistical methods, and the sample preparation procedures used for determining fatty acid and pigment and neurotransmitter concentrations. The author produced the entire English version of

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the thesis and is indebted to his supervisor, Prof. Alexander V. Oleskin, for producing the Russian version.

Publication of the results of the present work. The main points of the thesis were presented in 11 papers of which 7 papers are in journals recommended by the Degree-Awarding Commission (Council) MSU 015.5. The thesis author's contribution was 0.3 of 1.0 p.s. in article [1], 0.6 of 1.0 p.s. in article [2], 0.9 of 1.5 p.s. in article [3], 0.8 of 1.5 p.s. in article [4], 0.6 of 1.2 p.s. in article [5], 0.6 of 1.4 p.s. in article [6], 0.8 of 1.4 p.s. in article [7], and 1.0 of 1.8 p.s. in article [8], 0.4 of 0.8 p.s. in article [9], 0.4 of 0.8 p.s. in article [10], and 0.3 of 0.7 p.s. in article [11].

Publications in peer-reviewed scientific periodicals recommended by the Degree-Awarding Council of Moscow State University:

1. **Cao B.,** Chivkunova O.B., Fedorenko T.A., Solovchenko A.E., Lobakova E.S., Oleskin A.V. Neurochemical pollutants in aquatic ecosystems: modes of interaction with microalgae // Life of the Earth. — 2026. — vol. 48, № 1. – pp. 46-57. EDN: INBPXG. Impact factor 0.479 (РИНЦ). 0.3 of 1.0 p/s.

2. **Cao B.,** Oleskin A.V. Neurochemical pollutants in the aquatic medium: the results of studies with model organisms (microalgae) // Life of the Earth. — 2025. — vol. 47, № 4. pp. 550-562. EDN: PMKRCV. Impact factor 0.479 (РИНЦ). 0.6 of 1.0 p/s.

3. Олескин А.В., **Цао Б.** Взаимодействие нейротрансмиттеров с микроводорослями: концептуальные и практические аспекты // Вестник Московского университета. Серия 16: Биология. – 2023. – Т. 78, № 3. – С. 146–159. EDN: FEQZAF. Impact factor 0.443 (РИНЦ). 0.9 of 1.5 p/s.

4. **Цао Б.,** Федоренко Т.А., Чивкунова О.Б., Соловченко А.Е., Лобакова Е.С., Олескин А.В. Влияние биогенных аминов на накопление биомассы, содержание фотосинтетических пигментов и жирных кислот у цианобактерии *Limnospira platensis* IPPAS B-256 // Микробиология. — 2026. — Т. 95, № 1. — С. 68–84. EDN: ARCZUO. Impact factor 1.000 (РИНЦ). 0.8

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of 1.5 p/s. [Cao B., Fedorenko T.A., Chivkunova O.B., Solovchenko A.E., Lobakova E.S., Oleskin A.V. Impact of neurotransmitters on biomass accumulation, photosynthetic pigment content, and fatty acid composition of the cyanobacterium *Limnospira platensis* IPPAS B-256// Microbiology. — 2026. — vol. 95, no. 1. — pp. 82–95].

5. **Цао Б.**, Федоренко Т.А., Чивкунова О.Б., Соловченко А.Е., Лобакова Е.С., Олескин А.В. Влияние нейротрансмиттеров на содержание фотосинтетических пигментов у *Haematococcus lacustris* (штаммы IPPAS H-239 и ВМ-1) // Прикладная биохимия и микробиология. – 2025. – Т. 61, № 5. С.865-871. EDN: BOFTHW. Impact factor 1.087 (РИНЦ). 0.6 of 1.4 p/s. [**Cao B.,** Fedorenko T.A., Chivkunova O.B., Solovchenko A.E., Lobakova E.S., Oleskin A.V. Impact of neurotransmitters on the photosynthetic pigment content of the green microalga *Haematococcus lacustris* (strains IPPAS H-239 and BM-1) // Applied Biochemistry and Microbiology. – 2025. – vol. 61. № 5. – pp.865-871].

6. **Цао Б.,** Федоренко Т.А., Чивкунова О.Б., Соловченко А.Е., Лобакова Е.С., Олескин А.В. Влияние нейротрансмиттеров на состав жирных кислот и фотосинтетических пигментов зеленой микроводоросли *Scenedesmus quadricauda* // Прикладная биохимия и микробиология. – 2024. – Т. 60, № 5. С.487-498. EDN: QTJSYO. Impact factor 1.087 (РИНЦ). 0.8 of 1.4 p/s. [Cao B., Chivkunova O.B., Solovchenko A.E., Lobakova E.S., Oleskin A.V. Impact of neurotransmitters on the fatty acid composition and pigments of the green microalga *Scenedesmus quadricauda* // Applied Biochemistry and Microbiology. – 2024. – vol. 60. № 5. – pp.833–843].

7. Олескин А.В., **Цао Б.** Микроводоросли как объекты биомедицинских технологий: пробиотики, пребиотики, метабиотики // Прикладная биохимия и микробиология. — 2022. – Т.58, № 6. – С. 635-648. EDN: DITOVF. Impact factor 1.087 (РИНЦ). 1.0 of 1.8 p/s.). [Oleskin A.V., **Cao B.** Microalgae in terms of biomedical technology: probiotics, prebiotics, and metabiotics // Applied

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Biochemistry and Microbiology. – 2022. – vol. 58. № 6. – pp. 635–648].

Other publications concerning the subject of the thesis:

8. **Cao B.,** Chivkunova O.B., Solovchenko A.E., Lobakova E.S., Oleskin A.V. Neurotransmitters as ecopollutants: impact on the fatty-acid composition and photosynthetic pigment content of *Chlorella vulgaris* // Russian Journal of Ecosystem Ecology. — 2025. — vol. 10, № 4). – pp. 1-12. EDN: TDAXFF. Impact factor 0.500 (РИНЦ). 0.6 of 1.2 p/s.

9. Oleskin A. V., Postnov A. L., **Cao B.** Impact of biogenic amines on the growth of green microalgae // Journal of Pharmacy and Nutrition Sciences. – 2021. – vol. 11. – pp. 144–150. EDN: FQOETT. 0.4 of 0.8 p/s.

10. Oleskin A. V., Postnov A. L., **Cao B.** Impact of biogenic amines on the growth of a Chlorella vulgaris culture // Journal of Pharmacy and Nutrition Sciences. – 2021. – vol. 11. – pp. 49–53. EDN: CPYLNQ. 0.4 of 0.8 p/s.

11. **Cao B.,** Oleskin A. V., Vlasova T. A. Detecting biogenic amines in food and drug plants with HPLC: medical and nutritional implications // Journal of Pharmacy and Nutrition Sciences. – 2020. – vol. 10, №. 3. – P. 88–91. EDN: NVKLWL. 0.3 of 0.7 p/s.

Structure and size of the thesis. This work includes an Introduction, the Literature Review, Materials and Methods, Results (composed of 4 subsections), Discussion (not included in the synopsis), Conclusion, and the Reference List that counts 184 references, including 142 sources in English. The work is 229 pages long. It contains 37 figures (including graphs) and 55 tables.

Abbreviations: 5-HT, serotonin; ACh, acetylcholine; API, active pharmaceutical ingredient; BA, biogenic amine; DA, dopamine; DHA, docosahexaenoic acid; DMSO, dimethylsulfoxide; DOC, dissolved organic carbon; EPA, eicosapentaenoic acid; FA, fatty acid; HPLC, high-performance liquid chromatography; MUFA, monounsaturated fatty acid; NE, norepinephrine; PBR, photobioreactor; PUFA, polyunsaturated fatty acid; QS, quorum sensing; ROS,

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reactive oxygen species; SFA, saturated fatty acid.

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1. LITERATURE REVIEW

1.1. NEUROTRANSMITTERS AMONG NEW-GENERATION ECOTOXICANTS

The present work deals with the interaction of biogenic amines (functioning as neurotransmitters in the animal nervous system) with several important representatives of the phytoplankton of aquatic ecosystems, such as the green microalgae *Chlorella vulgaris, Scenedesmus quadricauda,* and *Haematococcus lacustris* as well as the cyanobacterium *Limnospira platensis*. The tested BAs form a potentially important part of the human-produced load for natural ecosystems and their components.

It was emphasized in the Introduction that humankind increasingly pollutes the environment with ecotoxicants. "An increasing global population is placing great strain on over 65% of the Earth's rivers with chemical pollution as one of the main causes of degradation and biodiversity loss in aquatic ecosystems" (Kay et al., 2017). An increasingly significant contribution is made by active pharmaceutic ingredients (APIs) contained in pharmaceuticals and personal care products (PPCPs). These substances are released into the environment with the wastewater of health care institutions and the facilities of pharmaceutical, cosmetic, and food industry; they also form a part of municipal waste. Importantly, "pharmaceuticals are routinely present in semi-rural and urban rivers and require management alongside more traditional pollutants" (Kay et al., 2017).

It was estimated that between 30 and 90% of the orally administered does of pharmaceuticals are excreted with the urine of patients utilizing them (Mudgal et al., 2013). Significant amounts of pharmaceuticals are used in veterinary medicine and enter the soil or the water via the animal organism. Pharmaceuticals are also released into the environment by wastewater treatment plants (WTPs), and they frequently pass their filters. They are present in WTP sludge and landfill leachate (Aydin et al., 2021).

In 2022, at least 23 representatives of pharmaceuticals were present in the

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environment at concentrations exceeding the safe levels, based on the data of the analysis performed in 104 countries and at 1052 points of the planet (Buozas-Monroy et al., 2022). Undoubtedly, these chemicals cause serious environmental damage. «In particular, chloropromazin and other preparations used in psychiatry (antipsychotics) accumulate in the food chains of natural ecosystems, modifying, even at low concentrations, the behavior of living organisms within these ecosystems» (Олескин, Постнов, 2022).

In spite of the relative instability of some pharmaceuticals (including the neurotransmitters considered below) in the environment, their ecotoxic potential depends on their capacity to affect living organisms and whole ecosystems at very low concentrations. If nanograms or micrograms of pharmaceuticals per liter are chronically released into the soil, water, and other media, they supplement the effects produced by the human-produced harmful cocktail and synergistically interact with them. In particular, this results in disrupting reproduction processes in amphibians, fish, molluscs, etc. (Chakraborty et al., 2023). For instance, hormones of the estradiol group cause feminization and ovary development in male fish; similar effects are exerted on frogs, alligators, or mussels (Chakraborty et al., 2023). Levonorgestrel, the active component of hormone-based prophylactics, accumulated in the rainbow trout organism that had been in contact with the wastewater of Swedish cities and reached concentrations of 8.5-12 ng/mL, which is close to the levonorgestrel level in the blood of its human consumers (Fick et al., 2010).

Generally, at least 9 types of pharmaceutical are classified as dangerous ecotoxicants. These are hormones, antibiotics, lipid metabolism regulators, beta-blockers, anticonvulsants, antineoplastics, and diagnostic contrast substances. A serious risk is posed by personal care products such as fragrances, preservatives, disinfectants, and sunscreen agents (Wang, Wang, 2016).

The effects of very low concentrations of some pharmaceuticals manifest themselves in aquatic ecosystems because they are identical with or homologous to

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signals that are produced by various hydrobionts. Many preparations exert a specific influence on the nervous cells of zooplankton or nekton representatives. Even aquatic organisms that lack a nervous system, including bacteria, algae, and invertebrates (sponges, cnidarians, combjellies, etc.) have receptors for such neuroactive pollutants, which accounts for their sensitivity to them. For instance, such widely used psychopharmacological preparations as Prozac (an antidepressant) and amphetamines (cognitive activity promoters) cause major changes in bacterial and algal associations within aquatic ecosystems (reviewed, Олескин, Постнов, 2022).

Acetylcholine, serotonin, dopamine, and other neurotransmitters are not officially considered as globally dangerous ecotoxicants, and they are relatively unstable in the environment. Nonetheless, these BAs are constantly released into the environment with wastewater and, moreover, secondarily produced by natural ecosystem components under the influence of other APIs, as emphasized in the Introduction.

Apart from neurotransmitters per se, of ecotoxicological interest are substances that interact with them their agonists, antagonists (blockers), re-uptake inhibitors, etc. For instance, it was revealed that propranolol, a β-adrenoreceptor blocker, is released into the water and reduces the capacity of fish to spawn. Serotonin re-uptake inhibitors citalopram and fluoxetine are used as antidepressants: however, they also enter natural aquatic ecosystems and significantly affect locomotive behavior in aquatic vertebrates and invertebrates (Sehonova et al., 2018).

In addition to the information provided in the Introduction, it should be noted that neuroactive substances including BAs have already been tested in published work using aquatic organisms such as zooplantktonic invertebrates. For instance, “norepinephrine produces a lethal toxic effect on the zooplankton representatives *Ceriodaphnia dubia* and *Daphnia magna*; one half of the tested daphnia die at ambient norepinephrine concentrations (the LD_{50} doses) of 46 and 38 μM, respectively. Even at much lower (sublethal) concentrations, norepinephrine

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disrupts the reproductuion process and the stages of individual development (e.g., the molting) in the tested species of daphnia" (Олескин, Постнов, 2022). To reiterate, all these ecotoxic substances, notwithstanding, are produced by ecosystem components as signals and regulatory agents. This is the reason why many ecosystem components have specific receptors for binding neurotransmitters, and this accounts for the significant effects of neurotransmitters at quite low concentrations ("trace amounts") and makes neurotransmitters still more important in ecotoxicological terms.

It was demonstrated that the addition of another neurotransmitter, dopamine, changes that pattern of the response of the water flea *Daphnia magna* to a lack of food (microalgae) in the medium: with dopamine, the water fleas grow and maturate as if food were plentiful, even though the food deficiency decreases the growth rate and slows down maturation without dopamine (Issa et al., 2020). These findings highlight the regulatory effects of neurotransmitters in water inhabitants that are exemplified by zooplankton representatives.

Therefore, the literature review part of this work gives much attention to BAs as signal substances that function in diverse ecosystems, including those of water bodies. The review expounds the hypothesis that BAs form a part of ecosystem-level signals (ecomones, Олескин, Постнов, 2022) that are capable of coordinating the functioning of entire aquatic ecosystems. This will be followed by a brief description of the main characteristics of the model organisms utilized in this work for testing BAs. The organisms will also be considered in biotechnological terms, forasmuch as the species of microalgae and cyanobacteria under study are of practical importance as producers of valuable substances that find application as pharmaceuticals, nutraceuticals, and cosmetics. Accordingly, neurotransmitters play a dual role in this context: (i) they are ecotoxins that can disrupt the functioning of aquacultural systems with microalgae; and (ii) they can stimulate the synthesis of valuable products by microalgae.

The above data additionally emphasize the reason why even very low

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concentrations (trace amounts) of neurotransmitters are of interest in ecotoxicological terms: the neurotransmitters are "erroneously" perceived by natural ecosystem components as their own endogenous signals or regulatory agents (or their homologs) and, therefore, they exert a specific influence on those components.

It is the "molecular mimicry" of human-produced neuroactive pollutants that resemble endogenous signals and regulators which is a prerequisite for their sufficiently strong ecotoxic effects even at very low concentrations. Such effects were demonstrated in the experimental section of the present work in which green microalgae and cyanobacteria were utilized.

1.2. COMMUNICATION IN NATURAL ECOSYSTEMS: THE ROLE OF NEUROTRANSMITTERS

"Many natural ecosystems are known to be characterized by complex cooperation and competition among a plethora of coexisting and interacting components, such as populations or associations of living organisms. They produce chemical signals that are recognized by other representatives of the same ecosystem. The signals exert multifarious regulatory effects on them, operating as pheromones, allomones, kairomones, synomones, etc". (Олескин, Постнов, 2022, С.6).

In aqueous ecosystems, "chemically mediated interactions strongly affect population structure, community organization, and ecosystem function" (Hay, 2009). Chemical communication influences "foraging strategies, feeding choices, commensal associations, selection of mates and habitats, competitive interactions, and transfer of energy and nutrients within and among ecosystems" (Hay, 2009).

In the literature, evidence has been presented that there are signal substances sensed by many ecosystem components and promote the coordination of their behaviors. Such signals are exemplified by dimethylsulfide that is produced by microalgae. It attracts the alga-consuming zooplankton (e.g., shrimps and krill) as

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well as the birds that eat the zooplankton (petrels, albatroses, prions, etc.) (Hay, 2009).

This work will focus on one specific subclass of signals that function in aquatic ecosystems. These are neurotransmitters that transmit impulses between nervous cells (neurons) or from a neuron to muscular/glandular cell that effectuates a neuron's command. As already emphasized above, these signal substances happen to accumulate in natural aquatic ecosystems because they are human-produced pollutants.

Neurotransmitters are subdivided into several groups including (1) biogenic amines (BAs) suvh as catecholamines (dopamine, norepinephrine, and epinephrine), serotonin, histamine, octopamine, tyramine, and others; (2) amino acids (aspartic, glutamic, and γ-aminobutyric acid, glycine, and others); (3) peptides including endorphins, enkephalins, dynorphins, substance P, etc.; (4) "gasotransmitters" including nitric oxide, carbon monoxide, and hydrogen sulfide; and (5) purines, e.g., adenosine and ATP. Many neurotransmitters combine several different functions: they are not only impulse-transmitting agents but also hormones and/or local tissue factors (histohormones). Moreover, neurotransmitters include evolutionarily conserved compounds that fulfil communicative and regulatory functions in representatives of various types of animals (Дубынин и др., 2010), plants (Рощина, 1991), fungi (Бузников, 1987, 2007), and protozoans (Бузников, 1987, 2007).

In the following, the main focus will be on BAs. They bind to specific receptors in various tissues and organs of the animal organism. However, to reiterate, they are called neurotransmitters because they interact with the receptors of nervous cells. A nervous cell (neuron) releases a neurotransmitter molecule that crosses the narrow gap (synaptic cleft) separating the neuron from its neighbor, the recipient of the neurotransmitter. It binds to the neighbor's *postsynaptic* receptor. Alternatively, the neurotransmitter-releasing cell may re-uptake it via a *presynaptic* receptor. There is large number of neurotransmitter receptors, and each neurotransmitter can

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bind to several different receptor types. For instance, serotonin (5-hydroxytryptaime, 5HT) can bind to receptors 5-HT1a, 5-HT1b, etc. The binding of all BAs proceeds with the involvement of the receptor family GPCR (G-ProteinCoupledReceptors). These are transmembrane proteins that are composed of 7 amphipathic α-helices, with the C end sticking out of the cell and the N end located inside it. All receptors of this family are related to the small GTPAse (G protein) family. The ligand-binding domain is in the center of the receptor molecule within the membrane matrix, and amino acids from several α-helices are implicated in binding neurotransmitters. Upon binding a ligand, the receptor changes its conformation, and the G protein coupled to it acquires GTPase activity.

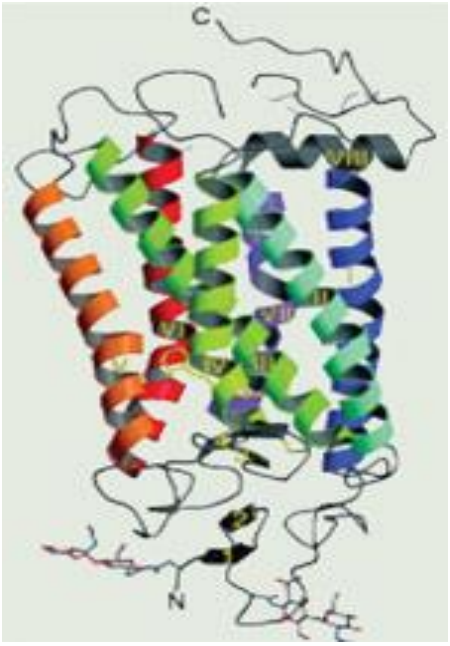

Fig. 1. The GPCR structure (according to: Filmore, 2004, modified).

G protein targets include adenylate cyclase, nucleotide cyclodiesterase, phospholipase C, and other synthetases (or esterases) that are referred to as secondary messengers. One of the most important messengers is cAMP that functions not only in higher animals but also in social protozoans such as

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myxomycetes. Upon binding a hormone or other mediator, an activated G protein molecule is capable to activate several target enzymes; one target enzyme can produce a large number of secondary messengers. Therefore, the reception of a signal results in the operation of at least two different signal transmission and amplification cascades. This provides for a high sensitivity of cells to very low hormone concentrations. All GPCR proteins are characterized by a highly conserved amino acid composition. The only homologues of this protein family are bacteriorhodopsin and halorhodopsin that function as light-dependent proton (or sodium) pumps of haloarchaeans. However, they do not belong to the GPCR, family, since their operatuion does not involve G proteins.

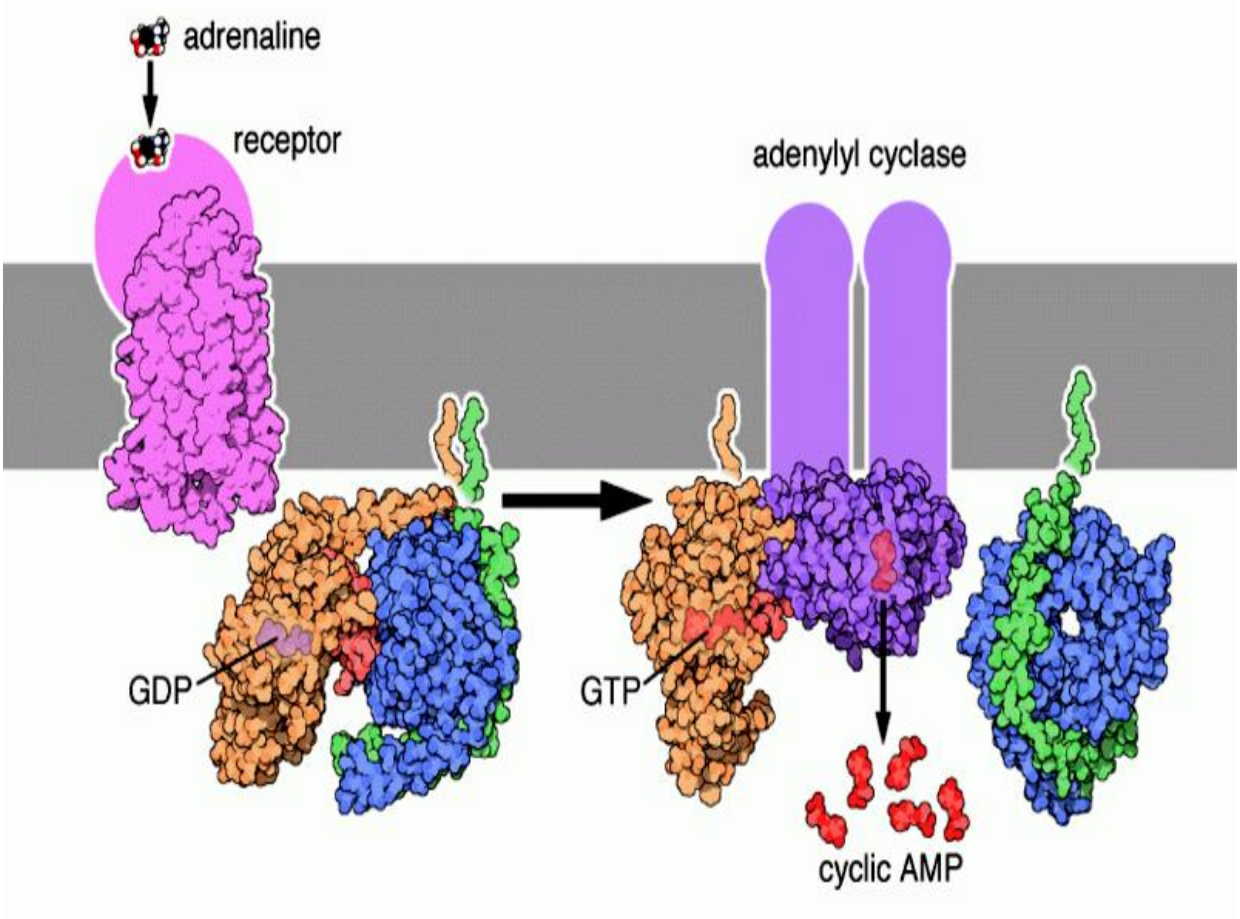

Fig. 2. Signal transduction by an α-adrenoreceptor (according to: Filmore, 2004, modified).

The following is a brief discussion of the properties and functions of the main BAs, taking account of their neurotransmitter activity in higher animals and

humans; subsequently, the BAs' ecosystem-related roles will be considered.

1.2.1. Catecholamines (dopamine, norepinephrine, and epinephrine) derive from the amino acid tyrosine; its hydroxylation results in producing dihydroxyphenylalanine (DOPA). DOPA is the direct precursor of catecholamines.

The synthesis of catecholamines (Fig. 3) is catalyzed by the following enzymes: tyrosine hydroxylase (1), DOPA decarboxylase (2), dopamine hydroxylase (3), and phenylethanolamine-N-methyltransferase (4). Catecholamine degradation involves the operation of two enzymes, monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT).

In mammals, *dopamine (DA)* works as a hormone, antagonizing prolactine that promotes milk production by mammary gland cells. Besides, DA is an important neurotransmitter. It enables transferring impulses from neuron to neuron via a synaptic cleft. DA is extensively used in three different parts of the brain: the substantia nigra, the midbrain tegument, and the hypothalamic nuclei. DA is virtually absent from the peripheral nervous system.

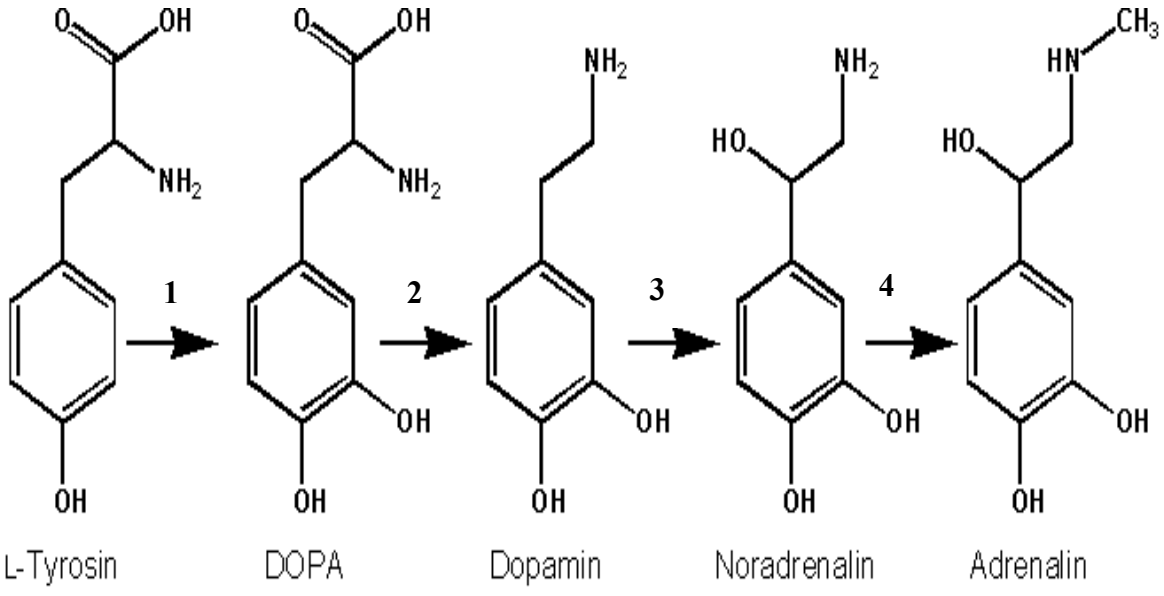

Fig. 3. Catecholamine synthesis. For enzyme designations (1—4) see text.

DA is necessary for maintaining the normal respiratory activity level and the state of wakefulness, as well as a high tone of the brain zones responsible for

sensory reception, motor coordination, memory, emotions, control of locomotive activity, and the inhibition of involuntary movements. The DA level affects the activity of the hypothalamus and the pituitary gland.

DA activities depend on DA binding to five subtypes of G protein-coupled D receptors that are denoted as D1-5. The receptors either activate (D1 and D5) or, conversely, inhibit (D2-4) adenylate cyclase, resulting in either increasing or decreasing the intracellular adenosinophosphate (cAMP) level. D1 and D2 are the most widespread receptor types. The recently described additional receptor, TAAR1 (trace amine-associated receptor 1) also influences intracellular adenylate cyclase activity (Grandy et al., 2016).

Insufficient DA concentrations in DA-dependent brain areas result in apathy and lack of initiative; at still lower DA concentrations, making active movements becomes impossible; for instance, a DA-deficient rat drowns if it plunges into water. DA deficiency that is caused by the destruction of the DA-producng brain structure (the substantia nigra of the midbrain) results in Parkinson's disease; its symptoms include inhibition of active movements, a high muscular tone, and involuntary tremor of the head and fingers. A decreased activity of the DA brain system is also characteristic of children suffering from attention deficit hyperactivity disorder (ADHD). ADHD partly dependns on hereditary factors, according to the data available in the literature. A remedy for ADHD, ritalin, increases the activity of the DA-dependent brain system (Masters, 2001).

Drugs that enhance the brain DA levels stimulate human physical and mental activity; some of them are addictive, exemplified by amphetamines that facilitate DA release into the synaptic cleft. They are one of the groups of drugs used in West Europe and North America in terms of the political protest subculture promoted by the Hippies of the 1960s and 1970s; this subculture was also based on using lysergic acid diethylamide (LSD) that is an antagonist of serotonin (see below). Amphetamines in terms of their wakefulness-stimulating role also made their contribution to the history of World War II. Subsequently, amphetamines were no

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longer in use because of the side effects of long-term treatment with them, including inevitable exhaustion and addiction development. Excessive DA concentrations are envisaged, in the literature, as a schizophrenia-specific symptom (reviewed, Олескин, 2007).

DA is degraded via oxidative deamination followed by aldehyde grouping oxidation and methyl group transfer, yielding the following three metabolites: DOPAC (3,4-dihydroxyphenylacetic acid), 3-MOT (3-metoxytryptamine), and HVA (homovanillic acid). DA is a highly evolutionarily conserved neurotransmitter that performs its functions even in primitive invertebrates (Бузников, 1987, 2007, and see the Results section for the author's own data on DA presence in daphnia). Plants are known to contain both free and bound (glycosylated) DA. Its maximum amounts are detected in banana fruits and in poppy plants (where it easily converts to morphine and other alcaloids). At a concentration of 5 mg/L, DA slows down the growth of the banana callus tissue, which is completely arrested by 50 mg/L DA (Рощина, 1991; Roschchina, 2010).

Norepinephrine (NE), another major neurotransmitter catecholamine, is also produced as a hormone by the adrenal medulla that also synthesizes epinephrine. NE functions as a neurotransmitter in the terminals of the sympathetic nervous system; is also operates in the central nervous system. NE effects are due to its interaction with adrenoreceptors, especially those of the α_1-subtype. In the capacity of a hormone, NE has vasoconstrictive activity; it elevates the blood pressure, which is used in intensive care wards. As mentioned above, NE results from DA hydroxylation. In an analogy to DA, NE is degraded in animal tissues with the formation of the following intermediate products: normetanephrine (3-metoxy-4-hydroxyphenylethanolamine), 3,4-dihydroxymandelic acid, and 3-metoxy-4-hydroxyphenylglycol. NE is implicated in effectuating *fight-or-flight* responses, albeit it to a lsser extent than epinephrine. Th blood NE level is increased by stress factors such as trauma, bleeding, burn injury as well as anxiety, fear, and nervous strain. The impact of DA on the heart is linked to its stimulatory influence on herat

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muscle adrenoreceptors, which increases the cardiac output. The hormonal effect of NE in terms of the stress response is supplemented by its neurotransmitter activity that is aimed at activating the brain under stress. Apart from central nervous system activation, NE increases locomotive activity, lowers the anxiety level, and promotes aggressive behaviors (Дубынин и др., 2010). NE prepares the brain for responding to new potentially dangerous stimuli. A high NE level promotes risky, novelty-seeking behaviors (reviewed, Олескин, 2007).

In pathogenic bacteria, catecholamines stimulate cell proliferation and the formation of toxins, adhesins, and other virulence factors (Lyte et al., 1996; Clarke et al., 2006). For instance, NE increases the adhesion of the pathogenic enterohemorragic strain of *E. coli* (EHEC) to the cecal (Chen et al., 2003), colonic (Green et al., 2004), and ileal (Vlisidou et al., 2004) mucosa. Catecholamines also promote the flagella-dependent motility of the EHEC bacterium (Sperandio et al., 2003).

1.2.2. Serotonin (5-hydroxytryptamine, 5-HT) belongs to the tryptamine subgroup of BAs. It forms in the animal organism (Fig. 4) from the amino acid tryptophan via its enzymatic hydroxylation and, thereupon, decarboxylation. In plant tissues, there is an alternative 5-HT synthesis pathway: Tryptophan → Tryptamine → 5-HT (Roshchina, 1991).

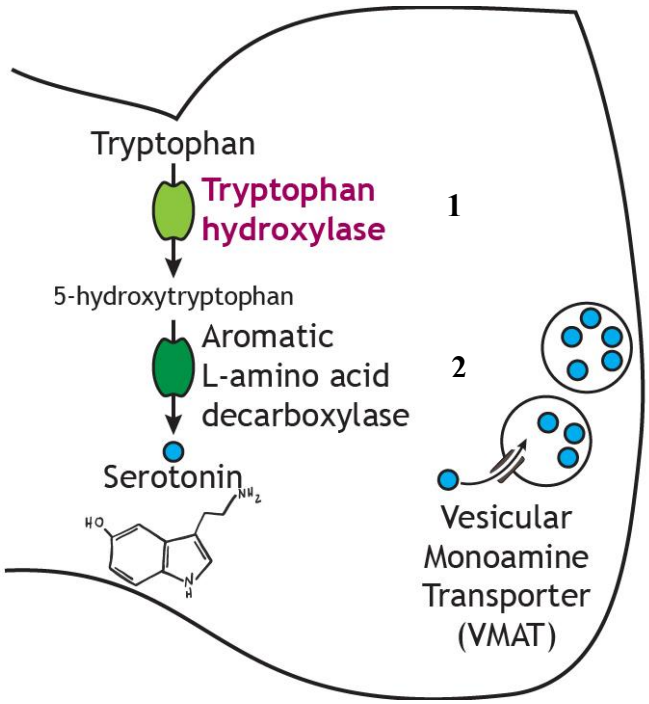

Fig. 4. Serotonin is synthesized through a two-step process. The rate-limiting step in the pathway is the conversion of tryptophan into 5-hydroxytryptophan by tryptophan hydroxylase. 'Serotonin Synthesis' by Casey Henley is licensed under a Creative Commons Attribution Non-Commercial Share-Alike (CC BY-NC-SA) 4.0 International License.

5-HT performs highly diverse physiological functions. It binds to several different types of 5-HT receptors. They differ in terms of the mechanism of action. Some of the receptors activate adenylate cyclase, producing cAMP, while others act on phospholipase C that catalyzes diacylglycerol and inositol triphosphate formation. Besides, there are receptors that serve as channels for K^+, Na^+, and Ca^{2+}. 5-HT works as a neurotransmitter in the central nervous system (CNS) (see Дубынин и др., 2010) that predominantly contains the 5-HT_{1a} and 5-HT_{1b} receptors A large number of serotonergic neurons were detected in the limbic system, the hypothalamus, the trigger zone, and other parts of the CNS. Along with DA, 5-HT is involved in the hypothalamic regulation of the endiocrine function of the pituitary gland. Stimulating the serotonergic pathways connecting the hypothalamus to the pituitary results in increasing the secretion of prolactine and some other hormones

of the front lobe of the pituitary; the effect of 5-HT is diametrically opposite to that produced by stimulating DA-dependent brain structures.

As a neurotransmitter, 5-HT is produced by the raphe nuclei of the brainstem and released into the synsptic clefts of almost all main brain structures. 5-HT inhibits the excessive spreading of excitation waves in the brain from a local activator. 5-HT is implicated in compartmentalizing the brain areas involved in perceiving stimuli: it helps *perception focusing.* 5-HT also "puts the brain asleep"; 5-HT-producing raphe nuclei are regarded as sleep centers in the brain (Дубынин и др., 2010). Blocking the inhibitory effect of 5-HT with LSD, an antagonist of 5-HT_2 receptors, disrupts the perception process and causes illusions and hallucinations (see Дубынин и др., 2010). 5-HT is additionally produced by the organism in the state of exstasy; its level is increased in euphoria and decreased in a depressive state. 5HT is synthesized by the pineal gland from tryptophan, an essential amino acid. 5-HT production is promoted by sunlight, its lack in winter often results in seasonal depression (Hart, 1998).

High 5-HT levels are typical of high-ranking individuals im monkey hierarchies. The dominant individual in a vervet group has more serum 5-HT and 5-hydroxyindoleacetic acid (5-HIAA), its oxidation product than the subordinate individuals. Placing the dominant individual into a separate enclosure prevents its interaction with subordinates and results in a gradual decrease in 5-HT level that finally equals that of a subordinate vervet (reviewed, Masters, 1994). Apart from the neurotransmitter function, 5-HT also works as a local histohormone produced by various tissues during local inflammation along with histamine (see below).

5-HT is chemically related to the plant growth hormone auxine (indole-3-acetic acid). In plants, 5-HT accelerates shoot growth and pollen germination (Рощина, 1991). The fungi of the genera *Psylocybe, Stropharia, Conocybe,* and *Panaeolus* contain such 5-HT-related substances as psilocin and psilocybin as well as some of their derivatives; they act as 5-HT antagonists in the human brain (by binding to 5-HT receptors without performing 5-HT functions), resulting in psychiatric

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problems with hallucinations. Some plants also contain such compounds and were traditionally used by indigenous tribes in various parts of the world because they work as excitatory drugs in the brain. Such hallucinogens may form a part of protective systems preventing plant consumption by herbivorous animals. Of much interest is the fact that plants contain tryptamine, the 5-HT precursor. Among edible plants, bananas are especially rich in BAs. Their fruits contain 5-HT, histamine, and other aliphatic amines as well as tyramine, phenylethylamine, DA, and NE. High 5-HT concentrations were detected in some legumes and in the peel of unripe pineapples (Рощина, 1991).

5-HT insignificantly stimulated the growth of the bacterium *Aeromonas hydrophila* and statistically verifiably increased the growth rate of the bacteria *Enterococcus faecalis*, *Escherichia coli,* and *Rhodospirillum rubrum* as well as the yeast *Candida guillermondii* and *Saccharomyces cerevisiae* (Страховская и др., 1993; Kinney et al., 1999; Олескин и др., 1998; Анучин и др., 2008; Маликина и др., 2010; Oleskin and Shenderov, 2020).

Interestingly, 5-HT also exerts a stimulatory influence on plants. For instance, it accelerates radish seed germination (Рощина, 1991); plausibly, the 5-HT effects in plants are due to the fact that 5-HT is chemically similar to auxin, as mentioned above. 5-HT is present in 37 plant species belonging to 17 families. 5-HT concentrations are particularly high in representatives of the families *Juglandaceae, Leguminosae (Fabaceae),* and *Lygophyllaceae*. 5-HT contained in the stinging hairs of the nettle and velvet beans *Mucuna pruriens* prevents animals from consuming these plants (Roshchina, 2010).

1.2.3. Histamine is produced by decarboxylating the amino acid histidine. It is bound by H receptors (H_1, H_2, H_3 etc.) in the animal organism. Histamine is a neurotransmitter in a limited part of the hypothalamus. Histamine is also an important histohormone (tissue hormone) involved in inflammation. Under normal conditions, histamine predominantly resides in a bound form in the organism.

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Pathological processes (anaphylactic shock, burn injury, frostbite, hay fever, urticaria, etc.) and some chemicals increase the level of free histamine. Histamine liberators include d-tubocurarine, morphine, iodine-containing X-ray-contrast preparations, high molecular weight compounds (polyglucine etc.), and other drug preparations. Unbound histamine is extremely active. It causes the spasm of smooth muscles (including those of the bronchi), capillary dilation, and blood pressure decrease; blood stagnation in capillaries and an increase in permeability of their walls bring about the swelling of adjacent tissues and blood thickening. The adrenal medulla is excited, resulting in epinephrine production; arterioles are constricted, and the heart beat is accelerated. Histamine also promotes gastric juice secretion (Болдырев и др., 2010; Дубынин и др., 2010).

Histamine accumulates in the tissues of many plant species. Much histamine is contained in the stinging hairs of the nettle and a number of other plants. From the ecological viewpoint, histamine serves as a protective factor together with 5-HT (see above). Histamine causes burns, pain, and allergic responses in plant-eating animals. Hot pepper plants contain up to 74.51 mg of histamine per 1 kg of biomass. Stress results in increasing the histamine concentration in plant tissues (Рощина, 1991). The data obtained in Dr. Oleskin's laboratory enable us to characterize histamine as an efficient stimulant of cell proliferation and biomass accumulation in a non-pathogenic strain of *E. coli* K-12; Анучин и др., 2008).

1.2.4. Acetylcholine. Historically, *acetylcholine (ACh)* was the first neurotransmitter to be discovered. In 1914 Arthur J. Ewins was the first to extract acetylcholine from nature. He identified it as the blood pressure-decreasing contaminant from some *Claviceps purpurea* ergot extracts, by the request of Henry Hallett Dale. In the same year, Dale described the effect of ACh on various types of peripheral synapses. In 1921, Otto Loewi established that the vagal nerve releases a substance that suppresses the functioning of the heart muscle. In 1926, Loewi and Navratil suggested the substance to be ACh. Later studies confirmed

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that ACh functions as a neurotransmitter. The operation of ACh in the animal nervous system is based on its binding to two receptor types: there are nicotin receptors (nAChR receptors responsible for nicotin addiction of tobacco smokers) and muscarin receptors (mAChR receptors; muscarin is contained in the fly agaric *Amanita muscari*) (Дубынин и др., 2010). ACh is synthesized by a number of microorganisms including representatives of the genera *Bacillus* and *Lactobacillus* (Wall et al., 2014; Johnson, Forster, 2018). As for unicellular eukaryotes, ACh was established to be synthesized by the protiozoan *Acanthamoeba* sp. (Baig et al., 2018).

ACh is contained in the tissues of at least 65 species of flowering plants belonging to 33 families; much ACl is present in the nettle (Ronga et al., 2019). In the weed plant *Arabidopsis thaliana,* ACh at high concentrations is contained in the seeds and the roots (Parker et al., 2008). The macroalgae *Sargassum tenerrimum* (Gupta et al., 2013) and *Laurencia obtusata* (Barwell, 1980) also contain ACh.

1.2.5. Role of neurotransmitters in aquatic ecosystems. It should be re-emphasized that the term *neurotransmitters* is somewhat arbitrary; it does not reflect the ubiquitous presence of the substances in question in living nature and their regulatory and communicative functions in various components of natural ecosystems. In a recent publication (Олескин, Постнов, 2022), it was suggested that neurotransmitters form a part of the pool of ecosystem-level signals that are produced and sensed by many ecosystem components and, therefore, are involved not only in pairwise interaction between a parasite and its host or between a predator and its prey. They also work at a more global level, functioning as intraspecies (pheromones) and interspecies (allomones, kairomones, synomones, etc.) signals. This gives grounds for the suggestion that neurotransmitters form a part of *ecomones*, i.e. ecosystem-orchestrating agents. To re-emphasize, this work only deals with the subgroup of neurotransmitters called biogenic amines.

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1.2.5.1. Neurotransmitters in the microbiota. Despite the general focus on microalgae in this work of note is the role of the microbiota in aquatic ecosystems. This issue was considered in the classical works by Sergey A. Zernov, the founder of the Hydrobiology Department of the Biology Faculty of Moscow State University. He stressed that the microbiota is the primary producer of organic substances in aquatic communities, and these substances partly result from chemosynthesis (Зёрнов, 1934). Over the last decades, hydrobiologists and ecologist were interested in the phenomenon called "the microbial loop". This is a special trophic chain in which dissolved organic carbon (DOC) is redirected to the main trophic levels via its incorporation into bacterial biomass; thanks to bacteria-consuming aquatic organisms, e.g., protozoans, DOC is made available for the conventional trophic chain including the phytoplankton, the zooplankton, and the necton (see Олескин, 2024).

The role of neurotransmitters among microbial signal molecules includes:

(1) their stimulatory effect on the growth of the cultures of various representatives of the microbiota. This point was underscored above. Neurotransmitter-stimulated microorganisms include waterbody ecosystem components. For instance, 5-HT stimulates the growth of a freshwater lake inhabitant, the purple phototrophic bactreium *Rhodospirillum rubrum,* and the water pollution bioindicator *E. coli* (Олескин и др., 1998). Additional examples are provided in the following quotation: "Norepinephrine, epinephrine, and dopamine stimulated the growth of *Vibrio parahaemolyticus* and *V. mimicus* but not *V. vulnificus* and *V. cholera*; norepinephrine inhibited the growth of *Mycoplasma hyopheunomiae*, suppressing the expression of genes involved in proliferation. Dopamine stimulated the proliferation of the yeast *Saccharomyces cerevisiae*; in contrast, norepinephrine caused no significant effect" (Олескин, Постнов, 2022, С. 7).

(2) neurotransmitter production and release by many microbiota representatives; these microbial neurotransmitters can interact with other

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ecosystem components. Using high-performance liquid chromatography (HPLC) with amperometric detection, it was revealed that catecholamines are contained in the cultures of a large number of pro- and eukaryotic microorganisms (Цавкелова и др., 2000). In terms of aquatic ecology, it is of much interest that micromiolar DA concentrations were detected in *Moganella morganii* (2.46 mg/L, i.e., ~16 μM), *Klebsiella pneumonia* (1.06 mg/L; 6.9 μM), and *Hafnia alvei* (0.73 mg/L; 4.7 μM) that were isolated from fish products (Özogul, 2004; Олескин, Постнов, 2022).

1.2.5.2. Neurotransmitters in the phytoplankton. This subsection only deals with relevant literature data because the author's own results are considered in great detail in the Results section below. A large number of algal species belonging to *Chlorophyta, Charophyta, Ochrophyta,* and *Rhodophyta* produce much DA, 5-HT, histamine, ACh, and tyramine. Up to 60—500 mg/g of histamine accumulate in the cells of the rred alga *Claviceps purpurea* (Олескин, Постнов, 2022).

The key enzyme of the acetylcholine synthesis pathway was detected in cyanobacteria (Smallman, Maneckjee, 1981), and some of them, e.g., those belonging to the genus *Oscillatoria*) contain ACh (Юрин, 2004).

Micrasterias denticulata, an unicellular green alga of the order Demidiales, is capable of light-dependent ACh production, according to the data obtained by HPLC with mass spectrometry (Schiechl et al., 2008; Lütz-Meindl, 2016). Substances that bind to ACh receptors (carbachol and nicotin) and those inpairing them (tubocurarine chloride and hexamethonium benzosulfate) suppress growth processes and cause major morphological alterations in the cells of this microalga if they are added at an early cultivation stage. For instance, nicotin inhibits the formation of the secondary cell wall. This gives grounds for the suggestion that *Micrasterias* possess cholinergic receptors involved in its growth and light-dependent differentiation (Schiechl et al., 2008; Lütz-Meindl, 2016). The ACh precursor choline was present at a concentration of N/D1-0.32% of dry weight in some microalgae belonging to the genera *Chlorophyta, Rhodophyta, Phaeophyta,* and *Euglenophyta* (Parsaiemehr et al., 2015).

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In the cells of some *Chlorella* species, ACh promotes the accumulation of lipids, including those containing unsaturating fatty acids, especially the biotechnologically important α-linolenic acid (Parsaiemehr et al., 2015).

Histochemical studies revealed the presence of endogenous 5-HT in the cells of the diatomic microalga *Ulnaria ulna*. Presumably, 5-HT is implicated in the stress responses of this microalga (Roshchina et al., 2022). In all likelihood, the stress responses involve the antioxidant effect of 5-HT or of melatonin that is produced from 5-HT via its oxidation and methoxylation. For comparison, it should be noted that 5-HT was also revealed in a macroalgal species, the charophyte *Chara australis* (Beilby et al., 2015).

Histamine and DA were histochemically detected in the cells of the diatomic microalga *Ulnaria ulna* (Roshchina et al., 2022). For comparison, it should be pointed out that DA is also synthesized by the green macroalga *Ulvaria obscura;* DA protects the alga against consumption by marine animals (Van Alstyne et al., 2018).

1.2.5.3. Neurotransmitters in the zooplankton. Planktonic animals that have a nervous system (even a diffuse neuronal network characteristic of a hydra) make good use of the function of neurotransmitters reflected in their name: neurotransmitters transmit impulses among neurons. However, even nervous system-lacking representatives of the zooplankton utilize neurotransmitters in terms of their communicative and regulatory functions. Proto-zoans possess neurotransmitter receptors, e.g., the infusorian *Paramecium primaurelia,* has both known types of ACh receptors (the nicotin and muscarin types) and also acetyl-choline esterase, the Ach-degrading enzyme. ACh suppresses conjugation in *P. prima-urelia.* DA is produced by another infusorian, *Tetrahymena pyriformis* (Roshchina, 2010).

DA is released into the water by some microalgae (see above) and therefore, can make an impact on the zooplankton. Such microalgal neurotransmitters should inteact, in a complex fashion with the neurotransmitters produced by zooplanktonic

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organisms per se. In the flatworm *Caenorhabditis elegans,* DA is released by nervous cells if the flatworm locates food; DA slows down the flatworm's movement. In the zooplanktonic crustacean *Daphnia magna*, DA added to the incubation medium prevents the growth rate decrease and maturation time prolongation caused by a lack of food (microalgal cells) (Issa et al., 2020).

Importantly, neurotransmitters are to be considered in conjunction with other chemical agents that also mediate interspecies interactions including those in a trophic chain. *Daphnia* also feed on the microalgae of the genus *Scenedesmus,* and daphnia-released urea induces the formation of compact cell colonies (cenobia) in *Scenedesmus subspicatum.* Large *Daphnia* species including *D. magna,* the subject under study in the work cited (Wiltshire, Lampert, 1999) easily use large cell aggregates as food. Presumably, urea production by the daphnia is useful for this predator because this substance makes microalgal cell groups much more compact, which facilitates food location and rapid consumption.

1.2.5.4. Neurotransmitters in higher plants. Higher plants are of interest within the context of this work insofar as they engage in interactions with microalgae and exchange communicative signals. This interaction with microalgae is possible both for water plants (duckweed, water lilies, etc.) and coastal plants that release signal substances into the water (see the subsection of the Results section dealing with neurotransmitters produced by coastal plants).

Recent research has demonstrated that neurotransmitters including ACh, DA, NE, 5-HT, histamine, and melatonin synthesized from 5-HT are contained in the tissues of many plant species (Рощина, 1991, 2010; Roschchina, 2010). For instance, high DA concentrations are characteristic of the banana peel and pulp that contain 0.8-5.6 g/kg and 0.025-1 g/kg of DA, respectively (Ramakrishna, Mukherjee, 2020). BAs are involved in root, stalk, leef, and flower formation, fruit maturation, pollen and seed germination, and senescence; BAs interact, in acomplex fashion, with other regulatiory substances (jasmonic and salicylic acid, nitric oxide, etc.) (Ramakrishna, Mukherjee, 2020; Dehghanian et al., 2024). For

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instance, ACh increases the growth rate of the tomato, wheat, radish, and other flowering plants. ACh facilitates flowering, accelerates stomatal movement, potentiates the effects produced by the photochrome system, downregulates the formation of ethylene, a gaseous plant hormone, inhibits leaf rolling (reviewed, Олескин, Постнов, 2022), and affects other leaf movements as well as the ion permeability of plant cell membranes (Tretyn, Kendrik, 1991). Addition of ACh to the cultivation medium of hydroponically grown *Arabidopsis thaliana* suppresses root hair growth. There is evidence of the involvement of ACh as a signal molecule in intraorganismic communication between the root and the shoot of *Vicia faba* (the kidney bean). Heating (heat shock) results in stimulating ACh accumulation in the stalks, leaves, and roots of plants (Cao et al, 2006; Ramakrishna, Mukherjee, 2020). Experiments revealed that ACh promotes root tip adherence to glass surfaces (Tretyn, Kendryk, 1991).

As for 5-HT, it is known to stimulate the growth of lateral roots (in *Arabidopsis thaliana*) and shoots (in *Mimosa pudica*). However, 5-HT produces a diametrically opposite effect on *A. thaliana* if its concentration is increased to 160 μM and above: high 5-HT concentrations suppress the growth of both primary and secondary (lateral) roots (Dehghanian et al., 2024).

NE and epinephrine substantially stimulate flower formation in the duckweed *Lemna paucicostata,* a component of many aquatic ecosystems*;* the duckweed and a number of other plant species possess adrenoreceptors that regulate cytoplasm movement and pollen and seed germination (Ramakrishna, Mukherjee, 2020).

Neurotransmitters are actively involved in plant responses to stress. Damaging potato leaves results in increasing their DA, NE, and epinephrine levels. A traumatized *Portulacca callus* cactus synthesizes additional DA amounts (reviewed, Олескин, Постнов, 2022). In a large number of plant species, DA influences the expression of stress response-related genes that are implicated in chlorophyll degradation, plant senescence, nitrate transport, auxine oxidation, and carbohydrate metabolism (Dehghanian et al., 2024).

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Histamine, an animal neurotransmitter and histohormone, was established to accumulate in the tissues of many plants. To reiterate, it is contained in the stinging hairs of the nettle and a number of other plants in which histamine works as a protector. If an animal attempts to eat a histamine-protected plant, the consequences will include local burns, pain, and allergic problems. Stress results in increasing histamine concentrations in plant tissues (Ramakrishna, Mukherjee, 2020).

Neurotransmitters are involved in allelopathic interactions among plants or between plants and animals. The death rate of ant larvae increases if they are given DA-cotaining food or velvet bean seeds that produce DA (Ramakrishna, Mukherjee, 2020).

1.2.5.5. Neurotransmitters in aquatic vertebrates (exemplified by fish). The functions of neurotransmitters in fish are similar to those in mammals. Moreover, fish include model organisms such as the zebrafish *Danio rerio;* they are frequently used as experimental subjects in research on the neurochemical mechanisms of the nervous system and especially the brain. Since this work deals with aquatic ecology and the role of microalgae in waterbodies, the following two aspects are to be emphasized:

1. Fish release neurotransmitters and their precursors and metabolites into the aquatic environment. This process is promoted by stress, especially if fish are injured by an attacking predator or during interindividual fighting. As pointed out above, neurotransmitters stimulate the growth and the production of many cell components of microalgae (the literature data briefly overviewed in this section will be supplemented with the author's own findings in the Results section below). It seems likely, therefore, that microalgae (and the neurotransmitter-stimulated microbiota) are "interested" in stressing and tramatizing fish, which results in releasing factors promoting their growth. From this perspective, neurotransmitters and, more specifically, BAs can be regarded as fish-produced kairomones.

2. Fish respond to neurotransmitters released into the water by other aquatic ecosystem components, including the aforementioned microalgae. Incubating the

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zebrafish in a DA-containing medium suppresses its reproductive activity, because DA is an antagonist of the reproduction-promoting neurohormone gonadotropin (Fontaine et al., 2013). In the long term, some microalgae are expected to decrease the number of algae-consuming predators by actively releasing DA. NE modifies the thermoregulatory mechanism of the nervous system of the goldfish *Carassius auratus*: they start avoiding warm water and gather in a cold water container (Wollmuth, 1987).

Oleskin and Postnov (Олескин, Постнов, 2022) place emphasis on the impact of human-caused neurochemical pollution on fish neurochemistry and behaviors. For instance, eels are under the influence of cocain that is present, at dangerous concentrations, in such rivers as the Themse in London or Arno near the Italian city Pisa. Their behavior is modified by cocaine, resulting in hyperactivity; migrating to the spawning site becomes impossible. Cocaine increases the DA concentration in the eel's brain, which prevents the sexual maturation of young individuals; in addition, the cortisol level is increased, and this prevents fat accumulation that normally takes place prior to eel migration (Learn, 2018).

The literature data on the role of neurotransmitters in the functioning of the main components of aquatic ecosystems are consistent with the hypothesis put forward by Oleskin and Postnov that neurotransmitters (exemplified by BAs) form a part of the pool of ecosystem-level signals, or *ecomones*. In the following, the focus will be on their impact on the phytoplankton and, more specifically, on green microalgae and cyanobacteria. Neurotransmitters are to be envisaged as human-produced ecotoxins. The pool of these compounds produced by pharmaceutical, cosmetic, and food industry supplements the neurotransmitter amounts synthesized by natural ecosystem components; these two neurotransmitter pools interact in a complex manner. Presumably, aquatic organisms mistake these human-produced ecotoxic "gifts" for their own endogenous signals and regulatory molecules; therefore, they specifically respond to very low (micromolar or submicromolar)

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doses of these ecotoxins. The experimental sections of this work will focus on the effects of such low neurotransmitter concentrations.

1.3. MICROALGAE AS RESEARCH SUBJECTS: THEIR ECOLOGICAL IMPORTANCE

In accordance with a somewhat arbitrary classification, algae are subdivided into two main groups: the macro- and the microalgae. While macroalgae represent macroscopic multicellular organisms that may be up to 65 meters long, microalgae are microscopic unicellular, colonial, or filamentous organisms; their size is ~ 1 to ~ 900 μ. In contrast to higher plants, microalgae lack roots, stalks, and leaves. Presumably, there are approximately 800,000 microalgal species, and at least 50,000 species have been described up to now (Ronga et al., 2019). In terms of taxonomy, manicroalgae are eukaryotes including representatives of the kingdom Viridiplantae such as green (division Chlorophyta) and red (division Rhodophyta) algae and of the kingdom Stramenophila (yellow-green algae including Ochrophyta and Prymnesiophyta). Other microalgae are prokaryotes, and they belong to cyanobacteria (divisions Nostocales, Oscillatoriales, etc.). (Белякова и др., 2006; Strassert et al., 2019). Microalgae carry out photosynthesis and are of paramount importance for life on Earth: they produce approximately 50% of all atmospheric oxygen and utilize huge amounts of carbon dioxide that otherwise causes the greenhouse effect.

The following is a brief discussion of the ecological role of these extremely diverse organisms that are spread worldwide and occupy various ecological niches including freshwater, brackish, and seawater habitats. Microalgae inhabit diverse water body types that can be dystrophic or eutrophic and contain various concentrations of organic com-pounds. Microalgal species may prefer low or, alternatively, high pH values; they com-prise thermo-, meso-, and cryophyles. Although many microalgal species form a part of the phytoplankton, water bodies

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also contain periphytic and benthic microalgae. Apart from water bodies, microalgae occur in soils, on the tree bark, and in other terrestrial habitats. The microalga *Chlamydomonas nivalis* colors the snow in the mountains red thanks to its carotenoids (Белякова и др., 2006). One of the groups of algae called prymnesiophytes (Class: Prymnesiophyceae), performs an important function with respect to the global carbon and sulfur turnover. Their calcified shells accumulate carbon from atmospheric CO_2 and form ocean bottom sediments and limestone and chalk deposits. The worldwide occurrence and global role of microalgae account for the importance of the chemical agents that operate as regulatory substances and/or signals both within the realm of algae and in terms of the ongoing dialogue between them and other forms of life.

Microalgae are involved in a complex gamut of interactions with other organisms including other components of aquatic ecosystems. The form "the basal link in the food chains including diverse consumers ranging from protozoans to cetaceans and are involved in complex self-regulatory mechanisms in natural ecosystems" (Гольдин, 2013). Food chains transmit microalga-formed toxins (microcystin, domoic acid, etc.) that are released during allelopathic interactions among microalgae and between them and representative of other taxa. Microalgal "water blooming" is caused by mass proliferation of, e.g., *Prymnesium parvum* associated with toxin production. It is "due to the protective response to the emergence of specific cyanobacterial species in the reservoir" (Гольдин, 2013).

Signal molecules are a prerequisite for multifarious symbiotic relationships between microalgae and other life forms. For instance, cyanobacteria and eukaryotic microalgae overgrow the surfaces of plant and animal organisms resulting in *exosymbiotic* systems. Green microalgae are detected in the hair of mammals, e.g., sloths.

Microalgae also exist *endosymbiotically,* in plant or animal tissues inside or outside their cells (intra- or extracellularly). Cyanobacteria form cyanellae, green microalgae produce zoochlorellae, and dynophytes give rise to zooxanthellae.

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Green microalgae are detected in the cells of protozoans, hydras, sponges, and flatworms (Белякова и др., 2006). The bark of trees is overgrown by the green microalgae *Pleurococcus vulgaris* and *Trentepolia piceana* and by representatives of the genera *Chlorella, Stichococcus,* and *Pleurastrum* as well as by some cyanpobacteria. Lichens are symbiotic systems that include cells of fungi (asco- or basidiomycetes) along with green microalgae (approximately 90% of the known lichens) or cyanobacteria (about 10% of lichens); green microalgae can be present in combination with cyanobacteria.

Cyanobacteria establish symbiotic relationships with liverworts, e.g., of the genera *Blasia* and *Clavicularia,* which are provided with nitrogen by cyanobacteria. The cyanobacterium *Anabaena azollae* is the symbiotic partner of the fern *Azolla filiculoides. "*Some gymnosperms, such as cycads (*Encephalartos* and others), and 40 species of the flowering plants *Gunnera* are involved in analogous nitrogen-fixing symbiotic systems with cyanobacteria" (Гольдин, 2013, С.59).

Symbiotic interactions can potentially result in parasitic relationships. The green algae of the genus *Cephaleuros* represent obligate parasites of coffee, tea, and other plants in which they cause the disease called rust (Белякова и др., 2006). The chlorophyll-lacking alga *Prototheca* is a parasite of animals and humans.

To sum up, it should be stressed that "linking together organisms at various levels from primary producers to those at the top of the trophic pyramide, including the inhabitants of freshwater and marine reservoirs, the coastal zone, of the hydrosphere and the continent, cyanobacteria and microalgae exert an influence on the dynamics of processes taking place in the environment" (Гольдин, 2013, С.50), including the ecosystems of diverse water bodies.

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1.4. BIOTECHNOLOGICAL POTENTIAL OF MICROALGAE

1.4.1. Microalgae at the intersection of ecotoxicology and biotechnology. The present work focuses on the ecotoxicological role of neurotransmitters; nonetheless, the fact should not be overlooked that the test subjects under study, i.e. microalgae including cyanobacteria, are of paramount biotechnological importance. Moreover, the ecotoxicological effects of the substances to be tested are of much interest, in terms of biotechnology, in two different aspects:

(1) the *negative* aspect: BAs that are present in wastewater may affect microalga-containing aquacultures maintained for biotechnological purposes and thereby disrupt microalga-dependent industrial processes;

2) the *positive* aspect: if studies on neurotransmitters in microalgal systems provide evidence for a stimulatory effect of these compounds on the growth and biochemical characteristics of microalgae, such data may find application in novel biotechnological developments aimed at boosting the yield of valuable products with low (inexpensive) concentrations of neurotransmitters (this issue will be revisited in Chapter 3, section 3.3).

1.4.1. Biotechnological applications of nicroalgae. Currently, microalgae are of paramount importance as sources of pharmaceuticals, nutraceuticals (e.g., food additives), cosmetics, biofuel, biofertilizers, animal feed, and wastewater treatment agents (Balasubramaniam et al., 2021). All aforementioned uses of microalgae are related to biotechnology as “industrial application of biological processes and agents on the basis of highly efficient forms of microorganisms and the cultures of plant/animal cells and tissues with desired properties" (Егоров и др., 1987), or, according to an alternative definition of biotechnology, of any technological application that “uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use” (UN Convention on Biological Diversity, 2022; see also: Springham et al., 1999, p. 1).

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Cyanobacteria include numerous biotechnologically important species (Table 1). Widely used producers of valuable compounds ranging from cosmetics and food additives to pharmaceutical preparations, animal feeds, and biofuel are exemplified by the representatives of the genus *Limnospira* (order Oscillatoriales; of note is *L. platensis*[1] used as food by Central American Aztecs and African tribes in the vicinity of Lake Chad), *Nostoc*; *N. commune* is currently utilized for the bioremediation of industrially polluted soil, Дидович и др., 2017), *Anabaena,* and *Aphanizomenon* (order Nostocales).

The following is an of necessity incomplete list of biotechnologically usable eukaryotic microalgae. The order Chlorophyta (green algae) forming a part of the kingdom Plantae includes widely used "working horses" of modern biotechnological labs such as representatives of the genera *Chlorella, Dunaliella, Scenedesmus, Haematococcus, Chlamydomonas, Botyococcus, Chlorococcum*, etc. that find application as pharmaceutical, nutraceutical, and cosmetic preparations. Of significant importance for biotechnology including its medical subfields are microscopic red algae (order Rhodophyta), e.g., the species of the genus *Porphyridium;* red algae also form a part of the kingdom Plantae (Белякова и др., 2006).

This kingdom does not include microalgae belonging to the alternative superkingdom Chromalveolata (Белякова и др., 2006) that has recently been replaced by the clade (supergroup) SAR (Harosa) (Cavalier-Smith, 2018; Strasser et al., 2019).

The same taxonomic "supergroup" is also exemplified by the kingdom Haptophyta with the representatives of the genus *Isochrysis* (order Prymnesiophyta) utilized in cosmetics (Белякова и др., 2006).

[1] The cyanobacterium was formerly called *Spirulina platensis* and later (before 2019) *Arthrospira platensis,* and the food additive produced from it in France starting from the early 1970's had the brandname Spirulina®.

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Table 1. Some biotechnologically important microalgae (including cyanobacteria).

Genus and representative species	Biotechnological applications (Феофилова, Мысякина, 2016; Mourelle et al., 2017; Camacho et al., 2019; Balasubramaniam et al., 2021; Khavari et al., 2021)
Cyanobacteria	
Limnospira *L. platensis*	Food additive, colorant, biofertilizer, medicine for cancer and allergy, biofuel (ethanol and H_2 production), cosmetics
Nostoc *N. commune*	Wastewater treatment, food additive, cosmetics, biofertilizer, biofuel
Anabaena *A. azollae*	Biofertilizer, biofuel
Aphanizomenon *A. flos-aquae*	Food additives (testing for toxicity required).
Green algae (Chlorophyta)	
Chlorella *C. vulgaris* *C. pyrenoidosa*	Food additive, colorant, animal feed, biofertilizer, immunostimulant, biofuel, cosmetics
Botryococcus *B. braunii*	Biofuel (bio-oil)
Scenedesmus *S. quadricauda* *S. obliquus*	Food additive, animal feed, biofertilizer, wastewater treatment, biofuel, cosmetics
Dunaliella *D. salina*	Food additive, β-carotene and glycerol source, animal feed, biofertilizer, cosmetics, cardioprotector
Haematococcus *H. lacustris*	Food additive, colorant, astaxanthin source, cosmetics, medicine for obesity
Chlamydomonas *C. reinhardii*	Biofuel (H_2 production), wastewater treatment
Chlorococcum *C. infusionum*	Astaxanthin source, biofuel (ethanol production)
Red algae (Rhodophyta)	
Porphyridium *P. cruentum*	Biofuel (ethanol production), cosmetics, cardioprotector, anti-inflammatory preparation
Ochraceous algae (Ochrophyta)	
Phaeodactylum *P. tricornutum*	Food additive, animal feed, medicine for obesity
Nitzschia *N. frigida*	Biofuel (biodiesel), wastewater treatment, medicine for obesity
Chaetoceros *C. affinis*	Animal feed, antimicrobial agent
Nannochloropsis *N. oculate*	Biofuel (biodiesel, bio-oil), food additive (ω–3), animal feed, biofertilizer, cosmetics, medicine for obesity
Haptophyte algae (Haptophyta)	
Isochrysis *I. galbana*	Animal feed, cosmetics, cardioprotector, anti-inflammatory preparation

It has been estimated that the global market of microalgal products in 2022 was worth 3.3 billion USD, and the most important contribution was made by pharmaceutical and food industry, taking into account the fact that people are concerned about their health and interested in environment-friendly alternatives to chemically synthesized products.

Attention should also be paid to increasingly widespread chronic diseases around the globe (Balsasubramaniam et al., 2021). The leading companies in the field of biotechnology include Algae Tec (Australia), Pond Biofuels Incorporated (Canada), Cyanotech (USA), and Algae Systems (USA).

Irrespective of their specific applications in biotechnology, microalgae *sensu lato* (including cyanobacteria) possess indisputable advantages over many other sources of biotechnological products. First, microalgae do not require large cultivation areas, e.g., with respect to fuel production, unlike more conventional fuel sources (maize, sugarcane, oil palms, etc.). Second, most microalgae are characterized by a high growth rate and easy cultivation scenarios (requiring only minerals, CO_2, and light), which is of paramount importance in terms of large-scale vaccine or monoclonal antibody production using genetically modified microalgae (Khavari et al., 2021).

Nonetheless, microalga-based biotechnological developments present serious difficulties. A large number of microalgal products do not have a sufficiently large global market; the production costs are too high, which calls for improving currently used biotechnological techniques. Food/drug safety is not always sufficiently guaranteed. Microalgal biotechnology should be in compliance with national and international legal norms, which is still problematic. Many customers are reluctant to use nontraditional food ingredients, e.g. chlorella biomass or extract, and drug preparations (Camacho et al., 2019). Challenging problems are associated with large-scale production using microalgae, i.e. with the transition from laboratory fermentors to pilot bioreactors and industrial setups.

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1.4.3. Cultivating microalgae for biotechnological purposes. An important stage of any microalga-based biotechnological project is *cultivation.* Microalgae are grown in photobioreactors (PBRs) or open natural waters (ponds, lakes and lagoons) and artificial (circular and raceway) ponds. Sunlight or artificial illumination can be utilized, and either batch or continuous cultivation can be carried out. The microalgae can grow on mineral medium (phototrophic, light-dependent cultivation) or they can utilize organic components in terms of a mixed photoheterotrophic cultivation mode that stimulates the growth of many microalgae (Balasubramaniam et al., 2021).

PBRs represent controlled, closed cultivation systems that reduce the likelihood of entry of foreign microorganisms. They are subdivided into tubular (a system of transparent plastic or glass tubes that maximize the intensity of illumination), vertical column-type (a glass column with a bottom nozzle supplying CO_2-enriched air) and flat-plate (including thin-walled polyethylene bags) setups (Dragone et al., 2010). The biomass yield in the PBRs is significantly higher than in in open ponds.

Nonetheless, cultivating microalgae in open ponds is a more economical method of large–scale biotechnological production. This is an example of *ecosystem biotechnology*: successful production largely depends on managing complex relationships between all components of the ecosystem to be created. If several algal species are grown in mixed culture (e.g., *Chlorella* plus *Scenedesmus*), competition between their populations may result in the elimination of one of the species or their sustainable coexistence. There are data, for instance, on antagonism between cyanobacteria (*Anacystis*) and green microalgae (*Chlorella, Scenedesmus, Chlamydomonas,* and *Haematococcus*) (Остроумов, 1986, 2006; Теличенко, Остроумов, 1990). However, mutually beneficial symbiosis is also possible. The development of the hydrocarbon producer *Botryococcus braunii* is stimulated by bacteria coexisting with the microalga (Сассон, 1987). Cultivating microalgae for biotechnological puroposes is followed by separating their biomass via aggregation

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(ultrasonic treatment or addition of flocculating agents), flotation (collecting the biomass in the surface film), centrifugation or filtration that requires ultrafiltration equipment with small (micron-sized) algal cells. The biomass will be usable for a longer period of time if it is dehydrated, i.e. spray-dried or freexe-dried (lyophilized), unless the goal is to immediately separate the target products, e.g. to extract carotenoids or other pigments as well as lipids and hydrocarbons (Balasubramaniam et al., 2021).

1.4.4. Synthetic biology: application to microalgal biotechnology. The prospects of modern biotechnology and especially its biomedical aspects are largely associated with *synthetic biology* as the engineering approach to the genome that can be modified and rearranged in order to change gene functions. This is an important step ahead compared to classical genetic engineering. In terms of aquatic ecology and world ocean conservation, of significant importance would be the creation of microalgae with genomes containing the genes of enzymes that are necessary for efficient degradation of all raw oil components, in case there were oil spills in a water body (Fabris et al., 2020). All these genes can be activated when needed "at the request" of customers if the microalgae are engineered to synthesize receptors for specific molecules, e.g., bacterial *quorum sensing* signals (Fabris et al., 2020).

The sequencing of microalgal genomes facilitated the development of genetic tools for the green alga *Chlamydomonas reinhardtii*, the stramenopiles *Phaeodactylum tricornutum* and *Nannochloropsis gaditana*, and the cyanobacterium *Synechocystis* sp. PCC 6803 (Fabris et al., 2020).

Researchers can develop specialized databases based on the following equation

G x E = P, where G is the genome, E the environment, and P the phenotype of a given microalgal species (resulting from interaction between genetic and environmental factors). This is *phenomics* that is based on extensive databases containing the important features (phenotypes) of microalgae. Phenomics enables

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target-oriented screening of natural and genetically modified (GM) organisms for obtaining optimum biotechnological producers that combine a high growth rate with a high specific yield of the target product, such as the antioxidant asthaxanthine that is expected to produce health-promoting effects (Fabris et al., 2020). Phenomics is still at an early stage of its development, as far as microalgal biotechnology is concerned. Recent progress in the more advanced *higher plant phenomics* highlights the potential of phenomics-related methodology and technology. For instance, a phenomic database enabled obtaining a mutant *Arabidopsis thaliana* plant that was better protected from pathogens than the wildtype and, in addition, was capable of more efficient photosynthesis-dependent growth (Fabris et al., 2020).

In addition, of significant practical importance is the *Internet of Things (IoT)* that involves automatons, sensors, and learning robots for developing self-adaptable biotechnological processes that respond to, and adjust to, any environmental changes; a biotechnological process is supplemented with its sensors-provided information-dependent *digital twin*, i.e., a virtual process that enables predicting the development of the actual process and making necessary technological changes for increasing the yield of biomass and target products and reducing the waste product amounts.

1.4.5. Biofuel. This topic is of relevance to *technical bioenergetics* (Самуилов, Олескин, 1994) as a field of biotechnology dealing with the efficient use of energy stored during photosynthesis, chemosynthesis, and other biological processes. This can be achieved by: (1) conversion of biomass accumulated as a result of photosynthesis into cheap and high–calorie fuels, such as biodiesel (alcohol esters of fatty acids), methane and other hydrocarbons, ethanol, butanol, acetone, dimethylfuran, etc.; (2) modification of the photosynthetic process, enabling us to use light energy with maximum efficiency to produce hydrogen or other fuels (Oleskin, Shenderov, 2020). In contrast to biofuel production based on terrestrial

plants (corn, sugar beet, and rapeseed) and wood-derived lignocellulose, algal biotechnology makes it possible to obtain biofuel using easily cultivable microalgae while economizing on water consumption and avoiding pesticide application (Феофилова, Мысякина, 2016; Hamed, 2016).

Many microalgae, including *Scenedesmus obliquus, Nannochloropsis* sp., *Chlorella vulgaris*, and *Nitzschia* sp., are characterized by a high lipid content (20-50% or more of dry weight; Cruz et al., 2018; Balasubramaniam et al., 2021). These lipids contain triacylglycerides. The substitution of methyl or ethyl alcohol for glycerol in their molecules is a method of producing biodiesel. A 30 times higher amount of biofuel can be obtained from 1 acre of area if algae, and not terrestrial plants, are cultivated (Феофилова, Мысякина, 2016). Microalgae have much potential as a raw material for the production of biodiesel (as well as for other types of biofuels), since the lipid content in their biomass is higher than that of conventional oilseed plants.

Biodiesel is renewable fuel. It has a relatively high ignition temperature, which makes it relatively safe. The advantages of biodiesel, in comparison with mineral diesel fuel, also include its low toxicity; it decomposes easily under natural conditions, producing very low amounts of soot, CO, SO_2, unburned hydrocarbons and solid particles during combustion. The use of biodiesel can dramatically reduce CO_2 emissions into the atmosphere. Finally, when biodiesel is burned as a mixture of esters, a pleasant fruity smell is felt (Феофилова, Мысякина, 2016; Balasubramaniam et al., 2021). Mixing biodiesel from algae with diesel or oil reduces engine wear compared to conventional diesel fuel and prolongs the service life of this engine (Dasan et al., 2019). The disadvantages of biodiesel include its high viscosity and insufficient resistance to oxidation during storage (Феофилова, Мысякина, 2016).

The cells of many microalgae, e.g., *Chlamydomonas reinhardtii,* contain large amounts of starch that is deposited in chloroplasts. Starch can be converterd to liquid biofuel composed of butanol, 2,3-butanediol, and especially ethanol

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(Самуилов, Олескин, 1994; Balasubramaniam et al., 2021).

Ethanol, an eco-friendly fuel that yields only CO_2 and H_2O, can be utilized in combustion engines per se; alternatuively, 10-20 per cent of ethanol are added to gasoline to produce gasohol. To produce ethanol as biofuel, the cell walls of microalgae (*Chlorella vulgaris, Scenedesmus abundans, S. obliquus, S. acuminatus, Porphyridium cruentum,* or *Limnospira platensis*) should be destroyed by, e.g., freezing-thawing. Thereupon, the polysaccharides and other polymers are to be degraded via acidic hydrolysis or treatment with enzymes sucg as amylase, cellulalse, or invertase; subsequently, ethanol is obtained by fermentation using the yeast *Saccharomyces cerevisiae* or the bacteria *Zymomonas mobilis, Clostridium thermocellum, Thermoanaerobium brockii,* and others (Balsubramaniam et al., 2021).

Biogas is an efficient kind of gaseous fuel with a high calorific value (21-33.5 kJ) that contains methane (50-70%), CO_2 (30-45%), and minor quantities of other gases других газов (nitrogen, hydrogen, ethane, ethylene, propane, and hydrogen sulfide). Biogas can be obtained via fermentation that relates to ecosystem biotechnology, similar to the aforementioned cultivation of algae in ponds. The three main stages of this process are (i) hydrolysing biopolymers (polysaccharides, proteins, etc.) to monomers; (ii) decomposing the monomers to organic acids and alcohols; and (iii) producing methane (methanogenesis). These three stages are frequently carried out by different microbial species. For instance, CH_4 formation per se is performed by methanogenic archaeans. It is, therefore, necessary, to orchestrate the cooperation of different microorganisms during the whole biogas production process; alternatively, different stages can be performed in different bioreactors (Самуилов, Олескин, 1994; Balasubramaniam et al., 2021). Biogas production from algal biomass is more efficient if the lipid content in algal biomass does not exceed 40%; the process efficiency is decreased by a high protein content in the microalgae, which results in a low carbon:nitrogen ratio (C/N). To increase the C/N value to a technological optimum level of 10-16, microalgal biomass is

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fermented in combination with, e.g., paper production wastewater (Singh et al., 2014). The thick strong cell wall of many microalgae impedes biogasification, but this problem can be eliminated by preheating the microalgae to 90ºC (Suganya et al., 2016).

Instead of fermentation, biogas can be obtained via biomass gasification, or pyrolysis at high temperatures (800-1000ºC) in the presence of catalysts[2], which results in producing *syngas* containing carbon monoxide, carbon dioxide, and hydrogen in addition to methane. Like biogas, syngas is utilized as biofuel, including its use for producing electricity (Wang et al., 2017).

Of significant importance are long-chain hydrocarbons (*bio-oil*) contained in the microalgae *Botryococcus, Isochrysis,* and *Nannochloropsis* in large quantities (up to 75-80% of dry weight). They are readily separated from biomas by centrifugation and can be used as motor fuel components or raw materials for chemical industry (Adman, Hossain, 2018).

The production of hydrogen using algae yields an absolutely eco-friendly biofuel with a high calorific value (143 kJ/g, which is 2.5 times higher than natural gas), which is easy to store and transport: this technology largely belongs to the future but is already being actively developed. The process is catalyzed by enzymes (hydrogenases or nitrogenases) and based on photosynthesis (water biophotolysis). Suitable algal species include the green microalgae *Chlorella vulgaris, Chlamydomonas reinhardtii*, and some other microalgal species, as well as the cyanobacteria *Anabaena* sp. and *Nostoc* sp. (Thapa et al., 2016).

It should also be noted that the biomass of many microalgae can be burned to produce heat or electricity; the disadvantage of the process is the high cost of the preparatory stages (for example, biomass drying; Aziz, Zainin, 2017).

[2] Alternatively, biomass pyrolysis can be carried out without catalysts if the temperature is still higher (1300ºC).

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1.4.6. Applications of microalgae in food industry. Colorants. The microalgae of the genera *Chlorella, Dunaliella,* and *Haematococcus,* as well as the cyanobacteria of the genera *Limnospira, Aphanizomenon,* and *Nostoc* are *generally regarded as safe (GRAS).* However, special test for toxicity should be conducted before using them as food, cosmetics, or pharmaceutical preparations (Варфоломеев, Вассерман, 2011; Chye et al., 2018). Microalgae receive the attention of many food biotechnologists as sources of essential amino acids, carbohydrates (glucose, starch, ect.), vitamins B_1, B_2, B_5, B_6, B_9, B_{12}, A, C, and E, biotin, carotenoids, and phycobiliproteins (in cyanobacteria) (Camacho et al., 2019; Scieszka, Klewicka, 2019; Khavari et al., 2021). Microalgae contain healthwise important long-chain polyunsaturated fatty acids (PUFAs) including (i) omega-6 (ω-6) acids such as linoleic, γ-linolenic, and arachidonic acid and (ii) omega-3 (ω-3) acids including α-linolenic, eicosapentaenoic, and dosohexaenoic acid.

Microalgae are easily digestible food components; they are characterized by a pleasant natural aroma, and some of them represent low-calorie food (Sompura et al., 2021). Liquid microalgal cultures and microalgal powders are increasingly used in food industry; they are utilized to supplement and vitaminize food items ranging from cheese and dairy products to pastries and sausages. Due to the presence of natural gelatinizing compounds (alginate, carrageenan, etc.), microalgae hold much value as food thickeners and stabilizers (Chye et al., 2018).

Limnospira platensis (formerly denoted as *Spirulina platensis*) with a high protein content (Balasubramaniam et al., 2021) and the eukaryotic green microalgae of the genus *Chlorella* (exemplified by *C. vulgaris*) will be considered in a special section herebelow (see Materials). In terms of producing proteins and other nutrients, special hopes are also pinned on *Dunaliella salina, Haematococcus pluvialis* (= *lacustris,* to be discussed in the Materials section)*, Phaeodactylum tricornutum,* and other microalgal species (Camacho et al., 2019). In Australia, research conducted by James Cook University/MBD Energy Research Facility

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revealed the presence of sufficiently high amounts of high-quality proteins in the biomass of *Scenedesmus* sp., *Nannochloropsis* sp., *Dunaliella* sp., and in a freshwater mixed culture (polyculture CPC) including *Schroederiella apiculate, Scenedesmus pectinatus, Tetraedrom minimum, Mesotaenium* sp., and *Desmodesmus* sp.; the resulting biomass was comparable, in terms of its nutritional value (including the essential amio acid index, EAAI) to commercial products obtained from *Limnospira* and *Chlorella* (Kumudha et al., 2015).

Notwithstanding these developments, the use of microalgae as a source of (crude) protein and other valuable products in food industry still has some disadvantages, mainly due to the insufficiently developed technology currently available for processing microalgae (Scieszka, Klewicka, 2019).

In general, microalga-based biotechnology is still underdeveloped globally, as it requires the development of new value chains with special attention to issues such as the cost of producing algal protein, food safety, the possibility of economically scaling production processes, and consumers' readiness for non-traditional microalga-produced items (Camacho et al., 2019).

The microalgae *Haematococcus pluvialis, Chlorella zofingiensis, Chlorococcum* sp.*, Coelastrella rubescens*, etc. contain the red pigment astaxanthin with high antioxidant activity, which plays an important role in food industry (as a non-toxic food colorant). Maximum amounts of astaxanthin are present in the encysted cells of *H. lacustris*, which are formed under unfavorable conditions, e.g, under bright light or at a high salinity.

Apart from astaxanthin, biotechnologically important microalgae pigments also include other carotenoids, such as β-carotene, lycopene, zeaxanthin, cataxanthin, etc. Intensely colored orange β-carotene is the precursor of vitamin A that is important for vision and the immune system, and has antioxidant properties; β-carotene also applies as a valuable food colorant (Варфоломеев, Вассерман, 2011; Balasubramaniam et al., 2021).

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The green microalga *Dunaliella salina* contains up to 12-14% β-carotene by dry weight and, in addition, up to 50% of glycerol (if cultivated with high concentrations of NaCl in the medium); glycerolis used as a raw material for the production of nitroglycerin and alkyd resins. β-Carotene from *D. salina* is produced by Earthrise Nutritionals (USA) and Nature Beta Technologies Cognis (Australia). Due to a lack of a rigid cell wall and a high protein content, the biomass of *Dunaliella* algae is easily digestable and finds application in bakery industry and as feed for fish and livestock (Camacho et al., 2019; Balasubramaniam et al., 2021).

The shades of red, orange, and yellow provided by carotenoids are supplemented with the green shades of microalgal chlorophylls as important food coloring agents. Chlorophyll *a* with a blue-green hue is produced by green microalgae (e.g. *Chlorella*) and cyanobacteria and widely used as a colorant due to its stability (Kent et al., 2015).

Food items can acquire various shades of blue if the pigments of cyanobacteria are utilized. For instance, *Limnospira platensis* produces phycobiliproteins (especially phycocyanin and allophycocyanin) that are water–soluble fluorescent pigments with antioxidant properties to be added to dairy products, ice cream, chewing gum, confectionery, soft drinks, and oat flakes (Dasgupta, 2015).

1.4.7. Animal feeds. The increasing demand for meat from the world's population necessitates the constant expansion of arable land allocated for fodder crops, especially soybeans. As an alternative strategy, a significant proportion of microalgal biomass production is now used as an additive to feed for cattle, goats, sheep, pigs, rabbits, birds, and fish, as well as marine invertebrates (Camacho et al., 2019; Balasubramaniam et al., 2021; Remize et al., 2021). *Limnospira* spp. is usable as food for cats, dogs, aquarium fish, ornamental birds, horses, and cows, especially because it improves the supply of essential fatty acids to their organisms. *Crypthecodiniun cohnii* and *Schizotrichium* sp. are used in poultry farming, especially for chickens, which can lay eggs enriched with omega-3 unsaturated

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fatty acids (Remize et al., 2021).

Cultivating algae in ponds in terms of aquaculture, an ecosystem technology briefly considered at the beginning of this review, is currently utilized to obtain feed biomass from such microalgae as *Nannochloropsis, Isochrysis, Pavlova, Phaeodactylum, Chaetoceros, Skeletonema, Thalassiosira,* and *Tetraselmis.* Of note are not only food ingredients but also feed components based on *Dunaliella salina;* its food/feed applications are largely due to a high protein (up to 57% of dry weight) and carbohydrate (up to 32%) content of its biomass. To re-iterate, the "work horses" of algal biotechnology *Chlorella vulgaris* and *Limnospira platensis* are very rich in proteins, and their amino acid composition is similar to that of soybean as staple protein-rich feed used in animal farming. Microalgae are an important source of essential fatty acids for fish; their organism is incapable of synthesizing unsaturated fatty acid species such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). *Nannochloropsis* sp. and *Isochrysis galbana* are widely used to enrich the biomass of the zooplankton in DHA and EPA; the zooplankton exemplified by rotifers is subsequently fed to fish or shrimps (Remize et al., 2021). The biomass of *C. vulgaris, L. platensis, Micractinium reisseri, Nannochloris bacillaris,* and *Tetracystis* sp. contains significant iron ion concentrations (Camacho et al., 2019; Balasubramamiam et al., 2021; Remize et al., 2021).

If cattle feed is supplemented even with small amounts of microalgal biomass, it exerts a positive influence on the physiological state of the animals involved: it improves the functioning of their immune system, increases their resistance to infections, and ameliorates the gut's functional state. Feed additives based on microalgae enhance the animal organism's resistance to viruses and bacteria, promote reproduction, and increase the weight of animals, including pigs and poultry. Adding *C. vulgaris* to cattle feed decreases the level of saturated fatty acids in the milk and increases the percentage of PULAs, especially DHA (Camacho et al., 2019). Microalgae represent valuable food for mussel larvae, crustaceans, and

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fish (Spoalore et al., 2006; Camacho et al., 2019; Remize et al., 2021).

Astaxanthin from microalgae such as *Haematococcus pluvialis* increases larvae's growth rate and viability in aquaculture; it improves their reproductive potential and the quality of the eggs of aquatic animals, due to astaxanthin's antioxidant activity. Astaxanthin efficiently increases the resistance of fish to infectious diseases and improves their immune responses (Camacho et al., 2019). Astaxanthin and other microalgal pigments find application in terms of improving the color of cultivable fish and invertebrates. Feeding the astaxanthin-containing microalgaeof the genus *Haematococcus* to salmon and molluscs results in intensifying the coloration of their tissues. When feeding fish with microalgae, the coloration of fish tissues changes due to the presence of carotenoids and, in the case of cyanobacteria, phycobiliproteins in algal biomass. The microalga *Haslea ostearia* is used to impart a bluish-green hue to the gills and labial tentacles of oysters (Варфоломеев, Вассерман, 2011).

1.4.8. Biofertilizers based on microalgae. *Bio-fertilizers* are products containing living microorganisms or natural substances that can improve the chemical and biological properties of soils, stimulate plant growth, and restore soil fertility. In the present-day world, bio-fertilizers compete with more widely used synthetic fertilizers. Obviously, the extensive use of synthetic agrochemicals has resulted in massive environmental deterioration worldwide. Suffice it to mention that lifeless zones are forming in the World Ocean; other consequences include eutrophication, decreased soil fertility, and biodiversity impoverisment (Garcia-Gonzalez, Sommerfeld, 2016).

Along with other types of bio-fertilizers and often in combination with them, bio-fertilizers based on microalgae and their components are aimed at increasing soil fertility and improving the utilization of macro- and microelements by plant crops (Guo et al., 2020), including nitrogen, phosphorus, potassium. Accordingly, microalgal biomass is to be considered an analogue of organic fertilizer that is

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slowly released into the environment. For example, *Limnospira* sp., was found to contain 6.70% nitrogen, 2.47% phosphorus, and 1.14% potassium by dry weight (Ronga et al., 2019). In addition, microalgae contain organic components such as polysaccharides, e.g. β-glucan that is characteristic of *Chlorella* and some other green microalgae. They can stimulate plant growth (Ronga et al., 2019).

There are two main approaches to the use of microalgae as bio-fertilizers. It is possible to increase the activity and growth of the microalgae that are already present in most soils. As a result, the soils get covered with a microalgal "green coating", which enables an increase in the yield of cultivated crops (Шалыго, 2019). The stimulation of the growth of aturally occurring microalgae is achieved by (i) introducing mineral elements (biogens) into the soil (or into the water if the microalgae are grown hydroponically), (ii) increasing the pH value (alkalinizing) by liming the soil, because its acidification suppresses the growth of beneficial cyanobacteria, and (iii) regulating the soil's water balance of the soil

The alternative approach is termed *algalization,* i.e., the treatment of agricultural plants with microalgal cultures, their extracts, or the biologically active substances (BASs) they contain. Microalgal preparations are introduced into the soil (i) prior to sowing the plants, (ii) in combination with them (alternatively, by incubating plant seeds in microalgal preparation-containing solutions), or (iii) at various plant development stages. It is also possible to spray such microalgal preparations over the leaves and other overground parts of plants (Шалыго, 2019; Guo et al., 2020; Bello et al., 2021).

Microalgae and their biomass and extracts obtained from it (using solvents under high pressure or carbon dioxide under supercritical conditions) are widely used to cultivate agricultural plants because they accelerate the germination of seeds and the further development of these plants. Microalgal extracts stimulate the development of seedlings, shoots, and root systems in various vegetable and grain crops, including radishes, lettuce, amaranth, white cabbage, Chinese cabbage, tomatoes, peppers, wheat, and rice (Bello et al., 2021). A relationship has been

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demonstrated between the application of microalga-based biofertilizers and improved utilization of nutrient medium components by plants with an increase in plant biomass yield (Garcia-Gonzalez, Sommerfeld, 2016). This reseach was conducted with such plants as rice, garlic, tomato, and fenugreek (Ronga et al., 2019). To accelerate plant growth, the following green microalgae and cyanobacteria were used: *Acutodermis dimorphus, Limnospira platensis, Anabaena azolla, Nostoc* sp., *Scenedesmus dimorphus*, and *Chlorella vulgaris* (Balasubramaniam et al., 2021).

Introduction of dry biomass of *Nannochloropsis* spp., *Ulothrix* spp., and spp. increased the concentrations of sugars and carotenoids in tomato fruit and improved their quality characteristics. Microalgal bio-fertilizers improved the quality of rose flowers, altering their carotenoid composition and enhancing the orange or yellow color of the petals. Spraying polysaccharides extracted from the culture of *Limnospira* spp. onto tomato and pepper leaves increased the size of their fruit by 20 and 30%, respectively (Ronga et al., 2019). An *Acutodermus dimorphus* culture, its extract, and dry biomass stimulated seed germination, plant growth, and flower formation in tomatoes in greenhouse conditions (Garcia-Gonzalez, Sommerfeld, 2016).

One of the most commonly used microalgae is *Chlorella*, which activates the growth and increases the yield of the above-ground part and grain of barley. Among the beneficial effects of chlorella, an increase in the content of humic acids in the soil should be noted (Шалыго, 2019). *C. vulgaris* culture suspension stimulates seed germination, shoot/root elongation, branching and flower formation in cucumbers and tomatoes (Balasubramaniam et al., 2021). *Hibiscus esculentus* germination and fruiting were accelerated by treating the soil and seeds with a *C. vulgaris* culture (Agwa et al., 2017).

A mixture of the extracts of *C. vulgaris* and *Scenedesmus quadricauda* stimulated the development of the root system in sugar beet seedlings cultivated in an aquatic environment (hydroponics) (Barone et al., 2018).

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Microalgae help plants overcome various stress factors, including extreme (high and low) temperatures, drought, excessive salinization of the environment, and deficiency of mineral components. Extracts of *Limnospira* sp. and *Chlorella* sp. mitigate the effects of stress caused by high salt concentrations and increase the survival of wheat plants under stressful conditions. The polysaccharide produced by *Dunaliella salina* reduces the effects of salt stress on tomato plants by increasing the concentrations of antioxidant enzymes, phenolic compounds, neophytadiene, tocopherol, stigmasterol, and 2,4-ditertbutylphenol in tomato tissues; these chemical factors contribute to the operation of the plant's protection system against oxidative stress (El Arrousi et al., 2018; Bello et al., 2021).

Importantly, many cyanobacteria, for example, *Anabaena* sp., *Nostoc* sp., and *Oscillatoria* sp., fix nitrogen from the air (Chittapun et al., 2018). The *Azolla* fern enters into symbiosis with the cyanobacterium *Anabaena azolla*, and this symbiotic system promotes the development of rice crops, since it fixes up to 120 kg of nitrogen per 1 hectare during the vegetation season. The introduction of nitrogen-fixing cyanobacteria increases the fertility of cotton plants on saline soils. Combined application of cyanobacteria of the genera *Nostoc, Cylindrospermum,* and *Anabaena* to 10 days-old cucumbers of the Mirabela variety in a greenhouse optimized the nitrogen content and pH value in the soil, resulting in stimulating cucumber growth and increasing the numbers of inflorescences and fruits (Шалыго, 2019).

Cyanobacteria colonize various tissues in the shoots and the root system; they fix nitrogen and phosphorus and releaseg siderophores chelating trace elements (iron, copper, etc.) in a form accessible to plants. Therefore, cyanobacteria improve plant growth, stimulate the plants' protective mechanisms, and increase soil fertility (Baulina, 2010; Balasubramaniam et al., 2021).

The beneficial effects of microalgal (including cyanobacterial) bio-fertilizers are largely due to their regulatory effect on plants. Microalgae produce plant hormones and other signaling substances, including auxins, cytokinins (especially

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cis-zeatin, which prevailed in the tested species of green microalgae, Ronga et al., 2019), gibberellins, betaniol, ethylene, brassinosteroids, salicylic acid and abscisic acid. This hormonal influence seems to account for the stimulatory effect of such microalgae, e.g., of *Scenedesmus* spp. The cyanobacterium *Aulosira fertilissima* affects the growth of flowers, stems and leaves in petunias, it also influences the development of rice seedlings (Ronga et al., 2019; Balasubramaniam et al., 2021).

As an environmentally friendly, economical and fairly effective alternative to chemical fertilizers, microalgae–based bio-fertilizers are increasingly appearing on the global market. They are typified by bio-fertilizers based on *Chlorella* spp. under the trade names Terradoc® (Mikroalg Food and Agriculture Industries, Turkey) and Terrasync® ("Accelergy Corporation", USA) (Balasubramaniam et al., 2021).

1.4.9. Microalgae and wastewater treatment. Alga-based wastewater treatment can be considered in terms of *phycoremediation*, an economically beneficial and eco-friendly biotechnology (Ahmad et al., 2020). It forms a part of *phytoremediation*, defined as the use of green plants and related microorganisms along with soil treatment and agronomic methods to bind, remove or neutralize toxic environmental pollutants (Das, 2018).

Conventionally, the process of municipal, industrial, and agricultural wastewater treatment can be subdivided into 4 stages: (1) the mechanical stage, i.e., the purification of wastewater with the removal of coarse and fine impurities using gratings, sieves, settling tanks, etc.; (2) the biological stage, the elimination of dissolved wastewater contaminants by a community of bacteria (along with protozoa and often multicellular plankton); they form the *activated sludge* or *biofilm* that takes up dissolved organic matter, oxidizing it to nitrates and phosphates; both aerobic and anaerobic bacteria can be used, depending on the presence or absence of oxygen in the mixture of activated sludge and wastewater; (3) the physico-chemical stage for post-purification of dissolved bio-purification products, such as nitrates, phosphates, residues of organic compounds, and also

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suspended solids (via flotation, sorption, ion exchange purification and other methods); and (4) disinfection of wastewater with the elimination of pathogenic microorganisms by means of, e.g., UV radiation (Das, 2018; Molazadeh et al., 2019; Ahmad et al., 2020). Introducing microalgae is a promising innovation that can modify the above procedures and enable combining stage 2 and 3.

During the stage of bio-purification (stage 2) based on aerobic degradation of organic substances and their nitrification (oxidation of organic pollutants and ammonium nitrogen), the necessary oxygen is traditionally supplied using an aerator. Replacing the aerator with photosynthetic microalgae transforms the wastewater treatment plant into a highly cost-effective, easily controlled system for cleaning municipal and industrial wastewater from organic matter. This is especially important in tropical countries where there is no constant supply of electricity (Molazadeh et al., 2021). Stabilization or storage ponds (Waste Stabilization Ponds, WSP) are used for wastewater treatment with combined cultures of bacteria and microalgae. Oxygen produced by algae under illumination is used by bacteria to break down organic matter, resulting in a decrease in integrated Biochemical Oxygen Demand (BOD). As mentioned above, the anaerobic bioremediation mode is also useful, and an extra pond with heterotrophic bacteria should be used (Das, 2018; Molazadeh et al., 2019; Ahmad et al., 2020).

A modification of the briefly described technology is a high-speed algal pond. The system contains a photobioreactor for growing algae and an oxidation pond, where microalgae generate oxygen. Bacteria decompose organic matter and make mineral components available for microalga, especially by converting ammonium nitrogen into nitrate (stage 3, see above). It is preferable to use filamentous microalgae (*Oscillatorium* or *Limnospira*) or those forming cell chains (cenobia, *Scenedesmus* sp.) because their biomass is easier to separate (Molazadeh et al., 2019). The species of the genera *Euglena, Oscillatoria, Chlamydomonas, Scenedesmus, Chlorella, Nitzschia, Navicula,* and *Stigeoclonium* are characterized by a high tolerance to organic pollutants in wastewater (Molazadeh et al., 2019).

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Apart from supplying oxygen supply at the stage of organic substance decomposition, microalgae are directly involved in degrading dangerous organic pollutants. The dye aniline that is used in the textile and food industries interacts with the microalgal cell wall, resulting in its neutralization (Balasubramaniam et al., 2021).

Waste products released by pharmaceutical and cosmetic industry enrich water with surfactants, hormones, antibiotics, plasticizers, antipyretics, antifungal drugs, as well as antidepressants and other psychotropic drugs that penetrate into natural reservoirs. It is assumed that "a wide range of aquatic organisms, including bacteria, algae, invertebrates, and fish, have receptors" for such neuroactive pollutants, which accounts for their sensitivity to such compounds (Rosi-Marchall et al., 2015). In particular, such widely used drugs as Prozac (an antidepressant) and amphetamine (cognitive activity enhancer) bring about substantial changes in the bacterial and algal communities of water ecosystems; the same substaces affect the growth rate of insects (Learn, 2018).

Microalgae are capable of completely or partially destroying pharmaceutical preparations and other organic pollutants. Under laboratory conditions (using open ponds and column photobioreactors with bubblers), it was revealed that microalgae are able to remove the antibiotics metoprolol, triclosan, and salicylic acid from water (eliminating more than 90% of their initial concentration) (Tolboom et al., 2019). The microalgae *Chlorella* sp. and *Scenedesmus* sp. purify water from phenolic compounds. *Chlorella pyrenoidosa* destroys, on average, 97.4% of phenol at its initial concentration of up to 0.8 g/l (Dayana Privadharshini, Bakthavatsalam, 2019; Leng et al, 2020).

Microalgae also carry out aforementioned stage (3) of wastewater treatment instead of (or along with) physical and chemical agents. Microalgae are able to completely sequester nitrogen and phosphorus because they utilize these elements. This is “double-effect technology”: in addition to wastewater treatment, valuable microalgal biomass is obtained (its biotechnological application was considered

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above, see Balasubramaniam et al., 2021).

Even at low temperatures, a group of researchers successfully applied a culture of microalgae (strain UHCC00027) to reduce the concentrations of N and P in wastewater to acceptable values. The microalgal biomass was used for the production of fuel (biodiesel, see above). However, the high unsaturated fatty acid content of the resulting biomass prevented meeting the international standards for biodiesel (Jämsä et al., 2017).

In addition to nitrogen and phosphorus, microalgae effectively remove other inorganic substances, such as sulfur and selenium compounds and, most importantly, heavy metals, including zinc, copper, lead, mercury, chromium, cadmium, nickel, iron, manganese, and vanadium (Ahmad et al., 2020). Microalgae can also be used to concentrate and purify precious metals (gold and silver) in solutions and to bind radioactive substances (radionuclides). Representatives of *Chlorella* and *Scenedesmus,* the "microalgae of choice" in terms of water purification, have a high affinity for polyvalent metal ions and, consequently, hold much practical value (Ahmad et al., 2020). Of note are also some other microalgae. Chromium is effectively removed from water by the cyanobacteria *Oscillatoria* spp. Lead is sequestered by the green algae *Chlamydomonas* spp. However, many metals are still better removed by the already mentioned staple biotechnological species *Chlorella vulgaris* (an efficient Cd, Cu, and Zn remover) and *Scenedesmus chlorelloides* (a Mo neutralizer) (Molazadeh et al., 2019).

Microalgae possess two mechanisms for removing heavy metals from water: (1) passive sorption by the surface structures of cells, resulting in the metals' chelation, complexation, and microdeposition by reagents released by cells and (2) active energy-dependent transport of metal ions into the cell. Active transport requires supplying the algae with necessary elements and fadjusting avorable temperature and pH values for them. In contrast, passive sorption does not imply active cell metabolism. It can be carried out by dead cells ("passive biomass"). They can be pretreated physically or chemically to promote the binding of metals or other

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pollutants (Ahmad et al., 2020).

The future development of phycoremediation should involve genetic engineering and proteomics to create highly cost-effective systems using microalgae with a high affinity for pollutants that can be selectively taken up and metabolized by the microalgae. Immobilizing microalgal cells is a promising approach. Microalgae can be incorporated (encapsulated) into synthetic (polyacrylamide) or natural polymer gels, including those synthesized by algae (alginate, carrageenan, and agar-agar). It is expected that immobilization will increase the photosynthetic activity and sorption capacity of algae, as well as their resistance to adverse conditions. It will prospectively enable us to compactly arrange phycoremediation facilities and to use available areas in a more economical fashion (Ahmad et al., 2020: Balasubramaniam et al., 2021).

1.4.10. Microalgae and cosmetics. Cosmetics include products aimed at improving the structure, morphology and appearance of the skin using excipients and active ingredients adapted to different types of the skin that may be normal, oily, sensitive, etc. (Mourelle et al., 2017)

Cosmetic preparations based on microalgae include their extracts (e.g., those used in beauty salons), alginic acids (used to make masks), and essential oils. Currently, algal cosmetics are gaining popularity. This is due to their effectiveness in the capacity of skin moisturizers (or, conversely, skin dryers), pigment (skin color) enhancers, rejuvenating agents, skin whiteners, and sunscreens (Варфоломеев, Вассерман, 2011; Mourelle et al., 2017). Microalgal components are added to hand and face creams, anti-aging ointments, wetting agents, skin irritation mitigaters, refreshing preparations, sunscreen lotions, and hair care agents (Bilal et al., 2017; Mourelle et al., 2017). The types of microalgae most commonly used in cosmetics include *Limnospira* sp., *Chlorella vulgaris, Dunaliella salina, Chondrus crispus, Mastocarpus stellatus, Ascophyllum nodosum,* and *Nannochloropsis oculata* (Bilal et al., 2017).

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Currently, large companies such as Louis Vuitton Moet Hennessy and Danial Jouvance (France) and AGI Dermatics (USA) productce cosmetics from microalgae. This is exemplified by Dermochlorella produced by Codif (France), which is an extract obtained from *Chlorella vulgaris*. It stimulates the synthesis of collagen as an integral part of the structural base (matrix) of the skin, promotes the regeneration of skin tissue, and causes wrinkles to smoothe (Mourelle et al., 2017).

Microalgal carotenoids are used as dyes in cosmetics, along with chlorophylls and phycobiliproteins (for these pigments, see the section on nutritional uses of algae above), which give cosmetics different shades of green, blue, yellow, orange, red, brown and serve as an alternative to synthetic dyes. The antioxidant effect of the aforementioned red pigment astaxanthin produced by *Haematococcus pluvialis* is 10 times stronger than those of beta-carotene and zeaxanthin and one hundred times stronger than that of tocopherol. The carotenoid β-cryptoxanthin (synthesized by *Dunaliella salina*), in addition to an antioxidant effect, stimulates the formation of hyaluronic acid in the skin. Therefore, it is considered an anti-aging agent (Mourelle et al., 2017; Balasubramaniam et al., 2021).

In addition to creating various shades of green, chlorophylls also have a deodorizing effect (masking unpleasant odors) They are also used in toothpastes and hygiene products. Fluorescent phycobiliproteins (phycoerythrin, phycocyanin, etc.) are used in lipsticks, eye shadows, and other cosmetics (Mourelle et al., 2017; Balasubramaniam et al., 2021).

In addition to colorants, algal pigments are used as tanning substances. The carotenoid canthaxanthin that is characteristic of *Chlorella* sp., *Haematococcus* sp., and *Nannochloropsis* sp., promotes tanning: this orange pigment is deposited in the skin and subcutaneous fat, giving it a darker shade. Another pigment, skytonemin (a dimeric alkaloid), can be used in sunscreen cosmetics because it produces a strong antioxidant and UV-protective effect. The whitening effect of cosmetics on human skin is characteristic of products from the biomass of microalgae *Haematococcus pluvialis* and *Nannochloropsis oculata,* due to the inhibition of

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tyrosinase activity that is necessary for the synthesis of the skin pigment melanin. Tyrosinase is inhibited by astaxanthin and zeaxanthin isolated from these algae (Mourelle et al., 2017; Balasubramaniam et al., 2021).

Polysaccharides contained in microalgae are characterized by moistening acitivity, which increases skin elasticity and luster and prevents premature skin aging and wrinkle formation (Wang et al., 2015).

Of significant importance are the potential applications of β-1,3-glucan that is characteristic of the microalgae of the genera *Chlorella, Skeletonema, Porphyridium,* and *Nostoc.* This polysaccharide exhibits considerable antioxidant, immunostimulant, and anti-inflammatory activities (Mourelle et al., 2017; Balasubramaniam et al., 2021).

1.4.11. Medical applications of microalgal biotechnology. Microalgae and their products hold much promise for revolutionizing pharmaceutical industry because they represent an environment-friendly alternative to chemically synthesized preparations used for treating or preventing diverse diseases including various types of diabetes, metabolic syndrome and obesity, cardio-vascular problems, malignant tumors, inflammatory processes, Alzheimer's disease, depression, and other psychiatric disorders, as well as various bacterial, fungal, and viral infections. The medically useful effects of microalgae are due to their components that exhibit antioxidant, light-protective, gel-forming, moistening, antimicrobial, and other activities. They are associated with the presence of polysaccharides (especially their sulfated derivatives), carotenoids, phycobiliproteins (in cyanobacteria), lipid components (especially polyunsaturated fatty acids (PUFAs)), vitamins, biogenic amines (catecholamines, serotonin, and histamine) and a large number of other chemical compounds (Варфоломеев, Вассерман, 2011; Mourelle et al., 2017; Sathasivam, Ki, 2018; Sathasivam et al., 2019; Choo et al., 2020; Balasubramaniam et al., 2021; Sompura et al., 2021).

Both separate microalgal components and whole biomass preparations find

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application as pharmaceuticals and preventives. *Chlorella* sp. biomass promotes the healing of gastric ulcers and wounds, accelerates muscular tissue synthesis, and increases the secretion of anabolic hormones. Per diem addition of 6 g of chlorella biomass to food substantially reduces the risk of pregnancy-associated complications (anemia, proteinuria, and edema) (Choo et al., 2020; Balasubramaniam et al., 2021).

In the capacity of food products or food additives, the microalgae of the genera *Chlorella, Dunaliella*, and *Haematococcus* and the cyanobacteria of the genera *Arthrospira, Aphanizomenon,* and *Nostoc* conform with the Generally Regarded As Safe (GRAS) designation but a prerequisite for their practical use is a test for toxicity (Варфоломеев, Вассерман, 2011; Sciezka, Klewicka, 2019). Microalgae are important protein sources: the global demand for algal protein exceeded 700 million USD in 2019, and it has continued increasing (Balasubramaniam et al., 2021). It was predicted that, by the mid-21st century, up to 18% of protein in the global market will be provided by algae (Caporgno, Mathys, 2018). Microalgae are valuable sources of essential amino acids, carbohydrates, e.g., glucose and starch, vitamins B1, B2, B5, B6, B9, B12, A, C, E, and biotin, as well as carotenoids and other nutritionally and pharmacologically important pigments, including the phycobiliproteins of cyanobacteria (Caporno, Mathys, 2018; Camacho et al., 2019; Khavari et al., 2021).

The main component of *Chlorella* biomass, β-1,3-glucan, is an immunostimulant and an antioxidant; it also decreases lipid concentrations in the blood (Варрфоломеев, Вассерман, 2011; Balasubramaniam et al., 2021). In the capacity of nutraceuticals and biologically active additives, *C. vulgaris* and other chlorella species are produced by such companies as Chlorella Manufacturing and Co. (Taiwan), Klötze (Germany), and Ocean Nutrition (Canada) (Balasubramaniam et al., 2021). In Russia, a popular trademark is Orgtium® (tableted chlorella;; the same trademark applies to *Limnospira* biomass denoted *Spirulina*). There is a Chinese analog, Natural Chlorella Tablet, that is manufactured by the Qingdao Vital

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Nutraceutical Ingredients Bioscience Co. company (Safi et al., 2014).

Of considerable interest is the use of polysaccharides, especially those with sulfate groups, that are obtained from microalgae including cyanobateria and possess antioxidant, anti-inflammatory, immunomodulating, and antiviral properties; they are also used for treating joint problems. Popular microalgae include *Tetraselmis* sp., *Isochrysis* sp., *Porphyridium cruentum,* and *Porphyridium purpureum,* in addition to various *Chlorella* species (Mourelle et al., 2017).

The advantages of chlorella biomass include high concentrations of ascorbic acid and K^+, Na^+, Mn^{2+}, and Ca^{2+} ions. *Chlorella vulgaris* competes with Arthrospira platensis in the capacity of a valuable vegetarian source of vitamin B_{12} contained in *Chlorella* biomass in the biologically active form of methylcobalamine that is suitable for the human organism (Kumudha et al., 2015). Cultivation at a low light intensity facilitates the accumulation of valuable PUFAs, in particular, α-linolenic acid, in *Chlorella* biomass (Balasubramaniam et al., 2021).

The global market features such microalgal pharmaceutically important substances as *astaxanthin* from *Haematococcus pluvialis* (= *lacustris*) under the trademark *Spirulina*® (Earth Spirulina Group, ES Co., South Korea) that is recommendable for decreasing the lipid content of the organism (Sathasivam et al., 2019), eicosapentaenoic acid from *Nannochloropsis* sp. (Almega®PL and Qualitas Health, USA) that reduces the cholesterol level in the blood (Rao et al., 2020; Balasubramaniam et al., 2021), and docosahexaenoic acid from *Schizochytrium limacinum* (Maris DHA Oil, IOI, Germany), a drug for rheumatoid arthritis (Dawczynski et al., 2018).

Apart from asthaxanthin, biotechnologically important microalgal pigments include other carotenoids, especially β-carotene, lycopene, zeaxanthin, canthaxanthin, etc. The intensely colored orange β-carotene is the precursor of visually and immunologically important vitamin A, and it also possesses antioxidant properties and is used as a valuable food dye (colorant) (Варфоломеев, Бассерман, 2011; Balasubramaniam et al., 2021). The green microalga *Dunaliella*

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salina contains up to 12–14% of β-carotene (by dry weight). β-Carotene from *D. salina* is produced by the companies Earthrise Nutritionals (USA) and Nature Beta Technologies Cognis (Australia). Due to a lack of a rigid cell wall and a high protein content, the biomass of the algae of the genus Dunaliella is easily ingested; it is used in baking industry and also as feed for fish and cattle (Camacho et al., 2019; Balasubramaniam et al., 2021; Oleskin, Shenderov, 2020; Олескин и др., 2020).

Microalgae are employed for preparing extracts (used, e.g., in beauty salons), alginic acids (as masks), and essential oils. The useful effects of microalgal preparations are due to their efficiency in the capacity of skin moisturizers (or, conversely, excessive liquid removers), thickeners, pigments, sunscreens, and rejuvenating and skin-bleaching agents (Варфоломеев, Вассерман, 2011; Mourelle et al., 2017; Balasubramaniam et al., 2021).

The products of genetically modified (GM) microalgae find increasingly wide application (Khavari et al., 2021). For instance, antibodies (Ramana et al., 2017), vaccines, erythropoietin, viral protein 28 (VP 28) (Yan et al., 2016), and immunoconjugated cytotoxins for target-oriented elimination of cancer cells (Fabris et al. 2020) were obtained from *Chlamydomonas reinhardtii* using genetic engineering techniques. Antibodies in the form that enables immediate introduction into the organism and elimination of pathogens therein are released into the medium by GM cells of *Phaeodactylum tricornutum* (Fabris et al., 2020).

Such microalgae can be considered candidate *new-generation probiotics,* based on the data presented in recent publications (summarized in: Шендеров и др., 2017; Oleskin, Shenderov, 2019, 2020; Shenderov et al., 2020).

2. MATERIALS AND METHODS

2.1. RESEARCH SUBJECTS

The model test subjects of the present work were representatives of green microalgae and cyanobacteria. In general, microalgae were discussed in the review section above from the ecological and biotechnological perspective. In the following, the tested microalgal species will be briefly described.

2.1.1. Prokaryote

The cyanobacterium *Limnospira platensis* IPPAS B-256 has the following taxonomic position: Domain Bacteria. Phylum Cyanobacteria Class Cyanophyceaea. Order Oscillatoriales. Family Microcoleaceae. Genus *Limnospira*. Species *Limnospira platensis*. Filamentous, motile (gliding motility). Phoautotrophic, non-nitrogen-fixing. Contains a circular chromosome with 6.8 million base pairs and 6631 genes (Fujisawa et al., 2010). The G+C content is 44.3%. Isolated from Chenghai Lake (程海) in China and from the soda lakes of East Africa. Active growth in media with high carbonate and bicarbonate concentrations. Growth optimum approximately 35 °C, pH 9-10.

L. platensis finds wide application in biotechnology, including its use as a food item or food additive; in this context, it is more often denoted as “Spirulina”. It is distinguished by a high protein content (up to 50-70% of dry weight) (Balasubramaniam et al., 2021) and holds much nutritional value that is partly due to the presence of minerals, vitamins, lipids with PUFAs, pigments, carbohydrates, and trace elements. *L. platensis* contains β-carotene, the precursor of vitamin A. It also contains 120-244 μg/g of dry weight of vitamin $B_{12,}$ although its functional usefulness for the human organism is still to be tested (Scieszka and Klewicka, 2019). Thanks to its valuable qualities, *L. platensis* biomass and its components have been granted an international priority status in the capacity of therapeutic food,

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biologically active additives (BAAs), and formula-feeding items produced by such leading companies as Saxony-Anhalt (Germany), Parry Nutraceuticals (India), and Japan Spirulina Co. Ltd (Japan). Another major company, Myanmar Spirulina (Myanmar) specializes in producing tablets, chips, pasta, and liquid extract from *L. platensis* (Balasubramaniam et al., 2021).

After the nuclear reactor disaster in Chernobyl, "Spirulina" was given to the victims because its antioxidant properties helped prevent the harmful effects of reactive oxygen species (Small, 2011). Proteins extracted from *L. platensis* can be used in food products as thickeners and stabilizers for preparing emulsions or foams. The blue pigment phycocyanin that forms a part of the light-harvesting complex of *L. platensis* can be utilized as a food colorant (Martelli et al., 2014). Since *L. platensis* cells contain hydrogenases that produce hydrogen, they are potentially usable for obtaining hydrogen as gaseous biofuel.

2.1.2. Eukaryotes

2.1.2.1. Microalgae

Chlorella vulgaris Beijer. Taxonomic position: Domain Viridoplantae (Green plants). Phylum Chlorophyta. Class Trebouxiphyceae. Order Chlorellales. Family Chlorellaceae. Genus *Chlorella.* Species *Chlorella vulgaris.* Detected in Precambrian fossils (Safi et al., 2014). Described in 1890 by Beijerinck as the first visible nucleus-containing microalga to be discovered. Chloroplast large in size. Occurs in fresh water and sea water reservoirs and in soil. An endosymbiont of freshwater invertebrates, e.g. the flatworms *Dalyellia viridis* and *Typhloplana viridata.*

C. vulgaris is widely used in biotechnology, owing to its high resistance to unfavoral conditions and invasive organims. The industrial efficiency in terms of production of various organic macromolecules (proteins, lipids, and starch) varies depending on the technology used for obtaining biomass (Safi et al., 2014). Under unfavorable conditions, the biomass yield decreases but the lipid and starch

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contents increase. Various cultivation methods, such as autotrophic, heterotrophic, and myxotrophic cultivation, were tested with *C. vulgaris*; autotrophic cultivation seems to be the preferable method because it does not require providing the microalga with costly organic carbon and only relies on inorganic sources of carbon (CO_2 or carbonates) and illumination for photosynthesis.

Similar to *Limnospira,* the green microalgae of the genus *Chlorella* are distinguished by a high content of easily digestible protein with a balanced amino acid ratio; the advantages of *Chlorella* include high biomass concentrations of ascorbic acid and K^+, Na^+, Mn^{2+}, and Ca^{2+} ions. *C. vulgaris* competes with *Limnospira platensis* in the capacity of a valuable source of vitamin B_{12} for vegans that is present in chlorella biomass in the biologically active form of methylcobalamine, which is useful for the human organism (Russian National Standard16634-1-2011, 2011). Cultivation at a low light intensity promotes the accumulation of valuable PUFAs, especially α-linolenic acid, in *Chlorella* sp. biomass (Balasubramaniam et al., 2021).

Scenedesmus quadricauda (Turp.) Breb. K-1149. Taxonomic position: Domain Viridoplantae (Green plants). Phylum Chlorophyta. Class Chorophyceae. Order Sphaeropleales. Family Scenedesmaceae. Genus *Scenedesmus.* Species *Scenedesmus quadricauda.* Forms flat groups of 2—6 cylindric cells (cennobia). Terminal cennobium cells with spines and bundles of chitin fibers. Immotile. Typical phytoplanktonic organisms frequently forming dense populations (Белякова и др., 2006).

Although this microalga is capable of producing several different kinds of biofuel, including hydrogen and ethanol, most attention is given to the use of microalgae of the genus p. *Scenedesmus* for producing biodiesel. Similar to many other microalga-based production processes, biofuel production causes challenging problems if carried out with large-scale industrial facilities. Supplying the algae with nutrients, implementing an efficient gas exchange system, and providing them with photosynthetically active radiation (PARs) present serious difficulties.

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Haematococcus lacustris (= pluvialis) strains IPPAS H-239 and BM-1. Taxonomic position: Domain Viridoplantae (Green plants). Phylum Chlorophyta. Class Chorophyceae. Order Chlamydomonadales. Family Haematococcaceae. Genus *Haematococcus.* Species *Haematococcus lacustris (=pluvialis).* Biflagellate cells with pronounced mucilagination of the internal cell wall level, stigmas, and pulsating vacuoles. Chloroplast bowl-shaped.

Under stress (bright light, high salinity), *H. pluvialis* forms cysts that have a red color due to the accumulation of the carotenoid pigment astaxanthin. Astaxanthin and other carotenoids protect cells from intense illumination and reactive oxygen species. Cysts germinate to form zoospores that gradually lose the red color because of a decrease in astaxanthin content (Белякова и др., 2006). *H. pluvialis* is widespread in regions with a temperate climate. Its astaxanthin-rich cysts account for blood-red colored films appearing at the bottom of drying natatorial, on rocks and sea shores, in fish cultivation ponds, and on house roofs.

Astaxanthin, as emphasized in the Review section of this work, is actively used in food industry as nontoxic food colorant. Astaxanthin from microalgae is produced by the Cyanotech Corporation (USA) and Aquasearch Algatechnologies (Israel) companies supplements the global astaxanthin market based on astaxanthin obtained from marine animals (salmon, shrimps, crabs, lobsters, etc.) and synthesized chemically by the DSM (the Netherlands), BASF (Switzerland), and Zhejiang NHU (浙江聂湖, China) companies. Microalgal astaxanthin is less toxic and possesses stronger antioxidant properties than its synthetic analog. *H. pluvialis* astaxanthin features the biologically active 3S, 3'S enantiomer, whereas synthetic astaxanthin represents a racemic mixture of three different enantiomers (Solovchenko, 2015; Camacho et al., 2019; Sciezka, Klewicka, 2019; Balasubramaniam et al., 2021; Khavari et al., 2021). Astaxanthin is utilized as a feed additive in aquaculture and in poultry farming. It is also used in cosmetics.

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Astaxanthin obtained from *H. lacustris* by extraction with supercritical carbon dioxide was granted the *novel food* status in the UK, according to the UK Food & Standards Agency; in the USA, microalgal astaxanthin is *Generally Regarded as Safe (GRAS)* in compliance with the decision of the US Food and Drug Administration (Shah et al., 2016).

2.1.2.2. Higher plants. Higher plants were used in the part of the present work that was concerned with endogenous BAs. They were determined in the following plants growing on the banks of lakes and ponds in the city parks of Shenzhen (Fig. 5):

1 . *Plumeria rubra* L. cv. *acutifolia.*

2. *Syzigium jambos* (L.) Alston

3. *Buxus megistophylla* (or *Euonymus japonicas* cv. *aureoma*)

4. *Cinnamomum bodinieri* Levl.

P. rubra L. cv. *acutifolia*, introduced from Central America and Mexico, is used in China as a medicinal and decorative plant. It is widely cultivated in subtropical and tropical climates, grows as a spreading tree (7–8 m high), and is flushed with fragrant flowers of shades of pink, white and yellow.

S. jambos (L.) Alston (rose apple) originated in Southeast Asia. It occurs widely elsewhere, having been introduced as an ornamental and fruit tree. A large shrub or small-to-medium-sized tree, typically 3 to 15 m high, with a tendency to low branching. Its leaves and twigs are glabrous. The flowers are in small terminal clusters, white or greenish white. In temperate regions the tree is summer-flowering. Several parts of the *S. jambos* tree are used as a tonic or a diuretic (Subhadrabandhu, 2001). The leaf decoction is applied to sore eyes, it also serves as an expectorant and is used in the treatment of rheumatism (World Agroforestry Center website).

The evergreen shrub *B. megistophylla* (boxwood) (2-12 m high) is native to western and southern Europe, southwest, southern and eastern Asia, Africa,

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Madagascar, northernmost South America, Central America, Mexico and the Caribbean. The leaves are opposite, rounded to lanceolate, and leathery. The flowers are small and yellow-green. *B. megistophylla* is only used in topiary and wood carving.

Fig. 5. Plant species used in this work for chromatographic analysis of endogenous BAs.1, *P. rubra*; 2, *S. jambos*; 3, *B. megistophylla*; 4, *C. bodinieri*.

C. bodinieri is a species native to southern China. A tree reaching 16 m (52 ft) it is typically found in situations with more light, such as alongside roads and streams or in open forests and thickets. Its essential oil is high in citral. It is used as a street tree in a number of southern Chinese cities. *C. bodinieri* belongs to the group of plants used to produce the spice cinnamon (Ravindran, 2003)

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2.1.2.3. Zooplankton

Water flea *Daphnia magna.* Freshwater crustacean of the genus *Daphnia* (Fig. 6). Males and females reach the size of 5 and 2 mm, respectively.

Fig. 6. *Daphnia magna.* Female with a clutch of asexual eggs. © Dieter Ebert, Basel, Switzerland

The body is protected by a semitranslucent chitin carapace. It has a ventral opening and five pairs of thoracic limbs, used to help the filtering process. Spike rows run along the back of the carapace. *D. magna* is a key species in many lentic habitats. It can be found in lakes and shallow ponds rich in organic matter sediment. It is an important primary consumer and prey of many planktivorous fishes.

2.2. METHODS

2.2.1. Cultivation. The research subjects were cultivated as follows.

The cyanobacterium *Arthrobacter platensis* strain IPPAS B-256 was aseptically cultivated in 250 mL flasks at a light intensity of 65 μmol. PAR photons m^{-2} s^{-1} with constant aeration with atmospheric air at 24°C in Zaroukk medium sterilized by autoclaving at 0.5 atm. The medium composition was as follows (Пиневич и др, 1970) (g/L): $NaHCO_3$ 16.8; $K_2HPO_4 \times 3H_2O$ 1.0; $NaNO_3$ 2.5; K_2SO_4 1.0; NaCl 1.0; $MgSO_4 \times 7H_2O$ 0.2 $CaCl_2 \times 2H_2O$ 0.04; Fe+EDTA 1.0 mL; microelement solution 1 1.0 mL; microelement solution 2 1.0 mL; the volume was brought up to 1L with distilled water. Preparing the $FeSO_4$-EDTA solution: 134 mL 1N KOH was supplemented with 30.2 g EDTA. The solution was diluted with water,

supplemented with 24.9 g $FeSO_4 \times 7H_2O$, and the volume was brought up to 1 L with distilled water. It was aerated with CO_2-free air for 12 hs. Microelement solution 1: H_3BO_3 2.86; $MnCl_2 \times 4H_2O$ 1.81; $ZnSO_4 \times 7H_2O$ 0.22; $CuSO_4 \times 5H_2O$ 0.08; MoO_3 0.015. Microelement solution 2: NH_4VO_3 0.023; $K_2Cr_2(SO_4)_4 \times 24H_2O$ 0.096; $NiSO_4 \times 7H_2O$ 0.048; $Na_2WO_4 \times 2H_2O$ 0.018; $Ti_2(SO_4)_3$ 0.040; $Co(NO_3)_2 \times 6H_2O$ 0.044.

The green microalgae *Chlorella vulgaris* Beijer и *Scenedesmus quadricauda* (Turp.) Breb. K-1149 were axenically cultivated in 250 mL flasks at a light intensity of 65 μmol. PAR photons.m^{-2} s^{-1} with constant aeration with atmospheric air at 24ºC in modified Tamiya medium; the medium composition was as follows (g/L): KNO_3 2.5; $MgSO_4 \times 7H_2O$ 1.25; KH_2PO_4 0.625; $FeSO_4 \times 7H_2O$ 0.003; EDTA 0.185; microelement solution 1 mL per 1 L (pH 6.0). The microelement solution was as follows (g/L): H_3BO_3: 2.86; $MnCl_2 \times 4H_2O$: 1.81; $ZnSO_4 \times 7H_2O$: 0.222; MoO_3 176.4 mL/10 L; NH_4VO_3: 229.6 mg/10 L; $CuSO_4 \times 5H_2O$: 0.01 mg/L; $Co(NO_3)_2 \times 4H_2O$ 0.146; KJ 0.083; $NaWO_4 \times H_2O$ 0.033; $NiSO_4(NH_4)SO_4 \times 6H_2O$ 0.198.

The green microalgae *Haematococcus lacustris (=pluvialis)* strains IPPAS H-239 were axenically cultivated in 25 mL tubes at a light intensity of 65 μmol. PAR photons.m^{-2} c^{-1} at 24ºC in BG-11 medium with the following composition (g/L): $NaNO_3$, 1.5; K_2HPO_4, 0.04 ; $MgSO_4 \times 7H_2O$, 0.075 ; $CaCl_2 \times 2H_2O$, 0.05 ; Na_2CO_3, 0.02; citric acid, 0.006 ; Na_2EDTA, N/D1; $FeC_6H_5O_7$, N/D6; and microelement solution, 1 mL/L. The microelement solution was as follows: H_3BO_3, 2.86; $MnCl_2 \times 4H_2O$, 1.81; $ZnSO_4 \times 7H_2O$, 0.22; $Na_2MoO_4 \times 2H_2O$, 0.4; $CuSO_4 \times 5H_2O$, 0.08; $Co(NO_3)_2 \times 7H_2O$, 0.05.

The inocula used in these experiments were microalgal cultures after several passages that reached the end of the exponential growth phase, i.e., day 3 of cultivation for *C. vulgaris*, day 4 for *S. quadricauda*, and day 7 for *H. lacustris* and *L. platensis*). Culture growth was estimated by direct cell count in the field of vision of a MIKMED var.2M-1500 microscope (LOMO, Russia) using a double-net Goryaev chamber (MiniMed, Russia); the results were expressed in millions of

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cells per 1 mL. In experiments with *C. vulgaris* and *S. quadricauda,* growth was also estimated nephelometrically from the culture's optical density (OD), based on calibration curves. The measuring wavelength was 750 nm. Optical density was measured using an SF-56 spectrophotometer (LOMO Joint-stock Company, Russia). The inoculum dose was 1.4×10^6 cells/mL (OD 0.12±0.01) for *C. vulgaris,* 0.59×10^6 cells/mL (OD 0.10±0.01) for *S. quadricauda*[3], and 1.2×10^6 trichomes for *L. platensis* (which corresponded to 0.39±0.08 mg/mL of dry weight). The inoculum dose varied between 14.6-20.8×10^6 cells for strain IPPAS H-239 and 12.0-17.5×10^6 cells for strain BM-1 of *H. lacustris.*

The cultures were grown until they reached the stationary growth phase (4 days of cultivation for *C. vulgaris,* 7 days for *S. quadricauda,* 14 days for *H. lacustris* and *L. platensis*). The selected time points for growth estimation (day 1, 2, 3, and 4 of cultivation for *C. vulgaris*; day 1, 2, 4, and 7 for *S. quadricauda;* day 7 and 14 for *H. lacustris* and for *L. platensis*) corresponded to the lag, early exponentional, late exponential, and stationary growth phase, respectively.[4]

2.2.2. Determining the effects of biogenic amines (BAs) on microalgal (cyanobacterial) biomass accumulation. The cultures of the tested microalgae (cyanobacteria) were supplemented with 0.1, 1, 10 or 100 μM of acetylcholine, dopamine, histamine, serotonin, and norepinephrine hydrochlorides added at inoculation as freshly prepared aqueous solutions. In preliminary experiments, it was established that supplementary addition of neurotransmitters during the cultivation failed to produce any significant extra effects (data not shown). According to the literature, neurotransmitters introduced after the initial growth phase are inefficient in terms of influencing the growth of various pro- and eukaryotic microbial cultures (see: Олескин и др., 1998; Анучин и др., 2008;

[3] The coefficients used to convert optical density values into cell numbers were obtained using the Goryayev chamber for counting cells.

[4] For *H. lacustris* and *L. platensis,* the two points corresponded to the exponential and stationary phase, respectively

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Маликина и др., 2010). The control culture was supplemented with an equal volume of water. All neurotransmitters were Analytic Grade (Sigma, USA).

Each experiment on the impact of BAs on culture growth, as well as on the fatty-acid composition of cell lipids and photosynthetic pigment content (see next subsections) was independently conducted 4—5 times, the tables and graphs contain averaged values and standard deviations (SDs). No positive control experiments were conducted because pure substances (not complex mixtures characteristic of biological materials) were used.

2.2.3. Determining the effects of BAs on the fatty-acid composition of cell lipids and photosynthetic pigment content in microalgal (cyanobacterial) cells. This research was conducted in collaboration with Prof. A.E. Solovchenko and Drs.T.A. Fedorenko and O.B. Chivkunova. The analysis of the fatty-acid composition of cell lipids and photosynthetic pigments was based on the method described in (Solovchenko et al., 2015). In stationary-phase cultures, the cells were pelleted by centrifugation, transferred to a glass–glass homogenizer and disrupted in a chloroform–methanol (10 mL, 2:1, v/v) mixture. The lipid fraction that also contained pigments was extracted. The chlorophyll *a* and *b* content and the total carotenoid concentration in *C. vulgaris* and *S. quadricauda* were determined with a Cary 300 Bio spectrophotometer (USA) using absorption coefficients coefficients for their chloroform solutions (Well, 1994). Fatty acid determination were performed using gas chromatography of their methyl esters (Kates, 1986). The methyl esters were separated and identified according to retention times of pure standards (Sigma, St. Louis, MO, USA) and by characteristic mass spectra obtained with an Agilent 7890 gas chromatograph equipped with a 30-m HP5MS UI capillary column coupled with an Agilent 5970 mass-selective detector (Agilent, Santa Clara, CA, USA).

As for *L. platensis* and *H. lacustris*, the total chlorophyll and carotenoid fraction was extracted with dimethylsulfoxide (DMSO) for 5 min at 70ºC to determine pigment concentrations. They were spectrophotometrically estimated in DMSO

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extracts using the following coefficients given in (Solovchenko et al., 2015):

Chlorophyll *a* concentration, $C_{Chl\,a}$ (mg/L) = $13.34\times A_{666} - 4.85\times A_{650}$.

Chlorophyll *b* concentration, $C_{Chl\,b}$ (mg/L) = $24.58\times A_{650} - 6.65\times A_{666}$.

Carotenoid concentration, C_{Car} (mg/L) = $(1000\times A_{480} - 1.29\ C_{Chl\,a} - 53.76\times C_{Chl\,b})/220$

where A is light absorption at respective wavelengths.

2.2.4. Determining endogenous neurotransmitters in the tissues of coastal plants, microalgae, and water fleas. Plant leaf samples of the species briefly described above (see Fig. 5) were collected close to the water front of ponds and lakes in the Dayun Park (Shenzhen). Based on the data presented in the literature (Рощина, 1991; Roshchina, 2010), we assumed that maximum concentrations of neurotransmitters are typically present in plant tissues during the hot tropical summer season. Summer weather promotes plant metabolic activities and, therefore, maximizes the involvement of regulatory substances that include biogenic amines. Therefore, plant leaf samples were collected at the beginning of June (in 2019). It is also known that neurotransmitters tend to accumulate in plant tissues towards the end of the day, as a result of the functioning of the photosynthetic machinery in the daytime. This is the reason why we harvested the leaves between 6 and 7 pm local time. To minimize the effect of storage conditions on biogenic amine concentrations, we stored the leaf samples, after collecting them, at -10ºC for maximally 2 days in polyethylene bags

In this work, frozen leaf samples were disintegrated using a mortar with Quartz sand at 0oC in 0.1 N HCl ; the debris were removed by centrifugation (20,000 g, 20 min). The disintegrated samples were transferred to a solution containing 0.1 N $HClO_4$ (20 mL per 1 g of biomass) and 0.5 μM 3,4-dioxybenzylamine as an internal standard for subsequent determination of BAs and their precursors and metabolites by high-performance liquid chromatography (HPLC) with amperometric detection.

The samples of *microalgae* were prepared for HPLC as follows. The cells were separated from the medium during the stationary growth phase (day 7 of cultivation

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in *S. quadricauda,* day 4 in *C. vulgaris*) by centrifugation for 10 minutes at 8000 g at the characteristic time points given aboveв. The culture liquid was collected to determine its BA content.

The pelleted biomass was resuspended in distilled water and recentrifuged for 10 minutes at 8000 g to remove the culture liquid. The biomass was cooled to 0ºC and thereupon sonicated (22 kHtz) for 7-8 minutes, intact cells and cell fragments were removed by recentrifugation for 15 minutes at 10000 g; the resulting supernatant was examined microscopically to guarantee the complete disintegration of all microalgal cells. The disintegrated biomass was resuspended in a solution with the same composition as that for plant leaves (see above).

To determine BAs in *water flea (Daphnia magna)* samples, 30-35 adult water fleas were separated from water (the samples were taken from the Glubokoe Lake in the Moscow Region[5]) with a paper filter, rinsed with distilled water, and disintegrated in a mortar with quartz sand at a temperature of 0° C in 0,1 N HCl; the debris were removed by centrifugation (20 000 g, 20 min).

The BAs were separated by HPLC with an amperometric detection system (Oleskin et al., 2014). A LC-304T chomatographer (BAS, West Lafayette, USA) with a Rheodyne 7125 injector was used; the volume of the loop used for applying samples was 20 μl. The tested biogenic amines were separated on a reverse-phase ReproSil-Pur column (ODS-3, 4x100 mm, 3 μ; Dr. Majsch GMBH, Elsico, Moscow). A PM-80 pump (BAS, USA) was used; the elution rate of the mobile phase was 1.0 ml/min at a pressure of 200 atm. The mobile phase contained 0.1 M citrate-phosphate buffer with 1.1 mM octanesulfonic acid, 0.1 mM EDTA, and 9% acetonitrile (pH 3.0). The measurements were carried out using an LC-4B electrochemical detector (BAS, USA) with a glass-carbon electrode (+0.85 V) against an Ag/AgCl reference electrode. The samples were scanned with the

[5] The water flea was identified as *Daphnia magna* by Dr. Andrey Postnov, Dr. Daria Gershkovich, and other staff members of the General Ecology and Hysrobiology Department of the Biology Faculty of MSU prior to the beginning of this work

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Multichrome 1.5 (Ampersand) hardware—software system. All reagents used for the assay were of analytical grade. The chromatographer was calibrated using a mixture of the tested biogenic amines; the concentrations of all these substances were 0.5 μM. The amine concentrations contained in the samples were calculated by the internal standard method that is based on determining the ratio between the peak area in the standard mixture and that in the samples (Oleskin et al., 2014).

2.2.5. Data treatment. The data presented in the form of histograms or in the tables below are arithmetic averages. Independent experiments were conducted 4-5 times, independent measurements in each experiment were done at least 3 times. The data obtained for each experimental variant were averaged, and standard deviations were calculated.

The hypothesis of the normal distribution of data for each variant (these were 12-16 values) was tested using the Statistics program, and the skewness and kurtosis of the data distribution curves were estimated. With their proximity to zero (and an unimodal data distribution), the distribution was considered normal, and the Student's *t*-test was applied using Microsoft Excel. The significance of the differences was assessed at p 0.05. Some data groups did not conform with the normal distribution pattern and were statistically analyzed using IBM SPSS Statistics version R27 0.1.0. If there were three or more independent data groups, independent samples were used in accordance with the Kruskal-Wallis H-test (Kruskal and Wallis, 1952; Cohen, 1988). In this situation, the standard (mean-square) deviation was calculated but the credibility of the data obtained was verified using the results of the H-test.

In the results of the Kruskal-Wallis test, the designation *Sig* stands for *significance* and is equivalent to the *p*-value in the Student's *t*-test in that it quantifies the strength of evidence against the null hypothesis (H_0). If *Sig* < the preset significance level (commonly $\alpha = 0.05$), the H_0 hypothesis is subject to rejection, indicating significant differences between the data groups to be compared.

3. EFFECTS OF BIOGENIC AMINES ON THE GROWTH PARAMETERS OF MICROALGAE

The data obtained in this work are separately presented for each of the experimental subjects under study; these data will be summed up in the Conclusions section.[6]

3.1. GREEN MICROALGAE

3.1.1. Impact of BAs on the growth of *Chlorella vulgaris* Beijer. The dynamics of the growth of *C. vulgaris* cultures with the biogenic amines 5-HT, histamine, DA, NE, ACh, and without them is presented in Figures 7-11. The selected time points (1, 2, 3, and 4 days of cultivation) corresponded to the lag phase, the early exponential phase, the late exponential phase, and the stationary phase, respectively.

3.1.1.1. Effect of serotonin (5-HT) on the growth of C. vulgaris. 5-HT produced no statistically significant effect on the growth of the *C. vulgaris* culture at the lowest tested concentration, 1 μM (Fig.7). However, the medium concentration of 5-HT, 10 μM, caused a slight (≈ 12%) but statistically verifiable increase in biomass yield after 4 days of cultivation. This marginal stimulation was not observed with the highest tested 5-HT concentration (100 μM). Towards the end of the cultivation period (after 4 days), the precipitation of a significant part of *C. vulgaris* biomass

[6] The results presented in this section are published in the work: **Cao B.,** Oleskin A.V. Neurochemical pollutants in the aquatic medium: the results of studies with model organisms (microalgae) // Жизнь Земли. — 2025. — Т. 47. № 4. С.550-562. DOI: 10.29003/m4988.0514-7468.2025_47_4/550-562. ИФ РИНЦ: 0.386 (1.0 p.s./0.6 p.s.). EDN: PMKRCV and in the supplementary publications: 1. **Цао Б.**, Федоренко Т.А., Чивкунова О.Б., Соловченко А.Е., Лобакова Е.С., Олескин А.В. Влияние биогенных аминов на накопление биомассы, содержание фотосинтетических пигментов и жирных кислот у цианобактерии *Limnospira platensis* IPPAS B-256 // Микробиология. — 2026. — Т. 95, № 1. — С. 68–84. DOI: 10.1134/S0026261725602921 ИФ РИНЦ: 1.034 (1.5 p.s./0.8 p.s.) [**Cao B.**, Fedorenko T.A., Chivkunova O.B., Solovchenko A.E., Lobakova E.S., Oleskin A.V. Impact of neurotransmitters on biomass accumulation, photosynthetic pigment content, and fatty acid composition of the cyanobacterium *Limnospira platensis* IPPAS B-256// Microbiology. — 2026. — Vol. 95, no. 1. — P. 82–95. DOI: 10.1134/S0026261725602921 SJR: 0.347 (1.5 p.s./0.8 p.s.); Oleskin A. V., Postnov A. L., Cao B. Impact of biogenic amines on the growth of green microalgae // J. Pharm. Nutr. Sci. — 2021. — V. 11. — P. 144–150. DOI: 10.29169/1927-5951.2021.11.17. SNIP: 0.193 (0.8 p.s./0.5 p.s.); Oleskin A. V., Postnov A. L., Cao B. Impact of biogenic amines on the growth of a *Chlorella vulgaris* culture // J. Pharm. Nutr. Sci. — 2021. — V. 11. — P. 49–53. DOI: 10.29169/1927-5951.2021.11.07. SNIP: 0.193 (0.8 p.s. /0.5 p.s.).

was documented. This pointed to a toxic effect of 5-HT at high concentrations.

3.1.1.2. Effect of histamine on the growth of C. vulgaris. This derivative of the amino acid histidine proved to be the most efficient growth stimulator at the low and the medium concentration (1 and 10 μM, respectively). The increase in biomass manifested itself at the early growth stages; the maximum effect was produced towards the end of the cultivation period (day 4), amounting to ≈100% of the control value. Histamine failed to exert its stimulatory effect when applied at the highest (100 μM) concentration; moreover, it inhibited the culture's growth towards the end of its cultivation (Fig. 8).

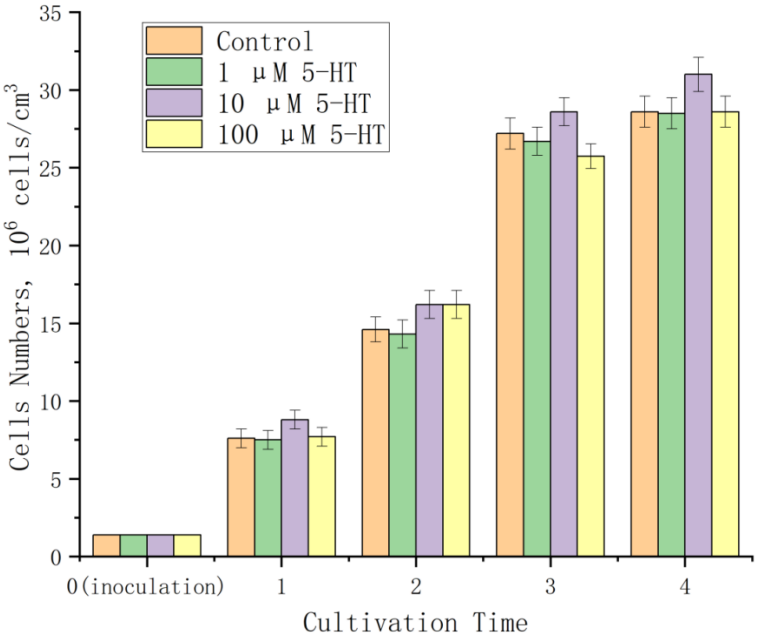

Fig. 7. Effect of serotonin (5-HT) on the growth of *C. vulgaris*. The figure contains cell numbers (millions of cells per 1 cm^3); averaged results of 4-5 repeats are given. All data obtained are statistically **significant**, according to the Student's *t* test at p =0.05.

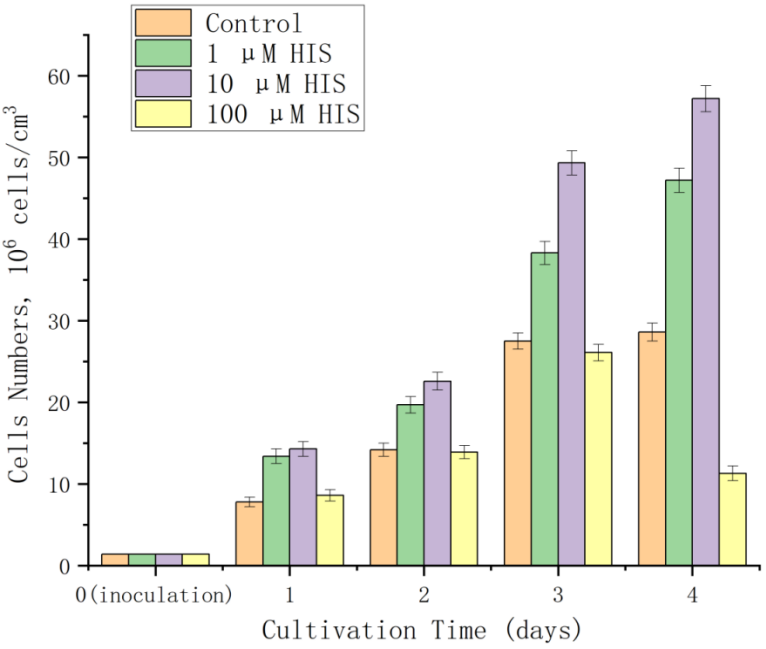

Fig. 8. Effect of histamine (His) on the growth of *C. vulgaris*. The figure contains cell numbers (millions of cells per 1 cm^3); averaged results of 4-5 repeats are given. All data obtained are statistically **significant**, according to the Student's *t* test at p =0.05.

3.1.1.3. Effect of dopamine (DA) on the growth of C. vulgaris. This neurotransmitter and lactation-inhibiting hormone of mammals that is produced from tyrosine, markedly (by over 50%) increased the cell number in the *C. vulgaris* culture at early cultivation stages (1-2 days), but this increase became less significant at later cultivation stages, suggestive of a growth-accelerating, rather than stimulatory, action on the culture (Fig. 9). This effect was characteristic of 1 and 10 μM DA, but the growth increase was not observed at the highest tested concentration (100 μM); presumably, another, inhibitory, effect caused by this DA concentration overrode the accelerating effect.

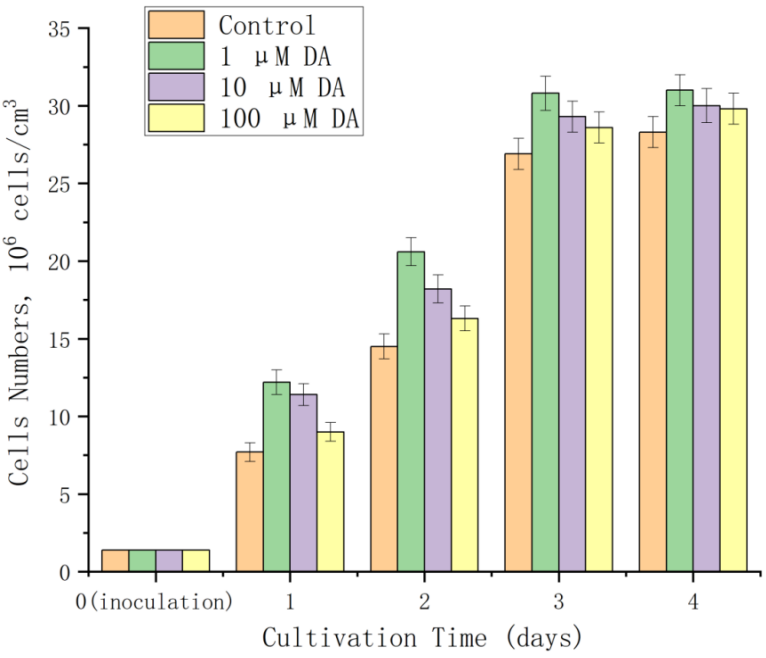

Fig. 9. Effect of dopamine (DA) on the growth of *C. vulgaris.* The figure contains cell numbers (millions of cells per 1 cm^3); averaged results of 4-5 repeats are given. All data obtained are statistically **significant**, according to the Student's *t* test at p =0.05.

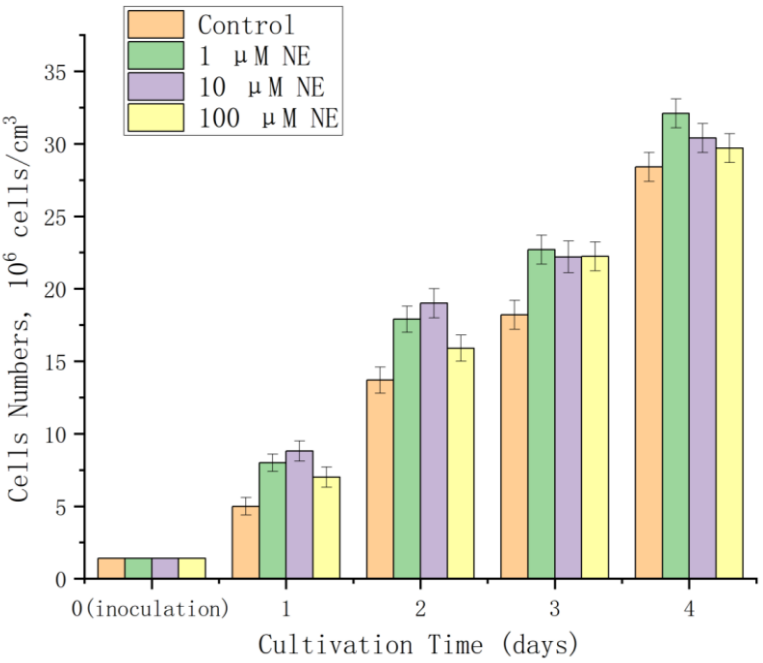

Fig. 10. Effect of norepinephrine (NE) on the growth of *C. vulgaris*. The figure contains cell numbers (millions of cells per 1 cm^3); averaged results of 4-5 repeats are given. All data obtained are statistically **significant**, according to the Student's *t* test at p =0.05.

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3.1.1.4. Effect of norepinephrine (NE) on the growth of C. vulgaris. As shown in Fig. 10, NE only increased the biomass yield by 13%, compared to the control culture, by day 4 of cultivation, i.e. by the onset of the stationary growth phase. The stimulatory effect was more significant (~37%) at the beginning of the exponential phase (day 2 of cultivation). Like DA, it tended to accelerate the growth of *C. vulgaris* rather than increase the final biomass yield. It was to be expected that NE would behave like DA, since these two compounds are structurally similar (NE has an extra hydroxy group in the side chain).

3.1.1.5. Effect of acetylcholine (ACh) on the growth of C. vulgaris. Fig. 11 demonstrates that ACh at a concentration of 1 μM and especially 10 μM increases the cell numbers both at the early and the late growth stages. The maximum stimulatory effect (with 10 μM ACh) amounted to approximately 40% after 1 day of cultivation and to 31% towards the end of day 4 as the culture was reaching the plateau level on the growth curve.

Therefore, among the tested BAs, catecholamines (DA and NE) predominantly exerted a growth-accelerating effect without a significant increase in growth plateau level (on day 4); the rest of the BAs produced a real stimulatory effect with an increase in cell number attainable during the growth of the *C. vulgaris* culture. Histamine exerted the strongest effect, increasing the biomass yield twofold. These effects were detected with 1 and/or 10 μM but not 100 μM BAs.

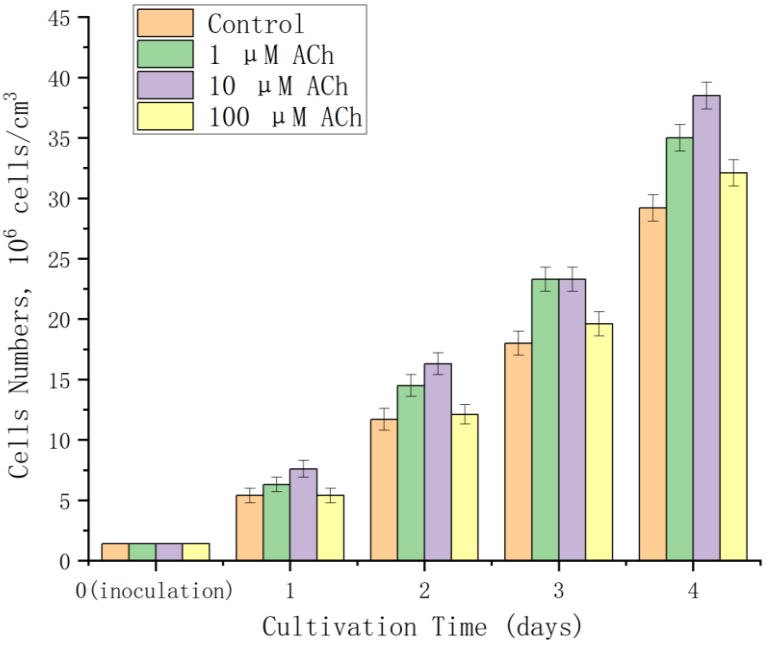

Fig. 11. Effect of acetylcholine (ACh) on the growth of *C. vulgaris*. The Figure contains cell numbers (millions of cells per 1 cm^3); averaged results of 4-5 repeats are given. All data obtained are statistically **significant**, according to the Student's *t* test at p =0.05.

3.1.2. Effects of BAs on the growth of *Scenedesmus quadricauda* (Turp.) Breb. K-1149. The dynamics of the growth of *S. quadricauda* cultures with the biogenic amines 5-HT, histamine, DA, NE, ACh, and without them is presented in Figures 12-16. The selected time points (1, 2, 4, and 7 days of cultivation) corresponded to the lag phase, the early exponential phase, the late exponential phase, and the stationary phase, respectively. The culture typically contains cenobia, i.e., groups of several cells forming a chain; each cenobium counted as 2,3, or 4 cells, depending on the cell number it included.

3.1.2.1. Effect of serotonin (5-HT) on the growth of S. quadricauda. 5-HT at concentrations of 1 and 10 μM brought about an approximately 30% increase in *S. quadricauda* biomass yield, according to the data on the cell number in the algal culture towards the end of the culture's growth period (day 7 of cultivation, Fig. 12). Stimulation was not caused by 100 μM 5-HT.

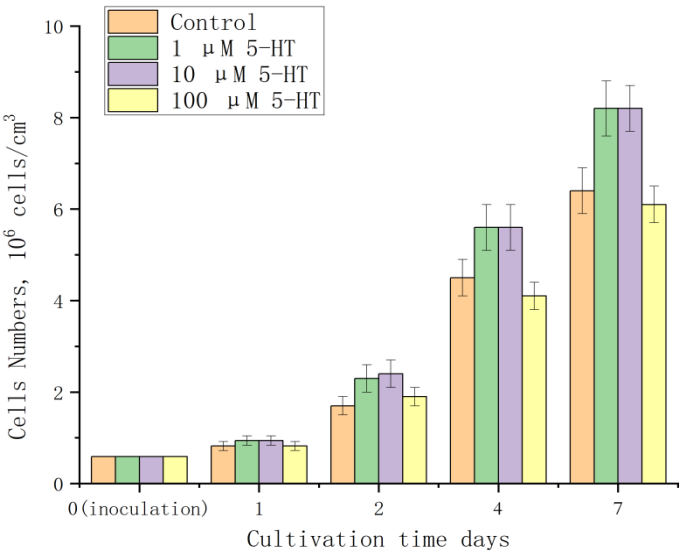

Fig. 12. Effect of serotonin (5-HT) on the growth of *S. quadricauda.* The Figure contains cell numbers (millions of cells per 1 cm^3); averaged results of 4-5 repeats are given. All data obtained are statistically **significant**, according to the Student's *t* test at p =0.05.

3.1.2.2. Effect of histamine on the growth of S. quadricauda. Histamine was the strongest growth stimulant among the biogenic amines at all tested concentrations, and the maximum (~65%) growth stimulation was achieved at a concentration of 10 μM. The increase in growth rate already manifested itself at early growth stages (Fig. 13) but it was especially prominent towards the end of the growth period. To re-iterate, a still stronger effect was produced by histamine on *C. vulgaris* (see above).

3.1.2.3. Effect of dopamine (DA) on the growth of S. quadricauda. DA significantly increased the *S. quadricauda* biomass yield. The averaged stimulatory effect at DA concentrations of 1 and 10 μM was 36% and 45% of the control, respectively, on day 7 of cultivation. In contrast to *S. quadricauda,* DA only accelerated *C. vulgaris* growth rather than increased the final biomass yield (see above). No growth increase was observed at the highest tested concentration (100 μM) in both algae; presumably, the inhibitory effect of dopamine overrode the accelerating effect.

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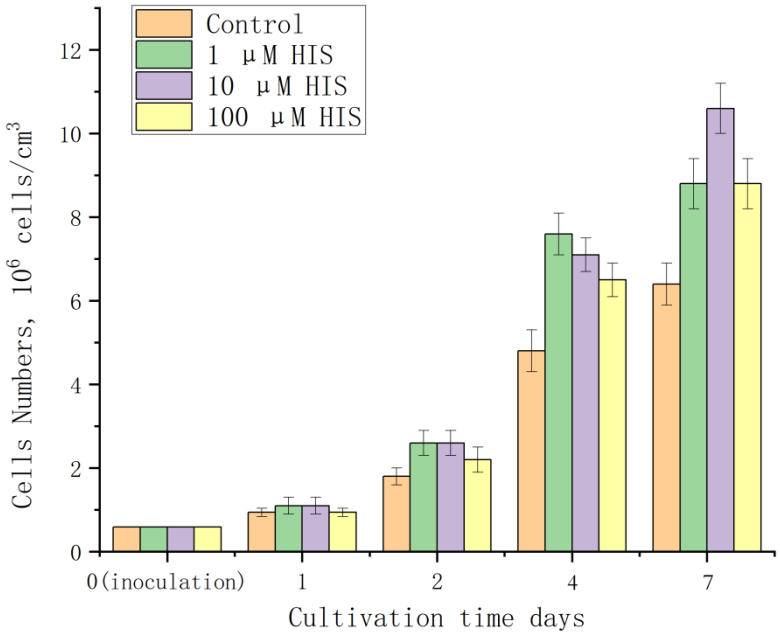

Fig. 13. Effect of histamine (His) on the growth of *S. quadricauda.* The Figure contains cell numbers (millions of cells per 1 cm^3); averaged results of 4-5 repeats are given. All data obtained are statistically **significant**, according to the Student's *t* test at p =0.05.

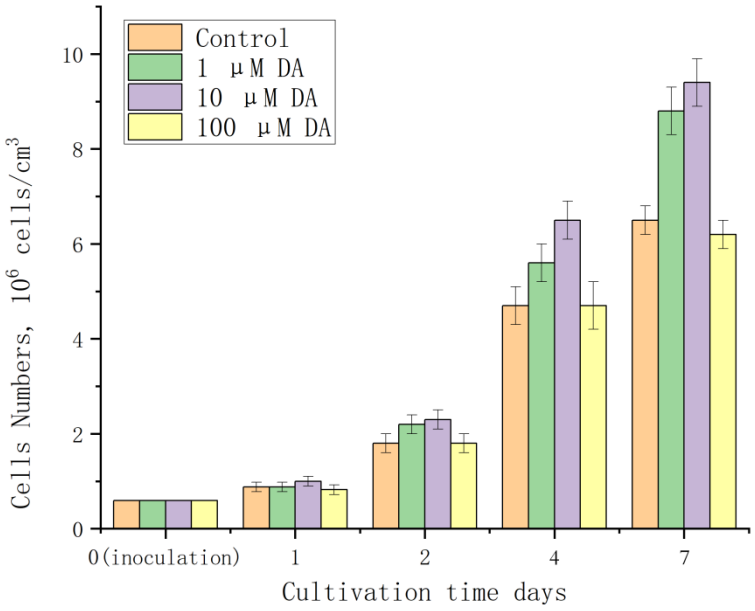

Fig. 14. Effect of dopamine (DA) on the growth of *S. quadricauda.* The Figure contains cell numbers (millions of cells per 1 cm^3); averaged results of 4-5 repeats are given. All data obtained are statistically **significant**, according to the Student's *t* test at p =0.05.

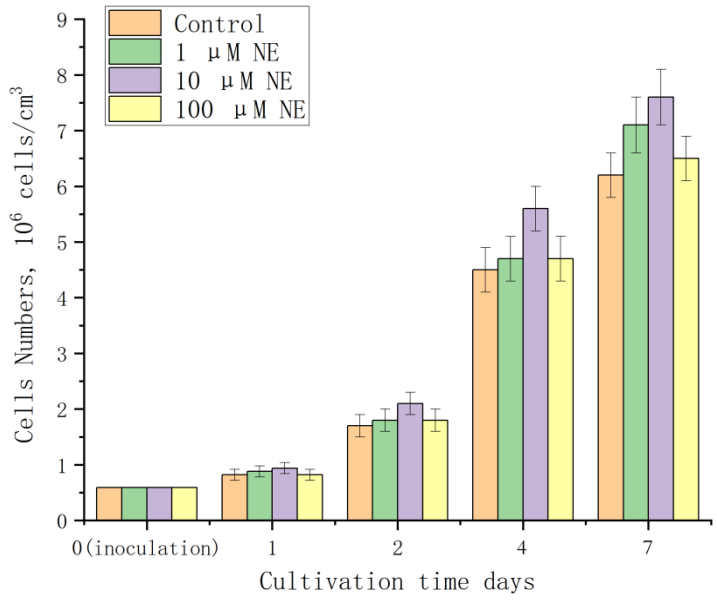

Fig. 15. Effect of norepinephrine (NE) on the growth of *S. quadricauda*. The Figure contains cell numbers (millions of cells per 1 cm^3); averaged results of 4-5 repeats are given. All data obtained are statistically **significant**, according to the Student's *t* test at *p* =0.05.

3.1.2.4. Effect of norepinephrine (NE) on the growth of S. quadricauda. NE stimulated the growth of *S. quadricauda* by 30% at concentrations of 1 and 10 μM; 100 μM NE failed to exert any effect (Fig. 15).

3.1.2.5. Effect of acetylcholine (ACh) on the growth of S. quadricauda. At a concentration of 10 μM, ACh brought about a moderate but statistically verifiable increase in *S. quadricauda* biomass yield, based on the data obtained on the cell numbers in the microalga's culture towards the end of the cultivation period (day 7). Growth stimulation was insignificant with 1 and 100 μM ACh (Fig. 16).

To sum up, all tested BAs at concentrations of 1 and 10 μM brought about an increase in cell number in the *S. quadricauda* culture, and this effect persisted until the end of the cultivation period when the growth plateau was reached (on day 7), whereas DA and NE only produced a growth-accelerating effect in the *C. vulgaris* culture (see above).

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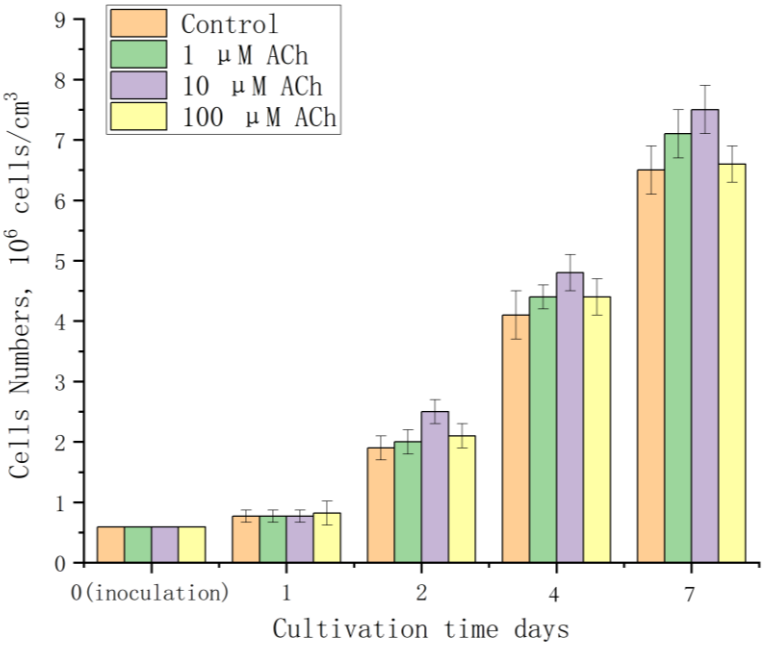

Fig. 16. Effect of acetylcholine (ACh) on the growth of *S. quadricauda.* The Figure contains cell numbers (millions of cells per 1 cm^3); averaged results of 4-5 repeats are given. All data obtained are statistically **significant**, according to the Student's *t* test at *p* =0.05.

3.1.3. Effects of BAs on the growth and cell differentiation of *Haematococcus lacustris* IPPAS H-239 and BM-1. This microalga is known to transition from the vegetative (green-colored, chlorophyll-containing) to the resting (red-colored, carotenoid-accumulating) stage via an intermediate palmelloid stage that first loses its flagella and acquires a rounded shape (vegetative palmella, Fig. 17, left) and thereupon starts producing secondary carotenoid-containing lipid droplets in its cytoplasm until it is almost completely filled with the red pigment (Fig. 17, right); this transition is promoted by stress factors such as high light and high salinity. In the following experiments, the total cell numbers and the percentages of the three developmental stages were estimated under the influence of BAs.

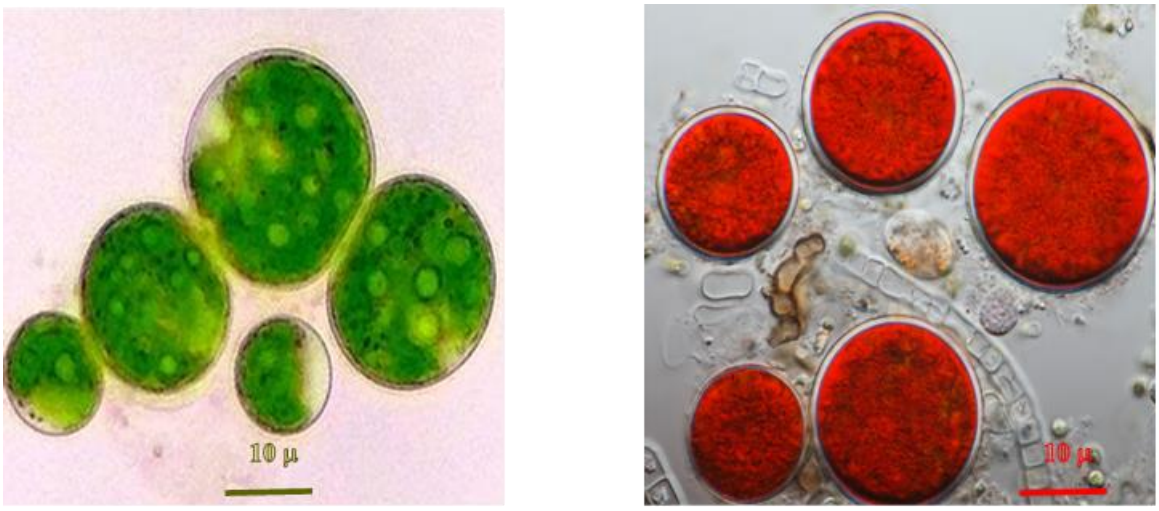

Fig. 17. Vegetative palmelloid (left) and encysted (with astaxanthin-filled lipid droplets in the cytoplasm) (right) *H. lacustris* cells. By Frank Fox - http://www.mikro-foto.de, CC BY-SA 3.0 de, https://commons.wikimedia.org/w/index.php?curid=20220268

3.1.3.1. Effect of serotonin (5-HT). Tables 2 and 3 contain the results of experiments in which *H. lacustris* strains IPPAS B-239 and BM-1 were cultivated for 14 days with and without 5-HT, the "green", transitional, and "red" (carotenoid-accumulating) cell numbers (millions per 1 cm^3) are given (see Fig. 17). It follows from the Tables that:

- 5-HT increased the total cell number in strain BM-1 (especially 10 µM 5-HT) but not strain IPPAS B-239 of *H. lacustris*.
- 5-HT at all tested concentrations decreased the vegetative ("green") cell numbers; "green" cells were not detectable at all in the strain IPPAS B-239 culture treated with 5-HT on inoculation.
- 5-HT also decreased the number of intermediate palmelloid ("brown") cells in strain BM-1 and, to a lesser extent, in strain IPPAS B-239 in which 5-HT caused a transient increase in palmelloids on day 7 of cultivation.
- 5-HT significantly increased the encysted ("red") cell number in the BM-1 strain, whereas the increase in encysted cell number in strain IPPAS B-239 in the presence of 5-HT was not statistically verifiable.

3.1.3.2. Effect of histamine. It was established that (Table 4 and 5) histamine, to a

still greater extent than 5-HT, increased the total cell numbers in both tested *H. lacustris* strains, i.e. functioned as a culture growth stimulant, in an analogy to its effect on *C. vulgaris* and *S. quadricauda* (see above). The stimulatory effect of histamine apparently overrode its cell differentiation-inducing effect: an increase in the numbers of all three cell types occurred without any significant change in the ratio between them.

3.1.3.3. Effect of dopamine (DA). DA (Table 6 and 7) also exerted a stimulatory influence manifesting itself in an increase in total cell number. The DA effect on the contents of individual cell types was strain-specifiic. In strain IPAS B-239, vegetative ("green") and palmelloid ("brown") cells disappeared by day 14 of cultivation even at the lowest tested DA concentration (0.1 μM) with a proportionate increase in the number of encysted cells that provided for a total increase in cell number in the IPPAS B-239 culture. In contrast, DA increased the vegetative cell cell content by day 14 of cultivation, concomitantly decreasing the palmelloid cell content. The encysted cell number was not augmented by DA.

3.1.3.4. Effect of norepinephrine (NE). Unlike DA, NE failed to significantly increase the total cell number in strain IPPAS B-239 and BM-1. In strain IPPAS B-239, NE brought the vegetative cell number down to zero by day 14, while the palmelloid cell number was increased and the encysted cell number virtually unchanged. In contrast to strain IPPAS B-239, 1 and 10 μM NE increased the vegetative cell number while somewhat decreasing the palmelloid cell number and producing virtually no effect on the encysted cell number (Table 8 and 9).

3.1.3.5. Effect of acetylcholine (ACh). ACh increased the total cell number while also augmenting the vegetative and palmelloid cell numbers in both *H. lacustris* strains. In strain BM-1, ACh somewhat decreased (especially at a concentration of 1 μM) the encysted cell number. In strain IPPAS B-239, ACh failed to exert any considerable influence on the encysted cell number.

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Table 2. Effect of serotonin (5-HT) on the growth and cell differentiation of *H. lacustris* IPPAS B-239.

	Cell numbers, millions of cells per 1 cm^3								
		Control		**5-HT, 0.1 μM**		**5-HT, 1 μM**		**5-HT, 10 μM**	
	Day 0	Day 7	Day 14	Day 7	Day 14	Day 7	Day 14	Day 7	Day 14
Vegetative	14.7 ± 0.5	8.5 ± 0.3	1.2 ± 0.3	7.4 ± 0.3	N/D	8.6 ± 0.1	N/D	10.8 ± 0.5	N/D
Palmelloid	N/D	4.9 ± 0.3	5.7 ± 0.3	5.9 ± 0.3	5.1 ± 0.5	5.2 ± 0.5	5.8 ± 0.5	6.1 ± 0.5	4.0 ± 0.3
Encysted	N/D	N/D	13.5 ± 0.7	N/D	12.2 ± 0.7	N/D	12.8 ± 0.7	N/D	14.6 ± 0.5
Total	14.6 ± 0.7	13.4 ± 0.7	20.3 ± 0.5	13.3 ± 0.5	17.2 ± 0.8	13.8 ± 0.9	18.7 ± 0.8	16.8 ± 0.7	18.6 ± 0.5

The Table contains cell numbers (millions of cells per 1 cm^3) after 0 (inoculating), 7, and 14 days of cultivation; averaged results of 4-5 repeats are given. N/D, not detected. Note: the stage denoted as *Palmelloid*[7] contained some red inclusions in the cytoplasm but was not yet filled with them, unlike the stage denoted as *Encysted.* The Kruskal-Wallis test results with independent samples for the experimental and control groups: Vegetative-day 7: Sig.=0.024 < 0.05, ruling the null hypothesis out, Vegetative-day 14: Sig.=0.048 < 0.05, ruling the null hypothesis out. Palmelloid – day 7: Sig.=0.043 < 0.05, ruling the null hypothesis out, Palmelloid – day 14 : Sig.=0.036 < 0.05 ruling the null hypothesis out. Encysted - day14 : **Sig.=0.080 > 0.05, conforming with the null hypothesis**. Therefore, only the difference between the control and experimental data groups for encysted cells is **not** statistically verifable.

[7] *H. lacustris* cells that acquired the "palmelloid" habit (lacked flagella, had a rounded shape, and became relatively large) but still retained the green color and did not contain visually noticeable carotenoid inclusions (see Fig. 7, left) were classified as *vegetative* cells in all Tables of this subsection (Tables 12-16).

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Table 3. Effect of serotonin (5-HT) on the growth and cell differentiation of *H. lacustris* BM-1.

	Cell numbers, millions of cells per 1 cm^3								
		Control		**5-HT, 0.1 μM**		**5-HT, 1 μM**		**5-HT, 10 μM**	
	Day 0	Day 7	Day 14	Day 7	Day 14	Day 7	Day 14	Day 7	Day 14
Vegetative	12.0 ± 0.5	1.5 ± 0.1	1.0 ± 0.1	1.2 ± 0.1	0.3 ± 0.1	0.9 ± 0.1	1.3 ± 0.1	1.1 ± 0.2	0.3 ± 0.1
Palmelloid	N/D	13.7 ± 0.5	10.6 ± 0.5	16.3 ± 0.5	8.3 ± 0.5	11.6 ± 0.5	6.9 ± 0.5	14.7 ± 0.7	10.8 ± 0.5
Encysted	N/D	0.7 ± 0.1	8.8 ± 0.1	1.0 ± 0.1	14.8 ± 0.5	1.2 ± 0.5	14.5 ± 0.5	0.7 ± 0.1	17.9 ± 0.7
Total	12.0 ± 0.5	15.9 ± 0.7	20.4 ± 0.7	18.4 ± 0.7	23.5 ± 0.7	13.7 ± 0.7	22.8 ± 0.7	16.4 ± 0.5	28.9 ± 0.5

The Table contains cell numbers (millions of cells per 1 cm^3) after 0 (inoculating), 7, and 14 days of cultivation; averaged results of 4-5 repeats are given. N/D, not detected.

The Kruskal-Wallis test results with independent samples for the experimental and control groups. Vegetative-day 7: Sig.=0.033 < 0.05, ruling the null hypothesis out, Vegetative-day 14: Sig.=0.024 < 0.05, ruling the null hypothesis out. Palmelloid – day 7: Sig.=0.019 < 0.05, ruling the null hypothesis out, Palmelloid – day 14: Sig.=0.024 < 0.05 ruling the null hypothesis out. Encysted – day 7: Sig.=0.117 > 0.05, **conforming with the null hypothesis**, Encysted – day 14: Sig.=0.037 < 0.05 ruling the null hypothesis out. Therefore, only the differences between the experimental and control groups for encysted cells are **not significant.**

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Table 4. Effect of histamine (His) on the growth and cell differentiation of *H. lacustris* IPPAS B-239

.	**Cell numbers, millions of cells per 1 cm^3**								
		Control		**His, 0.1 μM**		**His, 1 μM**		**His, 10 μM**	
	Day 0	Day 7	Day 14	Day 7	Day 14	Day 7	Day 14	Day 7	Day 14
Vegetative	11.00 ± 0.5	2.4 ± 0.3	0.3 ± 0.1	1.4 ± 0.3	0.6 ± 0.1	2.8 ± 0.1	0.6 ± 0.1	1.9 ± 0.1	1.4 ± 0.3
Palmelloid	N/D	8.4 ± 0.5	2.4 ± 0.5	10.3 ± 0.5	4 ± 0.5	11.4 ± 0.3	2.6 ± 0.3	9.5 ± 0.5	2.0 ± 0.3
Encysted	N/D	14.5 ± 0.7	20.5 ± 0.7	16.6 ± 0.7	27.5 ± 0.7	17.0 ± 0.7	25.9 ± 0.7	20.2 ± 0.7	23.8 ± 0.5
Total	11.0 ± 0.5	25.2 ± 0.7	23.1 ± 0.7	28.2 ± 0.7	32.1 ± 0.7	30.8 ± 0.5	29.1 ± 0.7	31.6 ± 0.7	27.1 ± 0.7

The Table contains cell numbers (millions of cells per 1 cm^3) after 0 (inoculating), 7, and 14 days of cultivation; averaged results of 4-5 repeats are given. N/D, not detected.

The Kruskal-Wallis test results with independent samples for the experimental and control groups. Vegetative-day 7 Sig.=0.017 < 0.05, Vegetative-day 14: Sig.=0.024 < 0.05. Palmelloid – day 7: Sig.=0.019 < 0.05, Palmelloid – day 14: Sig.=0.045 < 0.05. Encysted – day 7: Sig.=0.024 < 0.05, Encysted – day 14 : Sig.=0.016 < 0.05. All data groups rule the null hypothesis out. Therefore, the differences between the experimental and control data groups for PUFAs are significant

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Table 5. Effect of histamine (His) on the growth and cell differentiation of *H. lacustris* BM-1.

	Cell numbers, millions of cells per 1 cm^3								
		Control		**His, 0.1 μM**		**His, 1 μM**		**His, 10 μM**	
	Day 0	Day 7	Day 14	Day 7	Day 14	Day 7	Day14	Day7	Day 14
Vegetative	17.0 ± 0.5	6.5 ± 0.3	0.4 ± 0.3	5.6 ± 0.1	0.8 ± 0.3	4.8 ± 0.1	1.5 ± 0.1	6.3 ± 0.1	1.1 ± 0.1
Palmelloid	N/D	9.6 ± 0.5	4.3 ± 0.5	13.3 ± 0.7	5.3 ± 0.3	16.2 ± 0.3	3.5 ± 0.5	16.4 ± 0.5	2.8 ± 0.5
Encysted	N/D	8.2 ± 0.5	9.8 ± 0.7	11 ± 0.7	14.4 ± 0.7	11.5 ± 0.5	12.8 ± 0.7	12.5 ± 0.5	15.4 ± 0.5
Total	17.0 ± 0.5	24.3 ± 0.5	14.6 ± 0.7	29.9 ± 0.7	20.4 ± 0.7	32.5 ± 0.5	17.8 ± 0.3	35.2 ± 0.7	19.3 ± 0.7

The Table contains cell numbers (millions of cells per 1 cm^3) after 0 (inoculating), 7, and 14 days of cultivation; averaged results of 4-5 repeats are given. N/D, not detected.

The Kruskal-Wallis test results with independent samples for the experimental and control groups. Vegetative-day 7: Sig.=0.022 < 0.05, Vegetative-day 14: Sig.=0.025 < 0.05. Palmelloid – day 7: Sig.=0.024 < 0.05, Palmelloid – day 14: Sig.=0.023 < 0.05. Encysted – day 7: Sig.=0.025 < 0.05, Encysted – day 14: Sig.=0.019 < 0.05. All data groups rule the null hypothesis out. Therefore, the differences between the experimental and control data groups are significant.

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Table 6. Effect of dopamine (DA) on the growth and cell differentiation of *H. lacustris* IPPAS B-239.

	Cell numbers, millions of cells per 1 cm^3								
		Control		**DA, 0.1 μM**		**DA, 1 μM**		**DA, 10 μM**	
	Day 0	Day 7	Day 14	Day 7	Day 14	Day 7	Day 14	Day 7	Day14
Vegetative	14.5 ± 0.5	0.6 ± 0.1	1.1 ± 0.3	0.2 ± 0.1	N/D	0.1 ± 0.3	0.1 ± 0.3	0.1 ± 0.1	N/D
Palmelloid	N/D	6.5 ± 0.5	1.1 ± 0.3	6.3 ± 0.5	N/D	5.1 ± 0.3	N/D	3.7 ± 0.3	N/D
Encysted	N/D	16.3 ±0.7	20.6 ± 0.5	19.4 ± 0.7	32.1 ± 0.7	17.5 ± 0.5	27.6 ± 0.7	14.9 ± 0.3	20.4 ± 0.5
Total	14.5 ± 0.5	23.4 ± 0.7	22.9 ± 0.7	25.9 ± 0.7	32.1 ± 0.7	22.8 ± 0.7	27.6 ± 0.7	18.8 ± 0.5	20.4 ± 0.5

The Table contains cell numbers (millions of cells per 1 cm^3) after 0 (inoculating), 7, and 14 days of cultivation; averaged results of 4-5 repeats are given. N/D, not detected. The Kruskal-Wallis test results with independent samples for the experimental and control groups: Vegetative-day 7: Sig.=0.060 > 0.05, **conforming with the null hypothesis.** Palmelloid – day 7: Sig.=0.055 > 0.05, **conforming with the null hypothesis.** Encysted – day 7: Sig.=0.066 > 0.05, **conforming with the null hypothesis.** Encysted – day 14 : Sig.=0.024 < 0.05 ruling the null hypothesis out. Therefore, only the data concerning the encysted cells on day 14 of cultivation are statistically verifable.

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Table 7. Effect of dopamine (DA) on the growth and cell differentiation of *H. lacustris* BM-1.

	Cell numbers. millions of cells per 1 cm^3								
	Day 0	**Control**		**DA, 0.1 μM**		**DA, 1 μM**		**DA, 10 μM**	
		Day 7	Day 14	Day 7	Day 14	Day 7	Day 14	Day 7	Day 14
Vegetative	13.8 ± 0.5	7.9 ± 0.3	1.0 ± 0.3	7.7 ± 0.3	1.7 ± 0.5	7.2 ± 0.1	1.2 ± 0.1	6.3 ± 0.1	2.9 ± 0.1
Palmelloid	N/D	7.6 ± 0.3	1.6 ± 0.5	8.5 ±0.5	2.0 ± 0.5	8.9 ± 0.1	3.3 ± 0.1	10.9 ± 0.5	2.6 ± 0.3
Encysted	N/D	12.4 ± 0.5	15.8 ± 0.5	10.7 ± 0.5	14.6 ± 0.5	11.3 ± 0.7	13.5 ± 0.7	10.6 ± 0.5	15.8 ± 0.5
Total	13.8 ± 0.5	27.9 ± 0.7	18.3 ± 0.5	26.8 ± 0.7	18.3 ± 0.7	27.3 ± 0.7	17.9 ± 0.5	27.8 ±0.5	21.3 ± 0.5

The Table contains cell numbers (millions of cells per 1 cm^3) after 0 (inoculating). 7. and 14 days of cultivation; averaged results of 4-5 repeats are given. N/D, not detected. The Kruskal-Wallis test results with independent samples for the experimental and control groups: Vegetative-day 7: Sig.=0.024 < 0.05, ruling the null hypothesis out, Vegetative-day 14: Sig.=0.05, ruling the null hypothesis out. Palmelloid – day 7: Sig.=0.022 < 0.05, ruling the null hypothesis out, Palmelloid – day 14: Sig.=0.031 < 0.05 ruling the null hypothesis out. Encysted – day 7: Sig.=0.072 > 0.05, **conforming with the null hypothesis**, Encysted – day 14: Sig.=0.029 < 0.05 ruling the null hypothesis out, therefore, only the differences between the experimental and control groups for encysted cells on day 7 are **not significant**.

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Table 8. Effect of norepinephrine (NE) on the growth and cell differentiation of *H. lacustris* IPPAS B-239.

	Cell numbers. millions of cells per 1 cm^3								
		Control		**NE. 0.1 μM**		**NE. 1 μM**		**NE. 10 μM**	
	Day 0	Day 7	Day 14	Day 7	Day 14	Day 7	Day 14	Day7	Day 14
Vegetative	16.3 ±0.5	1.7 ± 0.3	0.3 ± 0.1	0.9 ± 0.5	N/D	0.8 ± 0.1	N/D	0.3 ± 0.1	N/D
Palmelloid	N/D	4.5 ± 0.5	2.8 ± 0.2	5.8 ± 0.7	4 ± 0.5	3.7 ± 0.3	4.7 ± 0.3	4.4 ± 0.5	4.8 ± 0.3
Encysted	N/D	14.8 ± 0.7	15.9 ± 0.5	14.0 ± 0.5	13.8 ± 0.7	14.0 ± 0.7	14.4 ± 0.5	15.3 ± 0.7	15.3 ± 0.5
Total	16.3 ± 0.5	20.9 ± 0.7	18.9 ± 0.7	20.8 ± 0.7	17.8 ± 0.7	18.4 ± 0.5	19.1 ± 0.5	20.5 ± 0.7	20.1 ± 0.7

The Table contains cell numbers (millions of cells per 1 cm^3) after 0 (inoculating). 7. and 14 days of cultivation; averaged results of 4-5 repeats are given. The Kruskal-Wallis test results with independent samples for the experimental and control groups: Vegetative-day 7 : Sig.=0.032 < 0.05, ruling the null hypothesis out. Palmelloid – day 7: Sig.=0.035 < 0.05, ruling the null hypothesis out, Palmelloid – day 14 : Sig.=0.035 < 0.05 ruling the null hypothesis out. Encysted – day 7: Sig.=0.111 > 0.05, **conforming with the null hypothesis**. Encysted – day 14 : Sig.=0.034 < 0.05 ruling the null hypothesis out, therefore, only the differences between the experimental and control groups for encysted cells on day 7 are **not significant.**

To sum up, an increase in total cell number in the cultures of IPPAS B-239 and BM-1 was only brought about by 5-HT and ACh. In contrast, DA, NE, and histamine decreased this number. In spite of this difference, all tested neurotransmitters lowered the vegetative ("green") cell numbers in the cultures of IPPAS B-239 and BM-1 in favor of the shares of intermediate (palmelloid, "brown") and resting (encysted, "red") cells. BAs differed in their impact on the ratio between these two cell types. 5-HT, histamine, and DA caused a decrease in the share of

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palmelloid cells in favor of that of resting cells. NE and ACh elevated the numbers of both resting and palmelloid cells (this effect was only detected in BM-1 with ACh). An interpretation of these effects is given in the Discussion section.

Table 9. Effect of norepinephrine (NE) on the growth and cell differentiation of *H. lacustris* BM-1.

	Cell numbers. millions of cells per 1 cm^3								
		Control		**NE, 0.1 μM**		**NE, 1 μM**		**NE, 10 μM**	
	Day 0	Day 7	Day 14	Day 7	Day 14	Day 7	Day 14	Day7	Day14
Vegetative	12.8 ± 0.5	0.3 ± 0.1	0.1 ± 0.1	0.6 ± 0.1	N/D	0.3 ± 0.1	0.3 ± 0.1	0.9 ± 0.1	2.1 ± 0.1
Palmelloid	N/D	3.2 ± 0.5	4.1 ± 0.5	3.6 ± 0.3	3.8 ± 0.1	3.5 ± 0.2	2.8 ± 0.5	3.3 ± 0.3	2.7 ± 0.5
Encysted	N/D	12.7 ± 0.1	16.9 ± 0.1	8.6 ± 0.3	17.2 ± 0.7	9.5 ± 0.5	18.9 ± 0.5	10.3 ± 0.7	16.2 ± 0.7
Total	12.8 ± 0.5	16.2 ± 0.7	21.1 ± 0.7	12.8 ± 0.5	20.9 ± 0.5	13.3 ± 0.7	22.0 ± 0.7	14.4 ± 0.7	18.9 ± 0.5

The Table contains cell numbers (millions of cells per 1 cm^3) after 0 (inoculating). 7. and 14 days of cultivation; averaged results of 4-5 repeats are given. The Kruskal-Wallis test results with independent samples for the experimental and control groups: Vegetative-day 7 : Sig.=0.024 < 0.05, ruling the null hypothesis out. Palmelloid – day 7: Sig.=0.522 > 0.05, **conforming with the null hypothesis**, Palmelloid – day 14: Sig.=0.036 < 0.05 ruling the null hypothesis out. Encysted – day 7: Sig.=0.019 < 0.05, ruling the null hypothesis out, Encysted – day 14 : Sig.=0.045 < 0.05 ruling the null hypothesis out., therefore, only the differences between the experimental and control groups for palmelloid cells on day 7 are **not significant.**

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Table 10. Effect of acetylcholine (ACh) on the growth and cell differentiation of *H. lacustris* IPPAS B-239.

	Cell numbers. millions of cells per 1 cm^3								
	Day 0	**Control**		**ACh, 0.1 μM**		**ACh, 1 μM**		**ACh, 10 μM**	
		Day 7	Day 14	Day 7	Day 14	Day 7	Day14	Day 7	Day14
Vegetative	18.5 ± 0.5	0.8 ± 0.1	0.4 ± 0.1	1.0 ± 0.1	0.8 ± 0.3	0.3 ± 0.1	0.5 ± 0.3	1.5 ± 0.1	0.3 ± 0.1
Palmelloid	2.0 ± 0.5	8.1 ± 0.5	9.0 ± 0.5	10.1 ± 0.5	11.8 ± 0.3	9.0 ± 0.3	10.0 ± 0.5	7.9 ± 0.5	7.4 ± 0.5
Encysted	0.3 ± 0.1	10.3 ± 0.5	12.4 ± 0.5	12.3 ± 0.5	15.3 ± 0.3	9.3 ± 0.5	13.3 ± 0.5	9.8 ± 0.3	13.6 ± 0.5
Total	20.8 ± 0.5	19.2 ± 0.7	21.8 ± 0.7	23.3 ± 0.7	27.9 ± 0.5	18.7 ±0.7	23.8 ± 0.7	19.3 ± 0.5	21.3 ± 0.5

The Table contains cell numbers (millions of cells per 1 cm^3) after 0 (inoculating). 7, and 14 days of cultivation; averaged results of 4-5 repeats are given. N/D, not detected. The Kruskal-Wallis test results with independent samples for the experimental and control groups: Vegetative-day 7: Sig.=0.017 < 0.05, ruling the null hypothesis out, Vegetative-day 14: Sig.=0.139 > 0.05, **conforming with the null hypothesis**. Palmelloid – day 7: Sig.=0.024 < 0.05, ruling the null hypothesis out, Palmelloid – day 14 : Sig.=0.017 < 0.05, ruling the null hypothesis out. Encysted – day 14 : Sig.=0.031 < 0.05, ruling the null hypothesis out, therefore, only the differences between the experimental and control groups for vegetative cells on day 14 are **not significant.**

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Table 11. Effect of acetylcholine (ACh) on the growth and cell differentiation of *H. lacustris* BM-1.

	Cell numbers. millions of cells per 1 cm^3								
		Control		**ACh, 0.1 μM**		**ACh, 1 μM**		**ACh, 10 μM**	
	Day 0	Day 7	Day 14	Day 7	Day 14	Day 7	Day 14	Day7	Day14
Vegetative	17.5 ± 0.5	0.5 ± 0.1	N/D	0.7 ± 0.1	0.3 ± 0.1	0.5 ± 0.1	0.1 ± 0.1	0.4 ± 0.1	N/D
Palmelloid	N/D	3.3 ± 0.1	2.9 ± 0.3	4.5 ± 0.3	7.1 ± 0.3	8.1 ± 0.1	8.2 ± 0.3	8.3 ± 0.3	8.6 ± 0.3
Encysted	N/D	16.1 ± 0.7	18.2 ± 0.7	14.7 ± 0.7	17.6 ± 0.5	11.4 ± 0.7	14.8 ± 0.7	13.0 ± 0.5	17.0 ± 0.5
Total	17.5 ± 0.5	19.9 ± 0.7	21.1 ± 0.7	19.9 ± 0.7	24.9 ± 0.7	20.0 ± 0.7	23.0 ± 0.7	21.8 ± 0.7	25.6 ± 0.5

The Table contains cell numbers (millions of cells per 1 cm^3) after 0 (inoculating). 7. and 14 days of cultivation; averaged results of 4-5 repeats are given. N/D, not detected.

The Kruskal-Wallis test results with independent samples for the experimental and control groups. Vegetative-day 7: Sig.=0.081 > 0.05, **conforming with the null hypothesis.** Palmelloid – day 7: Sig.=0.022 < 0.05, ruling the null hypothesis out, Palmelloid – day 14: Sig.=0.019 < 0.05, ruling the null hypothesis out. Encysted – day 7: Sig.=0.017 < 0.05, ruling the null hypothesis out, Encysted – day 14: Sig.=0.032 < 0.05, ruling the null hypothesis out, therefore, only the differences between the experimental and control groups for vegetative cells on day 7 are **not significant.**

3.2. CYANOBACTERIUM

This section is concerned with the effect of BAs on the growth of *Limnospira platensis* IPPAS B-256. It was established in preliminary experiments using a Goryaev chamber for counting cells that the control batch culture of *L. platensis* (without additions) reaches the maximum number of trichomes (hair-like structures composed of several cells) by day 7 of cultivation. Subsequently, the trichome number somewhat decreased. After day 7, the biomass dry weight per 1 cm^3 of

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culture volume continued increasing (gradually tapering off) and reached its maximum level on day 14 of cultivation. Apparently, the trichome number decrease was outweighed by an increase in trichome size, so that the biomass content could further increase.

The characteristic points on day 7 and 14 were chosen for estimating the influence of exogenous BAs on the dry weight of biomass, trichome number, photosynthetic pigment content (see next subsection). The fatty acid content (see below) was determined only when the biomass content reached the plateau level (on day 14 of cultivation).

3.2.1. Influence of BAs on the dry weight of *L. platensis* biomass. The bar chart of the subsection (Fig. 18-22) contains data on the influence of exogenous neurotransmitters at concentrations of 0.1, 1, and 10 μM on *L. platensis* biomass content. Upon inoculation, the dry weight was 0.38 g/mL in all experimental systems; it increased, on average, to 0.93±0.07, whereafter the growth started decelerating and reached a plateau level of 16.1±0.08 by day 14.

3.2.1.1. Serotonin (5-HT) caused a statistically significant increase in biomass dry weight on day 7 of cultivation; the biomass yield was elevated by 63% and 41% at 5-HT concentrations of 0.1 and 1 μM, respectively. This increase was not observed on day 14 (Fig. 18), i.e., 5-HT at this concentration was characterized by a culture development-accelerating rather than a true growth-stimulating effect. On day 7 and 14, the maximum 5-HT concentration (10 μM) verifiably decreased the biomass yield. The inhibitory effect of a comparatively high serotonin concentration was earlier attributed to an uncoupling action of 5-HT on biomembranes, which was demonstrated by measuring the membrane potential in the work (Олескин и др., 1998).

*3.2.1.2. Histamine***.** On day 7 of *L. platensis* cultivation, a 28% and 25% increase in biomass production was detected with 1 and 10 μM histamine, respectively (Fig. 19). Histamine failed to increase the dry weight value on day 14, i.e. it accelerated the culture growth dynamics without changing the plateau level on the growth

curve, similar to 5-HT.

3.2.1.3. Dopamine (DA) at all tested concentrations brought about a slight but statistically valid decrease in biomass yield on day 7 that tapered off by day 14 (Fig. 20); it seems likely, therefore, that DA *decelerated* the development of the batch culture of *L. platensis.*

3.2.1.4. Norepinephrine (NE) is distinguished from dopamine by only one extra OH-grouping in the side chain. It failed to exert a development-decelerating influence (Fig. 21), suggestive of a highly specific mode of action of DA.

3.2.1.5. Acetylcholine (ACh) brought about a statistically valid ~36.5% increase in dry weight on day 7, compared to the control culture. However, this effect was only exerted by ACh at a concentration of 1 μM (Fig. 22). On day 14, this increase was reduced to statistically insignificant 5%. Therefore, ACh at this concentration behaved as growth-accelerarting agent. Statistically unverifiable was the 17% increase in biomass dry weight on day 14 that was caused by 0.1 μM ACh.

To sum up, the data obtained indicate that, among the tested neurotransmitters, ACh, histamine, and 5-HT increased, whereas DA decreased the biomass content of the *L. platensis* culture on day 7 of cultivation. These effects tapered off by day 14 of cultivation.

3.2.2. Influence of BA on trichome proliferation in *L. platensis*. As mentioned in the Materials and Methods section above, the filamentous cyanobacterium *L. platensis* forms trichomes, and their number reached a maximum level (3.92±0.28) in the control culture by day 7 of cultivation and subsequently somewhat decreased (to 3.50±0.12) by day 14, despite the continued biomass yield increase; this was explained above by suggesting an increase in trichome size and cell number therein.

3.2.2.1. Serotonin (5-HT) slightly decreased the trichome number on day 7 and prevented its reduction that took place in the control culture by day 14 (with 0.1 and 10 μM 5-HT, the trichome number on day 14 did not significantly differ from

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that on day 7, Fig. 23), i.e. 5-HT prevented the "aging-related" decrease in trichome number.

3.2.2.2. Histamine reduced (by ~16% at concentrations of 0.1 and 10 μM) the trichome number on day 7 of cultivation. Unlike 5-HT, histamine did not increase the trichome number on day 14 (Fig. 24).

3.2.2.3. Dopamine (DA) elicited a more substantial decrease in trichome number in comparison to histamine and 5-HT; like these neurotransmitters, DA maintained the trichome number on day 14 at the level that was characteristic of day 7 (Fig. 25).

3.2.2.4. Norepinephrine (NE) caused no significant change in trichome number on day 7 (Fig. 26) but raised it to the day 7 level (0.1 and 1 μM NE); this level was even exceeded with 10 μM NE.

3.2.2.5. Acetylcholine (ACh) somewhat decreased (from 4.09±0.10 to 3.74±0.10 with 1 μM ACh) the trichome number in the *L. platensis* culture on day 7 of cultivation (Fig. 27), in spite of an increase in biomass content (see preceding subsection). However, the trichome number on day 14 exceeded that in the control culture with 0.1 and 1 μM ACh: the trichome number virtually remained at the level characteristic of the 7-day culture.

Therefore, all tested neurotransmitters except NE lowered the trichome number on day 7 of cultivating *L. platensis*; nonetheless, all these substances except histamine prevented the decrease in trichome number that proceeded in the control culture between day 7 and 14 of cultivation.

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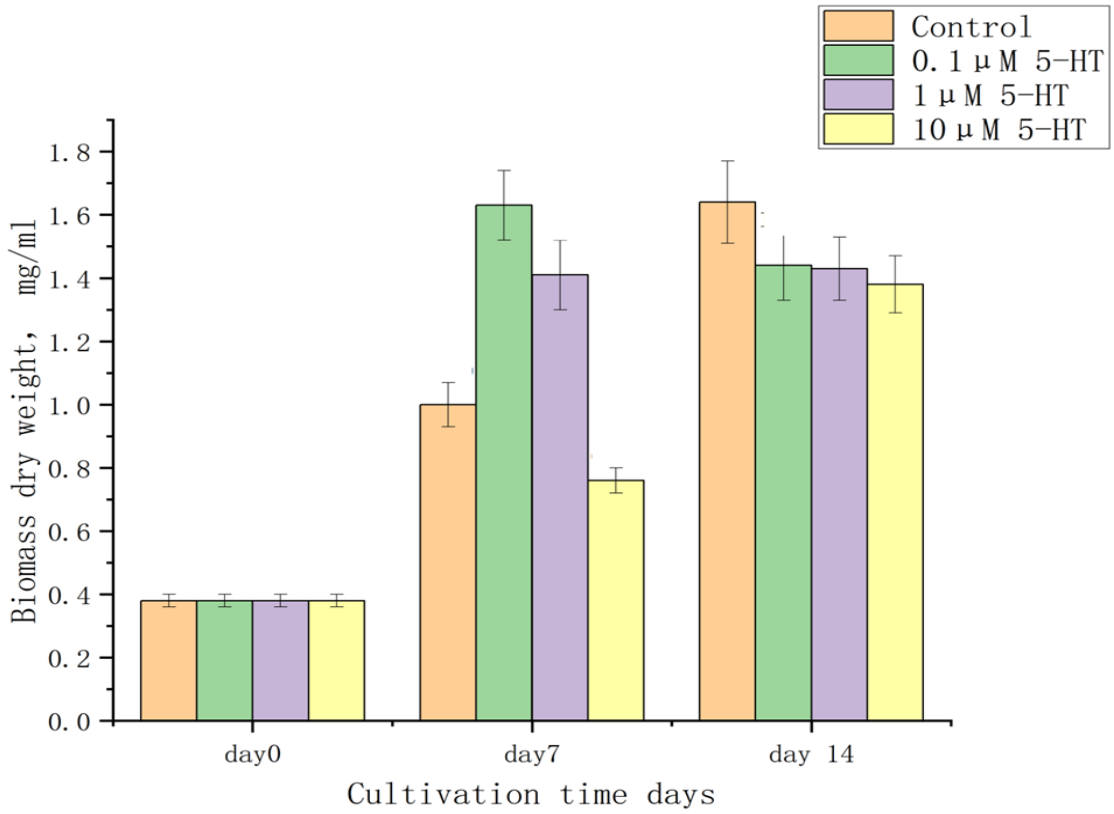

Fig. 18. Influence of serotonin (5-HT) on biomass accumulation by *L. platensis.* All data obtained are statistically **significant**, according to the Student's *t* test at p =0.05.

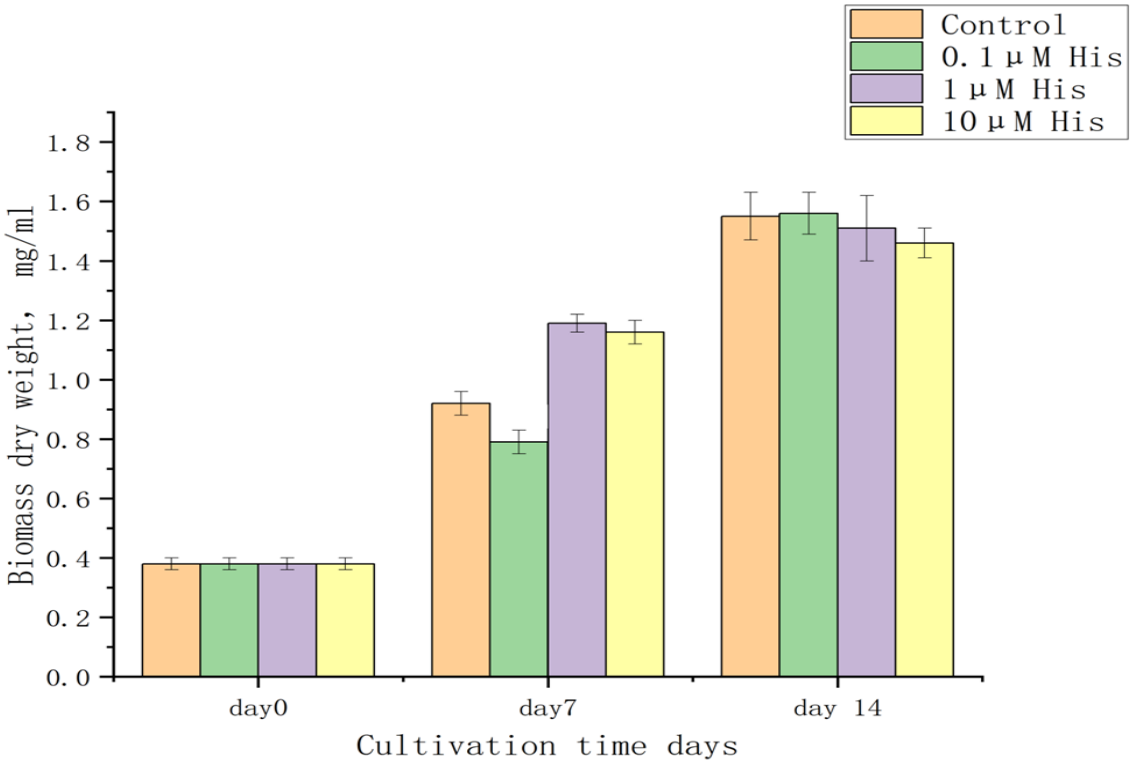

Fig. 19. Influence of histamine (His) on biomass accumulation by *L. platensis.* All data obtained are statistically **significant**, according to the Student's *t* test at p =0.05.

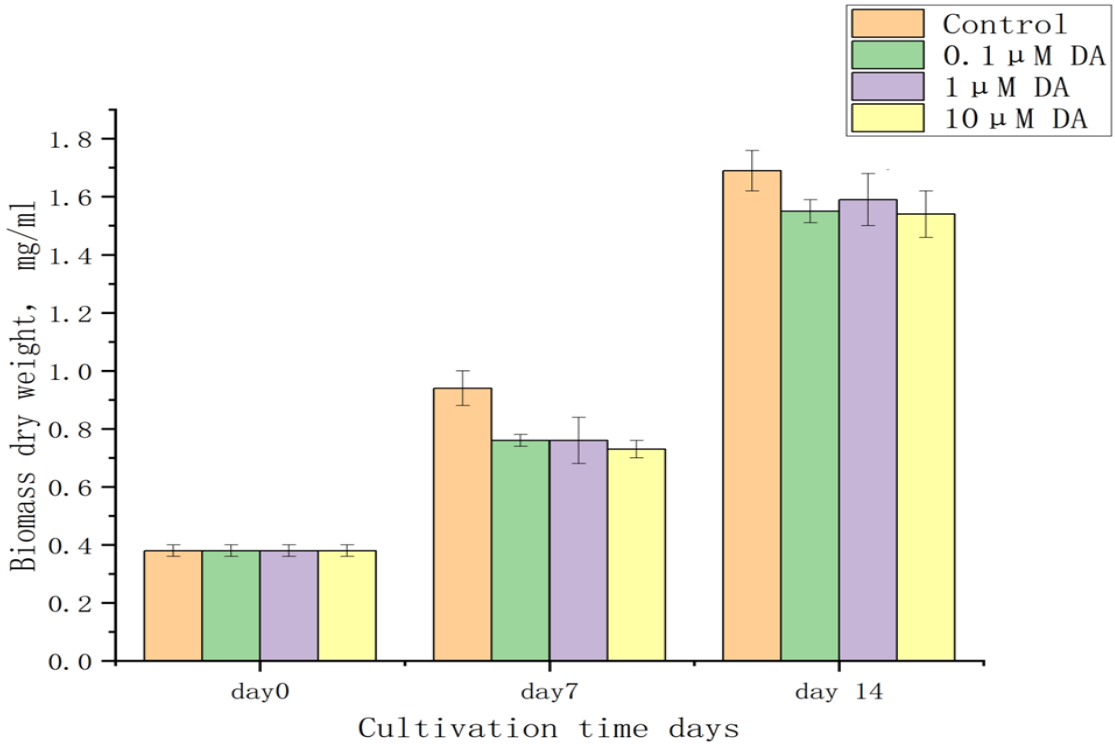

Fig. 20. Influence of dopamine (DA) on biomass accumulation by *L. platensis.* All data obtained are statistically **significant**, according to the Student's *t* test at p =0.05.

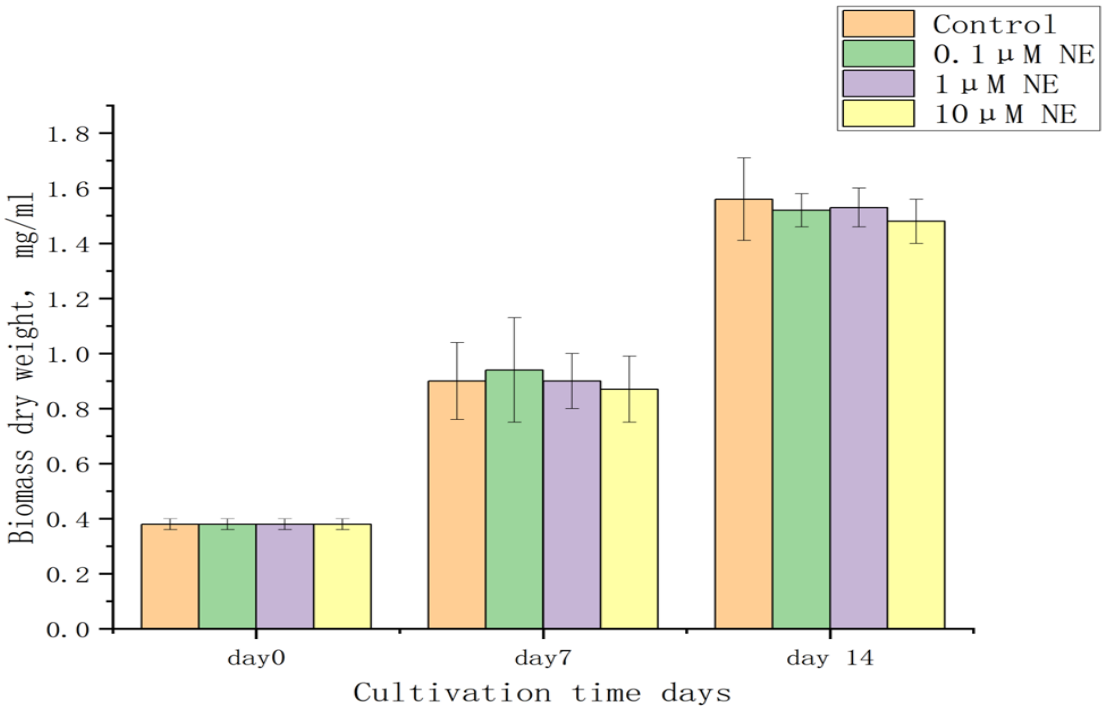

Fig. 21. Influence of norepinephrine (NE) on biomass accumulation by *L. platensis.* All data obtained are statistically **significant**, according to the Student's *t* test at p =0.05.

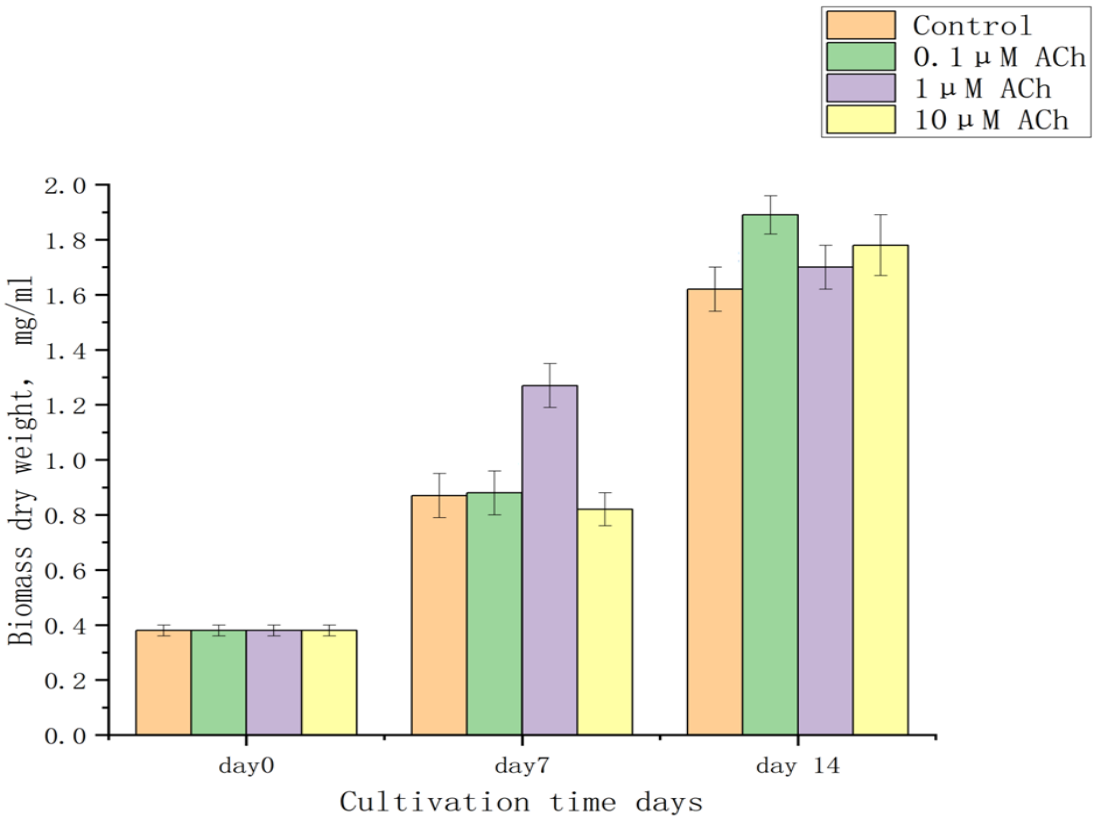

Fig. 22. Influence of acetylcholine (ACh) on biomass accumulation by *L. platensis.* All data obtained are statistically **significant**, according to the Student's *t* test at p =0.05.

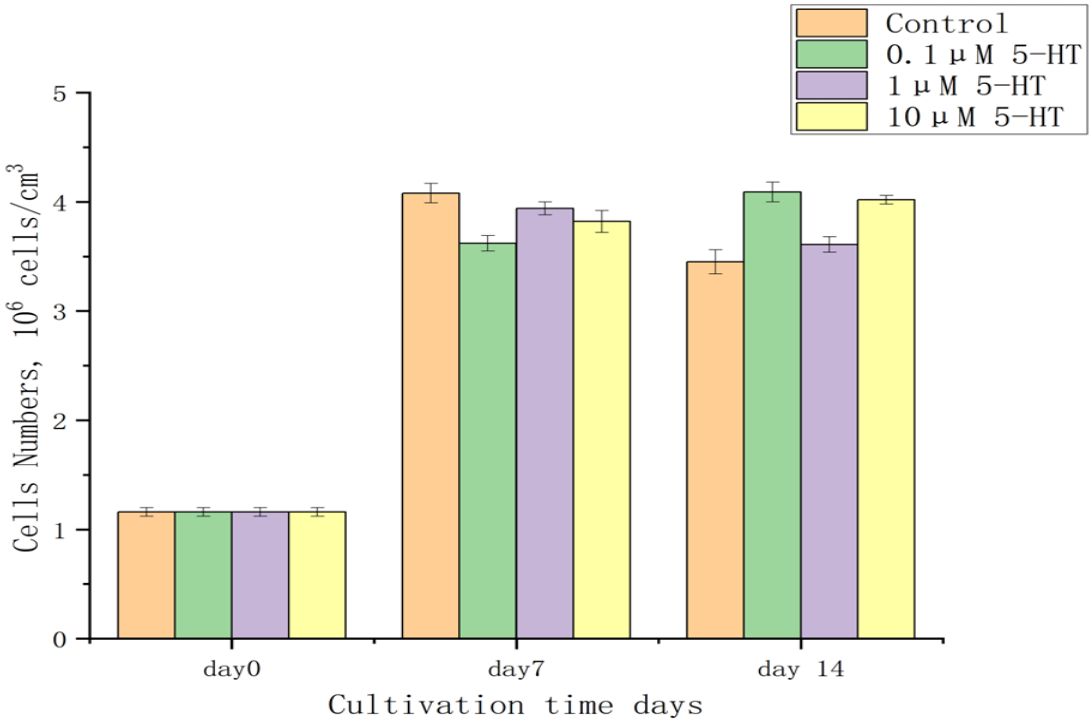

Fig. 23. Influence of serotonin (5-HT) on trichome proliferation in *L. platensis.* All data obtained are statistically **significant**, according to the Student's *t* test at p =0.05.

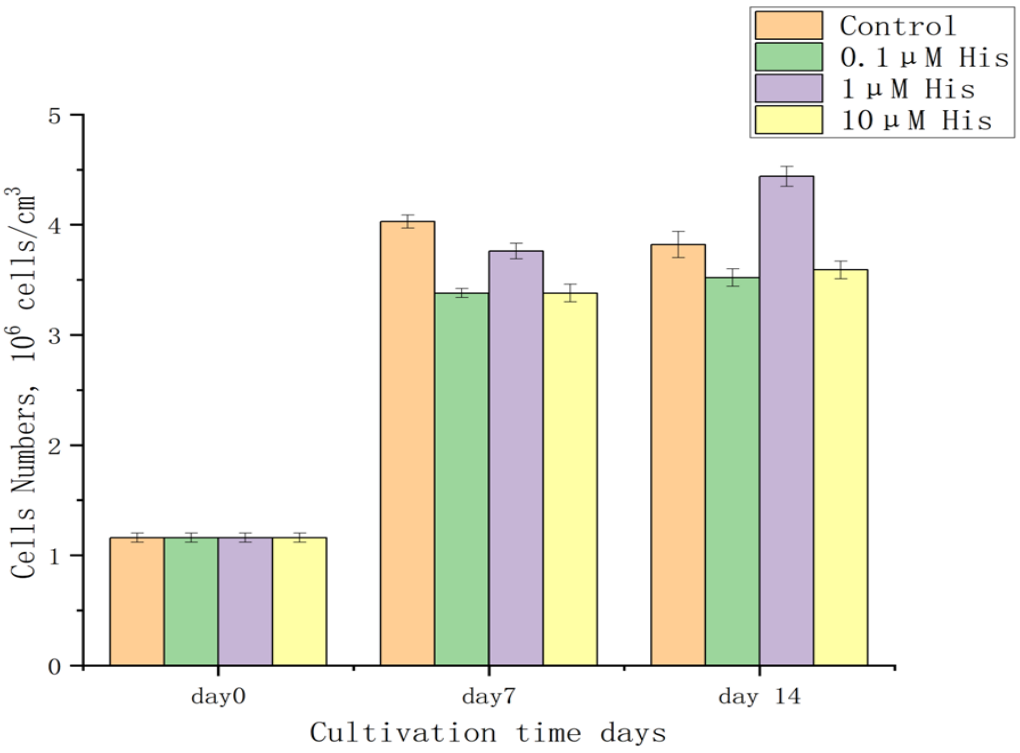

Fig. 24. Influence of histamine (His) on trichome proliferation in *L. platensis.* All data obtained are statistically **significant**, according to the Student's *t* test at p =0.05.

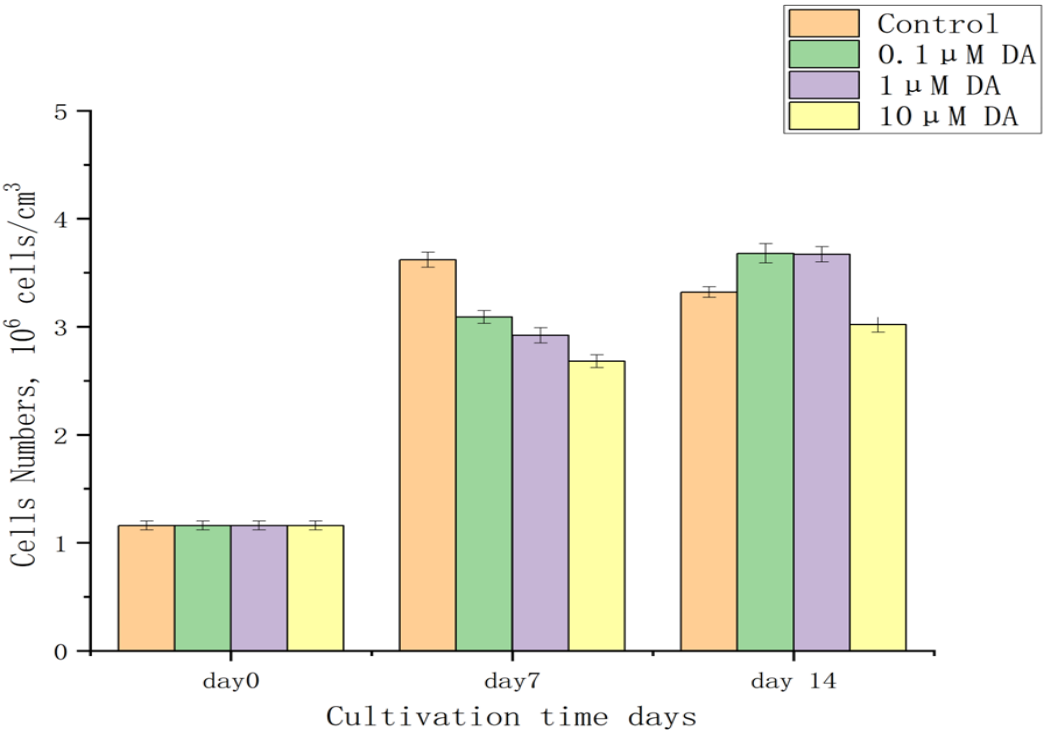

Fig. 25. Influence of dopamine (DA) on trichome proliferation in *L. platensis.* All data obtained are statistically **significant**, according to the Student's *t* test at p =0.05.

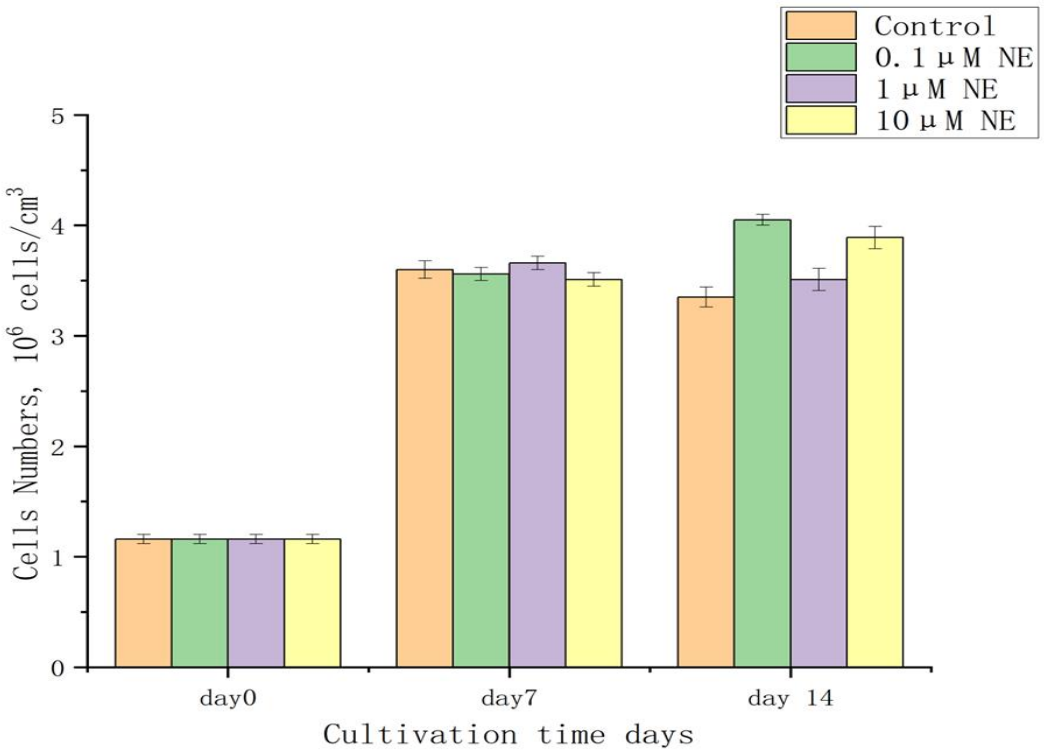

Fig. 26. Influence of norepinephrine (NE) on trichome proliferation in *L. platensis.* All data obtained are statistically **significant**, according to the Student's *t* test at p =0.05.

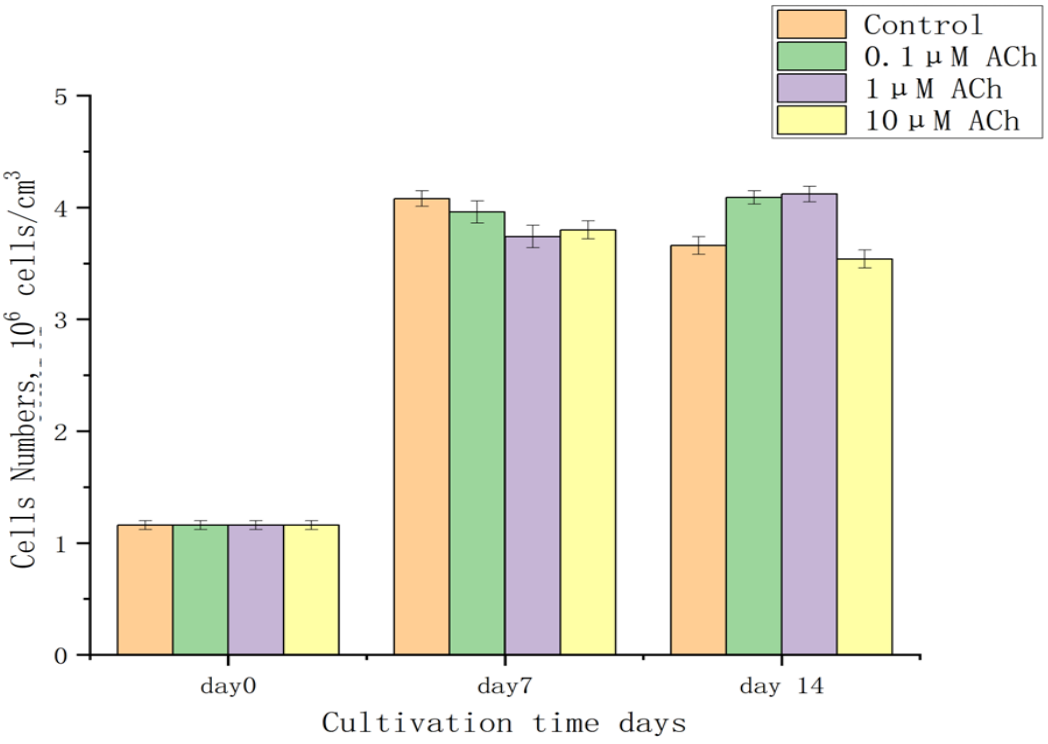

Fig. 27. Influence of acetylcholine (ACh) on trichome proliferation in *L. platensis***.** All data obtained are statistically **significant**, according to the Student's *t* test at p =0.05.

3.3. CONCLUSIONS

Recent publications on the ecotoxicological implications of active pharmaceutical ingredients (APIs) emphasize that low API concentrations (trace amounts) are detectable even as far as at a distance of 5 km (in river catchments, e.g., in tested areas in the UK) from the points at which they are released, e.g., in water that has passed wastewater treatment plants and still contains some APIs (Kay et al., 2017).

3.3.1. Summarizing the growth effects of neurotransmitters. The present work provides evidence that low (submicromolar or micromolar) concentrations of neurotransmitters exert significant, mainly stimulatory, effects on the growth (based on cell numbers) and accumulation of fatty acids in the lipids and of photosynthetic pigments in the photosystems of microalgae including cyanobacteria. The growth stimulation maximally results in a twofold increase in cell numbers, as is the case with *Chlorella vulgaris* treated with histamine (that is characteristic the wastewater of food-producting plants because it results from the bacterial fermentation of histidine contained in proteins). Presumably, neurotransmitters entering aquatic ecosystems can cause phytoplankton "blooming" that is fraught with eutrophication.

The results of the present work also support the idea that biogenic amines form a part of the pool of eviolutionarily conserved signals that exert their influence, apart from the animal world (where they function as neurotransmitters and hormones), on a wide variety of other organisms including the microbiota and the phytoplankton of waterbodies.

The literature data indicate that BA, especially catecholamines (DA. NE. and epinephrine) promote the growth of diverse pathogenic, opportunistic, and saprotrophic bacteria such as *Yersinia enterocolitica*, the pathogenic and symbiotic strains of *E. coli, Shigella* spp., *Salmonella* spp., *Pseudomonas aeruginosa, Bordetella pertussis, B. bronchiseptica, Aeromonas hydrophila, Helicobacter pylori, Haemophilus influenza, Klebsiella pneumonia, Listeria monocytogenes, Campylobacter jejunii, Borrelia burgdorfii, Mycoplasma hyopneumoniae,* and

Staphylococcus epidermidis as well as the yeast *Saccharomyces cerevisiae*. 5-HT also increases the growth rate of bacteria (*Enterococcus faecalis, E. coli,* and *Rhodospirillum rubrum*) and yeasts (*Candida guillermondii* and *S. cerevisiae*) (reviewed, Oleskin, Shenderov, 2020).

During the course of the present graduate work, it was revealed that BAs produce stimulatory effects, at relatively low concentrations, on the tested representatives of microalgae of several different taxonomic groups, such as *Chlorella vulgaris* (class Trebouxiophyceae)*, Scenedesmus quadricauda* (class Chlorophyceae, order Sphaeroleales), and *Haematococcus lacustris* (class Chlorophyceae, order Chlamydomonadales), as well as a prokaryote, the cyanobacterium *Limnospira platensis* (kingdom/domain Bacteria, phyla Cyanobacteria, class Cyanophyceae, order Oscillatoriales)

Among the BAs used in this work, histamine proved to be the strongest growth stimulator. and the cell number increase caused by histamine amounted to ~65% in *S. quadricauda* (Fig. 13), almost 100% of the control value in *C. vulgaris* (Fig. 8), 34% (Table 4) and ~40% (Table 5) in the strains *H lacustris* IPPAS B-203 and BM-1, respectively.

In the cyanobacterium *L. platensis,* not separate cells but whole chains of them, trichomes, were counted during the experiment, and histamine even somewhat reduced their number on day 7 of cultivation (see Fig. 24 above). However, if the biomass content was estimated from dry weight data, then histamine was also to be considered a biomass accumulation stimulant (or, at least, accelerator, Fig. 19).

Hypothetically. the mode of action of histamine in microalgae is based on its binding to specific receptors on the cell membranes of microalgae. in an analogy to other unicellular organisms (reviewed, Oleskin, Shenderov, 2020; Олескин и др., 2020; Олескин, 2024). Receptor-based mechanisms of action in microbial cells were posited in the literature with respect to catecholamines and other BAs (Lyte, 2016).

ACh also markedly stimulated the growth of *C. vulgaris* (Fig. 11), in conformity

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with the literature data on its impact on a related algal species. *C. sorokiniana* (Czerpak et al., 2003). ACh to a somewhat lesser extent stimulated the growth of *S. quadricauda* (Fig. 16). ACh also increased the total cell number in both tested *H. lacustris* strains (Table 10 and 11). Finally, ACh brought about a statistically verifiable increase in biomass content (based on dry weight) in the cyanobacterium *L. platensis* (Fig. 22), although it failed to increase the trichome number and even decreased it (Fig. 27). Unlike histamine and ACh, NE only produced a very weak stimulatory effect on the cultures of *C. vulgaris* and *S. quadricauda* (Fig. 10 and 15); this effect was also completely absent with *H. lacustris* (Table 8 and 9) and *L. platensis* (Fig. 21).

3.3.2. Plausible mechanisms of action of neurotransmitters. The brief reminder of some of the growth-related effects of BAs that were discussed in detail above, should serve as a primer for considering the possible mechanisms of action of these neurotransmitters. In the review section of this thesis, it was noted that there are relatively few recent publications on the effects of neurotransmitters in microalgae (Czerpak et al., 2003; Schiechl et al., 2008; Parsaiemehr et al., 2015; Van Alstyne et al., 2018; Roshchina et al., 2022; Zhao et al., 2025a).

However, some important studies have been conducted on the impact on microalgae (Piotrowska-Niczyporuk, Bajguz, 2014; Yu et al., 2022; Qiu et al., 2025; Zhao et al., 2025b) including cyanobacteria (Tiwari et al., 2020) of phytohormones that are chemically and functionally similar to neurotransmitters. At low concentrations, various subgroups of phytohormones (auxins, gibberellins, cytokinins, brassinosteroids, ethylene, abscisic, salicylic, and jasmonic acid, etc.) exert a stimulatory influence on the growth of microalgae, biosynthesis of valuable products by them, and their stress resistance (Цавкелова и др., 2006а, б; Qiu et al., 2025).

A recently investigated hormone, GR24, that belongs to strigolactones (plant root hormones that suppress branching and promote interactions between plants

and arbuscular fungi) stimulates, at nanomolar concentrations, the growth and photosynthetic activity of *Chlorella vulgaris*. GR24 was revealed to facilitate wastewater bioremediation with the removal of polluting antibiotics from the water by means of a symbiotic system including the microalga, the fungus *Clonotactys rosea* and the bacterium S395-2 (Zhao et al., 2025b).

In the literature, several possible mechanisms of stimulatory action of phytohormones on microalgal growth were posited.

3.3.2. 1. Antioxidant effect that is due to (a) the activation of enzymes that quench reactive oxygen species (ROS) and (b) an increase in the concentrations of antioxidants such as ascorbic acid and glutathione. For instance, it was demonstrated that the auxins indole-3-acetic acid. indole-3-butyric acid. and phenylacetic acid. as well as their synthetic analog 1-naphthaleneacetic acid stimulated *C. vulgaris* growth at concentrations of 0.1-1.0 μM (Piotrowska-Niczyporuk, Bajguz. 2014). These effects were attributed to the activation of antioxidant systems by these hormones since they were revealed to increase ascorbate peroxidase, catalase, and superoxide dismutase activities and to decrease lipid peroxidation and H_2O_2 accumulation. The neurotransmitters that were utilized in this work are chemically similar to auxins; this primarily concerns 5-HT whose oxidation results in producing 5-hydroxyindole-3-acetic acid. a substituted auxin

3.3.2.2. Promotion of photosynthetic activity by increasing the photosynthetic pigment content, which, in turn, implies an increase in gene expression related to chlorophyll and carotenoid synthesis. In addition to increasing antioxidant activity, auxins cause an increase in photosynthetic pigment production (Piotrowska-Niczyporuk, Bajguz, 2014).

3.3.2.3. Neurotransmitters' influence on the dynamics and rhythms of development of microalgal cultures. Phytohormones are known to exert a regulatory influence on biorhythms and the dynamics of "age-related" stages in the development of plants. For instance, abscisic acid and ethylene promote leaf shedding, suppress growth processes, and accelerate plant senescence (Цавкелова и др., 2006а, б).

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There are analogous examples concerning microalgae. Of note is a study that dealt with serotonin and its derivative melatonin. In the cells of the charophyte *Chara australis,* diurnal oscillations in serotonin and melatonin concentrations were documented, and the oscillation rhythm was different under long day (12 hs light:12 hs dark) and short day (9hs light:15 hs dark) conditions (Van Alstyne et al., 2018).

Presumably, the effects of neurotransmitters are due to mechanisms that are analogous to those listed above.

First, serotonin and other BAs are capable of quenching reactive oxygen species and preventing the peroxidation of unsaturated fatty acids in lipids. It is likely, therefore, that antioxidant activity (item 1) contributes to the stimulatory action of some of the tested BAs on the content of biomass, photosynthetic pigments, and fatty acids. Even though antioxidant activity was not monitored in the present work, account should be taken of the analogous stimulatory action of phytohormones, incliding auxins, on the enzymatic and non-enzymatic systems of ROS quenching in microalgae.

Second, the data on an increase in photosysnthetic pigment content in the presence of neurotransmitters directly support the suggestion contained in item 2. This suggestion will be discussed below in the relevant subsection of Chapter 5.

Third, the effects of neurotransmitters on the dynamics of trichome numbers in the cyanobacterium under study (Fig. 23-27) seems to be of relevance to item 3 in the list above. In the literature on microalgae, including the author's published work (Cao et al, 2024), the suggestion has been put forward that neurotransmitters decelerate the growth stages of batch cultures, prolonging the earlier stages with active protosynthesis and cell proliferation. In terms of this suggestion, it is possible to account for the decrease in trichome number on day 7 of cultivation and continued trichome proliferation up to day 14, even though the trichome number clearly tends to decrease in the control culture. The same suggestion in conjunction with the data of this work on eukaryotic microalgae enables explaining the increase in PUFA percentage with some of the tested BAs. Prolonging the earlier

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development stages results in increased accumulation of unsaturated faty acids that are characteristic of these stages (to be further discussed in the relevant subsection below).

It follows from the data obtained in the present work that the tested neurotransmitters exert their effects at low concentrations (0.1-10 μM). Such neurotransmitter concentrations are sufficient for their specific functions that are based, in an animal organism, on interactions between neurotransmitters and respective receptors (Дубынин и др., 2010). For instance, DA at submicromolar concentrations efficiently binds to $D_{1\text{-}5}$ receptors and the chemically related NE to α- and β-adrenoreceptors.

Cyanobacteria have not yet received sufficient attention in the literature on neurotransmitter effects on microorganisms. Nevertheless, detailed research was conducted with other prokaryotes within the framework of an increasingly popular subfield of microbiology that is referred to as *microbial endocrinology* (Lyte, 2016; Oleskin, Shenderov, 2020). Some neurotransmitters (dopamine, morepinephrine, serotonin, etc.) function as quorum sensing (QS) signals in prokaryotes. DA and NE are functionally analogous to QS signal AI-3. Similar to this signal, they bind to receptor histidine kinases QseC and QseE in *Escherichia coli* and other intestinal bacteria. Such specific signal-receptor interaction stimulates the expression of gene groups (operons) that are mandatory for growth processes and the synthesis of products involved in the microbiota-host interaction or in the regulation of the functioning of natural microbial associations.

The data that neurotransmitters at (sub)micromolar concentrations considerably influence the fatty acid and photosynthetic pigment content in *L. platensis* give grounds for the suggestion that this cyanobacterium possesses a quorum sensing system with receptors that bind neurotransmitters as specific signals. Presumably, they activate enzymatic and non-enzymatic antioxidant protective systems and, in addition, promote the expression of fatty acid desaturase genes, resulting in increasing the fatty acid desaturation degree.

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In this context, further research should address the interaction of neurotransmitters with the operons that are responsible for the synthesis and metabolism of fatty acids and photosynthetic pigments. For comparison, the addition of naphthyl acetic acid (an auxin) to the culture of *C. vulgaris* was established to 7.1-fold increase the expression of the genes encoding acetyl-CoA synthase, a key enzyme in the lipid biosynthesis pathway (Zhao et al., 2022).

Research on bacteria (e.g., *E. coli,* Олескин и др., 1998) and eukaryotic microalgae (*C. vulgaris, Scenedesmus quadricauda,* and *Haematococcus lacustris,* the data of this graduate work) revealed that relatively high concentrations of some neurotransmitters (10-100 μM) were less stimulatory to microalgal growth than lower concentrations (0.1-1 μM). For instance, acetylcholine (Fig. 22) increased the biomass yield on day 7 of cultivation at a concentration of 1 μM but not 10 μM. Presumably, neurotransmitters at relatively high concentrations produce nonspecific toxic effects since they form ions that can uncouple membrane phosphorylation. To re-emphasize, serotonin was earlier revealed to collapse the membrane potential of *Rhodospirillum rubrum* at concentrations of 10 μM and above (Олескин и др., 1998).

Interestingly, phytohormones including auxins also stimulate microalgal growth at low concentrations; higher auxin concentrations suppress growth and elicit cell death (Singh et al., 2020; Abd-El-Aziz et al., 2025).

3.3.3. Possible role of neurotransmitters as cross-species signals and as potential ecopollutants in aquatic ecosystems. In the literature, there are extensive data indicating that many algal species, including a number of representatives of Chlorophyta, Charophyta, Ochrophyta, and Rhodophyta, synthesize large quantities of such neuroactive amines as DA, 5-HT, histamine, tyramine, and ACh (Herrmann, Jüttner, 1977; Van Alstyne et al., 2018). For instance, histamine is present at concentrations up to 60-500 mg/g of biomass in the red alga *Claviceps purpurea* (Van Alstyne et al., 2018). However, in this work

the analysis of the content of disintegrated algal cells and the cultural liquid of *S. quadricauda* and *C. vulgaris* using HPLC revealed zero concentrations of 5-HT, NE, DA, and the dopamine precursor DOPA in these microalgae (see the final subsection of the Results section). This fact seems to rule out the suggestion that these neurochemicals can fulfill an autoregulatory function.

It seems likely, therefore, that BAs and, presumably, other neuroactive compounds function as interspecies rather than intraspecies signals. The microalgal growth-promoting neurochemicals NE, DA, and 5-HT are produced by other components of natural aqueous ecosystems in which microalgae form a part of the phytoplankton. For instance, they all are synthesized by various representatives of the microbiota (reviewed, Oleskin and Shenderov, 2020) and, therefore, the impact of these compounds on microalgae can be considered in terms of chemical interaction between microalgae and other microorganisms. This interaction is apparently beneficial for microalgae with respect to their growth that is stimulated by BAs.

High DA concentrations are also released by such macroalgae as *Ulvaria* spp. In the literature, evidence has been presented that *Ulvaria*-produced dopamine functions as an antimicrobial agent and as a kairomone, i.e., a protective substance preventing the consumption of *Ulvaria* by sea urchins, gastropods, and arthropods (Van Alstyne et al., 2019; Ramakrishna, Mukherjee, 2020). It follows that this macroalga is expected to stimulate microalgal growth as long as the DA concentration does not exceed the threshold (10 μM in the experiments shown in Fig. 9) above which DA ceases to exert a stimulatory influence on microalgae. The species *U. obscura* forms a dense population and causes the formation of "green tides" releasing high DA concentrations (Van Alstyne et al., 2019). Accordingly, the *U. obscura* population should be surrounded by two distinct concentric zones in a water body: (i) the distant zone in which the DA is diluted to low (1-10 μM) levels and green microalgae are expected to increase their growth rate and (2) the proximate zone in which no microalgal growth stimulation should be observed;

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moreover, microalgal growth will be inhibited in this area.

Interestingly, not only *Ulvaria* but also other macroalgae, e.g., *Nitella flexilis* and C*hara vulgaris,* and also higher plants growing on the water surface such as the duckweed (*Lemna minor*) produce biogenic amines and release into the environment (Ramakrishna, Mukherjee, 2020).

As for the role of neurotransmitters as new-generation ecopollutants, it seems likely that similar zones with different dopamine levels and diametrically opposite effects on the phytoplankton are formed by wastewater released by pharmaceutical or food industrial facilities if it contains dopamine.

Submicromolar or micromolar concentrations of DA, NE, and 5-HT are produced by a number of coastal plant species exemplified by tropical trees and shrubs growing around lakes and ponds in southern China, namely, in Shenzhen as shown in the present work (Table 53). According to recent literature data, many higher plants both contain neurochemicals and respond to them (Oleskin, Shenderov, 2018). BAs produce strong stimulatory effects on the seeds of *Raphanus sativus*, the vegetative microspores of *Equisetum arvense*, and the pollen of *Hippeastrum hybridum* (Рощина, 1991), in an analogy to the microalgae investigated in the present work.

Aqueous ecosystems typically contain invertebrates that also release neurochemicals (reviewed, Грицай, 2017). This work contains data on DA formation by the water flea *Daphnia magna,* a representative of the zooplankton (see below, Chapter 6, Table 52). Hypothetically, the predator *D. magna* produces a substance that stimulates the growth of its prey (microalgae) if released into the water by excretion or as a result of injury sustained by *D. magna*. Moreover, DA promotes cell aggregation in *C. vulgaris* (unpublished data obtained in collaboration with a student, Jia Hui). Plausibly, the water flea prefers consuming compact cell pellets (“Chlorella hamburgers”) rather than small separate *C. vulgaris* cells (Олескин, 2024)

Stress factors including traumas caused by predator attacks or intraspecies

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conflicts can result in extra amounts of histamine. 5-HT. and catecholamines being released into the water by aquatic animals. In light of the data presented in this work, stress- (trauma-)associated BAs are expected to promote microalgal growth in waterbodies; microalgae seem to be "interested" in representatives of the zooplankton and nekton being stressed or traumatized. This is in compliance with an ancient Chinese philosophical principle: *the suffering of some components of a system contributes to the flourishing of its other components.*

In similar fashion, the gut bacteria of the human organism. including useful probiotic species and strains. accelerate their development in the presence of BAs and other neurotransmitters. These neurotransmitters are more efficiently produced and released into the intestinal lumen during local inflammation, i.e., the gut microbiota is "interested" in keeping the intestines chronically inflamed (reviewed, Oleskin, Shenderov, 2020).

Special attention should be given to another important green microalgal species, *Haematococcus lacustris (= pluvialis)*. This predominantly freshwater microalga inhabiting ephemeral rain pools and natural and artificial ponds (Shah et al., 2016) is of indisputable ecological importance as a component of the phytoplankton that enters into complex interactions with its other constituents. For instance, representatives of the genus *Haematococcus* are exposed to the toxic action of substances (allomones) released by the cyanobacterium *Anacystis nidulans* (reviewed, Остроумов, 1986).

3.3.4. Biotechnological implications. Using stress effects. The species *H. lacustris* holds much biotechnological value since it synthesizes practically useful products including PUFA-rich lipids and especially the carotenoid astaxanthin, the "super-antioxidant" already considered in this work in the Materials and Methods chapter (Chapter 2). "As societies nowadays are looking toward "green" solutions, natural astaxanthin from *H. pluvialis* seems to be more favorable than its synthetic counterpart due to structure, function, application, and security" (Shah et al., 2016,

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p. 6-7). The applications of astaxanthin in health care and cosmetics should be re-emphasized. Unlike other carotenoids, astaxanthin is not a vitamin precursor; therefore. even its long-term intake does not result in hypervitaminosis (excessive vitamin production) (Solovchenko, 2015). It has been estimated that the global astaxanthin market has the potential to reach a valuation of US $2.57 billion in 2025 and $ 3.40 billion by 2027 (Kim et al., 2022; Zhao et al., 2025a).

Active astaxanthin accumulation by encysted cells is preceded by stress-stimulated transformation of the “green” cells to non-motile palmelloid cells containing an increasing amount of astaxanthin against the background of still significant chlorophyll content (the so called “brown” cells) (Solovchenko, 2015). They convert to “red” (encysted) cells containing up to 3-5% of astaxanthin by dry weight, which accounts for 80-99% of the total carotenoid content (Shah et al., 2016).

In this work, two subsections are devoted to astaxanthin, dealing, respectively, with the effect of neurotransmitters (BA) on (1) the ratio of astaxanthin-loaded encysted cells and other cell types and (2) the content of astaxanthin in secondary carotenoids.

The results obtained (Tables 2-11) should be considered in the context of literature data on the effects of low-molecular-weight hormones and other regulatory substances on the synthesis of astaxanthin by microalgae. According to the literature, plant hormones involved in the response of plants to stress (abscisic and jasmonic acid) as well as growth regulators (gibberellic and salicylic acid as well as brassinosteroids) dramatically increase the yield of astaxanthin in the culture of *H. lacustris*. Salicylic acid is particularly effective: at concentrations of 25 or 50 mg/L, it stimulates astaxanthin production, while at the same time enhancing the transcription of eight carotenoids involved in astaxanthin synthesis (Gao et al., 2012; Shah et al., 2016).

Important data on the effect of DA on *H. lacustris* have been presented recently. Against the background of high salinity stress caused by adding 1 g/L NaCl, 33 μM

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DA brings about a weak (7.63%) additional stimulation of *H. lacustris* biomass accumulation but significantly (by 41.25%) increases the astaxanthin content in culture cells, as well as astaxanthin productivity (by 52.04%), compared to the system containing NaCl only. DA stimulates lipid synthesis in cells (Zhao et al., 2025a). This is obviously due to the fact that astaxanthin accumulates in cytoplasmic lipid droplets; moreover, some stages of its biosynthesis are carried out in the droplets (Solovchenko, 2015). DA also lowers the level of reactive oxygen species (ROS) in the cells (Zhao et al., 2025a).

It was revealed that melatonin, a serotonin derivative, also increases the astaxanthin yield in the *H. lacustris* culture. This effect is potentiated by the addition of 3-methyladenine that inhibits autophagy, i.e. the process in which a cell destroys its own components in a specific organelle, the lysosome (Zhao et al., 2021).

As mentioned above, experiments with *C. vulgaris* (Fig.7-11) and *S. quadricauda* (Fig. 12-16) indicated that all tested neurotransmitters stimulated the growth of the cultures of these microalgae (judging by an increase in their cell numbers relative to the control cultures).

In similar fashion (Tables 2-11), an increase in the total cell numbers of strains IPPAS B-239 and BM-1 was detected in the presence of all tested neurotransmitters except 5-HT (with strain IPPAS B-239) and NE (with both strains) that failed to exert any significant effects. It seems likely that the impact of the neurotransmitters on microalgal culture growth results from the superposition of two neurotransmitter effects:

(1) the stimulatory effect that also causes the cell number increase observed in *C. vulgaris* and *S. quadricauda* cultures (see above) and is presumably due to the binding of the neurotransmitters to specific receptors on microalgal cells, in an analogy to animal nervous cells (this suggestion will be revisited below, in the subsection on the BA impact on the fatty-acid composition of microalgal lipids); this effect plausibly prevailed with histamine as the most efficient growth stimulant

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in *H. lacustris* (and other microalgae) that apparently did not "meddle into" the dynamics of the vegetative–palmelloid–encysted cell transition, and, therefore, did not significantly influence the ratio between these cell types.

(2) the stress effect that brings about microalgal growth inhibition and, in addition, accelerates the conversion of vegetative cells into encysted cells via the intermediate palmelloid stage. This effect is presumably similar to that of high salinity, high light intensity and other stress factors described in the literature that also promote microalgal cells' transition to the resting state with a high carotenoid content. Presumably, it was the stress effect that prevented a total cell number increase with 5-HT and NE.

In terms of the stress effect, neurotransmitters can be classified into (a) the substances promoting the accumulation of "red" cells corresponding to the final stage, i.e., increasing the encysted cell numbers; these include 5-HT (in strain BM-1 only) and DA (in strain IPPAS B-239 only) and (b) those increasing the palmelloid cell percentage, such as ACh (in both strains) and NE (in stage IPPAS B-239) that locked the cell conversion dynamics at the intermediate "brown" stage.

The aforementioned work dealing with the salicylic acid (SA) impact on astaxanthin synthesis (Gao et al., 2012) emphasized the "induction of astaxanthin accumulation by SA without any other stimulus". As for the effect of 5-HT (serotonin) in strain BM-1, the words of the work cited above (Gao et al., 2012) on the effect of salicylic acid on astaxanthin production can be modified as follows: "induction of astaxanthin accumulation by 5-HT… without any other stimulus has an attractive application potential in astaxanthin production with *H. lacustris*".

In the cyanobacterium *L. platensis,* the growth-related effects of the tested BAs were monitored using two criteria: (i) dry weight and (ii) cell chain (trichome) number. In the control cultures, the dry weight kept increasing until the plateau was reached by day 14 of cultivation, whereas the trichome number peaked on day 7, and this coincided with achieving the maximum chlorophyll *a* content. Subsequently, the trichome number somewhat decreased. Under these conditions,

ACh, histamine, and 5-HT increased and DA reduced the biomass content of the *L. platensis* culture on day 7 of cultivation. All BAs except NE decreased the trichome number on day 7. These effects tapered off by day 14, i.e. the neurotransmitters changed not the plateau level but the tempo in which it was reached: the growth dynamics of the culture was either accelerated (by ACh, histamine, and 5-HT), or decelerated (by DA).

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4. EFFECTS OF BIOGENIC AMINES ON THE FATTY-ACID COMPOSITION OF MICROALGAL LIPIDS[8]

4.1. GREEN MICROALGAE

4.1.1. Effects of BAs on the fatty-acid composition of the lipids of *C. vulgaris.*

The microalga under study is characterized by a high lipid content, including triacylglycerols that accumulate during the later stages of development of a batch culture (Dasan et al., 2019; Balasubramaniam et al., 2021; Hu et al., 2023). Triacylglycerol esterification results in producing environment-friendly biodiesel fuel that can help a country's economy become partly independent of non-renewable energy sources. At the earlier stages, microalgal lipids are enriched in

[8] The results presented in this section have been published in the work: **Cao B.,** Chivkunova O.B., Fedorenko T.A., Solovchenko A.E., Lobakova E.S., Oleskin A.V. Neurochemical pollutants in aquatic ecosystems: modes of interaction with microalgae // Жизнь Земли. — 2026. — Т. 48. № 1. С.16-29. DOI: 10.29003/m28/0514-7468. ИФ РИНЦ: 0.386 (1.0 p.s/0.6 p.s) and in the supplementary publications: **Цао Б.**, Федоренко Т.А., Чивкунова О.Б., Соловченко А.Е., Лобакова Е.С., Олескин А.В. Влияние биогенных аминов на накопление биомассы, содержание фотосинтетических пигментов и жирных кислот у цианобактерии *Limnospira platensis* IPPAS B-256 // Микробиология. — 2026. — Т. 95, № 1. — С. 68–84. DOI: 10.1134/S0026261725602921 ИФ РИНЦ: 1.034 (1.5 p.s./0.8 p.s.) [**Cao B.,** Fedorenko T.A., Chivkunova O.B., Solovchenko A.E., Lobakova E.S., Oleskin A.V. Impact of neurotransmitters on biomass accumulation, photosynthetic pigment content, and fatty acid composition of the cyanobacterium *Limnospira platensis* IPPAS B-256// Microbiology. — 2026. — Vol. 95, no. 1. — P. 82–95. DOI: 10.1134/S0026261725602921 SJR: 0.347 (1.5 p.s./0.8 p.s.); **Cao B.**, Chivkunova O.B., Solovchenko A.E., Lobakova E.S., Oleskin A.V. Neurotransmitters as ecopollutants: impact on the fatty-acid composition and photosynthetic pigment content of *Chlorella vulgaris* // Russian J. Ecosystem Ecol. — 2025. — V. 10(4). P. 10-21. DOI: 10.21685/2500-0578-2025-4-4. РИНЦ: 0.850 (1.2 p.s./0.6 p.s.); **Цао Б.,** Федоренко Т.А., Чивкунова О.Б., Соловченко А.Е., Лобакова Е.С., Олескин А.В. Влияние нейротрансмиттеров на содержание фотосинтетических пигментов у *Haematococcus lacustris* штаммы IPPAS H-239 и BM-1 // Прикл. биохим. микробиол. – 2025. – Т. 61(5). С.865-871. DOI: 10.31857/S0555109925050046 ИФ РИНЦ: 0.992 (1.4 p.s./0.6 p.s.). [**Cao B.,** Fedorenko T.A., Chivkunova O.B., Solovchenko A.E., Lobakova E.S., Oleskin A.V. Impact of neurotransmitters on the photosynthetic pigment content of the green microalga *Haematococcus lacustris* (strains IPPAS H-239 and BM-1) // Appl. Biochem. Microbiol. – 2025. – Vol. 61(5). – P.865-871. DOI: 10.1134/S0003683825601155. SJR: 0.294 (1.4 p.s./0.6 p.s.)]; **Цао Б.**, Федоренко Т.А., Чивкунова О.Б., Соловченко А.Е., Лобакова Е.С., Олескин А.В. Влияние нейротрансмиттеров на состав жирных кислот и фотосинтетических пигментов зеленой микроводоросли *Scenedesmus quadricauda* // Прикл. биохим. микробиол. – 2024. – Т. 60, № 5. С.378-389. DOI: 10.31857/S0555109924050064. ИФ РИНЦ: 0.992. (1.4 p.s./0.8 p.s.) [**Cao B.**, Chivkunova O.B., Solovchenko A.E., Lobakova E.S., Oleskin A.V. Impact of neurotransmitters on the fatty acid composition and pigments of the green microalga *Scenedesmus quadricauda* // Appl. Biochem. Microbiol. – 2024. – Vol. 60(5). – P.833–843. DOI: 10.1134/S0003683824604554 SJR: 0.294 (1.4 p.s./0.8 p.s.)].

unsaturated lipid acids that find application in pharmaceutical and cosmetical industry (see the literature review section of this work). Of much interest are substances that stimulate the formation of biotechnologically important lipid components because they can potentially optimize the fatty-acid composition of the lipids for practical purposes.

4.1.1.1. Effect of serotonin (5-HT) on the fatty-acid composition of C. vulgaris lipids. Table 12 contains the total fatty acid content (per 1 g of dry weight) and the percentages of various fatty acid species in *C. vulgaris* cultures grown with and without 5-HT. The data obtained boil down to the following points:

- 5-HT decreased the total fatty acid content in *C. vulgaris* lipids.
- Based on the results of the Kruskal-Wallis test, it only insignificantly increased the polyunsaturated fatty acid (PUFA) percentage, at the same time, 5-HT brought down to zero the contribution of γ-linolenic acid; with 100 μM serotonin, the lipids also lacked 20:2, 20:3, and 20:4 fatty acids.
- 5-HT verifiably lowered the monounsaturated fatty acid (MUFA) and, to a lesser extent, saturated fatty acid (SFA) percentage (although this does not concern arachic acid).

4.1.1.2. Effect of histamine. It is evident from Table 13 that

- Histamine significantly decreased the total fatty-acid content in the cells.
- Histamine increased the percentage of PUFAs and decreased that of SFAs (at a concentration of 10 μM); as for individual fatty acid species, the percentages of stearic and myristic acid were reduced by 45% and 18%, respectively, with 1 μM histamine of some unsaturated fatty acids.
- Based on the results of the Kruskal-Wallis test, histamine only caused an insignificant decrease in MUFA percentage.

4.1.1.3. Effect of dopamine (DA). The data shown in Table 14 are as follows:

- DA at a concentration of 10 μM significantly increased the total fatty-acid content in *C. vulgaris* cells.

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• The DA-caused slight decrease in PUFA content proved insignificant statistically.

• DA produced no verifiable effect on the MUFA and SFA percentages

4.1.1.4. Effect of norepinephrine (NE). The data obtained are as follows (Table 15):

• NE at a concentration of 1 μM slightly increased, and at higher concentrations decreased the total fatty-acid content, these effects being statistically significant

• NE only insignificantly (according to the Kruskal-Wallis test results) increased the PUFA percentage and decreased the MUFA and SFA percentages

4.1.1.5. Effect of acetylcholine (ACh). From Table 16, the following conclusions can be drawn:

• ACh at a concentration of 10 μM statistically significantly increased the fatty-acid content of *C. vulgaris* cells, in compliance with the literature data on a different *Chlorella* species, *C. sorokiniana* (Parsaiemehr et al., 2015) as well as with our work on the microalga *S. quadricauda* (see next subsection); in contrast, ACh at a higher concentration (100 μM) brought about a decrease in fatty-acid content.

• ACh increased the percentage of such unsaturated fatty acids as oleic and α-linolenic acid.

• ACh decreased the percentages of saturated and monounsaturated fatty acids (SFAs and MUFAs, respectively)

According to the data presented in this subsection, ACh (at a concentration of 10 μM only), DA, and NE (at a concentration of 1 μM only) increased the total fatty acid content in *C. vulgaris* cells. 5-HT and histamine decreased this content. ACh and histamine statistically verifiably augmented the PUFA percentage at the expense of the SFA and/or MUFA percentage. 5-HT significantly reduced the MUFA and SFA share, although the proportional PUFA increase proved insignificant, based on Kruskal-Wallis test results. DA and NE failed to exert a statistically verifiable effect on the ratio between fatty acid types.

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Table 12. Fatty acid content of *C. vulgaris* cultures grown with or without serotonin (5-HT).

Fatty acid	**Proportion in total fatty acids, %**			
	Control	**5HT, 1 μM**	**5HT,10 μM**	**5HT, 100 μM**
Myristic acid 14:0	0.37±0.05	0.41±0.05	0.35±0.04	0.31±0.04
Myristoleic acid14:1	0.20±0.03	0.17±0.03	0.46±0.04	1.06±0.04
Pentadecanoic acid15:0	2.08±0.1	2.14±0.1	2.14±0.1	1.53±0.08
Pentadecenoic acid15:1	0.22±0.04	0.16±0.03	0.17±0.04	0.10±0.02
Palmitic acid 16:0	24.9±0.1	23.2±0.1	23.5±0.1	23.2±0.1
Palmitoleic acid 16:1	2.38±0.04	1.99±0.04	1.94±0.03	2.01±0.04
Hexadecadienoic acid 16:2	3.50±0.07	3.84±0.08	3.93±0.07	4.45±0.09
Hexadecatrienoic acid 16:3	13.3±0.05	17.4±0.06	16.4±0.06	17.1±0.06
Stearic acid 18:0	3.10±0.03	1.58±0.04	1.58±0.04	1.59±0.03
Oleic acid 18:1	5.91±0.06	3.37±0.05	4.45±0.05	3.49±0.05
Linoleic acid 18:2	17.7±0.10	16.7±0.09	17.0±0.11	18.0±0.10
γ-Linolenic acid 18:3	0.14±0.03	N/D	N/D	N/D
α-Linolenic acid 18:3	23.1±0.4	26.3±0.5	25.9±0.5	25.5±0.6
Arachidic acid 20:0	0.25±0.04	0.34±0.04	0.36±0.05	0.34±0.03
Eicosaenoic acid 20:1	0.35±0.05	0.20±0.04	0.17±0.03	0.20±0.03
Eicosadienoic acid 20:2	0.09±0.02	0.10±0.02	0.14±0.03	N/D
Eicosatrienoic acid 20:3	0.18±0.03	0.10±0.02	0.14±0.02	N/D
Arachidonic acid 20:4	0.17±0.03	0.14±0.02	0.15±0.03	N/D
Eicosapentaenoic acid 20:5	1.66±0.09	1.71±0.1	2.02±0.1	0.91±0.08
Heneicosanoic acid 21:0	0.32±0.03	0.08±0.06	0.17±0.04	0.18±0.02
Behenic acid 22:0	N/D	N/D	N/D	N/D
Saturated fatty acids (SFAs)	**31.0±1.0**	**27.8±0.9**	**28.1±1.0**	**27.1±0.9**
Monounsaturated fatty acids (MUFAs)	**9.06±0.5**	**5.88±0.6**	**6.19±0.55**	**6.9±0.6**
Polyunsaturated fatty acids (PUFAs)	**59.9±1.4**	**66.3±1.5**	**65.7±1.7**	**66.0±1.4**
Total fatty acid content, mg/g of dry weight (on day 3 of cultivation)	***84.4±1.0***	***72.1±0.9***	***73.2±1.0***	***68.9±0.9***

Averaged values based on the results of 4-5 repeats are given. N/D. not detected. Kruskal-Wallis test results with independent samples for the experimental and control groups. SFAs: Sig.=0.047 < 0.05. MUFAs Sig.=0.041 < 0.05. PUFAs: **Sig.=0.082 > 0.05** Total fatty acid content: Sig.=0.024 < 0.05. The results for all the data groups except the PUFA data rule the null hypothesis out, therefore, only the differences between the experimental and control groups for PUFAs are **not significant.**

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Table 13. Fatty acid content of *C. vulgaris* cultures grown with or without histamine (His).

Fatty acid	**Proportion in total fatty acids, %**			
	Control	**His, 1 μM**	**His,10 μM**	**His, 100 μM**
Myristic acid 14:0	0.56±0.06	0.47±0.05	0.49±0.05	0.52±0.06
Myristoleic acid14:1	0.07±0.01	0.28±0.03	0.49±0.03	N/D
Pentadecanoic acid15:0	1.35±0.15	1.98±0.19	2.10±0.18	1.92±0.20
Pentadecenoic 15:1	0.80±0.07	0.81±0.08	0.73±0.08	1.79±0.09
Palmitic acid16:0	24.7±0.7	26.6±0.6	31.3±0.9	25.0±0.7
Palmitoleic acid 16:1	2.47±0.09	1.93±0.08	2.31±0.08	2.06±0.077
Hexadecadienoic acid 16:2	4.63±0.08	5.45±0.09	5.67±0.09	6.45±0.10
Hexadecatrienoic acid 16:3	6.69±0.10	7.41±0.09	7.79±0.11	8.69±0.12
Stearic acid 18:0	4.15±0.08	2.73±0.06	3.44±0.08	2.18±0.06
Oleic acid 18:1	21.1±0.7	7.44±0.50	8.59±0.54	8.47±0.49
Linoleic acid 18:2	14.2±0.6	16.2±0.7	13.1±0.6	16.3±0.8
γ-Linolenic acid 18:3	N/D	0.65±0.01	0.37±0.01	0.48±0.01
α-Linolenic acid 18:3	14.8±0.8	16.3±0.9	15.2±0.8	18.0±0.7
Arachidic acid 20:0	0.38±0.05	0.81±0.06	0.78±0.06	0.89±0.07
Eicosaenoic acid 20:1	0.93±0.08	1.98±0.09	0.45±0.07	0.48±0.07
Eicosadienoic acid 20:2	0.19±0.04	1.08±0.03	0.57±0.03	N/D
Eicosatrienoic acid 20:3	0.12±0.05	0.56±0.06	0.67±0.07	N/D
Arachidonic acid 20:4	0.10±0.01	0.90±0.02	0.58±0.02	N/D
Eicosapentaenoic acid 20:5	3.20±0.18	5.91±0.20	4.88±0.19	6.30±0.18
Heneicosanoic acid 21:0	0.50±0.05	0.30±0.05	0.42±0.06	0.47±0.06
Behenic acid 22:0	N/D	0.17±0.02	N/D	N/D
Saturated fatty acids (SFAs)	**31.6±0.9**	**33.1±1.0**	**38.6±1.0**	**30.9±0.9**
Monounsaturated fatty acids (MUFAs)	**23.7±0.7**	**11.7±0.6**	**11.6±0.7**	**11.9±0.7**
Polyunsaturated fatty acids (PUFAs)	**43.9±1.1**	**54.4±1.2**	**48.9±1.1**	**56.3±1.2**
Total fatty acid content, mg/g of dry weight (on day 3 of cultivation)	***79.3±1.0***	***44.2±1.0***	***43.7±0.9***	***49.7±1.1***

Averaged values based on the results of 4-5 repeats are given. N/D. not detected. Kruskal-Wallis test results with independent samples for the experimental and control groups. SFAs: Sig.=0.031 < 0.05. MUFAs **Sig.=0.082 > 0.05**. PUFAs: Sig.=0.019 < 0.05 Total fatty acid content: Sig.=0.024 < 0.05. The results for all the data groups except the MUFA data rule the null hypothesis out, therefore, only the differences between the experimental and control groups for MUFAs are **not significant**

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Table 14. Fatty acid content of *C. vulgaris* cultures grown with or without dopamine (DA)

Fatty acid	**Proportion in total fatty acids, %**			
	Control	**DA, 1 μM**	**DA,10 μM**	**DA, 100 μM**
Myristic acid 14:0	0.31±0.05	0.32±0.06	0.30±0.05	0.40±0.06
Myristoleic acid14:1	0.11±0.02	0.13±0.02	0.14±0.02	0.23±0.03
Pentadecanoic acid15:0	1.80±0.2	1.81±0.2	1.89±0.2	1.93±0.3
Pentadecenoic acid 15:1	0.27±0.03	0.42±0.04	0.22±0.03	0.67±0.04
Palmitic acid 16:0	23.7±0.6	23.6±0.6	24.1±0.7	25.4±0.7
Palmitoleic acid 16:1	1.26±0.08	1.57±0.09	1.58±0.09	1.50±0.08
Hexadecadienoic acid 16:2	3.53±0.40	3.59±0.36	3.60±0.38	3.31±0.40
Hexadecatrienoic 16:3	13.2±0.6	13.1±0.5	13.5±0.7	13.4±0.6
Stearic acid 18:0	1.62±0.06	1.53±0.06	1.79±0.077	1.22±0.06
Oleic acid 18:1	3.87±0.08	3.78±0.08	4.95±0.09	3.62±0.09
Linoleic acid 18:2	15.9±1.0	17.1±1.1	16.5±1.0	15.8±0.09
γ-Linolenic acid 18:3	0.26±0.07	0.21±0.07	0.20±0.08	N/D
α-Linolenic acid 18:3	25.4±0.7	26.0±0.8	25.1±0.9	29.2±0.9
Arachidic acid 20:0	0.56±0.08	0.48±0.09	0.46±0.08	0.54±0.09
Eicosaenoic acid 20:1	0.32±0.05	0.23±0.04	0.25±0.06	0.19±0.03
Eicosadienoic acid 20:2	0.12±0.04	0.13±0.04	0.13±0.02	0.06±0.02
Eicosatrienoic acid 20:3	0.16±0.07	N/D	N/D	N/D
Arachidonic acid 20:4	0.13±0.05	0.06±0.04	N/D	N/D
Heneicosanoic acid 21:0	0.24±0.04	0.21±0.03	0.24±0.03	0.26±0.04
Behenic acid 22:0	N/D	N/D	N/D	N/D
Saturated fatty acids (SFAs)	**28.2±1.0**	**28.0±0.9**	**28.7±0.9**	**29.7±1.0**
Monounsaturated fatty acids (MUFAs)	**5.7±0.5**	**6.1±0.6**	**7.1±0.6**	**6.2±0.5**
Polyunsaturated fatty acids (PUFAs)	**66.0±1.4**	**65.9±1.6**	**64.1±1.4**	**64.1±1.5**
Total fatty acid content, mg/g of dry weight (on day 3 of cultivation)	***60.7±1.1***	***59.7±1.0***	***68.5±1.2***	***61.7±1.0***

Averaged values based on the results of 4-5 repeats are given. N/D. not detected. Kruskal-Wallis test results with independent samples for the experimental and control groups. SFAs: **Sig.=0.294 > 0.05**. MUFAs **Sig.=0.142 > 0.05.** PUFAs: **Sig.=0.168 > 0.05** Total fatty acid content: Sig.=0.042 < 0.05. The results for all the data groups except the total fatty acid data **conform with** the null hypothesis, therefore, only the differences between the experimental and control groups for total fatty acid content are significant.

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Table 15. Fatty acid content of *C. vulgaris* cultures grown with or without norepineprine (NE)

Fatty acid	**Proportion in total fatty acids, %**			
	Control	**NE, 1 μM**	**NE,10 μM**	**NE, 100 μM**
Myristic acid 14:0	0.47±0.04	0.49±0.04	0.50±0.05	0.60±0.05
Myristoleic acid14:1	0.26±0.03	0.22±0.03	0.31±0.04	0.21±0.02
Pentadecanoic acid15:0	2.21±0.20	2.08±0.20	2.42±0.21	2.31±0.22
Pentadecenoic acid 15:1	1.18±0.14	0.60±0.11	0.71±0.12	1.07±0.13
Palmitic acid 16:0	29.3±0.66	25.7±0.64	30.6±0.65	30.5±0.66
Palmitoleic acid 16:1	1.56±0.09	1.36±0.08	1.94±0.09	1.90±0.09
Hexadecadienoic acid 16:2	3.66±1.00	3.45±0.97	2.87±0.96	3.02±0.98
Hexadecatrienoic acid 16:3	11.0±1.4	11.5±1.3	7.62±1.00	8.63±1.00
Stearic acid 18:0	2.77±0.30	1.90±0.25	2.75±0.30	2.00±0.22
Oleic acid 18:1	4.64±0.09	3.33±0.09	4.80±0.10	4.56±0.10
Linoleic acid 18:2	16.8±1.0	17.6±1.1	17.8±1.0	18.7±0.9
γ-Linolenic acid 18:3	0.24±0.09	0.1±0.04	N/D	N/D
α-Linolenic acid 18:3	20.3±0.35	25.9±0.40	20.7±0.36	22.2±0.40
Arachidic acid 20:0	0.66±0.09	0.56±0.08	0.65±0.08	0.56±0.09
Eicosaenoic acid 20:1	0.30±0.04	0.25±0.04	0.50±0.05	0.21±0.03
Eicosadienoic acid 20:2	0.08±0.01	0.16±0.02	0.34±0.02	0.11±0.01
Eicosatrienoic acid 20:3	0.08±0.01	0.25±0.01	0.14±0.02	0.09±0.01
Arachidonic acid 20:4	N/D	0.13±0.02	0.07±0.02	0.12±0.03
Heneicosanoic acid	0.26±0.05	0.32±0.05	0.20±0.04	0.27±0.04
Behenic acid 22:0	N/D	N/D	N/D	N/D
Saturated fatty acids (SFAs)	**35.7±1.0**	**31.0±0.9**	**37.1±1.0**	**36.2±1.1**
Monounsaturated fatty acids (MUFAs)	**7.9±0.5**	**5.8±0.4**	**8.3±0.5**	**7.9±0.4**
Polyunsaturated fatty acids (PUFAs)	**56.4±1.3**	**63.3±1.4**	**54.6±1.3**	**55.8±1.2**
Total fatty acid content, mg/g of dry weight (on day 3 of cultivation)	***60.7±1.0***	***68.8±1.3***	***54.3±1.0***	***53.1±0.9***

Averaged values based on the results of 4-5 repeats are given. N/D. not detected. Kruskal-Wallis test results with independent samples for the experimental and control groups. SFAs: **Sig.=0.057 > 0.05.** MUFAs **Sig.=0.086 > 0.05.** PUFAs: **Sig.=0.052 > 0.05** Total fatty acid content: Sig.=0.019 < 0.05. The results for all the data groups except the total fatty acid data **conform with** the null hypothesis, therefore, only the differences between the experimental and control groups for MUFAs are significant

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Table 16. Fatty acid content of *C. vulgaris* cultures grown with or without acetylcholine (ACh)

Fatty acid	**Proportion in total fatty acids, %**			
	Control	**ACh, 1 μM**	**ACh,10 μM**	**ACh, 100 μM**
Myristic acid 14:0	1.33±0.07	1.00±0.07	0.82±0.06	0.74±0.06
Myristoleic acid14:1	0.63±0.08	0.39±0.08	0.19±0.07	0.09±0.05
Pentadecanoic acid15:0	1.44±0.10	1.30±0.10	1.15±0.09	1.37±0.10
15:1	1.31±0.08	1.35±0.08	1.76±0.11	1.44±0.09
Palmitic acid16:0	21.7±0.4	20.8±0.4	22.2±0.5	19.9±0.3
Palmitoleic acid 16:1	1.99±0.09	1.63±0.09	1.93±0.09	1.50±0.07
Hexadecadienoic acid 16:2	5.27±0.36	5.55±0.39	4.80±0.40	5.04±0.41
Hexadecatrienoic acid 16:3	8.79±0.60	9.48±0.59	10.2±0.61	11.0±0.61
Stearic acid 18:0	2.98±0.55	3.04±0.56	1.91±0.58	1.52±0.58
Oleic acid 18:1	10.8±0.7	9.78±0.6	10.2±0.5	8.38±0.6
Linoleic acid 18:2	17.5±0.9	17.6±1.0	16.7±1.0	16.5±0.9
γ–Linolenic acid 18:3	N/D	N/D	N/D	N/D
α-Linolenic acid 18:3	19.9±1.1	21.3±1.3	22.2±1.3	24.1±1.5
Arachidic acid 20:0	0.18±0.03	0.10±0.02	0.18±0.03	N/D
Eicosaenoic acid 20:1	0.56±0.80	0.24±0.70	0.17±0.65	N/D
Eicosadienoic acid 20:2	0.15±0.03	0.15±0.04	0.08±0.02	0.16±0.03
Eicosatrienoic acid 20:3	0.22±0.10	0.22±0.12	0.16±0.11	0.19±0.12
Arachidonic acid 20:4	0.16±0.02	0.27±0.03	N/D	N/D
Eicosapentaenoic acid 20:5	4.52±0.43	5.51±0.44	5.47±0.46	7.82±0.47
Heneicosanic acid 21:0	0.41±0.80	0.20±0.81	0.28±0.71	0.32±0.72
Behenic acid 22:0	0.13±0.02	0.16±0.02	N/D	N/D
Saturated fatty acids (SFAs)	**28.2±0.9**	**26.6±0.8**	**26.6±0.9**	**23.9±0.8**
Monounsaturated fatty acids (MUFAs)	**15.3±0.8**	**13.4±0.7**	**13.9±0.7**	**11.4±0.7**
Polyunsaturated fatty acids (PUFAs)	**56.5±1.0**	**60.0±1.1**	**59.5±1.1**	**64.7±1.2**
Total fatty acid content, mg/g of dry weight (on day 3 of cultivation)	***62.4±2.0***	***60.6±1.9***	***69.5±1.9***	***54.7±1.9***

Averaged values based on the results of 4-5 repeats are given. N/D, not detected. Kruskal-Wallis test results with independent samples for the experimental and control groups. SFAs: Sig.=0.043 < 0.05. MUFAs Sig.=0.031 < 0.05. PUFAs: Sig.=0.024 < 0.05 Total fatty acid content: Sig.=0.024 < 0.05. The results for all the groups rule the null hypothesis out, which indicates significant differences between the data groups to be compared (at α 0.05).

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4.1.2. Effects of BAs on the fatty acid composition of *S. quadricauda* lipids.

4.1.2.1. Effect of serotonin (5-HT). Table 17 below contains the total fatty acid content (per 1 g of dry weight) and the percentages of various fatty acid species in *C. vulgaris* cultures grown with and without 5-HT. From this table. the following conclusions can be drawn:

- 5-HT at concentrations of 10 and 100 μM slightly (by 16% and 12%. respectively) decreased the total fatty acid concentrations in *S. quadricauda* cells
- 10-100 μM 5-HT marginally decreased the percentages of linoleic and α-linolenic acid while slightly increasing the percentages of monounsaturated fatty acids
- 1-10 μM 5-HT appreciably increased the percentages of all tested saturated fatty acids; for instance. the myristic and stearic acid contents were elevated by 52% and 86%. respectively. with 1 μM 5-HT.

4.1.2.2. Effect of histamine. Table 18 demonstrates that

- Histamine at concentrations of 1 and 10 μM marginally (by maximally 13% with 1 μM histamine) increased the total fatty acid concentrations in the cells;
- Histamine at all tested concentrations somewhat increased the percentages of some of the PUFAs (16:2. 16:3. 16:4. and α-linolenic acid)
- It decreased the percentages of all monounsaturated and especially saturated acids; for instance. the percentage of stearic and myristic acid was reduced by 45% and 42%. respectively. with 1 μM histamine.

4.1.2.3. Effect of dopamine (DA).

- DA at concentrations of 10 and especially 100 μM drastically decreased the total fatty acid concentrations in *S. quadricauda* cells (see Table 19);
- Among PUFAs, DA increased the percentages of α-linolenic and linoleic (10 μM dopamine only) acid; the 16:3 and 16:4 contents were decreased
- The same DA concentration substantially increased the percentages of monoun-

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saturated and especially of saturated fatty acids; for instance, the stearic and palmitic acid contents became 81% and 54% higher with 10 μM DA than in the control.

4.1.2.4. Effect of norepinephrine (NE). Compared to DA, NE produces a considerably weaker stimulatory effect on the growth of *S. quadricauda* (see above, Table 10) and also *C. vulgaris* (Table 5). The data of the present work indicate that (Table 20):

- NE brought about no significant changes in total fatty acid content
- Among PUFAs, 10 μM NE increased the percentage of the most unsaturated 16:4 fatty acid species, and 1 μM NE slightly augmented that of α-linolenic acid
- Low (1 μM) NE concentrations tended to increase, and higher (10 and 100 μM) to decrease, the percentages of some saturated fatty acids (myristic and stearic acid).

4.1.2.5. Effect of acetylcholine (ACh). It is evident from Table 21 that

- ACh at concentrations of 10 and 100 μM increased the total fatty acid concentrations in the cells by 65% and 58%. respectively;
- ACh increased the percentages of linoleic and α-linolenic acid. maximally by 56% and 27%. respectively
- ACh decreased the percentages of all saturated (myristic. stearic. and palmitic acid) and some monounsaturated (myristoleic and palmitoleic) fatty acids.

To sum up, the neurotransmitters tested in this work can be subdivided into two groups: (i) ACh and histamine that tend to increase the fatty acid content and also the ratio between PUFAs and more saturated fatty acid species and (ii) 5-HT and DA that, conversely, decrease the total fatty acid content and increase the percentage of saturated (and, in some cases, monounsaturated) fatty acids. Being chemically similar to DA, NE, nonetheless, tends to increase the percentage of some PUFAs at the expense of more saturated fatty acid species, similar to ACh and histamine.

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Table 17. Fatty acid content of *S. quadricauda* cultures grown with or without serotonin (5-HT).

Fatty acid	**Proportion in total fatty acid, %**			
	Control	**5-HT, 1 μM**	**5-HT, 10 μM**	**5-HT,100 μM**
Myristic acid 14:0	0.48±0.04	0.73±0.06	0.63±0.05	0.48±0.05
Myristoleic acid 14:1	0.15±0.01	0.28±0.03	0.18±0.02	0.15±0.01
Pentadecanoic acid 15:0	1.30±0.08	1.38±0.08	1.16±0.10	1.30±0.08
Pentadecenoic acid15:1	2.22±0.13	1.99±0.11	1.74±0.12	2.22±0.13
Palmitic acid 16:0	20.68±0.29	22.78±0.24	22.47±0.26	22.68±0.28
Palmitoleic acid 16:1	4.39±0.08	5.00±0.09	5.46±0.09	4.39±0.08
Hexadienoic acid 16:2	1.54±0.02	1.58±0.03	1.71±0.03	1.54±0.02
Hexatrienoic acid 16:3	5.71±0.41	4.56±0.39	5.75±0.40	5.71±0.39
Hexatetraenoic acid 16:4	11.90±0.10	10.57±0.09	13.47±0.10	11.90±0.11
Stearic acid 18:0	1.15±0.08	2.14±0.09	1.89±0.08	1.15±0.07
Oleic acid 18:1	8.28±0.10	9.92±0.11	9.93±0.12	8.28±0.10
Linoleic acid 18:2	9.90±0.09	9.20±0.08	8.71±0.08	7.87±0.09
α-Linolenic acid 18:3	27.44±1.1	29.53±1.2	25.41±1.0	25.12±1.0
Saturated fatty acids (SFAs)	**23.6±0.5**	**27.0±0.5**	**26.1±0.5**	**25.6±0.5**
Monounsaturated fatty acids (MUFAs)	**15.04±0.32**	**17.19±0.34**	**17.31±0.35**	**15.04±0.32**
Polyunsaturated fatty acids (PUFAs)	**56.49±1.72**	**55.44±1.79**	**55.05±1.61**	**52.14±1.61**
Total fatty acid content, mg/g of dry weight (on day 7 of cultivation)	**79.9±4.9**	**73.1±4.6**	**67.0±4.4**	**70.0±4.4**

Averaged values based on the results of 4-5 repeats are given. N/D. not detected. Kruskal-Wallis test results with independent samples for the experimental and control groups. SFAs: Sig.=0.023 < 0.05. MUFAs Sig.=0.037 < 0.05. **PUFAs: Sig.=0.161 > 0.05** Total fatty acid content: Sig.=0.05 ⩽ 0.05. The results for all the data groups except the PUFA data rule the null hypothesis out, therefore, only the differences between the experimental and control groups for PUFAs are **not significant.** Note: the table and those herebelow do not contain data on the percentages of vaccenic (18:2), γ-linolenic (18:3), 20:1, and other minor fatty acids that may be included in the tables with data on other microalgal species

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Table 18. Fatty acid content of *S. quadricauda* cultures grown with or without histamine (His).

Fatty acid	**Proportion in total fatty acids, %**			
	Control	**His, 1 μM**	**His,10 μM**	**His,100 μM**
Myristic acid 14:0	0.60±0.04	0.39±0.03	0.41±0.03	0.45±0.04
Myristoleic acid 14:1	0.10±0.01	0.14±0.02	0.10±0.02	0.07±0.01
Pentadecanoic acid 15:0	1.62±0.11	1.47±0.12	1.51±0.11	1.41±0.11
Pentadecenoic acid 15:1	1.63±0.12	1.17±0.10	1.30±0.10	1.62±0.11
Palmitic acid 16:0	18.45±0.59	14.97±0.55	15.42±0.54	16.42±0.56
Palmitoleic acid 16:1	3.55±0.03	3.11±0.02	3.00±0.02	3.18±0.03
Hexadienoic acid 16:2	1.22±0.08	1.50±0.09	1.46±0.08	1.52±0.07
Hexatrienoic acid 16:3	5.70±0.31	4.69±0.28	6.62±0.30	6.25±0.31
Hexatetraenoic acid 16:4	13.80±0.40	17.07±0.45	16.04±0.41	15.35±0.40
Stearic acid 18:0	1.10±0.07	0.60±0.04	0.61±0.04	0.71±0.05
Oleic acid 18:1	9.32±0.48	7.26±0.49	7.25±0.45	7.77±0.48
Linoleic acid 18:2	9.23±0.39	9.48±0.44	9.44±0.43	9.94±0.45
α-Linolenic acid 18:3	31.90±1.6	36.59±1.7	35.30±1.5	33.74±1.4
Saturated fatty acids (SFAs)	**21.77±0.81**	**17.43±0.74**	**17.95±0.72**	**18.99±0.76**
Monounsaturated fatty acids (MUFAs)	**14.6±0.64**	**11.68±0.63**	**11.65±0.59**	**12.64±0.63**
Polyunsaturated fatty acids (PUFAs)	**61.85±1.78**	**69.33±1.96**	**68.86±1.72**	**66.8±1.63**
Total fatty acid content, mg/g of dry weight (on day 7 of cultivation)	***76.9±4.7***	***86.9±5.0***	***85.9±4.9***	***83.9±4.7***

Averaged values based on the results of 4-5 repeats are given. Kruskal-Wallis test results with independent samples for the experimental and control groups. SFAs: Sig.=0.031 < 0.05. MUFAs Sig.=0.043 < 0.05. PUFAs: Sig.=0.041 < 0.05 Total fatty acid content: **Sig.=0.161 > 0.05**. The results for all the data groups except the total fatty acid content data rule the null hypothesis out, therefore, only the differences between the experimental and control groups for the total fatty acid content are **not significant**

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Table 19. Fatty acid content of *S. quadricauda* cultures grown with or without dopamine (DA)

Fatty acid	**Proportion in total fatty acids. %**			
	Control	**DA, 1 μM**	**DA,10 μM**	**DA, 100 μM**
Myristic acid 14:0	0.47±0.04	0.37±0.03	0.80±0.04	0.63±0.03
Myristoleic acid 14:1	0.10±0.01	0.11±0.02	0.22±0.03	0.24±0.03
Pentadecanoic acid 15:0	1.23±0.11	0.95±0.10	1.61±0.10	1.61±0.12
Pentadecenoic acid 15:1	2.13±0.13	1.59±0.12	2.43±0.13	2.57±0.12
Palmitic acid 16:0	17.24±0.60	18.10±0.61	26.55±0.66	22.88±0.65
Palmitoleic acid 16:1	4.47±0.05	4.33±0.04	5.28±0.05	4.47±0.04
Hexadienoic acid 16:2	2.80±0.09	3.73±0.10	3.75±0.10	3.29±0.10
Hexatrienoic acid 16:3	9.48±0.61	8.46±0.62	3.76±0.58	4.28±0.59
Hexatetraenoic acid 16:4	9.74±0.55	9.41±0.57	5.74±0.48	7.62±0.49
Stearic acid 18:0	0.85±0.08	0.82±0.06	1.54±0.07	1.54±0.09
Oleic acid 18:1	8.72±0.50	9.84±0.48	14.17±0.59	14.59±0.60
Linoleic acid 18:2	12.60±0.44	14.34±0.48	17.02±0.49	15.53±0.50
α-Linolenic acid 18:3	26.57±1.7	24.73±1.6	13.80±1.7	17.66±1.5
Saturated fatty acids (SFAs)	**19.79±0.83**	**20.24±0.80**	**30.5±0.87**	**26.66±0.89**
Monounsaturated fatty acids (MUFAs)	**15.42±0.69**	**15.87±0.66**	**22.1±0.8**	**21.87±0.79**
Polyunsaturated fatty acids (PUFAs)	**61.19±1.39**	**60.67±1.37**	**44.07±1.35**	**48.38±3.18**
Total fatty acid content, mg/g of dry weight (on day 7 of cultivation)	***79.7±4.5***	***76.5±5.0***	***41.9±3.9***	***21.5±3.8***

Averaged values based on the results of 4-5 repeats are given. Kruskal-Wallis test results with independent samples for the experimental and control groups. SFAs: Sig.=0.024 < 0.05. MUFAs Sig.=0.036 < 0.05. PUFAs: Sig.=0.029 < 0.05 Total fatty acid content: Sig.=0.024 < 0.05. The results for all the groups rule the null hypothesis out, therefore, all differences between the data groups to be compared are significant (at α 0.05).

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Table 20. Fatty acid content of *S. quadricauda* cultures grown with or without norepinephrine (NE)

Fatty acid	**Proportion in total fatty acids. %**			
	Control	**NE, 1 μM**	**NE, 10 μM**	**NE, 100 μM**
Myristic acid 14:0	0.53±0.05	0.60±0.06	0.42±0.05	0.39±0.04
Myristoleic acid 14:1	0.17±0.02	0.15±0.02	0.16±0.03	0.15±0.02
Pentadecanoic acid 15:0	1.48±0.12	1.32±0.12	1.19±0.11	1.13±0.11
Pentadecenoic acid 15:1	2.47±0.15	2.16±0.14	1.77±0.14	1.72±0.12
Palmitic acid 16:0	19.43±0.65	19.62±0.66	19.33±0.66	19.77±0.65
Palmitoleic acid 16:1	3.87±0.06	3.68±0.05	3.41±0.04	3.61±0.04
Hexadienoic acid 16:2	1.15±0.08	1.06±0.07	0.98±0.06	1.39±0.07
Hexatrienoic acid 16:3	5.07±0.51	5.04±0.50	5.10±0.50	5.74±0.54
Hexatetraenoic acid 16:4	12.97±0.60	13.56±0.59	14.30±0.63	13.19±0.58
Stearic acid 18:0	1.29±0.09	1.42±0.10	1.04±0.09	1.05±0.08
Oleic acid 18:1	9.98±0.60	10.12±0.64	8.89±0.61	9.83±0.59
Linoleic acid 18:2	9.33±0.48	8.81±0.46	8.78±0.45	10.30±0.49
α-Linolenic acid 18:3	29.15±1.9	29.38±1.8	31.57±2.0	28.75±1.5
Saturated fatty acids (SFAs)	**22.73±0.91**	**22.96±0.94**	**21.98±0.91**	**22.34±0.88**
Monounsaturated fatty acids (MUFAs)	**16.49±0.83**	**16.11±0.85**	**14.23±0.82**	**15.31±0.77**
Polyunsaturated fatty acids (PUFAs)	**57.67±3.57**	**57.85±3.42**	**60.73±3.64**	**59.73±3.18**
Total fatty acid content, mg/g of dry biomass (on day 7 of cultivation)	**79.7±4.5**	**84.8±4.8**	**74.3±4.6**	**76.1±4.8**

Averaged values based on the results of 4-5 repeats are given. N/D. not detected. Kruskal-Wallis test results with independent samples for the experimental and control groups. **SFAs: Sig.=0.051 > 0.05**. MUFAs Sig.=0.044 < 0.05. **PUFAs: Sig.=0.764 > 0.05** Total fatty acid content: Sig.=0.018 < 0.05. The results for MUFA groups and total fatty acid content group rule the null hypothesis out and only these results are significant.

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Table 21. Fatty acid content of *S. quadricauda* cultures grown with or without acetylcholine (ACh)

Fatty acid	**Proportion in total fatty acids. %**			
	Control	**ACh, 1 μM**	**ACh, 10 μM**	**ACh,100 μM**
Myristic acid 14:0	0.54±0.05	0.39±0.04	0.27±0.04	0.25±0.03
Myristoleic acid 14:1	0.12±0.01	0.11±0.01	0.07±0.01	0.08±0.01
Pentadecanoic acid 15:0	1.61±0.09	1.37±0.08	1.22±0.08	1.27±0.09
Pentadecenoic acid 15:1	2.46±0.14	2.16±0.13	2.22±0.14	2.37±0.13
Palmitic acid 16:0	18.68±0.23	16.96±0.20	14.46±0.19	12.94±0.20
Palmitoleic acid 16:1	6.25±0.08	5.67±0.08	4.83±0.06	4.09±0.07
Hexadienoic acid 16:2	2.64±0.02	3.78±0.03	2.90±0.02	2.25±0.02
Hexatrienoic acid 16:3	8.21±0.39	9.10±0.40	11.31±0.39	9.61±0.40
Hexatetraenoic acid 16:4	8.00±0.09	7.31±0.08	7.06±0.08	9.34±0.09
Stearic acid 18:0	0.90±0.07	0.64±0.07	0.37±0.04	0.32±0.04
Oleic acid 18:1	10.35±0.09	11.27±0.10	9.42±0.08	10.59±0.09
Linoleic acid 18:2	10.67±0.08	13.64±0.07	17.08±0.10	12.13±0.10
α-Linolenic acid 18:3	26.50±1.0	27.56±1.1	28.75±1.1	34.09±1.0
Saturated fatty acids (SFAs)	**21.73±0.44**	**19.36±0.39**	**16.32±0.35**	**14.78±0.36**
Monounsaturated fatty acids (MUFAs)	**19.18±0.32**	**19.21±0.32**	**16.54±0.29**	**17.13±0.3**
Polyunsaturated fatty acids (PUFAs)	**56.02±1.58**	**61.39±1.68**	**67.1±1.69**	**67.42±1.61**
Total fatty acid content, mg/g of dry biomass (on day 7 of cultivation)	**75.1±4.8**	**78.6±4.9**	**123.7±5.1**	**118.7±5.0**

Averaged values based on the results of 4-5 repeats are given. Kruskal-Wallis test results with independent samples for the experimental and control groups. SFAs: Sig.=0.016 < 0.05. MUFAs Sig.=0.026 < 0.05. PUFAs: Sig.=0.024 < 0.05 Total fatty acid content: Sig.=0.036 < 0.05. The results for all the groups rule the null hypothesis out, therefore, the diffeences between the control and experimental data are significant.

Comparison between the results of these experiments with those with *C. vulgaris* (see above) demonstrates that similar trends are observable in both algae with respect to the effects of BAs. However, some BAs exert diametrically opposite effects on the two species that belong to different taxonomic groups of algae.

4.1.3. Effects of BAs on the fatty acid composition of *H. lacustris* lipids. As noted above in the subsection on the BA effects on *C. vulgaris* and *S. quadricauda,*

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BAs can be broken up into two different subgroups, depending on the ratio between saturated and unsaturated fatty acids in their lipids. As for the two tested *H. lacustris* strains, the effects of BAs towards the end of the cultuivation period (by day 14) were generally less pronounced than with the microalgal species described above.

4.1.3.1. Serotonin (5-HT). Tables 22 and 23 contain the contents of various fatty acids per 1 g of dry weight in the samples of *H. lacustris* cultures (strains IPPAS H-239 and BM-1) grown with 0.1, 1, and 10 μM 5–HT or without it (the control systems) for 14 days. The following conclusions can be drawn:

- `5-HT at a concentration of 0.1 μM marginally (by 15.6%) increased and at a concentration of 10 μM decreased (by 22.6%) the total fatty acid content in strain IPPAS H-239; in strain BM-1, a decrease in total fatty acid content was noted; it was statistically verifiable with 0.1 and 10 μM 5-HT;
- 5-HT in both *H. lacustris* strains failed to exert any significant influence on the ratio between saturated (SFAs), mono- (MUFAs), and polyunsaturated (PUFAs) fatty acids in the cell lipids;
- The 5-HT effects on the percentages of fatty acid species boiled down to an increase in the share of myristic (14:0) and myristoleic (14:1) acid and the disappearance of the minor pentadecane acid (in strain IPPAS H-239 only). With strain BM-1, no stati-stically significant changes occurred under the influence of 5-HT except a slight de-crease in 14:1 and 18:1 content at the maximum tested 5-HT concentration (10 μM).

4.1.3.2. Histamine. Unlike 5-HT, histamine was found to increase the total fatty acid content (Tables 24 and 25) in strain IPPAS H-239 (by 22.5% with 1 μM histamine) and especially in strain BM-1 (more than twofold with 1 μM histamine). This might be correlated with a substantial increase in secondary carotenoid formation (see below) under the influence of histamine, which should also result in stimulating the formation of lipid droplets. They harbor astaxanthin in the cytoplasm of *H. lacustris* cells that undergo the transition to the encysted state

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(Solovchenko, 2015).

As for the percentages of individual fatty acids, no statistically verifiable effects were documented for strain IPPAS H-239, except for a slight but statistically significant increase in PUFA percentage at the expense of the SFA and MUFA shares. The linolenic acid (18:3) percentage increased in the presence of 1 and 10 μM histamine in strain BM-1; the same histamine concentrations caused a marginal decrease in the content of heicosanoic acid.

4.1.3.3. Dopamine (DA). Of note is a progressive decrease in total fatty acid content in the cell lipids of strains IPPAS H-239 and BM-1 with an increase in DA concentration from 0.1 to 10 μM (Table 26 and 27). In strain IPPAS H-239, a statistically significant increase in SFA level and a decrease in PUFA level were documented with 1 μM DA; in contrast, a minimally verifiable decrease in SFA content and an increase in PUFA content were detected in strain BM-1.

DA produced diametrically opposite effects in the two tested strains on the percentages of myristic (14:0) and myristoleic (14:1) acids: they increased in IPPAS H-239 and decreased in BM-1. In strain IPPAS H-239, a drop in the content of two minor fatty acid species, arachidonic (20:4) and behenic (22:0) acid, was observed. DA decressed the percentages of palmitoleic (16:1), stearic (18:0), and behenic acid. The contents of the polyunsaturated linolenic and eicosadienoic (20:2) acid were increased, i.e. a shift in fatty-acid profile favoring PUFA accumulation was established.

4.1.3.4. Norepinephrine (NE). Despite its chemical similarity to DA, NE appreciably increased the total fatty acid content in strains IPPAS H-239 and BM-1 (Table 28 and 29).

In both tested *H. lacustris* strains, NE at the maximum concentration (10 μM) slightly increased the PUFA percentage at theexpense of the SFA percentage.

NE decreased the percentages of myristic, myristoleic, and eicosapentaenoic acid in both tested strains. In strain IPPAS H-239, NA also decreased the percentage

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of the saturated stearic and behenic acid, while increasing those of eicosatrienoic and eicosadienoic acids belonging to PUFAs. In strain BM-1, a decrease in the percentages of pentadecenic (15:1) and palmitoleic (16:1) acids with an increase in that of linolenic acid was documented.

4.1.3.5. Acetylcholine (ACh). Of note is an increase in total fatty acid content in both tested strains (Tables 30 and 31). Similar to other BAs, ACh caused a decrease in myristic acid percentage. In strain IPPAS H-239, the percentages of the monounsaturated palmitoleic and the saturated stearic acid decreased, while those of specific PUFAs like linolenic and eicosadienoic acid increased (without any effect on the total PUFA percentage). In strain BM-1, the effects of ACh on the contents of various fatty acid species were more complex and no significant influence on the SFA:MUFA:PUFA ratio was detected.

In summary, ACh proved to be the most reliable stimulant of fatty-acid synthesis in microalgae because it also significantly increased the fatty acid content of both tested *H. lacustris* strains, in an analogy to the microalgae (*C. vulgaris* and *S. quadricauda*) tested in the preceding subsections. Similar to *S. quadricauda, H. lacustris* responded to histamine by augmenting its fatty-acid content. Two chemically similar catecholamines, DA and NE, produced diametrically opposite effects on *H. lacustris* fatty acid content; it was increased by NE and decreased by DA. This pointed to a specific, plausibly receptor-dependent, mode of action of the catecholamines. Despite its stimulatory action on the total production of fatty acids, ACh failed to produce, in this system, any verifiable effect on the SFA:MUFA:PUFA ratio, nor did histamine and 5-HT. NE caused a slight but statistically significant increase in PUFA percentage in both strains, and DA in BM-1 only.

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Table 22. Fatty acid content of *H. lacustris* IPPAS H-239 cultures grown with or without serotonin (5-HT)

Fatty acid	Proportion in total fatty acids, %			
	Control	5-HT, 0.1μM	5-HT,1 μM	5-HT,10 μM
Myristic acid 14:0	0.96±0.1	2.49±0.4	1.70±0.4	2.27±0.4
Myristoleic acid 14:1	0.13±0.1	0.17±0.1	0.20±0.1	0.16±0.1
Pentadecanoic acid 15:0	0.34±0.1	0.36±0.1	0.33±0.1	0.35±0.1
Pentadecenoic acid 15:1	0.06±0.05	0.12±0.1	0.03±0.02	0.11±0.1
Palmitic acid 16:0	26.92±2.0	26.17±2.0	26.52±2.0	26.18±1.0
Palmitoleic acid 16:1	1.75±0.4	1.89±0.4	1.37±0.1	1.61±0.1
Hexadienoic acid 16:2	0.42±0.1	0.41±0.1	0.40±0.1	0.44±0.1
Hexatrienoic acid 16:3	1.42±0.4	1.30±0.4	1.40±0.4	1.42±0.4
Hexatetraenoic acid 16:4	4.58±1.0	4.14±1.0	4.15±0.8	3.99±0.8
Stearic acid 18:0	1.23±0.1	1.58±0.1	1.31±0.1	1.41±0.1
Oleic acid 18:1	19.23±2.0	19.30±2.0	19.62±1.0	19.64±1.0
Linoleic acid 18:2	20.27±1.0	19.69±2.0	20.57±2.0	20.13±2.0
Linolenic acid 18:3	14.90±1.0	13.87±1.0	14.19±0.8	14.17±0.8
Arachic acid 20:0	1.44±0.1	1.34±0.1	1.36±0.1	1.29±0.1
Eicosenoic acid 20:1	2.79±0.4	2.58±0.4	2.62±0.1	2.54±0.1
Eicosadienoic acid 20:2	0.17±0.05	0.17±0.05	0.21±0.05	0.21±0.02
Eicosatrienoic acid 20:3	0.14±0.02	0.21±0.05	0.23±0.03	0.22±0.1
Arachidonic acid 20:4	0.61±0.1	0.62±0.1	0.75±0.1	0.85±0.2
Eicosapentaenoic acid 20:5	1.08±0.2	2.05±0.4	1.41±0.4	1.61±0.4
Henicosanic acid 21:0	0.48±0.1	0.48±0.1	0.54±0.1	0.54±0.2
Behenic acid 22:0	1.08±0.1	1.04±0.1	1.09±0.1	0.90±0.1
Saturated fatty acids (SFAs)	**32.4 ± 1.6**	**33.5 ± 1.6**	**32.8 ± 1.5**	**32.9 ± 1.0**
Monounsaturated fatty acids (MUFAs)	**23.9 ± 1.5**	**24.1 ± 1.4**	**23.8 ±1.3**	**24.0 ± 1.3**
Polyunsaturated fatty acids (PUFAs)	**43.6 ± 1.9**	**42.5 ± 2.0**	**43.3± 1.7**	**43.0± 1.8**
Total fatty acid content. mg/g of dry biomass (on day 14 of cultivation)	**76.0 ± 2.0**	**87.9 ± 4.0**	**83.8 ± 3.0**	**58.9± 4.5**

Averaged values based on the results of 4-5 repeats are given. N/D. not detected. Kruskal-Wallis test results with independent samples for the experimental and control groups. **SFAs: Sig.=0.887 > 0.05. MUFAs Sig.=0.935 > 0.05. PUFAs: Sig.=0.764 > 0.05** Total fatty acid content: Sig.=0.019 < 0.05. The results for all the groups are **not significant** except total fatty acid content

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Table 23. Fatty acid content of *H. lacustris* BM-1 cultures grown with or without serotonin (5-HT).

Fatty acid	**Proportion in total fatty acids, %**			
	Control	**5-HT, 0.1μM**	**5-HT, 1 μM**	**5-HT, 10μM**
Myristic acid 14:0	0.73±0.1	0.91±0.1	0.91±0.1	0.52±0.1
Myristoleic acid 14:1	0.56±0.1	0.55±0.1	0.61±0.1	0.55±0.1
Pentadecanoic acid 15:0	0.20±0.05	0.18±0.05	0.18±0.05	0.15±0.05
Pentadecenoic acid 15:1	0.07±0.04	0.05±0.03	N/D	N/D
Palmitic acid 16:0	27.30±2.0	29.94±2.0	27.78±2.0	27.48±2.0
Palmitoleic acid 16:1	2.12±0.4	1.99±0.4	2.41±0.4	2.09±0.4
Hexadienoic acid 16:2	1.44±0.1	1.27±0.1	1.44±0.2	1.59±0.1
Hexatrienoic acid 16:3	2.06±0.4	1.79±0.2	2.06±0.4	2.25±0.4
Hexatetraenoic acid 16:4	3.20±0.5	2.87±0.4	3.33±0.4	3.35±0.4
Stearic acid 18:0	2.53±0.3	2.83±0.4	2.58±0.4	2.26±0.4
Oleic acid 18:1	20.02±1.0	18.56±0.8	19.66±1.0	17.99±0.8
Linoleic acid 18:2	21.46±2.0	21.00±1.0	20.83±1.0	22.71±2.0
Linolenic acid 18:3	11.41±1.1	11.61±0.78	10.95±0.67	12.16±1.23
Arachic acid 20:0	1.60±0.4	1.45±0.1	1.64±0.4	1.48±0.1
Eicosenoic acid 20:1	2.38±0.4	2.19±0.4	2.39±0.8	2.38±0.8
Eicosadienoic acid 20:2	0.37±0.1	0.29±0.1	0.27±0.1	0.28±0.1
Eicosatrienoic acid 20:3	0.21±0.1	0.25±0.1	0.19±0.1	0.19±0.1
Arachidonic acid 20:4	0.49±0.2	0.42±0.2	0.53±0.4	0.50±0.4
Eicosapentaenoic acid 20:5	0.36±0.2	0.39±0.2	0.74±0.3	0.61±0.2
Heneicosanic acid 21:0	0.54±0.15	0.62±0.2	0.56±0.2	0.48±0.2
Behenic acid 22:0	0.96±0.1	0.85±0.1	0.94±0.2	0.97±0.3
Saturated fatty acids (SFAs)	**33.9 ± 1.5**	**36.8 ± 1.6**	**34.6 ± 1.4**	**33.3 ± 1.1**
Monounsaturated fatty acids (MUFAs)	**25.2±1.1**	**23.3 ± 1.2**	**25.1 ± 1.1**	**23.0 ± 1.0**
Polyunsaturated fatty acids (PUFAs)	**41.0 ± 1.7**	**39.9 ± 1.1**	**40.3± 1.66**	**43.6 ± 1.9**
Total fatty acid content. mg/g of dry biomass (on day 14 of cultivation)	***86.7±2.0***	***73.1±2.0***	***85.4±1.0***	***76.9±2.0***

Averaged values based on the results of 4-5 repeats are given. N/D. not detected.

Kruskal-Wallis test results with independent samples for the experimental and control groups. SFAs: Sig.=0.035 < 0.05. MUFAs Sig.=0.025 < 0.05. PUFAs: Sig.=0.047 < 0.05 Total fatty acid content: Sig.=0.026 < 0.05. The results for all the groups rule the null hypothesis out, which here and below indicates significant differences between the data groups to be compared (at p 0.05) as long as their SD ranges do not overlap

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Table 24. Fatty acid content of H. lacustris IPPAS H-239 cultures grown with or without histamine (His)

Fatty acid	**Proportion in total fatty acids, %**			
	Control	**His, 0.1 μM**	**His, 1 μM**	**His, 10 μM**
Myristic acid 14:0	1.36±0.1	1.39±0.1	1.46±0.1	1.17±0.1
Myristoleic acid 14:1	0.24±0.05	0.31±0.05	0.33±0.05	0.33±0.05
Pentadecanoic acid 15:0	0.78±0.1	0.76±0.1	0.66±0.1	0.67±0.1
Pentadecenoic acid 15:1	0.27±0.05	0.24±0.05	0.25±0.04	0.24±0.04
Palmitic acid 16:0	26.77±2.0	27.36±2.0	26.45±2.0	26.51±2.0
Palmitoleic acid 16:1	2.16±0.6	1.76±0.5	1.95±0.8	1.68±0.8
Hexadienoic acid 16:2	0.43±0.1	0.41±0.1	0.43±0.1	0.44±0.1
Hexatrienoic acid 16:3	1.36±0.4	1.43±0.4	1.55±0.4	1.60±0.4
Hexatetraenoic acid 16:4	3.75±0.8	3.78±0.8	3.89±0.8	3.97±0.8
Stearic acid 18:0	2.38±0.3	2.17±0.4	2.15±0.4	1.77±0.4
Oleic acid 18:1	19.89±1.0	19.85±1.0	19.84±1.0	19.30±1.0
Linoleic acid 18:2	19.36±2.0	19.36±2.0	19.62±2.0	20.37±2.0
Linolenic acid 18:3	15.00±0.8	14.74±0.8	15.12±0.8	15.41±1.0
Arachic acid 20:0	1.28±0.1	1.28±0.1	1.23±0.1	1.31±0.1
Eicosenoic acid 20:1	2.51±0.4	2.54±0.4	2.54±0.4	2.60±0.4
Eicosadienoic acid 20:2	0.17±0.03	0.29±0.04	0.33±0.05	0.36±0.05
Eicosatrienoic acid 20:3	0.15±0.03	0.15±0.02	0.14±0.03	0.16±0.02
Arachidonic acid 20:4	0.45±0.1	0.49±0.1	0.44±0.1	0.46±0.1
Eicosapentaenoic acid 20:5	0.55±0.1	0.54±0.1	0.55±0.1	0.55±0.1
Henicosanic acid 21:0	0.32±0.04	0.33±0.04	0.27±0.05	0.27±0.05
Behenic acid 22:0	0.82±0.1	0.81±0.1	0.81±0.1	0.84±0.1
Saturated fatty acids (SFAs)	**33.7± 1.7**	**34.1 ± 1.8**	**33.0 ± 1.6**	**32.5 ± 1.7**
Monounsaturated fatty acids (MUFAs)	**25.1±1.1**	**24.7 ± 1.0**	**24.7 ± 2.2**	**23.9 ± 1.2**
Polyunsaturated fatty acids (PUFAs)	**41.0 ± 1.7**	**39.9 ± 1.1**	**40.3 ± 1.6**	**43.6 ± 1.9**
Total fatty acid content. mg/g of dry biomass (on day 14 of cultivation)	**86.2±2.0**	**89.2±2.0**	**105.7±2.0**	**67.6±2.0**

Averaged values based on the results of 4-5 repeats are given. Kruskal-Wallis test results with independent samples for the experimental and control groups. SFAs: **Sig.=0.764 > 0.05. MUFAs Sig.=0.789 > 0.05**. PUFAs: Sig.=0.047 < 0.05. Total fatty acid content: Sig.=0.019 < 0.05. The results for SFA and MUFA groups are **not significant.**

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Table 25. Fatty acid content of *H. lacustris* BM-1 cultures grown with or without histamine (His)

Fatty acid	**Proportion in total fatty acids, %**			
	Control	**His, 0.1 μM**	**His, 1 μM**	**His, 10 μM**
Myristic acid 14:0	1.42±0.4	1.10±0.1	1.17±0.1	1.03±0.1
Myristoleic acid 14:1	0.24±0.04	0.24±0.04	0.27±0.05	0.25±0.05
Pentadecanoic acid 15:0	0.70±0.1	0.60±0.1	0.62±0.1	0.55±0.1
Pentadecenoic acid 15:1	0.17±0.04	0.18±0.03	0.19±0.04	0.18±0.04
Palmitic acid 16:0	31.22±2.0	30.01±2.0	29.14±2.0	28.52±2.0
Palmitoleic acid 16:1	2.45±0.8	1.93±0.4	1.80±0.4	1.71±0.4
Hexadienoic acid 16:2	1.01±0.1	1.03±0.1	1.10±0.1	1.22±0.2
Hexatrienoic acid 16:3	1.89±0.4	1.99±0.4	2.20±0.8	2.19±0.8
Hexatetraenoic acid 16:4	2.87±0.8	3.05±1.0	3.55±1.0	3.53±1.0
Stearic acid 18:0	2.95±0.8	2.50±0.8	2.21±0.4	2.11±0.4
Oleic acid 18:1	20.64±2.0	19.98±1.0	19.21±1.0	19.44±1.0
Linoleic acid 18:2	19.68±2.0	21.15±2.0	21.11±2.0	21.55±2.0
Linolenic acid 18:3	9.10±0.8	10.32±0.8	11.30±0.8	11.63±0.8
Arachic acid 20:0	1.41±0.1	1.50±0.1	1.55±0.1	1.59±0.1
Eicosenoic acid 20:1	1.81±0.4	2.03±0.4	2.12±0.4	2.08±0.4
Eicosadienoic acid 20:2	0.19±0.04	0.21±0.05	0.22±0.05	0.22±0.05
Eicosatrienoic acid 20:3	0.21±0.04	0.20±0.03	0.18±0.04	0.18±0.03
Arachidonic acid 20:4	0.33±0.1	0.38±0.1	0.41±0.1	0.35±0.1
Eicosapentaenoic acid 20:5	0.53±0.1	0.44±0.1	0.50±0.1	0.51±0.1
Henicosanic acid 21:0	0.59±0.1	0.44±0.1	0.34±0.06	0.32±0.06
Behenic acid 22:0	0.60±0.1	0.74±0.1	0.81±0.1	0.84±0.4
Saturated fatty acids (SFAs)	**38.9±2.0**	**36.9±1.8**	**35.8±2.0**	**35.0±1.9**
Monounsaturated fatty acids (MUFAs)	**25.3±1.4**	**24.4±1.5**	**23.6±1.3**	**23.7±1.4**
Polyunsaturated fatty acids (PUFAs)	**35.8±1.9**	**38.7±1.8**	**40.6±2.0**	**41.4±2.1**
Total fatty acid content. mg/g of dry biomass (on day 14 of cultivation)	***70.2±2.0***	***141.4±2.0***	***155.5±2.0***	***58.4±2.0***

Averaged values based on the results of 4-5 repeats are given. Kruskal-Wallis test results with independent samples for the experimental and control groups. SFAs: **Sig.=0.108 > 0.05.** MUFAs Sig.=0.043 < 0.05. PUFAs: Sig.= 0.05. Total fatty acid content: Sig.=0.016 < 0.05. The results for the SFA group are **not significant**.

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Table 26. Fatty acid content of *H. lacustris* IPPAS H-239 cultures grown with or without dopamine (DA)

Fatty acid	**Proportion in total fatty acids, %**			
	Control	**DA, 0.1 μM**	**DA, 1 μM**	**DA, 10 μM**
Myristic acid 14:0	2.84±0.4	2.55±0.4	4.26±0.8	1.06±0.4
Myristoleic acid 14:1	0.15±0.04	0.14±0.04	0.22±0.04	N/D.
Pentadecanoic acid 15:0	0.62±0.1	0.59±0.1	0.81±0.1	0.54±0.1
Pentadecenoic acid 15:1	0.13±0.03	0.18±0.05	0.17±0.05	0.27±0.05
Palmitic acid 16:0	24.63±2.0	25.19±2.0	27.28±2.0	25.91±2.0
Palmitoleic acid 16:1	1.77±0.4	1.84±0.4	2.51±0.4	1.80±0.4
Hexadienoic acid 16:2	0.26±0.05	0.29±0.05	0.30±0.1	0.38±0.1
Hexatrienoic acid 16:3	1.50±0.4	1.52±0.4	1.50±0.4	1.70±0.4
Hexatetraenoic acid 16:4	4.67±0.8	4.79±0.8	4.56±1.0	4.79±1.0
Stearic acid 18:0	1.82±0.4	1.60±0.4	1.83±0.4	1.52±0.4
Oleic acid 18:1	17.85±1.0	17.69±1.0	16.79±1.0	18.04±1.0
Linoleic acid 18:2	19.48±2.0	19.58±2.0	18.20±1.0	20.63±2.0
Linolenic acid 18:3	16.58±1.0	17.33±1.0	15.43±1.0	17.15±1.0
Arachic acid 20:0	1.02±0.1	1.01±0.1	0.86±0.1	1.13±0.1
Eicosenoic acid 20:1	2.84±0.4	2.87±0.4	2.65±0.4	2.82±0.4
Eicosadienoic acid 20:2	0.30±0.05	0.41±0.1	0.34±0.05	0.23±0.04
Eicosatrienoic acid 20:3	0.69±0.1	0.65±0.1	0.48±0.1	0.50±0.1
Arachidonic acid 20:4	1.23±0.4	1.24±0.4	0.99±0.1	0.53±0.1
Eicosapentaenoic acid 20:5	0.92±0.1	0.11±0.02	0.23±0.02	0.35±0.02
Henicosanic acid 21:0	0.23±0.03	0.25±0.03	0.35±0.03	0.32±0.03
Behenic acid 22:0	0.48±0.05	0.15±0.05	0.23±0.05	0.34±0.07
Saturated fatty acids (SFAs)	**31.6±1.9**	**31.3±1.8**	**35.6±1.9**	**30.8±1.7**
Monounsaturated fatty acids (MUFAs)	**22.7±1.6**	**22.7±1.4**	**22.3±1.5**	**22.9±1.4**
Polyunsaturated fatty acids (PUFAs)	**45.6±2.0**	**45.9±2.1**	**42.0±2.0**	**46.2±1.8**
Total fatty acid content. mg/g of dry biomass (on day 14 of cultivation)	***65.6±2.0***	***51.6±2.0***	***43.7±2.0***	***35.9±2.0***

Averaged values based on the results of 4-5 repeats are given. N/D, not detected. Kruskal-Wallis test results with independent samples for the experimental and control groups. SFAs: Sig.=0.029 < 0.05. MUFAs **Sig.=0.779 > 0.05**. PUFAs: Sig.=0.031 < 0.05 Total fatty acid content: Sig.=0.016 < 0.05. The differences between the control and experimental data for MUFAs are **not significant.**

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Table 27. Fatty acid content of *H. lacustris* BM-1 cultures grown with or without dopamine (DA)

Fatty acid	**Proportion in total fatty acids, %**			
	Control	**DA, 0.1 μM**	**DA, 1 μM**	**DA, 10 μM**
Myristic acid 14:0	1.86±0.1	0.84±0.1	1.19±0.1	1.47±0.1
Myristoleic acid 14:1	0.39±0.04	0.22±0.04	0.30±0.05	0.26±0.05
Pentadecanoic acid 15:0	0.78±0.1	0.84±0.1	0.67±0.1	0.81±0.1
Pentadecenoic acid 15:1	0.25±0.04	0.21±0.03	0.20±0.04	0.20±0.04
Palmitic acid 16:0	28.91±2.0	27.86±2.0	27.44±2.0	32.34±2.0
Palmitoleic acid 16:1	2.88±0.8	1.62±0.4	2.00±0.4	1.73±0.4
Hexadienoic acid 16:2	1.11±0.1	1.33±0.1	1.24±0.1	1.15±0.1
Hexatrienoic acid 16:3	1.88±0.1	2.09±0.4	2.10±0.4	1.62±0.1
Hexatetraenoic acid 16:4	3.00±0.4	3.35±0.8	3.40±0.8	2.35±0.4
Stearic acid 18:0	3.28±0.4	2.21±0.4	2.45±0.4	3.09±0.4
Oleic acid 18:1	20.78±1.0	19.33±1.0	19.94±1.0	20.46±1.0
Linoleic acid 18:2	20.14±1.0	21.68±1.0	21.29±1.0	19.51±1.0
Linolenic acid 18:3	6.95±0.8	10.70±0.8	11.62±0.8	9.92±0.8
Arachic acid 20:0	1.46±0.4	1.75±0.4	1.62±0.4	1.35±0.5
Eicosenoic acid 20:1	1.80±0.4	1.93±0.4	2.01±0.8	1.44±0.4
Eicosadienoic acid 20:2	0.20±0.04	0.91±0.05	0.21±0.03	0.20±0.03
Eicosatrienoic acid 20:3	0.21±0.04	0.23±0.05	0.20±0.04	0.28±0.05
Arachidonic acid 20:4	0.37±0.1	0.49±0.1	0.38±0.1	0.34±0.1
Eicosapentaenoic acid 20:5	2.62±0.8	0.72±0.1	0.58±0.1	0.54±0.1
Henicosanic acid 21:0	0.37±0.1	0.88±0.1	0.31±0.1	0.45±0.1
Behenic acid 22:0	0.75±0.1	0.79±0.1	0.83±0.1	0.50±0.1
Saturated fatty acids (SFAs)	**37.41 ± 1.2**	**35.17 ± 1.2**	**34.51 ± 1.2**	**40.01 ± 1.3**
Monounsaturated fatty acids (MUFAs)	**26.1 ± 1.3**	**23.31 ± 1.9**	**24.45 ± 2.3**	**23.83 ± 1.8**
Polyunsaturated fatty acids (PUFAs)	**36.4 ± 1.3**	**41.5 ± 1.4**	**41.02 ± 1.3**	**35.91 ± 1.6**
Total fatty acid content. mg/g of dry biomass (on day 14 of cultivation)	**62.5 ± 2.0**	**54.8 ± 2.0**	**57.5±2.0**	**51.6 ± 2.0**

Averaged values based on the results of 4-5 repeats are given. Kruskal-Wallis test results with independent samples for the experimental and control groups. SFAs: Sig.=0.031 < 0.05. MUFAs **Sig.=0.294 > 0.05.** PUFAs: Sig.=0.036 < 0.05 Total fatty acid content: Sig.=0.023 < 0.05. The differences between the control and experimental data for MUFAs are **not significant**.

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Table 28. Fatty acid content of *H. lacustris* IPPAS H-239 cultures grown with or without norepinephrine (NE)

Fatty acid	Proportion in total fatty acids, %			
	Control	NE, 0.1 μM	NE, 1 μM	NE, 10 μM
Myristic acid 14:0	1.33±0.1	1.27±0.1	1.18±0.1	0.97±0.1
Myristoleic acid 14:1	0.66±0.05	0.22±0.03	0.33±0.05	0.26±0.05
Pentadecanoic acid 15:0	0.22±0.04	0.82±0.04	0.83±0.1	0.68±0.1
Pentadecenoic acid 15:1	0.17±0.03	0.19±0.02	0.19±0.02	0.17±0.02
Palmitic acid 16:0	25.90±2.0	27.41±2.0	27.24±2.0	25.38±2.0
Palmitoleic acid 16:1	1.58±0.4	1.99±0.4	1.82±0.4	1.92±0.4
Hexadienoic acid 16:2	0.31±0.05	0.35±0.05	0.29±0.04	0.29±0.04
Hexatrienoic acid 16:3	1.52±0.1	1.64±0.1	1.55±0.1	1.49±0.1
Hexatetraenoic acid 16:4	4.99±1.0	5.23±1.0	4.99±0.8	4.91±0.8
Stearic acid 18:0	2.88±0.4	1.67±0.4	1.63±0.4	1.73±0.4
Oleic acid 18:1	16.48±1.0	16.70±1.0	17.21±1.0	17.10±1.0
Linoleic acid 18:2	18.75±2.0	19.38±2.0	19.48±2.0	20.18±2.0
Linolenic acid 18:3	15.64±1.0	15.66±1.0	15.47±1.0	16.11±1.0
Arachic acid 20:0	1.01±0.1	0.98±0.1	0.94±0.1	1.00±0.1
Eicosenoic acid 20:1	2.86±0.4	2.74±0.4	2.74±0.4	2.84±0.4
Eicosadienoic acid 20:2	0.21±0.05	0.34±0.05	0.39±0.05	0.51±0.05
Eicosatrienoic acid 20:3	0.18±0.03	0.34±0.03	0.19±0.02	0.44±0.03
Arachidonic acid 20:4	0.85±0.1	0.88±0.1	0.85±0.1	0.81±0.1
Eicosapentaenoic acid 20:5	3.37±0.4	1.27±0.4	1.96±0.4	2.64±0.4
Henicosanic acid 21:0	0.34±0.04	0.47±0.04	0.44±0.04	0.40±0.04
Behenic acid 22:0	0.73±0.05	0.44±0.05	0.28±0.05	0.16±0.05
Saturated fatty acids (SFAs)	**32.4±1.0**	**33.1 ± 1.7**	**32.5 ± 1.7**	**30.3 ± 1.0**
Monounsaturated fatty acids (MUFAs)	**21.7±1.9**	**21.8 ± 1.8**	**22.3 ± 1.2**	**22.3 ± 1.9**
Polyunsaturated fatty acids (PUFAs)	**45.8±1.7**	**45.1 ± 1.7**	**45.2 ± 1.5**	**47.38 ± 1.5**
Total fatty acid content. mg/g of dry biomass (on day 14 of cultivation)	**59.9±2.0**	**81.9±2.0**	**64.6±2.0**	**71.3±2.0**

Averaged values based on the results of 4-5 repeats are given. Kruskal-Wallis test results with independent samples for the experimental and control groups. SFAs: Sig.=0.028 < 0.05. MUFAs **Sig.=0.901 > 0.05**. PUFAs: Sig.=0.036 < 0.05 Total fatty acid content: Sig.=0.016 < 0.05. The differences between the control and experimental data for MUFAs are **not significant**.

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Table 29. Fatty acid content of *H. lacustris* BM-1 cultures grown with or without norepinephrine (NE)

Fatty acid	**Proportion in total fatty acids, %**			
	Control	**NE, 0.1 μM**	**NE, 1 μM**	**NE, 10 μM**
Myristic acid 14:0	1.29±0.1	0.83±0.1	0.99±0.1	0.76±0.1
Myristoleic acid 14:1	0.29±0.05	0.26±0.05	0.25±0.05	0.23±0.05
Pentadecanoic acid 15:0	0.73±0.1	0.66±0.1	0.69±0.1	0.61±0.1
Pentadecenoic acid 15:1	0.14±0.02	0.19±0.02	0.11±0.02	0.10±0.02
Palmitic acid 16:0	27.44±2.0	27.31±2.0	26.76±2.0	26.20±2.0
Palmitoleic acid 16:1	2.11±0.1	1.60±0.1	1.84±0.1	1.66±0.1
Hexadienoic acid 16:2	1.14±0.1	1.24±0.1	1.20±0.1	1.24±0.1
Hexatrienoic acid 16:3	2.08±0.4	2.17±0.4	2.20±0.4	2.20±0.4
Hexatetraenoic acid 16:4	4.50±0.8	4.67±0.8	4.87±0.8	4.68±0.8
Stearic acid 18:0	2.51±0.4	1.89±0.4	2.16±0.4	1.90±0.4
Oleic acid 18:1	18.11±1.0	17.26±1.0	17.46±1.0	17.93±1.0
Linoleic acid 18:2	20.45±2.0	21.27±2.0	21.11±2.0	22.02±2.0
Linolenic acid 18:3	12.47±1.0	13.48±0.8	13.47±1.0	13.57±1.0
Arachic acid 20:0	1.40±0.1	1.52±0.1	1.46±0.1	1.53±0.1
Eicosenoic acid 20:1	2.38±0.4	2.56±0.4	2.53±0.4	2.50±0.4
Eicosadienoic acid 20:2	0.33±0.05	0.35±0.05	0.35±0.05	0.34±0.05
Eicosatrienoic acid 20:3	1.00±0.1	1.13±0.1	1.10±0.1	1.12±0.1
Arachidonic acid 20:4	0.53±0.1	0.57±0.1	0.57±0.1	0.52±0.1
Eicosapentaenoic acid 20:5	0.51±0.05	0.47±0.05	0.31±0.05	0.33±0.05
Henicosanic acid 21:0	0.36±0.04	0.32±0.04	0.31±0.04	0.32±0.04
Behenic acid 22:0	0.23±0.05	0.26±0.05	0.24±0.05	0.26±0.05
Saturated fatty acids (SFAs)	**34.0±1.0**	**32.8 ± 1.8**	**32.6 ± 1.7**	**31.6 ± 1.1**
Monounsaturated fatty acids (MUFAs)	**23.0±1.6**	**21.9 ± 1.8**	**22.2 ± 1.6**	**22.4 ± 1.6**
Polyunsaturated fatty acids (PUFAs)	**43.0±1.1**	**45.3 ± 1.4**	**45.2 ± 1.6**	**46.0 ± 1.0**
Total fatty acid content. mg/g of dry biomass (on day 14 of cultivation)	***87.0±3.0***	***83.6±3.0***	***91.5±3.0***	***97.9±3.0***

Averaged values based on the results of 4-5 repeats are given. Kruskal-Wallis test results with independent samples for the experimental and control groups. SFAs: Sig.=0.016 < 0.05. MUFAs: **Sig.=0.764 > 0.05.** PUFAs: Sig.=0.048 < 0.05 Total fatty acid content: Sig.=0.023 < 0.05. The differences between the control and experimental data for MUFAs are **not significant.**

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Table 30. Fatty acid content of *H. lacustris* IPPAS H-239 cultures grown with or without acetylcholine (ACh)

Fatty acid	**Proportion in total fatty acids, %**			
	Control	**ACh, 0.1 μM**	**ACh, 1 μM**	**ACh, 10 μM**
Myristic acid 14:0	3.09±0.4	1.11±0.1	1.36±0.1	2.60±0.4
Myristoleic acid 14:1	0.35±0.1	1.14±0.1	0.32±0.1	0.31±0.1
Pentadecanoic acid 15:0	0.33±0.1	0.33±0.1	0.35±0.1	0.35±0.1
Pentadecenoic acid 15:1	0.22±0.05	0.06±0.04	0.10±0.05	0.13±0.05
Palmitic acid 16:0	28.89±2.0	29.69±2.0	30.09±2.0	28.67±2.0
Palmitoleic acid 16:1	2.96±0.4	2.11±0.4	2.27±0.4	1.90±0.4
Hexadienoic acid 16:2	0.44±0.1	0.56±0.1	0.49±0.1	0.48±0.1
Hexatrienoic acid 16:3	1.26±0.4	1.39±0.4	1.34±0.4	1.39±0.4
Hexatetraenoic acid 16:4	3.46±0.8	3.94±0.8	4.12±0.8	4.25±0.8
Stearic acid 18:0	2.15±0.4	1.49±0.4	1.41±0.4	1.49±0.4
Oleic acid 18:1	18.75±1.0	19.72±1.0	18.62±1.0	18.30±1.0
Linoleic acid 18:2	16.74±1.0	18.07±1.0	18.31±1.0	18.01±1.0
Linolenic acid 18:3	11.43±0.8	13.09±0.8	13.47±0.8	13.39±0.8
Arachic acid 20:0	1.21±0.4	1.33±0.4	1.44±0.4	1.42±0.4
Eicosenoic acid 20:1	2.29±0.4	2.55±0.4	2.68±0.4	2.67±0.4
Eicosadienoic acid 20:2	0.32±0.1	0.40±0.1	0.99±0.1	1.00±0.1
Eicosatrienoic acid 20:3	0.15±0.02	0.11±0.02	0.12±0.02	0.09±0.02
Arachidonic acid 20:4	0.53±0.1	0.58±0.1	0.65±0.1	0.65±0.1
Eicosapentaenoic acid 20:5	4.70±0.4	1.11±0.4	1.16±0.4	2.21±0.4
Henicosanic acid 21:0	0.69±0.1	0.77±0.1	0.72±0.1	0.70±0.1
Behenic acid 22:0	0.05±0.03	0.43±0.03	N/D	N/D
Saturated fatty acids (SFAs)	**36.4±1.8**	**35.2±1.9**	**35.4±1.7**	**35.2±1.9**
Monounsaturated fatty acids (MUFAs)	**24.6±1.5**	**25.6±1.4**	**24.0±1.5**	**23.3±1.6**
Polyunsaturated fatty acids (PUFAs)	**39.0±1.9**	**39.3±2.0**	**40.6±2.0**	**41.5±1.8**
Total fatty acid content. mg/g of dry biomass (on day 14 of cultivation)	***76.4±3.0***	***91.6±3.0***	***85.2±3.0***	***81.3±3.0***

Averaged values based on the results of 4-5 repeats are given. N/D, not detected. Kruskal-Wallis test results with independent samples for the experimental and control groups. SFAs: Sig.=0.043 < 0.05. MUFAs **Sig.=0.516 > 0.05**. PUFAs: Sig.=0.036 < 0.05 Total fatty acid content: Sig.=0.023 < 0.05. The differences between the control and experimental data for MUFAs are **not significant**.

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Table 31. Fatty acid content of *H. lacustris* BM-1 cultures grown with or without acetylcholine (ACh)

Fatty acid	Proportion in total fatty acids, %			
	Control	ACh, 0.1 μM	ACh, 1 μM	ACh, 10 μM
Myristic acid 14:0	2.63±0.4	2.12±0.4	1.90±0.4	1.03±0.1
Myristoleic acid 14:1	0.32±0.05	0.36±0.05	0.37±0.05	0.27±0.05
Pentadecanoic acid 15:0	0.32±0.05	0.44±0.05	0.24±0.05	0.45±0.05
Pentadecenoic acid 15:1	0.17±0.02	0.07±0.02	0.09±0.02	0.16±0.02
Palmitic acid 16:0	28.19±2.0	27.78±2.0	27.56±2.0	28.76±2.0
Palmitoleic acid 16:1	2.89±0.4	2.55±0.4	2.31±0.4	1.61±0.4
Hexadienoic acid 16:2	0.92±0.1	0.89±0.1	0.92±0.1	0.82±0.1
Hexatrienoic acid 16:3	1.88±0.4	1.73±0.4	2.10±0.4	1.97±0.4
Hexatetraenoic acid 16:4	3.65±0.8	3.27±0.8	4.02±0.8	4.03±0.8
Stearic acid 18:0	2.59±0.4	2.81±0.4	2.90±0.4	2.81±0.4
Oleic acid 18:1	17.33±1.0	17.55±1.0	16.84±1.0	17.31±1.0
Linoleic acid 18:2	18.59±1.0	18.58±1.0	18.99±1.0	20.87±1.0
Linolenic acid 18:3	12.35±0.8	12.20±0.8	12.53±0.8	12.30±0.8
Arachic acid 20:0	1.42±0.1	1.38±0.1	1.39±0.1	1.49±0.1
Eicosenoic acid 20:1	2.90±0.4	2.81±0.4	2.87±0.4	2.86±0.4
Eicosadienoic acid 20:2	0.87±0.1	0.89±0.1	0.26±0.1	0.23±0.1
Eicosatrienoic acid 20:3	0.23±0.05	0.25±0.05	0.22±0.05	0.21±0.05
Arachidonic acid 20:4	0.71±0.1	0.65±0.1	0.85±0.1	0.65±0.1
Eicosapentaenoic acid 20:5	1.46±0.1	3.04±0.1	1.97±0.1	0.58±0.1
Henicosanic acid 21:0	0.58±0.1	0.61±0.1	0.47±0.1	0.39±0.1
Behenic acid 22:0	N/D	N/D	1.18±0.4	1.19±0.4
Saturated fatty acids (SFAs)	**35.7±1.9**	**35.1±2.0**	**35.6±2.1**	**36.1±1.8**
Monounsaturated fatty acids (MUFAs)	**23.6±1.3**	**23.3±1.5**	**22.5±1.5**	**22.2±1.4**
Polyunsaturated fatty acids (PUFAs)	**40.7±2.1**	**41.5±2.0**	**41.9±2.1**	**41.7±1.9**
Total fatty acid content. mg/g of dry biomass (on day 14 of cultivation)	***70.6±2.0***	***57.9±2.0***	***81.3±2.0***	***98.0±2.0***

Averaged values based on the results of 4-5 repeats are given. N/D, not detected. Kruskal-Wallis test results with independent samples for the experimental and control groups. SFAs: **Sig.=0.789 > 0.05**. MUFAs: **Sig.=0.764 > 0.05.** PUFAs: Sig.=0.016 < 0.05 Total fatty acid content: Sig.=0.016 < 0.05. The differences between the experimental and control data groups for SFAs and MUFAs are **not significant**.

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4.2. CYANOBACTERIUM

The cyanobacterium *L. platensis* under study is characterized by a high lipid content, including a sizable fraction of polyunsaturated fatty acids (PUFAs), especially α-linolenic acid that, to reiterate, is increasingly utilized in pharmaceutical preparations and cosmetics (Wu et al., 2016; Искусных и др., 2021). The results of chromatographic separation of fatty acids with their mass spectrometry on day 14 of cultivating *L. platensis* are presented herebelow.

4.2.1. Serotonin (5-HT) at a concentration of 0.1 μM verifiably (by ~19%) increased the total fatty acid content of *L. platensis* lipids (Table 32). Higher 5-HT concentrations, in contrast, decreased it; they also altered the ratio between their types: the contribution of the MUFAS was decreased and that of PUFAs increased.

4.2.2. Histamine failed to exert any significant influence on the total fatty acid content (Table 33) but the Kruskal-Wallis test demonstrated a slight but verifiable decrease in MUFA percentage at the lowest tested histamine concentration (0.1 μM).

4.2.3. Dopamine (DA) caused no statistically valid effects (Table 34).

4.2.4. Norepinephrine (NE) significantly decreased the total fatty acid content (by 12% with 1 μM NE), concomitantly reducing the MUFA and elevating the PUFA percentage, while producing no verifiable effect on the SFA contribution (Table 35).

4.2.5. Acetylcholine (ACh) at concentrations of 0.1 and 1 μM statistically significantly (based on the Kruskal-Wallis H test) increased the total fatty acid content per 1 g of dry weight (Table 36) without considerably changing the ratio between saturated, mono-, and polyunsaturated fatty acids (SFAs, MUFAs, and PUFAs, respectively).

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Table 32. Fatty acid content of *L. platensis* cultures grown with or without serotonin (5-HT).

Fatty acid	**Proportion in total fatty acids, %**			
	Control	**5-HT, 0.1 μM**	**5-HT, 1 μM**	**5-HT,10 μM**
Myristic acid 14:0	1.38 ± 0.90	1.92 ± 0.10	1.73 ± 0.19	2.09 ± 0.28
Myristoleic acid14:1	0.35 ± 0.05	0.36 ± 0.02	0.33 ± 0.03	0.43 ± 0.02
Pentadecanoic acid15:0	1.53 ± 0.14	1.34 ± 0.04	1.27 ± 0.03	1.82 ± 0.52
Pentadecenoic 15:1	0.77 ± 0.06	0.75 ± 0.06	0.47 ± 0.37	0.34 ± 0.18
Palmitic acid16:0	31.06 ± 0.92	30.28 ± 0.24	31.65 ± 0.72	32.3 ± 0.71
Palmitoleic acid 16:1	5.85 ± 0.16	5.77 ± 0.54	6.72 ± 0.27	7.43 ± 1.16
Stearic acid 18:0	3.1 ± 0.14	2.87 ± 0.05	2.63 ± 0.23	2.44 ± 0.01
Oleic acid 18:1	11.53 ± 0.11	11.11 ± 1.11	9.38 ± 0.64	8.17 ± 0.32
Linoleic acid 18:2	22 ± 0.17	22.34 ± 0.19	21.9 ± 0.07	22.23 ± 0.00
γ-Linolenic acid 18:3	14.33 ± 0.32	14.64 ± 1.01	16.46 ± 0.17	16.29 ± 0.26
α-Linolenic acid 18:3	0.67 ± 0.21	0.55 ± 0.18	0.49 ± 0.16	0.48 ± 0.04
Eicosaenoic acid 20:1	2.02 ± 0.16	2.23 ± 0.21	1.81 ± 0.09	1.52 ± 0.47
Heneicosanoic acid 21:0	5.4 ± 0.33	5.84 ± 0.30	5.16 ± 0.18	4.44 ± 0.61
Saturated fatty acids (SFAs)	**42.47 ± 2.43**	**42.25 ± 0.73**	**42.44 ± 1.35**	**43.09 ± 2.13**
Monounsaturated fatty acids (MUFAs)	**20.52 ± 0.54**	**20.22 ± 1.94**	**18.71 ± 1.40**	**17.89 ± 2.15**
Polyunsaturated fatty acids (PUFAs)	**37.00 ± 0.70**	**37.53 ± 1.38**	**38.85 ± 0.40**	**39.00 ± 0.30**
Total fatty acid content, mg/g of dry weight	***3.92 ± 0.20***	***4.65 ± 0.43***	***3.12 ± 0.55***	***2.98 ± 0.26***

Averaged values based on the results of 4-5 repeats are given. N/D, not detected**.** Kruskal-Wallis test results with independent samples for the experimental and control groups: SFAs: **Sig.=0.161 > 0.05**. MUFAs: Sig.=0.024 < 0.05. PUFAs Sig.=0.033 > 0.05. Total fatty acid content: Sig.=0.025 < 0.05. . The results for all the data groups except the SFA data rule the null hypothesis out, therefore, only the differences between the experimental and control groups for SFAs **are not significant** (at α 0.05)**.**

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Table 33. Fatty acid content of *L. platensis* cultures grown with or without histamine (His)

Fatty acid	**Proportion in total fatty acids, %**			
	Control	**His, 0.1 μM**	**His,1 μM**	**His,10 μM**
Myristic acid 14:0	1.45±0.28	1.54±0.31	1.6±0.05	1.67±0.14
Myristoleic acid 14:1	0.28±0.01	0.34±0.05	0.34±0.04	0.36±0.07
Pentadecanoic acid 15:0	0.99±0.06	1.19±0.14	1.36±0.18	1.27±0.13
Pentadecenoic 15:1	0.65±0.09	0.46±0.11	0.71±0.28	0.49±0.01
Palmitic acid16:0	33.05±0.04	32.49±0.32	31.84±0.53	31.1±1.07
Palmitoleic acid 16:1	6.96 ± 0.17	6.78 ± 0.41	7.05 ± 0.23	6.8 ± 0.17
Stearic acid 18:0	2.96 ± 0.13	2.61 ± 0.27	2.71 ± 0.32	2.76 ± 0.41
Oleic acid 18:1	8.63 ± 0.42	8.38 ± 0.07	8.42 ± 0.65	9.66 ± 0.09
Linoleic acid 18:2	21.78 ± 0.56	23.12 ± 0.20	21.96 ± 0.09	21.4 ± 0.87
γ-Linolenic acid 18:3	16.61 ± 0.38	16.29 ± 0.31	17.41 ± 1.11	17.05 ± 0.42
α-Linolenic acid 18:3	0.3 ± 0.00	0.38 ± 0.06	0.43 ± 0.07	0.4 ± 0.04
Eicosaenoic acid 20:1	1.61 ± 0.02	1.59 ± 0.04	1.54 ± 0.08	1.74 ± 0.08
Heneicosanoic acid 21:0	4.71 ± 0.21	4.84 ± 0.09	4.64 ± 0.11	5.31 ± 0.24
Saturated fatty acids (SFAs)	**43.16 ± 0.72**	**42.67 ± 1.13**	**42.15 ± 1.19**	**42.11 ± 1.99**
Monounsaturated fatty acids (MUFAs)	**18.13 ± 0.71**	**17.55 ± 0.68**	**18.06 ± 1.28**	**19.05 ± 0.42**
Polyunsaturated fatty acids (PUFAs)	**38.69 ± 0.94**	**39.79 ± 0.57**	**39.8 ± 1.27**	**38.85 ± 1.33**
Total fatty acid content, mg/g of dry weight	***3.53 ± 0.03***	***3.69 ± 0.15***	***3.12 ± 0.20***	***3.24 ± 0.54***

Averaged values based on the results of 4-5 repeats are given. N/D, not detected**.** Kruskal-Wallis test results with independent samples for the experimental and control groups: SFAs: **Sig.=0.218 > 0.05**. MUFAs: Sig.=0.025 < 0.05. PUFAs: **Sig.=0.270 > 0.05.** Total fatty acid content:: **Sig.=0.075 > 0.05**. The results for all the data groups except the PUFA data conform to the null hypothesis, therefore, only the differences between the experimental and control groups for PUFAs are significant (at α 0.05)**.**

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Table 34. Fatty acid content of *L. platensis* cultures grown with or without dopamine (DA)

Fatty acid	**Proportion in total fatty acids, %**			
	Control	**DA, 0.1 μM**	**DA, 1 μM**	**DA, 10 μM**
Myristic acid 14:0	2.09 ± 0.19	1.48 ± 0.79	1.74 ± 0.16	1.92 ± 0.26
Myristoleic acid14:1	0.4 ± 0.03	0.38 ± 0.04	0.39 ± 0.02	0.42 ± 0.09
Pentadecanoic acid15:0	1.52 ± 0.24	1.59 ± 0.17	1.46 ± 0.12	1.61 ± 0.31
Pentadecenoic 15:1	0.47 ± 0.13	0.58 ± 0.09	0.49 ± 0.16	0.53 ± 0.03
Palmitic acid16:0	33.07 ± 0.52	33.33 ± 0.34	32.24 ± 0.08	32.3 ± 0.68
Palmitoleic acid 16:1	7.61 ± 0.73	8.28 ± 0.06	7.39 ± 0.02	6.71 ± 1.47
Stearic acid 18:0	3.29 ± 0.06	2.79 ± 0.21	2.56 ± 0.09	2.81 ± 0.36
Oleic acid 18:1	10.14 ± 0.90	7.71 ± 0.83	8.17 ± 0.27	8.83 ± 0.86
Linoleic acid 18:2	20.41 ± 0.46	20.84 ± 0.06	21.52 ± 0.28	21.31 ± 1.04
γ-Linolenic acid 18:3	14.84 ± 1.06	17.49 ± 0.12	17.05 ± 0.27	16.83 ± 0.27
α-Linolenic acid 18:3	0.43 ± 0.05	0.41 ± 0.06	0.43 ± 0.07	0.36 ± 0.20
Eicosaenoic acid 20:1	1.58 ± 0.20	1.3 ± 0.04	1.75 ± 0.06	1.69 ± 0.36
Heneicosanoic acid 21:0	4.16 ± 0.16	3.82 ± 0.24	4.81 ± 0.23	4.69 ± 0.82
Saturated fatty acids (SFAs)	**44.13 ± 1.17**	**43.01 ± 1.75**	**42.81 ± 0.68**	**43.33 ± 2.43**
Monounsaturated fatty acids (MUFAs)	**20.2 ± 1.99**	**18.25 ± 1.06**	**18.19 ± 0.53**	**18.18 ± 2.81**
Polyunsaturated fatty acids (PUFAs)	**35.68 ± 1.57**	**38.74 ± 0.24**	**39 ± 0.62**	**38.5 ± 1.51**
Total fatty acid content, mg/g of dry weight	***3.26 ± 0.22***	***3.06 ± 0.21***	***2.94 ± 0.05***	***2.95 ± 0.05***

Averaged values based on the results of 4-5 repeats are given. N/D, not detected**.** Kruskal-Wallis test results with independent samples for the experimental and control groups: SFAs: **Sig.=0.161 > 0.05**. MUFAs: **Sig.=0.099 > 0.05**. PUFAs: Sig.=0.049 < 0.05. Total fatty acid content: **Sig.=0.094 > 0.05**. The results for all the data groups except the PUFA data conform to the null hypothesis, therefore, only the differences between the experimental and control groups for PUFAs are significant (at α 0.05)**.**

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Table 35. Fatty acid content of *L. platensis* cultures grown with or without norepinephrine (NE)

Fatty acid	**Proportion in total fatty acids, %**			
	Control	**NE, 0.1 μM**	**NE, 1 μM**	**NE, 10 μM**
Myristic acid 14:0	1.93 ± 0.13	1.85 ± 0.05	1.71 ± 0.21	1.68 ± 0.06
Myristoleic acid14:1	0.42 ± 0.02	0.41 ± 0.08	0.39 ± 0.06	0.36 ± 0.03
Pentadecanoic acid15:0	1.49 ± 0.13	1.46 ± 0.06	1.37 ± 0.15	1.32 ± 0.08
Pentadecenoic 15:1	0.19 ± 0.01	0.61 ± 0.05	0.44 ± 0.04	0.64 ± 0.00
Palmitic acid16:0	32.91 ± 0.50	32.24 ± 0.38	33.5 ± 0.93	33.11 ± 0.84
Palmitoleic acid 16:1	7.06 ± 0.03	6.62 ± 0.73	7.5 ± 0.13	7.25 ± 0.42
Stearic acid 18:0	3 ± 0.42	2.73 ± 0.12	2.79 ± 0.21	2.57 ± 0.11
Oleic acid 18:1	10.48 ± 0.58	10.47 ± 0.90	8.59 ± 0.05	8.55 ± 0.82
Linoleic acid 18:2	21.84 ± 0.48	22.24 ± 0.11	21.4 ± 0.49	22.14 ± 0.48
γ-Linolenic acid 18:3	14.2 ± 0.12	14.58 ± 0.47	15.86 ± 0.66	16.11 ± 0.47
α-Linolenic acid 18:3	0.4 ± 0.02	0.44 ± 0.07	0.39 ± 0.06	0.37 ± 0.01
Eicosaenoic acid 20:1	1.73 ± 0.08	1.72 ± 0.13	1.62 ± 0.09	1.54 ± 0.16
Heneicosanoic acid 21:0	4.35 ± 0.12	4.63 ± 0.10	4.45 ± 0.25	4.35 ± 0.37
Saturated fatty acids (SFAs)	**43.68 ± 1.30**	**42.91 ± 0.71**	**43.82 ± 1.75**	**43.03 ± 1.46**
Monounsaturated fatty acids (MUFAs)	**19.88 ± 0.72**	**19.83 ± 1.89**	**18.54 ± 0.37**	**18.34 ± 1.43**
Polyunsaturated fatty acids (PUFAs)	**36.44 ± 0.62**	**37.26 ± 0.65**	**37.26 ± 1.21**	**38.62 ± 0.96**
Total fatty acid content, mg/g of dry weight	***3.35 ± 0.28***	***3.59 ± 0.18***	***2.95 ± 0.07***	***3.14 ± 0.03***

Averaged values based on the results of 4-5 repeats are given. N/D, not detected**.** Kruskal-Wallis test results with independent samples for the experimental and control groups:: SFAs: **Sig.=0.248 > 0.05**. MUFAs: Sig.=0.038 < 0.05. PUFAs: Sig.=0.025 < 0.05. Total fatty acid content: Sig.=0.023 < 0.05. The results for all the data groups except the SFA data rule the null hypothesis out, therefore, only the differences between the experimental and control groups for SFAs **are not significant** (at α 0.05)**.**

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Table 36. Fatty acid content of *L. platensis* cultures grown with or without acetylcholine (ACh)

Fatty acid	**Proportion in total fatty acids, %**			
	Control	**ACh, 0.1 μM**	**ACh, 1 μM**	**ACh, 10 μM**
Myristic acid 14:0	1.04±0.45	0.68±0.12	0.57±0.21	0.65±0.1
Myristoleic acid14:1	0.2±0.02	0.21±0.01	0.19±0.01	0.2±0.03
Pentadecanoic acid15:0	0.39±0.13	0.26±0.03	0.26±0.05	0.24±0.02
Pentadecenoic 15:1	0.99±0.7	0.33±0.26	0.67±0.05	0.74±0.01
Palmitic acid16:0	32.43±0.64	33.65±0.74	30.14±0.37	29.6±0.81
Palmitoleic acid 16:1	7.3±0.14	7.52±0.01	6.16±0.3	6.16±0.47
Stearic acid 18:0	2.93±0.42	2.35±0.13	2.38±0.11	2.4±0.01
Oleic acid 18:1	8.88±0.75	7.56±0.1	10.29±0.4	10.5±0.63
Linoleic acid 18:2	22.75±0.76	23.63±0.47	23.99±0.53	24.18±0.4
γ-Linolenic acid 18:3	17.16±0.12	18.05±0.72	17.22±0.17	16.67±0.01
α-Linolenic acid 18:3	0.34±0.23	0.12±0.02	0.1±0.01	0.1±0.01
Eicosaenoic acid 20:1	1.44±0.07	1.41±0.03	2.2±0.02	2.34±0.14
Heneicosanoic acid 21:0	4.16±0.02	4.22±0.17	5.83±0.07	6.21±0.24
Saturated fatty acids (SFAs)	**40.95±1.66**	**41.16±1.19**	**39.18±0.81**	**39.1±1.17**
Monounsaturated fatty acids (MUFAs)	**18.81±1.68**	**17.03±0.4**	**19.51±0.77**	**19.94±1.27**
Polyunsaturated fatty acids (PUFAs)	**40.25±1.11**	**41.8±1.21**	**41.31±0.7**	**40.95±0.41**
Total fatty acid content, mg/g of dry weight	***7.10±0.69***	***8.94±0.14***	***8.76±0.11***	***7.72±0.81***

Averaged values based on the results of 4-5 repeats are given. N/D, not detected**.** Kruskal-Wallis test results with independent samples for the experimental and control groups: SFAs: Sig.=0.038 < 0.05. MUFAs: Sig.=0.016 < 0.05. PUFAs: **Sig.=0.064 > 0.05.** Total fatty acid content:: Sig.=0.029 < 0.05. The results for all the data groups except the PUFA data rule the null hypothesis out, therefore, only the differences between the experimental and control groups for PUFAs **are not significant** (at α 0.05)

Therefore, the tested neurotransmitters include two subgroups: (1) ACh and 5-HT that significantly increased the total fatty acid content in *L. platensis* lipids and (2) histamine and DA that produced no verifiable effect. NE was unique in that it decreased the total fatty acid content on day 14 of cultivation. Two neurotransmitters, 5-HT and NE, considerably increased the contribution of the biotechnologically important PUFAs.

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4.3. CONCLUSIONS

4.3.1. Summarized fatty-acid content data. To sum up, the present chapter is concerned with the effects of BAs on the fatty acid composition of the lipids of *C. vulgaris*, *S. quadricauda, H. lacustris* (two strains), and *L. platensis.* The following is to sum up the data of gas chromatography with mass spectrometry that were presented in the tables of the Results section.

It was revealed that ACh (Table 16), DA (Table 14), and NE (at a concentration of 1 μM, Table 15) increased the total fatty acid content of *C. vulgaris* cells whereas 5-HT (Table 12) and histamine (Table 13) decreased it. As for *S. quadricauda.* ACh (Table 21) and, to a lesser extent, histamine (Table 18) elevated the total fatty acid content while DA and. to a lesser extent. 5-HT lowered it.

In similar fashion (taking the phylogenetic relatedness of the genera *Scenedesmus* and *Haematococcus* into account), the tested strains IPPAS H-239 and BM-1 of *H. lacustris* were characterized by an increase in total fatty acid lipid content in the presence of ACh (Table 30 and 31) and histamine (Table 24 and 25), whereas DA (Table 26 and 27) decreased the total fatty acid content. Despite its similarity to DA, NE, nevertheless, incressed the fatty acid content in both strains.

The prokaryote *L. platensis* had its peculiarities. ACh also significantly raised its fatty acid content. However, in contrast to the eukaryotic microalgae, the second fatty acid synthesis stimulant was serotonin, whereas histamine and dopamine failed to exert a verifiable influence and norepinephrine decreased the total fatty acid amount (Table 32-36).

4.3.2. General trends. The data obtained on the percentages of various fatty acid species in *C. vulgaris* (Tables 12-16), *S. quadricauda* (Table 17-21), *H. lacustris* (Table 22-31), and *L. platensis* (Table 32-36) reveal a sophisticated pattern that reflects the specific features of each neurotransmitter and each of the microalgae.

The general trends revealed in the work on *C. vulgaris* and *S. qudricauda*

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(Table 37) are as follows:

1. ACh and histamine increased the percentage of unsaturated and decreased that of saturated fatty acids in the lipids of the microalgae under study.

2. DA and 5-HT decreased the percentage of unsaturated fatty acids (MUFAs and PUFAs) in *S. quadricauda*. In *C. vulgaris,* the effects of DA and 5-HT on the PUFA content failed to pass the Kruskal-Wallis H test.

3. NE exerted no significant effect on the two tested microalgal species.

Table 37. Summarized data on the impact of BAs on the fatty acid composition of the lipids of the tested microalgae (based on the data of Tables 17-36)

Characteristics	Species/strain of microalgae	Biogenic amines				
		5-HT	His	DA	NE	ACh
Total fatty acid content, mg/g of dry biomass	*C. vulgaris*	↘	↘	↗	↗	↗
	S.quadricauda	↘	↗	↘	—	↗
	IPPAS H-239	—	↗	↘	↗	↗
	BM-1	↘	↗	↘	↗	↗
	L. platensis	↗*	—	—	↘	↗
Percentage of unsaturated fatty acids	*C. vulgaris*	—	↗	—	—	↗
	S.quadricauda	↘	↗	↘	—	↗
	IPPAS H-239	—	—	—	↗	—
	BM-1	—	—	↗	↗	—
	L. platensis	↗**	↘	—	↗**	—
Percentage of saturated fatty acids	*C. vulgaris*	↘	↘	—	—	↘
	S.quadricauda	↗	↘	↗	—	↘
	IPPAS H-239	—	—	—	↘	—
	BM-1	—	—	↘	↘	—
	L. platensis	—	—	—	—	—

Designations: ↗. upward trends; ↘. downward trends; —. insignificant or ambiguous effects. IPPAS H-239 and BM-1, *H. lacustris* strains; * at a concentration of 0.1 μM only; ** only the PUFA fraction with a decrease in MUFA fraction

In the two *H. lacustris* strains under study, NE shifted the balance between saturated and unsaturated fatty acids towards the dominance of PUFAs, i.e. it behaved as a subgroup 1 substance in this system. The chemically related DA increased the PUFA share only in strain BM-1. 5-HT, ACh, and histamine failed to exert a statistically verifiable effect on the ratio between saturated and unsaturated fatty acid in the cell lipids of the tested *H. lacustris* strains.

As for the cyanobacterium *L. platensis,* two of the tested BAs, 5-HT and NE, significantly increased the PUFA share, whereas the other neurotransmitters produced no statistically valid effects.

4.3.3. Potential biotechnological use. From the biotechnological viewpoint, subgroup 1 (ACh, histamine, 5-HT in *C. vulgaris,* and NE in *H.* lacustris) holds some promise with regard to the production of PUFA-rich preparations for therapeutic or cosmetic purposes.

The potential competitiveness of the strategy of increasing the efficiency of microalgae as producers of valuable substances is due to the fact that neurotransmitters are relatively inexpensive if applied at sufficiently low concentrations. To cite an example: in order to supplement the algal culture in a 1 m^3 pilot photobioreactor with 10 μM acetylcholine, only 1.82 g of acetylcholine chloride are required. If this reagent is purchased from Sigma with Acros Organic as the intermediary (catalogue number A6625, CAS 60-31-1), the price of 25 g acetylcholine chloride is 5777 roubles as of May 18, 2026, and the extra expenses amount to 420 roubles, i.e., ~ 5.4 US $ (http://chimmed.ru), based on the official exchange rate. In these circumstances, the statistically verifiable increase in biomass yield with *C.vulgaris* (Table 6) and *S. quadricauda* (Table 11) accompanied by a significant rise in PUFA percentage in their lipids (Table 21 and 26) translates into a high extra profit exceeding the additional expenses by several orders of magnitude (for techno-economic analysis. see. e.g., Tredici et al., 2016; Fabris et al., 2020).

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As for the cyanobacterium *L. platensis,* creating an acetylcholine concentration of 0.1 μM that is sufficient for an approximately 26% increase in fatty acid content in its cells (see Table 36) in a 1 m^3 pilot bioreactor requires only 18.2 mg of acetylcholine chloride, the expenses do not exceed 0.4 roubles.

It is conceivable to explore new strategies of boosting the PUFA yield by combining neurotransmitters and stress factors, in an analogy to the aforementioned work on promoting the production of astaxanthin by *H. lacustris* by utilizing dopamine and high salinity stress (Zhao et al., 2025a).

Subgroup 2 neurotransmitters (DA and 5-HT in *S. quadricauda* and *C. vulgaris*) increase the total fatty acid content without verifiably changing the PUFA percentage. They are potentially applicable for practical developments aimed at producing biofuel. To re-emphasize, saturated and monounsaturated fatty acid-containing triacylglycerides hold special promise in terms of producing biodiesel (Феофилова, Мысякина, 2016).

In the work by Solovchenko et al. (2015), it was demonstrated that the total fatty acid content in the cells of the green microalga *Desmodesmus* sp. tends to decrease during cultivation. However. this decrease is staved off by several days if the CO_2 concentration in the air is increased to 20%; on day 6 of cultivation. the "high-CO_2" culture has an approximately 30% higher total fatty acid content than the control ("low-CO_2") microalgal culture. according to Fig. 1, b. of the work cited. Presumably. the effects of neurotransmitters of the first subgroup (acetylcholine, histamine, and, to a lesser extent, norepinephrine) are phenomenologically similar to those of high CO_2 concentrations. This suggestion is in line with our data that acetylcholine, histamine, and norepinephrine increase the percentage of PUFAs (that form a part of membrane lipids) at the expense of saturated and monounsaturated fatty acids characteristic of reserve lipids. The impact of these neurotransmitters on fatty acid composition apparently is analogous to that of high CO_2 reported in the work referred to (Solovchenko et al., 2015).

Batch cultures such as used in the present work are known to undergo several

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development stages. including the lag phase and the exponential. growth deceleration. and stationary stage (Love and Travisano. 2013). It seems likely that ACh, histamine, and, to a limited extent, NE are similar to CO_2 at high concentrations in that they prolong the earlier cultivation stages characterized by rapid cell division and expansion of chloroplast membranes harboring lipids rich in valuable PUFA content.

4.3.4. Hypothetic receptor-based mechanisms. Presumably, the unicellular photosynthetic pro- and eukaryotes investigated in the present work expresses receptors for neurotransmitters. For comparison. mammalian H_1 receptors to histamine couple to G_q which regulates Ca^{2+} mobilization. H_2 receptors couple to G_s to stimulate cyclic AMP, and H_3 and H_4 receptors couple to $G_{i/o}$ to inhibit cyclic AMP accumulation (Powell, 2007. p. 1). The cAMP messenger system may modulate the activity of desaturases involved in producing PUFAs, so as to maximize their yield.

The fact that chemically similar dopamine and norepinephrine have quite different effects with respect to fatty acid contents and pigment concentrations (see next subsection) is apparently consistent with our suggestion concerning a receptor-dependent mechanism of neurotransmitter action on microalgae. In mammalian cells. dopamine and norepinephrine exert dissimilar effects and bind to different receptor types (D_{1-5} receptors vs. α- and β-adrenoreceptors, respectively).

The hypothetical receptor-based mechanism of action of these neurotransmitters does not rule out their antioxidant system-stimulating effects. Such effects were established for serotonin and its derivative melatonin. e.g., in the diatomic alga *Ulnaria ulna* (Roshchina et al., 2022); they were suggested for chemically related auxins in the literature (Piotrowska-Niczyporuk and Bajguz, 2014). Moreover, it is via binding to receptors that antioxidant enzymatic (peroxidase-. catalase-. and superoxide dismutase-based) and non-enzymatic systems may become activated.

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A different mode of action seems to be characteristic of the second neurotransmitter subgroup including 5-HT and DA that tend to decrease the PUFA percentage in favor of saturated or monounsaturated fatty acids. If microalgal cells contain mammal-like receptors (or their analogs), they might downregulate fatty acid desaturases and/or inhibit their activity, resulting in accelerated algal culture development and an increased share of saturated reserve triacylglycerides.

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5. EFFECTS OF BIOGENIC AMINES ON THE PHOTOSYNTHETIC PIGMENT CONTENT OF MICROALGAL CELLS[9]

"Microbial oxygenic phototrophs (microalgae) are believed to be very efficient solar energy converters" (Masojídek et al., 2013, p.32). This is one of the reasons why investigating parameters related to photosynthetic efficiency is ecologically, environmentally, and also biotechnologically relevant. The following is the data of this work on photosynthetic pigments in the tested microalgae with and without neurotransmitters.

5.1. GREEN MICROALGAE

5.1.1 Effects of BAs on the photosynthetic pigment content of *C. vulgaris.*

5.1.1.1. Serotonin (5-HT) increased the chlorophyll *a+b* content and, to a lesser extent, the carotenoid content (at concentrations of 10 and 100 μM) (Fig. 28).

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[9] The results presented in this section have been published in the work: Cao B., Oleskin A.V. Neurochemical pollutants in the aquatic medium: the results of studies with model organisms (microalgae) // Жизнь Земли. — 2025. — Т. 47. № 4. С.550-562. DOI: 10.29003/m4988.0514-7468.2025_47_4/550-562. ИФ РИНЦ: 0.386 (1.0 printed sheets/0.6 printed sheets). EDN: PMKRCV and in the supplementary works: **Цао Б.**, Федоренко Т.А., Чивкунова О.Б., Соловченко А.Е., Лобакова Е.С., Олескин А.В. Влияние биогенных аминов на накопление биомассы, содержание фотосинтетических пигментов и жирных кислот у цианобактерии *Limnospira platensis* IPPAS B-256 // Микробиология. — 2026. — Т. 95, № 1. — С. 68–84. DOI: 10.1134/S0026261725602921 ИФ РИНЦ: 1.034 (1.5 p.s./0.8 p.s.) [**Cao B.,** Fedorenko T.A., Chivkunova O.B., Solovchenko A.E., Lobakova E.S., Oleskin A.V. Impact of neurotransmitters on biomass accumulation, photosynthetic pigment content, and fatty acid composition of the cyanobacterium *Limnospira platensis* IPPAS B-256// Microbiology. — 2026. — Vol. 95, no. 1. — P. 82–95. DOI: 10.1134/S0026261725602921 SJR: 0.347 (1.5 p.s./0.8 p.s.); **Cao B.**, Chivkunova O.B., Solovchenko A.E., Lobakova E.S., Oleskin A.V. Neurotransmitters as ecopollutants: impact on the fatty-acid composition and photosynthetic pigment content of *Chlorella vulgaris* // Russian J. Ecosystem Ecol. — 2025. — V. 10(4). P. 10-21. DOI: 10.21685/2500-0578-2025-4-4. РИНЦ: 0.850 (1.2 p.s./0.6 p.s.); **Цао Б.,** Федоренко Т.А., Чивкунова О.Б., Соловченко А.Е., Лобакова Е.С., Олескин А.В. Влияние нейротрансмиттеров на содержание фотосинтетических пигментов у *Haematococcus lacustris* штаммы IPPAS H-239 и BM-1 // Прикл. биохим. микробиол. – 2025. – Т. 61(5). С.865-871. DOI: 10.31857/S0555109925050046 ИФ РИНЦ: 0.992 (1.4 p.s./0.6 p.s.). [**Cao B.,** Fedorenko T.A., Chivkunova O.B., Solovchenko A.E., Lobakova E.S., Oleskin A.V. Impact of neurotransmitters on the photosynthetic pigment content of the green microalga *Haematococcus lacustris* (strains IPPAS H-239 and BM-1) // Appl. Biochem. Microbiol. – 2025. – Vol. 61(5). – P.865-871. DOI: 10.1134/S0003683825601155. SJR: 0.294 (1.4 p.s./0.6 p.s.)]; **Цао Б.**, Федоренко Т.А., Чивкунова О.Б., Соловченко А.Е., Лобакова Е.С., Олескин А.В. Влияние нейротрансмиттеров на состав жирных кислот и фотосинтетических пигментов зеленой микроводоросли *Scenedesmus quadricauda* // Прикл. биохим. микробиол. – 2024. – Т. 60, № 5. С.378-389. DOI: 10.31857/S0555109924050064. ИФ РИНЦ: 0.992. (1.4 p.s./0.8 p.s.) [**Cao B.**, Chivkunova O.B., Solovchenko A.E., Lobakova E.S., Oleskin A.V. Impact of neurotransmitters on the fatty acid composition and pigments of the green microalga *Scenedesmus quadricauda* // Appl. Biochem. Microbiol. – 2024. – Vol. 60(5). – P.833–843. DOI: 10.1134/S0003683824604554 SJR: 0.294 (1.4 p.s./0.8 p.s.)].

5.1.1.2. Histamine insignificantly increased the total chlorophyll content in *C. vulgaris* cells without considerably influencing their carotenoid content (Fig. 29).

5.1.1.3. Dopamine (DA) produced a minor but statistically verifiable effect on the chlorophyll content without affecting the carotenoid content (Fig. 30).

5.1.1.4. Norepinephrine (NE) increased the chlorophyll *a+b* content and to a lesser extent. the carotenoid content (at concentrations of 10 and 100 μM) (Fig. 31).

5.1.1.5. Acetylcholine (ACh) at the lowest concentration (1 μM) decreased the chlorophyll *a* content. thereby also reducing the total chlorophyll *a+b* amount; it also increased the carotenoid content. At a concentration of 10 μM. ACh markedly increased both the chlorophyll *a* and chlorophyll *b* content. whereas the carotenoid content was virtually the same as in the control samples (without acetylcholine). Finally, at the highest tested concentration (100 μM), the total chlorophyll content returned to its control level. and the carotenoid content was somewhat decreased (Fig. 32).

In summary, all tested substances increased, to some extent, the photopigment content and, therefore, probably the photosynthetic activity of *C. vulgaris* cells, which is consistent with the earlier work revealing the stimulatory effects of the neurotransmitters on the growth of this alga (see above). The data obtained are also in line with the literature data on the ACh effect on microalgae (Czehpak et al., 2003).

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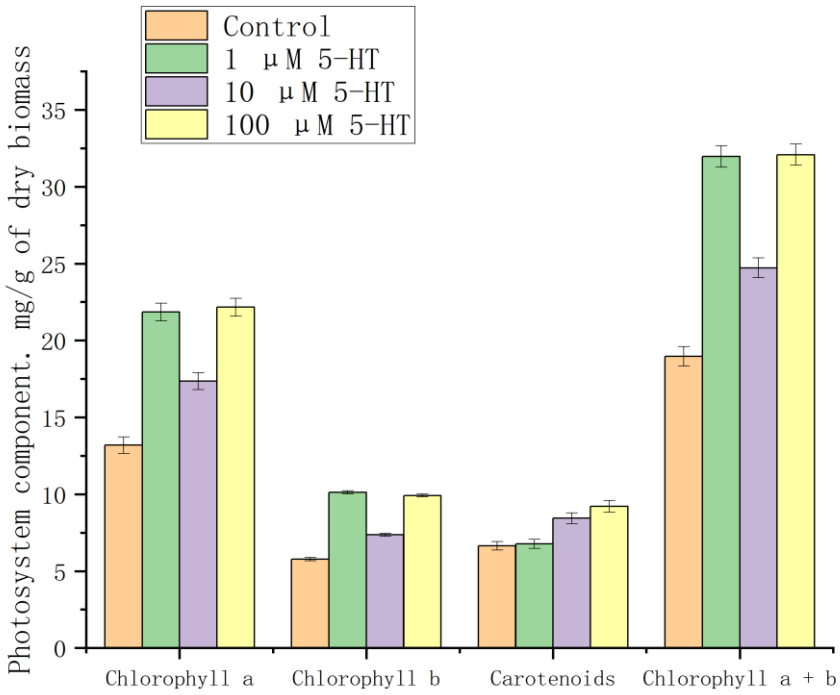

Fig. 28. Influence of serotonin (5-HT) on the pigment contents of *C. vulgaris* cells. The figure contains the averaged results of 4-5 independent replicates. The Kruskal-Wallis test results with independent samples for the experimental and control groups. Chl *a*: Sig.=0.024 < 0.05. Chl *b*: Sig.=0.019 < 0.05. Carotenoids: Sig.=0.024 < 0.05. Chl *a* + *b*: All data groups rule the null hypothesis out; therefore, the differences between all experimental and control data groups are significant.

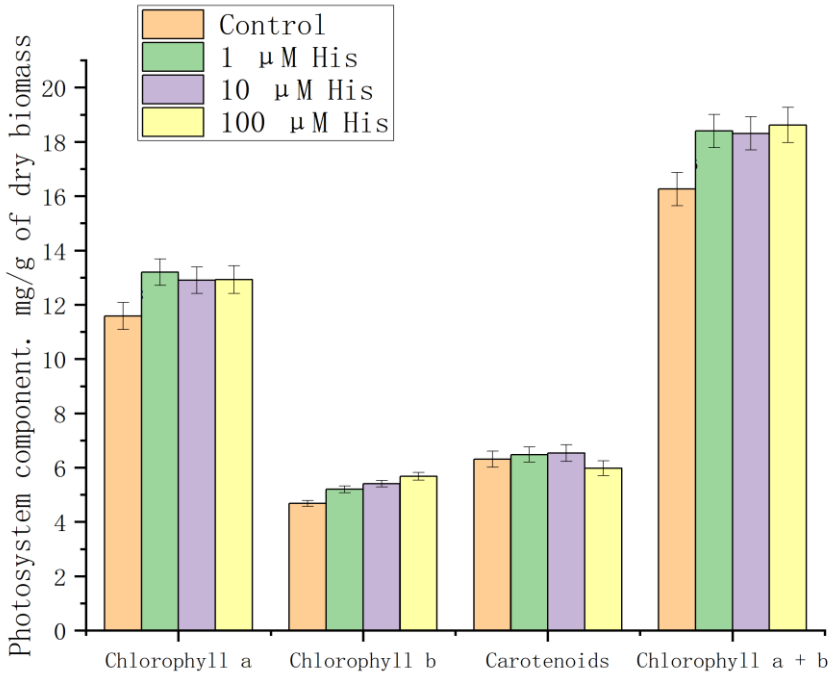

Fig. 29. Influence of histamine (His) on the pigment contents of *C. vulgaris* cells. The figure contains the averaged results of 4-5 independent replicates. The Kruskal-Wallis test results with independent samples for the experimental and control groups. Chl *a*: **Sig.=0.082 > 0.05, conforming with the null hypothesis.** Chl *b:* Sig.=0.019 < 0.05, ruling the null hypothesis out. Carotenoids: Sig.=0.016 < 0.05, ruling the null hypothesis out. Chl *a* + *b*: **Sig.=0.082 > 0.05, conforming with the null hypothesis.** Therefore, only the data for Chl *b* and carotenoids are statistically valid.

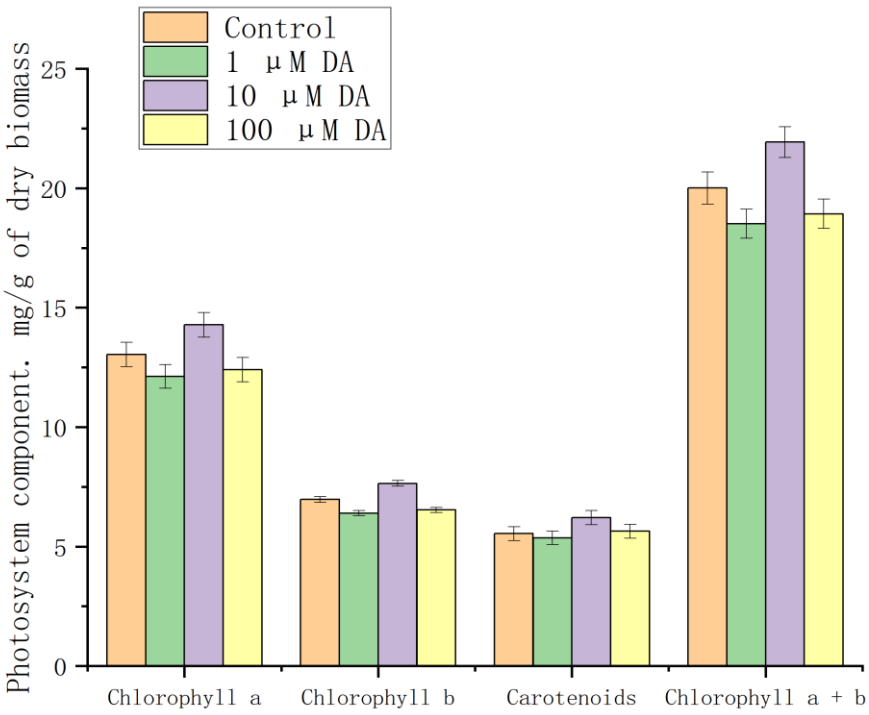

Fig. 30. Influence of dopamine (DA) on the pigment contents of *C. vulgaris* cells. The figure contains the averaged results of 4-5 independent replicates. The Kruskal-Wallis test results with independent samples for the experimental and control groups. Chl *a*: Sig.=0.041 < 0.05, ruling the null hypothesis out**.** Chl *b:* Sig.=0.019 < 0.05, ruling the null hypothesis out. Carotenoids: **Sig.=0.0103 > 0.05, conforming with the null hypothesis**. Chl *a* + *b*: Sig.=0.031 < 0.05, ruling the null hypothesis out. Only the carotenoid data are **not valid statistically.**

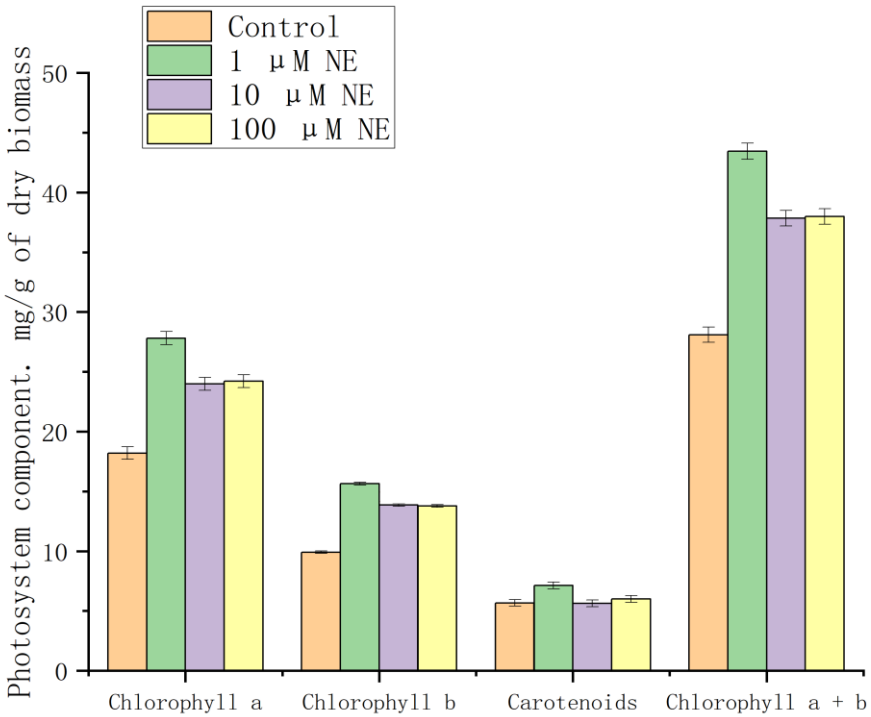

Fig. 31. Influence of norepinephrine (NE) on the pigment contents of *C. vulgaris* cells. The figure contains the averaged results of 4-5 independent replicates. The Kruskal-Wallis test results with independent samples for the experimental and control groups. Chl *a*: Sig.=0.024 < 0.05. Chl *b:* Sig.=0.024 < 0.05. Carotenoids: Sig.=0.041 < 0.05. Chl *a* + *b*: Sig.=0.024 < 0.05. The results for all the data groups rule the null hypothesis out and, therefore, are statistically verifiable.

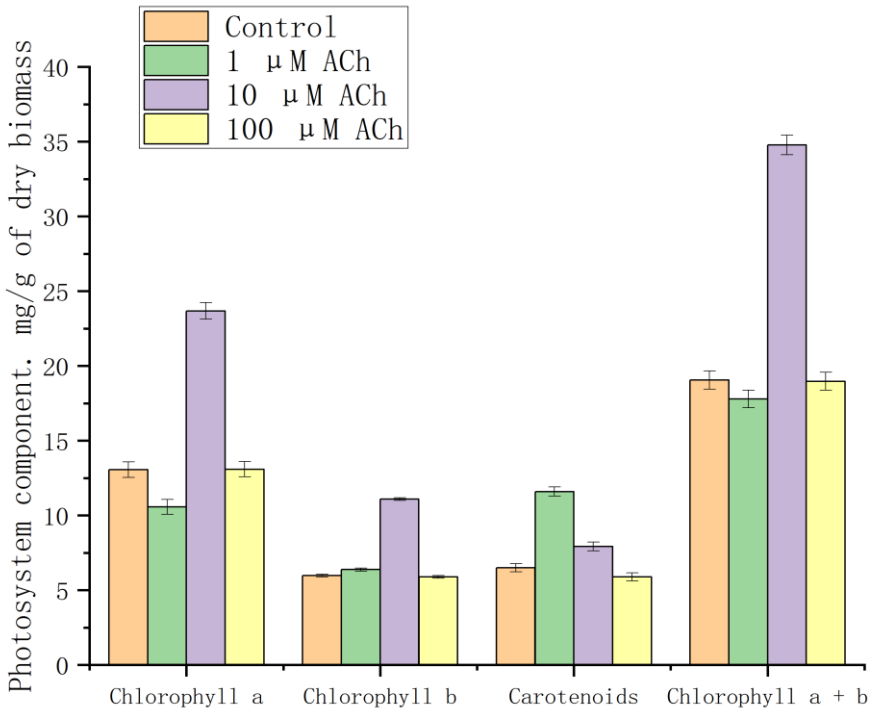

Fig. 32. Influence of acetylcholine (ACh) on the pigment contents of *C. vulgaris* cells. The figure contains the averaged results of 4-5 independent replicates. The Kruskal-Wallis test results with independent samples for the experimental and control groups. Chl *a*: Sig.=0.024 < 0.05. Chl *b:* Sig.=0.021 < 0.05. Carotenoids: Sig.=0.016 < 0.05. Chl *a* + *b*: Sig.=0.031 < 0.05. The results for all data groups rule the null hypothesis out and, therefore, are statistically valid.

5.1.2. Effects of BAs on the photosynthetic pigment content of *S. quadricauda.*

5.1.2.1. Serotonin (5-HT). As shown in Fig. 33, 5-HT failed to produce any significant effect on the tested photosynthetic variables. The maximum 5-HT concentration brought about an increase in the chlorophyll:carotenoid ratio. which seems to be attributable to a stress-related decrease in the number of light-trapping pigments per photosystem.

5.1.2.2. Histamine had almost no effect on total chlorophyll content. whereas the carotenoid content was marginally increased. The chlorophyll *a/b* ratio was somewhat skewed in favor of chlorophyll *a*, and the chlorophyll:carotenoid ratio in favor of carotenoids (Fig. 34). These changes might indicate an altered mode of

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operation of the microalgal photosystems and reflect a stress response caused by histamine, which plausibly also manifests itself in an increased PUFA percentage in the lipid fraction (see above).

5.1.2.3. Dopamine (DA) caused a proportionate decrease in the concentrations of chlorophylls *a* and *b* as well as of carotenoids (Fig. 35), indicative of a net decrease in photosystem number per cell. This is likely to diminish photosynthetic activity, and the results obtained are in line with the above data on a decrease in fatty acid content.

5.1.2.4. Norepinephrine (NE). In contrast to the chemically similar dopamine. norepinephrine at a concentration of 1 µM (but not at the higher tested concentrations) brought about a slight increase in the concentrations of chlorophylls *a* and *b* as well as of carotenoids (Fig. 36), suggestive of an overall increase in photosystem number per cell and. therefore. in photosynthetic activity.

5.1.2.5. Acetylcholine (ACh) increased the total chlorophyll *a* + chlorophyll *b* content by maximally 65% (with 10 µM ACh) without changing their ratio or that between the chlorophyll and carotenoid content (Fig. 37). Therefore, ACh was likely to increase algal photosynthesis efficiency, which seems to account for the above data on the stimulatory effect of ACh on biomass accumulation and especially fatty acid production in *S. quadricauda.*

In summary. ACh and, to a lesser extent, NE increased the total chlorophyll and carotenoid contents which were decreased by DA. These BAs produced similar effects on *C. vulgaris* (see above). However, in contrast to *C. vulgaris,* 5-HT and histamine in *S. quadricauda* were without effect on the total photosystem component content but histamine caused changes in photopigment ratios. suggestive of a stressor-type influence of this neurotransmitter.

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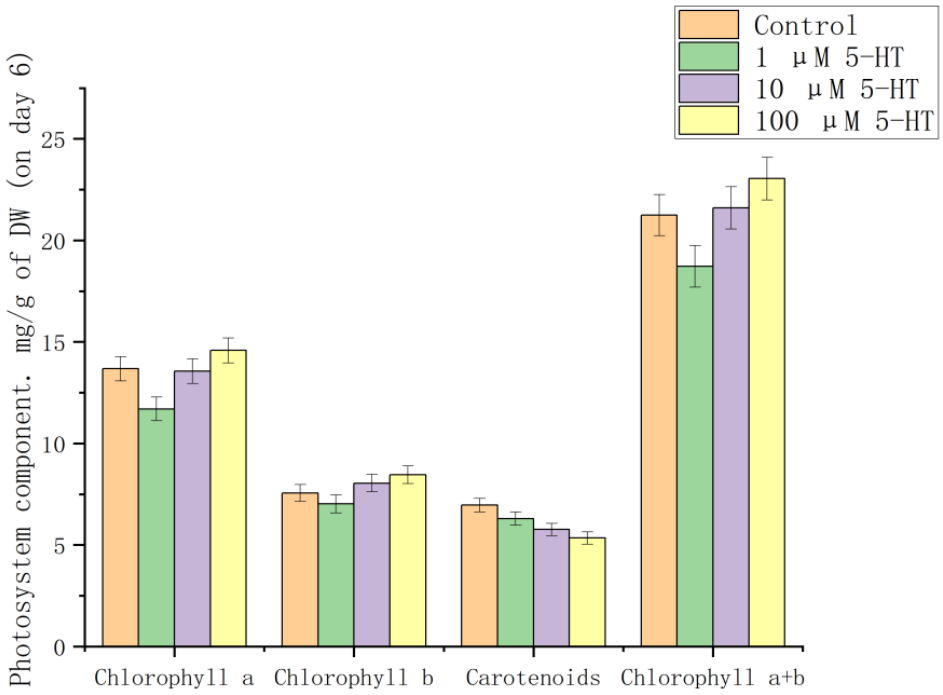

Fig. 33. Influence of serotonin (5-HT) on the pigment contents of *S. quadricauda* cells. The Figure contains the averaged results of 4-5 independent replicates. The Kruskal-Wallis test results with independent samples for the experimental and control groups. Chl *a*: Sig.=0.046 < 0.05. Chl *b:* **Sig.=0.764 > 0.05.** Carotenoids: Sig.=0.016 < 0.05. Chl *a* + *b*: Sig.=0.016 < 0.05. The results for all the data groups rule the null hypothesis out **except the Chl *b* content**, therefore, only the Chl *b* data are **not valid** statistically.

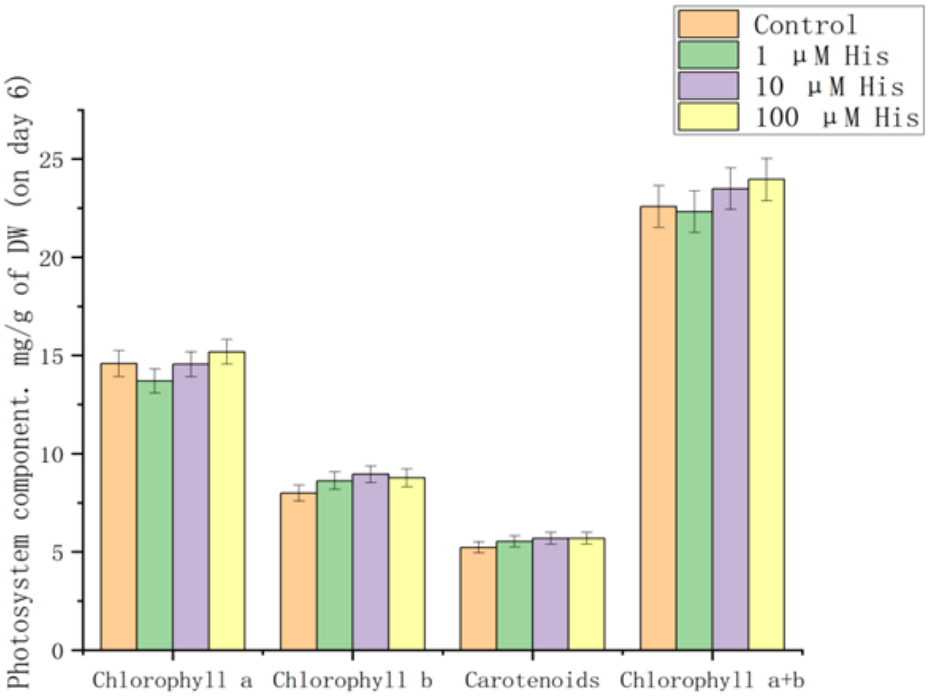

Fig. 34. Influence of histamine (His) on the pigment contents of *S. quadricauda* cells. The Figure contains the averaged results of 4-5 independent replicates. The Kruskal-Wallis test results with independent samples for the experimental and control groups. Chl *a*: Sig.=0.047 < 0.05. Chl *b:* **Sig.=0.161> 0.05**. Carotenoids: Sig.=0.048 < 0.05. Chl *a* + *b*: Sig.=0.028 < 0.05. The results for all the data groups rule the null hypothesis out **except the Chl *b* content**, therefore, only the Chl *b* data are **not valid** statistically.

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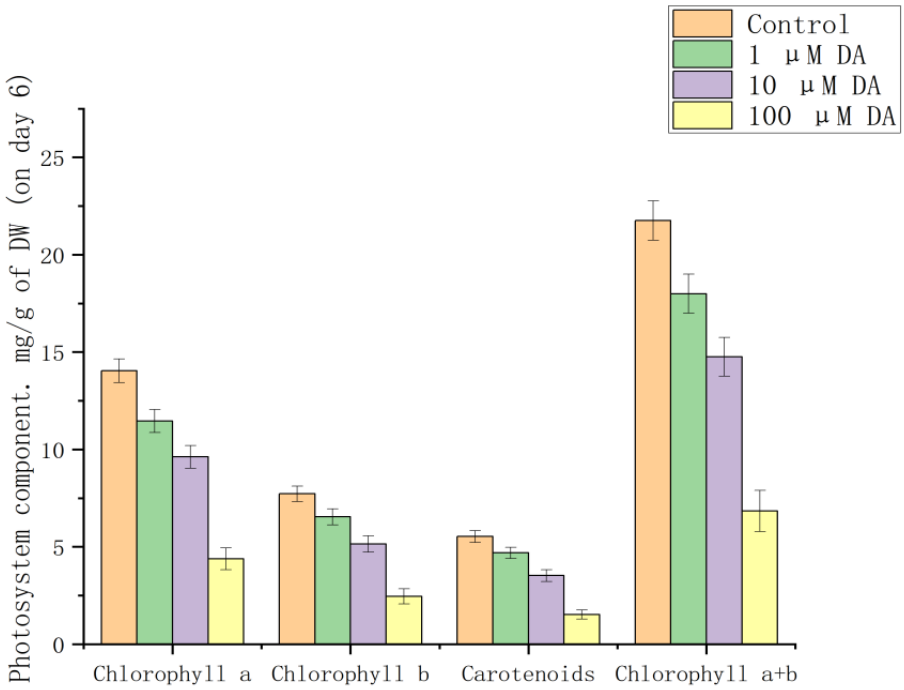

Fig. 35. Influence of dopamine (DA) on the pigment contents of *S. quadricauda* cells. The Figure contains the averaged results of 4-5 independent repeats. Kruskal-Wallis test results with independent samples for the experimental and control groups. Chl *a*: Sig.=0.016 < 0.05. Chl *b:* Sig.=0.016 < 0.05. Carotenoids: Sig.=0.016 < 0.05. Chl *a* + *b*: Sig.=0.016 < 0.05. The results for all the data groups rule the null hypothesis out and are statistically verifiable.

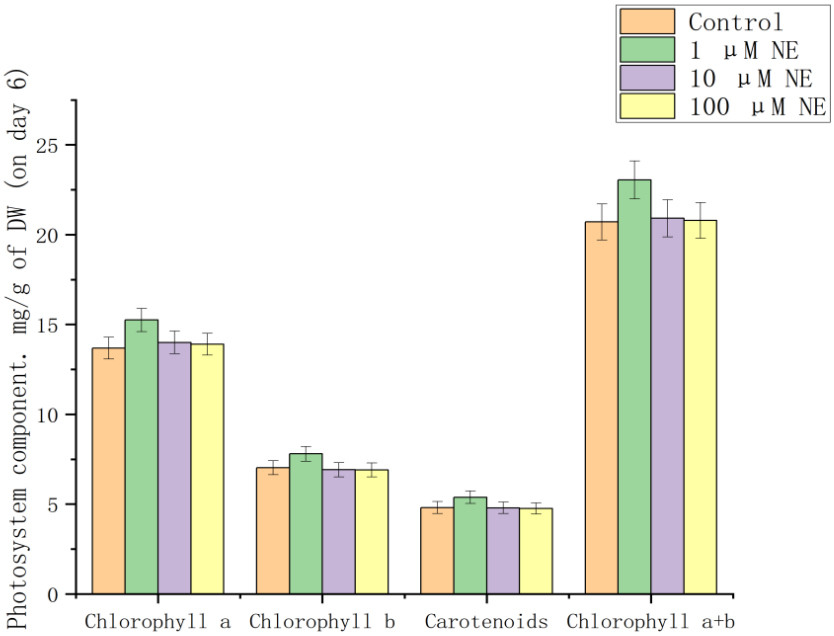

Fig. 36. Influence of norepinephrine (NE) on the pigment contents of *S. quadricauda* cells. The Figure contains the averaged results of 4-5 independent repeats. Kruskal-Wallis test results with independent samples for the experimental and control groups. Chl *a*: **Sig.=0.113 > 0.05.** Chl *b:* **Sig.=0.115> 0.05.** Carotenoids: Sig.=0.043 < 0.05. Chl *a* + *b*: Sig.=0.042 < 0.05. The results for Carotenoids and Chl *a* + *b* groups rule the null hypothesis out. The differences between the experimental and control data for the the Chl *a* and Chl *b* content are **not significant.**

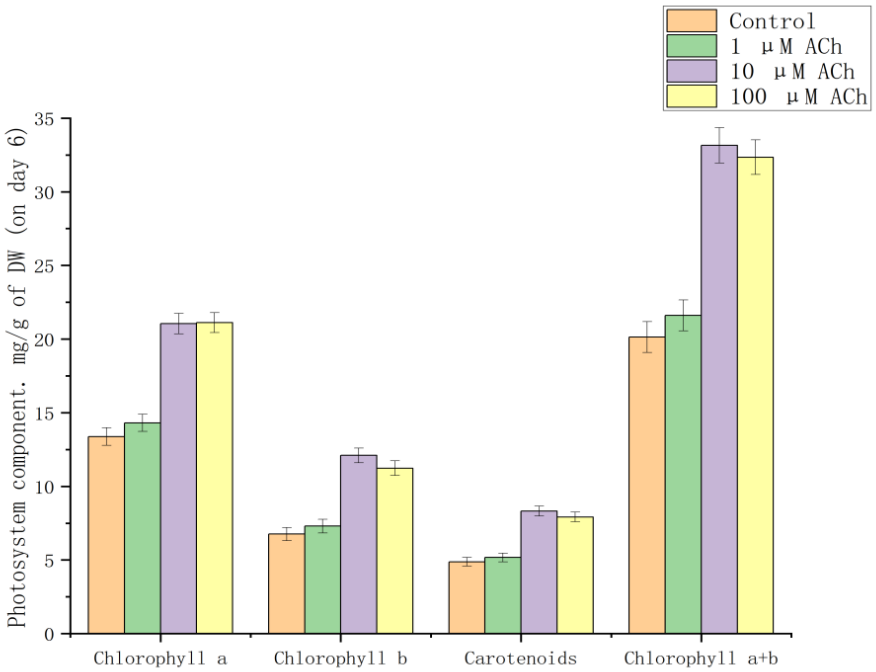

Fig. 37. Influence of acetylcholine (ACh) on the pigment contents of *S. quadricauda* cells. The Table contains the averaged results of 4-5 independent repeats. Kruskal-Wallis test results with independent samples for the experimental and control groups. Chl *a*: Sig.=0.029 < 0.05. Chl *b:* Sig.=0.023 < 0.05. Carotenoids: Sig.=0.027 < 0.05. Chl *a* + *b*: Sig.=0.029 < 0.05. The results for all the data groups rule the null hypothesis out and are valid statistically.

5.1.3. Effects of BA on the photosynthetic pigments of *Haematococcus lacustris.*

As mentioned above, the green microalga *H. lacustris* transitions from the vegetative stage with green chlorophyll-containing cells to the resting carotenoid-accumulating reddish stage via the intermediate palmelloid stage with flagella-lacking rounded brown cells (Solovchenko, 2015). All tables of this subsection (Tables 38-47) include data on the dynamics of chlorophyll and carotenoid content change during the cultivation period of strains IPPAS B-239 and BM-1 without any additions (these are the table columns referring to the control systems). Comparison of the data corresponding to days 0 (immediately after the inoculation). 7. 14 of cultivation reveals a gradual decrease in chlorophyll *a* and *b* content with a concomitant increase in carotenoid concentration. What is the impact of

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neurotransmitters on these dynamics?

5.1.3.1. Serotonin. This biogenic amine inhibits the synthesis of carotenoids and chlorophylls on day 7 and 14 of cultivation of strain *H. lacustris* IPPAS B-239. compared to the control (Table 38). In contrast, 5-HT is virtually without effect on the carotenoid content and slightly increases the chlorophyll content in strain BM-1 (Table 39).

5.1.3.2. Histamine was also revealed to inhibit carotenoid and chlorophyll synthesis in strain HP-1 (Table 40). However, histamine brought about a significant stimulation of carotenoid and chlorophyll formation in strain BM-1 (Table 41), i.e., the effects of histamine proved to be strain-specific.

5.1.3.3. Dopamine inhibited carotenoid synthesis in HP-1 (Table 42) but, in contrast to 5-HT and histamine, DA somewhat increased the chlorophyll content in IPPAS B-239 cells on day 7 and 14 of cultivation. As for BM-1 (Table 43), DA stimulated chlorophyll and carotenoid synthesis to some extent (by approximately 15%).

5.1.3.4. Norepinephrine is only distinguished from DA by the presence of an extra hydroxy group in the side chain but its effect on *H. lacustris* significantly differed from that of DA. NE stimulated chlorophyll and carotenoid synthesis in both tested strains (Tables 44 and 45).

5.1.3.5. Acetylcholine (Tables 46 and 47) considerably stimulated chlorophyll and carotenoid synthesis in strains IPPAS B-239 and BM-1 of *H. lacustris.*

The difference between the neurotransmitter effects on the two tested strains of *H. lacustris* is attributable to the different conditions of their habitats: strain BM-1 was isolated from the brackish coastal water of the White Sea (Chekanov et al., 2015) and strain IPPAS H-239 was obtained from the collection of the Institute of Plant Physiology of the Russian Academy of Sciences. Despite the interstrain differences. some common patterns were revealed. For instance. acetylcholine and norepinephrine increase the chlorophyll and carotenoid content of both strains. These patterns will be addressed in the Conclusions section in connection with the effects of neurotransmitters on other microalgal species.

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Table 38. Effect of serotonin (5-HT) on carotenoid and chlorophyll content in *H. lacustris* IPPAS B-239 cells

	Content, mg/g of dry weight								
	Day 0	**Control**		**5-HT, 0.1 μM**		**5-HT, 1 μM**		**5-HT, 10 μM**	
		Day 7	Day 14	Day 7	Day 14	Day 7	Day14	Day 7	Day14
Chlorophyll *a*	1.9± 0.15	1.29± 0.15	1.12± 0.10	1.30± 0.10	1.13± 0.10	1.29± 0.10	1.05± 0.10	1.35± 0.10	1.29± 0.15
Chlorophyll *b*	0.97± 0.10	0.66± 0.10	0.59± 0.10	0.66± 0.10	0.61± 0.10	0.65± 0.10	0.55± 0.10	0.67± 0.10	0.64± 0.10
Chlorophyll *a* + *b*	2.87± 0.20	1.95± 0.15	1.71± 0.15	1.97± 0.15	1.74± 0.20	1.95± 0.20	1.60± 0.20	1.40± 0.15	1.05± 0.15
Carotenoids	2.86± 0.20	2.71± 0.20	5.18± 0.25	2.65± 0.20	5.91± 0.25	2.59± 0.20	5.26± 0.25	2.65± 0.20	3.39± 0.20

The averaged results of 3 repeats are presented. The Kruskal-Wallis test results with independent samples for the experimental and control groups: Chl *a*-day 7: Sig.=0.035 < 0.05, Chl *a*-day 14: Sig.=0.038 < 0.05. Chl *b*-day 7: **Sig.=0.789 > 0.05,** Chl *b*-day 14: **Sig.=0.764 > 0.05**. Chl *a*+*b*-day 7: Sig.=0.039 < 0.05, Chl *a*+*b*-day 14: **Sig.=0.086 > 0.05.** Carotenoids-day 7**: Sig.=0.789 > 0.05**, Carotenoids-day 14: Sig.=0.024 < 0.05. The results for Chl *b*, Chl *a*+*b*-day 14, Carotenoids-day 7 are **not statistically verifable**.

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Table 39. Effect of serotonin (5-HT) on carotenoid and chlorophyll content in *H. lacustris* BM-1 cells

	Content, mg/g of dry weight								
		Control		**5-HT, 0.1 μM**		**5-HT, 1 μM**		**5-HT, 10 μM**	
	Day 0	Day 7	Day 14	Day 7	Day 14	Day 7	Day14	Day 7	Day14
Chlorophyll *a*	2.97± 0.20	1.38± 0.15	0.85± 0.15	1.50± 0.20	0.86± 0.10	1.63± 0.20	0.98± 0.155	1.34± 0.20	0.98± 0.15
Chlorophyll *b*	1.30± 0.10	0.60± 0.10	0.39± 0.10	0.60± 0.15	0.43± 0.10	0.69± 0.15	0.44± 0.10	0.55± 0.10	0.47± 0.15
Chlorophyll *a* + *b*	4.27± 0.25	1.98± 0.20	1.23± 0.20	2.09± 0.25	1.29± 0.25	2.32± 0.25	1.42± 0.15	1.88± 0.15	1.44± 0.15
Carotenoids	0.69± 0.10	2.01± 0.20	2.23± 0.20	2.13± 0.20	2.21± 0.25	2.44± 0.25	2.42± 0.25	2.11± 0.20	2.24± 0.20

The averaged results of 3 repeats are presented. The Kruskal-Wallis test results with independent samples for the experimental and control groups. Chl *a*-day 7: Sig.=0.036 < 0.05, Chl *a*-day 14: Sig.=0.046 < 0.05. Chl *b*-day 7: **Sig.=0.698 > 0.05,** Chl *b*-day 14: Sig.=0.038 < 0.05. Chl *a*+*b*-day 7: **Sig.=0.200 > 0.05**, Chl *a*+*b*-day 14: **Sig.=0.396 > 0.05.** Carotenoids-day 7**:** Sig.=0.038 < 0.05, Carotenoids-day14: Sig.=0.038 < 0.05. The results for Chl *b*-day 7 and Chl *a*+*b* group are **not statistically verifiable.**

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Table 40. Effect of histamine (His) on carotenoid and chlorophyll content in *H. lacustris* IPPAS B-239 cells

	Content, mg/g of dry weight								
	Day 0	**Control**		**His, 0.1 μM**		**His, 1 μM**		**His, 10 μM**	
		Day 7	Day 14	Day 7	Day 14	Day 7	Day14	Day 7	Day14
Chlorophyll *a*	1.82± 0.15	1.23± 0.15	2.46± 0.15	1.23± 0.15	1.35± 0.15	1.22± 0.10	1.36± 0.10	1.18± 0.10	1.38± 0.15
Chlorophyll *b*	1.11± 0.15	0.69± 0.10	1.26± 0.10	0.66± 0.10	0.82± 0.10	0.66± 0.10	0.79± 0.10	0.67± 0.10	0.77± 0.10
Chlorophyll *a* + *b*	2.93± 0.20	1.92± 0.20	3.72± 0.25	1.89± 0.15	2.17± 0.20	1.88± 0.15	2.15± 0.20	1.84± 0.15	2.15± 0.20
Carotenoids	0.64± 0.10	2.94± 0.20	6.57± 0.30	2.96± 0.20	5.61± 0.25	3.15± 0.20	4.99± 0.20	2.90± 0.20	5.35± 0.25

The averaged results of 3 repeats are presented. The Kruskal-Wallis test results with independent samples for the experimental and control groups. Chl *a*-day 7: Sig.=0.031 < 0.05, Chl *a*-day 14: Sig.=0.029 < 0.05. Chl *b*-day 7: **Sig.=0.789 > 0.05,** Chl *b*-day 14: Sig.=0.028 < 0.05. Chl *a*+*b*-day 7: Sig.=0.038 < 0.05, Chl *a*+*b*-day 14: Sig.=0.048 < 0.05**.** Carotenoids-day 7**:** Sig.=0.047 < 0.05, Carotenoids-day 14: Sig.=0.023 < 0.05. The results for all the data groups rule the null hypothesis out and are valid statistically **except the Chl *b*-day 7 group**.

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Table 41. Effect of histamine (His) on carotenoid and chlorophyll content in *H. lacustris* BM-1 cells

	Content, mg/g of dry weight								
		Control		**His, 0.1 μM**		**His, 1 μM**		**His, 10 μM**	
	Day 0	Day 7	Day 14	Day 7	Day 14	Day 7	Day14	Day 7	Day14
Chlorophyll *a*	1.93± 0.10	1.20± 0.10	1.13± 0.10	1.47± 0.10	1.89± 0.15	1.77± 0.15	1.88± 0.15	1.86± 0.15	1.96± 0.15
Chlorophyll *b*	1.06± 0.10	0.50± 0.10	0.59± 0.10	0.62± 0.10	1.08± 0.15	0.80± 0.15	1.07± 0.15	0.85± 0.10	1.03± 0.15
Chlorophyll *a* + *b*	2.99± 0.25	1.70± 0.15	1.73±0. 25	2.09± 0.15	2.97± 0.15	2.57± 0.20	2.94± 0.20	2.71± 0.20	2.98± 0.20
Carotenoids	0.57± 0.10	1.86± 0.10	3.36± 0.20	2.25± 0.20	6.40± 0.20	2.25± 0.20	6.54± 0.20	2.25± 0.15	7.09± 0.15

The averaged results of 3 repeats are presented. The Kruskal-Wallis test results with independent samples for the experimental and control groups. Chl *a*-day 7: Sig.=0.024 < 0.05, Chl *a*-day 14: Sig.=0.028 < 0.05. Chl *b*-day7: Sig.=0.039 < 0.05**,** Chl *b*-day14: Sig.=0.028 < 0.05. Chl *a*+*b*-day 7: Sig.=0.024 < 0.05, Chl *a*+*b*-day 14: Sig.=0.029 < 0.05**.** Carotenoids-day 7**: Sig.=0.095 > 0.05**, Carotenoids-day 14: Sig.=0.024 < 0.05. The results for all the data groups rule the null hypothesis out and are statistically valid **except the carotenoids-day 7 group**.

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Table 42. Effect of dopamine (DA) on carotenoid and chlorophyll content in *H. lacustris* IPPAS B-239 cells

	Content, mg/g of dry weight								
		Control		**DA, 0.1 μM**		**DA, 1 μM**		**DA, 10 μM**	
	Day 0	Day 7	Day 14	Day 7	Day 14	Day 7	Day14	Day 7	Day14
Chlorophyll *a*	1.83± 0.15	1.76± 0.15	1.33± 0.15	1.22± 0.10	1.26± 0.10	1.30± 0.10	1.26± 0.10	1.26± 0.15	1.33± 0.10
Chlorophyll *b*	0.65± 0.10	0.68± 0.10	0.62± 0.10	0.22± 0.05	0.44± 0.10	0.30± 0.10	0.51± 0.10	0.48± 0.15	0.55± 0.15
Chlorophyll *a* + *b*	2.48± 0.20	2.45± 0.20	1.95± 0.20	2.45± 0.15	1.70± 0.20	1.59± 0.20	1.77± 0.20	1.74± 0.25	1.88± 0.20
Carotenoids	2.85± 0.20	3.26± 0.20	3.09± 0.20	2.04± 0.15	2.66± 0.20	2.52± 0.20	2.86± 0.15	2.28± 0.20	2.85± 0.20

The averaged results of 3 repeats are presented. The Kruskal-Wallis test results with independent samples for the experimental and control groups. Chl *a*-day 7: **Sig.=0.092 > 0.05**, Chl *a*-day 14: **Sig.=0.817 > 0.05**. Chl *b*-day 7: Sig.=0.034 < 0.05**,** Chl *b*-day 14: Sig.=0.039 < 0.05. Chl *a*+*b*-day 7: Sig.=0.038 < 0.05, Chl *a*+*b*-day 14: Sig.=0.046 < 0.05**.** Carotenoids-day 7**:** Sig.=0.023 < 0.05, Carotenoids-day 14: Sig.=0.030 < 0.05. The results for all the data groups rule the null hypothesis out and are statistically valid **except the Chl *a* group**.

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Table 43. Effect of dopamine (DA) on carotenoid and chlorophyll content in *H. lacustris* BM-1 cells

	Content. mg/g of dry weight								
	Day 0	**Control**		**DA, 0.1 μM**		**DA, 1 μM**		**DA, 10 μM**	
		Day 7	Day 14	Day 7	Day 14	Day 7	Day 14	Day 7	Day14
Chlorophyll *a*	2.34± 0.20	1.20± 0.20	1.23± 0.15	1.59± 0.15	1.46± 0.15	1.04± 0.10	1.42± 0.10	1.28± 0.10	1.52± 0.15
Chlorophyll *b*	1.23± 0.15	0.55± 0.15	0.67± 0.15	0.74± 0.10	0.77± 0.10	0.43± 0.10	0.77± 0.15	0.57± 0.10	0.78± 0.15
Chlorophyll *a* + *b*	3.57± 0.20	1.75± 0.20	1.90± 0.20	2.34± 0.20	2.23± 0.20	1.47± 0.15	2.19± 0.15	1.85± 0.20	2.30± 0.20
Carotenoids	0.59± 0.10	1.91± 0.20	4.44± 0.20	2.21± 0.20	4.44± 0.20	1.70± 0.15	5.07± 0.20	1.96± 0.15	4.07± 0.20

The averaged results of 3 repeats are presented. The Kruskal-Wallis test results with independent samples for the experimental and control groups. Chl *a*-day 7: Sig.=0.041 < 0.05, Chl *a*-day 14: Sig.=0.032 < 0.05. Chl *b*-day 7: **Sig.=0.099 > 0.05,** Chl *b*-day 14: **Sig.=0.796 > 0.05**. Chl *a*+*b*-day 7: Sig.=0.031 < 0.05, Chl *a*+*b*-day 14: Sig.=0.042 < 0.05**.** Carotenoids-day 7**:** Sig.=0.035 < 0.05, Carotenoids-day 14: Sig.=0.042 < 0.05. The results for all data groups rule the null hypothesis out and arestatistically valid **except the Chl *b* group**.

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Table 44. Effect of norepinephrine (NE) on carotenoid and chlorophyll content in *H. lacustris* IPPAS B-239 cells

	Content, mg/g of dry weight								
	Day 0	**Control**		**NE, 0.1 μM**		**NE, 1 μM**		**NE, 10 μM**	
		Day 7	Day 14	Day 7	Day 14	Day 7	Day14	Day 7	Day14
Chlorophyll *a*	1.91± 0.15	1.33± 0.15	1.76± 0.15	1.33± 0.15	1.22± 0.10	1.26± 0.10	1.30± 0.15	1.26± 0.15	1.26± 0.10
Chlorophyll *b*	0.85± 0.10	0.55± 0.10	0.68± 0.15	0.62± 0.10	0.22± 0.10	0.44± 0.10	0.30± 0.10	0.51± 0.10	0.48± 0.15
Chlorophyll *a* + *b*	2.76± 0.20	1.88± 0.15	2.44± 0.20	1.95± 0.15	1.44± 0.15	1.70± 0.15	1.60± 0.15	1.78± 0.15	1.74± 0.15
Carotenoids	0.73± 0.10	2.85± 0.20	3.26± 0.20	3.09± 0.25	2.04± 0.20	2.66± 0.20	2.52± 0.20	2.86± 0.20	2.28± 0.20

The averaged results of 3 repeats are presented. The Kruskal-Wallis test results with independent samples for the experimental and control groups. Chl *a*-day 7: **Sig.=0.817 > 0.05**, Chl *a*-day 14: **Sig.=0.086 > 0.05**. Chl *b*-day 7: Sig.=0.028 < 0.05, Chl *b*-day 14: Sig.=0.038 < 0.05. Chl *a*+*b*-day 7: Sig.=0.028 < 0.05, Chl *a*+*b*-day 14: Sig.=0.035 < 0.05**.** Carotenoids-day 7**:** Sig.=0.036 < 0.05, Carotenoids-day 14: Sig.=0.023 < 0.05. The results for all the data groups rule the null hypothesis out and are statistically valid **except the Chl *a* group**.

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Table 45. Effect of norepinephrine (NE) on carotenoid and chlorophyll content in *H. lacustris* BM-1 cells

	Content, mg/g of dry weight								
	Day 0	**Control**		**NE, 0.1 μM**		**NE, 1 μM**		**NE, 10 μM**	
		Day 7	Day 14	Day 7	Day 14	Day 7	Day14	Day 7	Day14
Chlorophyll *a*	2.58± 0.20	1.58± 0.15	1.74± 0.15	1.75± 0.15	2.06± 0.20	1.75± 0.15	1.98± 0.15	1.65± 0.15	2.10± 0.20
Chlorophyll *b*	1.05± 0.10	0.67± 0.10	0.63± 0.10	0.78± 0.10	0.72± 0.10	0.71± 0.10	0.81± 0.10	0.71± 0.10	0.83± 0.10
Chlorophyll *a* + *b*	3.63± 0.20	2.26± 0.20	2.37± 0.20	2.54± 0.20	2.78± 0.25	2.46± 0.20	2.79± 0.25	2.36± 0.20	2.93± 0.25
Carotenoids	0.79± 0.10	1.99± 0.15	3.03± 0.20	2.16± 0.20	3.42± 0.20	2.04± 0.15	3.47± 0.20	1.91± 0.15	3.81± 0.25

The averaged results of 3 repeats are presented. The Kruskal-Wallis test results with independent samples for the experimental and control groups. Chl *a*-day 7: Sig.=0.035 < 0.05, Chl *a*-day 14: Sig.=0.061 > 0.05. Chl *b*-day 7: Sig.=0.034 < 0.05, Chl *b*-day 14: Sig.=0.048 < 0.05. Chl *a*+*b*-day 7: Sig.=0.043 < 0.05, Chl *a*+*b*-day 14: Sig.=0.037 < 0.05. Carotenoids-day 7: Sig.=0.043 < 0.05, Carotenoids-day 14: Sig.=0.045 < 0.05. The results for all the data groups rule the null hypothesis out and are statistically valid except the Chl *a*-day14 group.

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Table 46. Effect of acetylcholine (ACh) on carotenoid and chlorophyll content in *H. lacustris* IPPAS B-239 cells

	Content, mg/g of dry weight								
	Day 0	**Control**		**ACh, 0.1 μM**		**ACh, 1 μM**		**ACh, 10 μM**	
		Day 7	Day 14	Day 7	Day 14	Day 7	Day14	Day 7	Day14
Chlorophyll *a*	1.87± 0.15	1.33± 0.15	1.17± 0.15	1.93± 0.20	1.92± 0.20	1.75± 0.15	1.83± 0.15	1.80± 0.15	1.29± 0.10
Chlorophyll *b*	0.83± 0.10	0.67± 0.10	0.56± 0.10	0.91± 0.15	0.92± 0.15	0.85± 0.15	0.85± 0.15	0.88± 0.15	0.63± 0.10
Chlorophyll *a* + *b*	2.71± 0.20	2.00± 0.20	1.74± 0.15	2.84± 0.20	2.85± 0.20	2.61± 0.20	2.68± 0.20	2.68± 0.20	1.91± 0.15
Carotenoids	0.53± 0.10	2.71± 0.20	5.73± 0.25	3.97± 0.25	8.01± 0.25	3.78± 0.20	7.91± 0.25	3.60± 0.20	6.02± 0.25

The averaged results of 3 repeats are presented. The Kruskal-Wallis test results with independent samples for the experimental and control groups. Chl *a*-day 7: **Sig.=0.700 > 0.05**, Chl *a*-day 14: Sig.=0.033 < 0.05. Chl *b*-day 7: **Sig.=0.238 > 0.05**, Chl *b*-day 14: Sig.=0.05. Chl *a*+*b*-day7: Sig.=0.047 < 0.05, Chl *a*+*b*-day 14: Sig.=0.029 < 0.05**.** Carotenoids-day 7**:** Sig.=0.047 < 0.05, Carotenoids-day 14: Sig.=0.029 < 0.05. The results for all the data groups rule the null hypothesis out and are statistically valid **except the Chl a-day7 and Chl b-day7 group**.

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Table 47. Effect of acetylcholine (ACh) on carotenoid and chlorophyll content in *H. lacustris* BM-1 cells

	Content, mg/g of dry weight								
	Day 0	**Control**		**ACh, 0.1 μM**		**ACh, 1 μM**		**ACh, 10 μM**	
		Day 7	Day 14	Day 7	Day 14	Day 7	Day14	Day 7	Day14
Chlorophyll *a*	4.81± 0.20	1.27± 0.15	1.05± 0.15	1.23± 0.15	0.71± 0.10	1.30± 0.15	1.45± 0.15	1.12± 0.10	1.90± 0.15
Chlorophyll *b*	1.27± 0.15	0.54± 0.10	0.53± 0.10	0.54± 0.10	0.36± 0.10	0.60± 0.10	0.65± 0.10	0.57± 0.10	0.82± 0.15
Chlorophyll *a* + *b*	7.08± 0.25	1.82± 0.15	1.57± 0.15	1.77± 0.15	1.07± 0.15	1.89± 0.20	2.11± 0.20	1.69± 0.15	2.72± 0.20
Carotenoids	1.34± 0.15	1.85± 0.15	2.58± 0.20	1.85± 0.15	2.02± 0.20	1.98± 0.20	2.99± 0.25	1.71± 0.15	4.01± 0.3

The averaged results of 3 repeats are presented. The Kruskal-Wallis test results with independent samples for the experimental and control groups. Chl *a*-day 7: Sig.=0.033 < 0.05, Chl *a*-day 14: Sig.=0.016 < 0.05. Chl *b*-day 7: Sig.=**0.789 > 0.05**, Chl *b*-day 14: Sig.=0.029 < 0.05. Chl *a*+*b*-day 7: **Sig.=0.579 > 0.05**, Chl *a*+*b*-day 14: Sig.=0.016 < 0.05**.** Carotenoids-day 7**:** Sig.=0.034 < 0.05, Carotenoids-day 14: Sig.=0.019 < 0.05. The results for all the data groups rule the null hypothesis out and are statistically valid **except those for the Chl b-day7 and Chl a+b-day7 groups**.

4.2. CYANOBACTERIUM

As pointed out above, the control culture of *L. platensis* reaches the maximum Chl *a* (16.49±1.5 mg/g of dry biomass) and carotenoid (8.38±1.0 mg/g of dry biomass) content by day 7 of cultivation, and it was this point that was chosen for measuring the effects of exogenous neurotransmitters, along with the day 14 point that is characterized by a decrease in endogenous Chl *a* content to 0.76±0.25 mg/g, while the carotenoid content was virtually at the day 7 level (their content on day 14 was 8.92±1.0 mg/g) in the control culture.

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5.2.1. Serotonin (5-HT). The only statistically valid effect produced by 5-HT on Chl *a* was increasing its content on day 7 by ~57% (at a 5-HT concentration of 10 μM), compared to the control culture. (Table 48). No increase in Chl *a* content occurred on day 14. Moreover, 1 μM 5-HT 2.5-fold decreased the Chl *a* content. 5-HT was revealed to increase the carotenoid content on day 7 (with 10 μM 5-HT only) and 14 (this increase was verifiable at 1 and 10 μM 5-HT).

5.2.2. Histamine. The effect of histamine on the Chl *a* content in *L. platensis* cells (Table 49) boiled down to its decrease on day 7 and increase on day 14, compared to the control Chl *a* content values. This effect wаз similar to that of ACh; however, histamine brought about a more significant decrease in Chl *a* content on day 7. As for the carotenoids, histamine increased their content on day 7 (at a concentration of 0.1 μM only) and 14.

5.2.3. Dopamine (DA). As shown in Table 50, DA increased the Chl *a* content in *L. platensis* on day 7 (by ~61% with 10 μM DA) without exerting a statistically significant effect on day 14 of cultivation. DA also augmented the carotenoid content but only on day 7 of cultivation.

5.2.4. Norepinephrine (NE). Despite its chemical similarity to DA, NE produced a different effect on the photosynthetic pigments of *L. platensis*. NE failed to significantly influence the Chl *a* content on day 7 and reduced it on day 14 at a concentration of 0.1 μM (Table 51). NE did not affect the carotenoid content.

5.2.5. Acetylcholine (ACh). This neurotransmitter lowered the Chl *a* content in *L. platensis* cells on day 7 of cultivation (Table 52) by 12.5% and 24.5%, respectively, at ACh concentrations of 0.1 and 1 μM, respectively; 10 μM was without effect. All ACh concentrations somewhat increased (statistically verifiably with 10 mM ACh only) the Chl *a* content on day 14 of cultivation.

On day 7 of cultivation, ACh was established to increase the carotenoid content. This increase in the concentration of the light-harvesting photosystem components could offset the aforementioned Chl *a* content decrease and account for the biomass

yield increase in the presence of ACh. By day 14, the carotenoid content decreased. It should be re-emphasized that, at this point, no statistically verifiable biomass yield increase was brought about by ACh (see Fig. 22).

To sum up, the effects of the neurotransmitters on the photosynthetic pigment content on day 7 of cultivation enable us to subdivide them into two subgroups: (1) ACh and histamine that decrease the Chl *a* and increase the carotenoid content and (2) 5-HT and DA that elevate both the Chl *a* and the carotenoid content. NE produced no effect at all.

Table 48. Impact of serotonin (5-HT) on the pigment content of *L. platensis*

Day of cultivation	**Photosynthetic pigment content, mg/g of dry biomass**								
	Day 0	**Control**		**5-HT, 0.1 μM**		**5-HT, 1 μM**		**5-HT, 10 μM**	
		7	14	7	14	7	14	7	14
Chlorophyll *a*	2.62 ± 0.02	13.92 ±1.73	0.15 ± 0.01	11.14 ±1.27	0.09 ± 0.07	13.11 ±1.21	0.06 ± 0.01	21.81 ±0.5	0.16 ± 0.09
Carotenoids	2.53 ± 0.02	7.35 ± 0.73	9.0 ± 0.74	5.95 ± 0.31	9.83 ± 0.81	6.73 ± 0.96	11.03 ± 0.22	11.58 ±0.52	11.49 ± 0.59

The Kruskal-Wallis test results with independent samples for the experimental and control groups. Chl *a*-day 7: Sig.=0.041 < 0.05, ruling the null hypothesis out, Chl *a*-day 14: **Sig.=0.184 > 0.05**, **conforming with the null hypothesis**. Carotenoids-day 7: Sig.=0.038 < 0.05, ruling the null hypothesis out, Carotenoids-day 14: Sig.=0.026 < 0.05 ruling the null hypothesis out. The data on Chl *a* on day 14 are **not valid** statistically

Table 49. Impact of histamine (His) on the pigment content of *L. platensis*

Day of cultivation	Photosynthetic pigment content, mg/g of dry biomass								
	Day 0	Control		His, 0.1 μM		His, 1 μM		His, 10 μM	
		7	14	7	14	7	14	7	14
Chlorophyll *a*	2.62± 0.02	18.24 ±0.97	0.1± 0.08	21.02 ±1.43	0.07± 0.01	15.43 ±0.65	0.28± 0.11	15.32 ±0.42	0.25± 0.05
Carotenoids	2.53± 0.02	8.32± 0.3	8.57± 1.04	11.97 ±0.16	9.54± 0.9	8.32± 0.13	9.78± 1.14	7.99± 0.23	10.15 ± 0.86

The Kruskal-Wallis test results with independent samples for the experimental and control groups. Chl *a*-day 7: Sig.=0.025 < 0.05, ruling the null hypothesis out, Chl *a*-day 14: Sig.=0.05, ruling the null hypothesis out. Carotenoids-day 7: Sig.=0.043 < 0.05, ruling the null hypothesis out, Carotenoids-day 14: **Sig.=0.303 > 0.05 conforming with the null hypothesis**.These data are **not verifiable** statistically

Table 50. Impact of dopamine (DA) on the pigment content of *L. platensis*

Day of cultivation	Photosynthetic pigment content, mg/g of dry biomass								
	Day 0	Control		DA, 0.1 μM		DA, 1 μM		DA, 10 μM	
		7	14	7	14	7	14	7	14
Chlorophyll *a*	2.62± 0.02	15.59 ±4.62	1.07± 0.47	18.32 ±1.76	0.8± 0.22	19.93 ±1.24	1.13± 0.24	25.07±1.08	1.18± 0.03
Carotenoids	2.53± 0.02	8.78± 2.63	9.3± 0.88	10.55 ±0.81	10.06± 0.53	12.6± 0.86	9.99± 1.52	13.34±0.36	10.08± 0.26

The Kruskal-Wallis test results with independent samples for the experimental and control groups. Chl *a*-day 7: **Sig.=0.062 > 0.05, conforming with the null hypothesis**, Chl *a*-day 14 : **Sig.=0.218 > 0.05, conforming with the null hypothesis.** Carotenoids-day 7: Sig.=0.033 < 0.05, ruling the null hypothesis out, Carotenoids-day 14: **Sig.=0.668 > 0.05, conforming with the null hypothesis**. Only the data on carotenoids (day 7) are statistically valid.

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Table 51. Impact of norepinephrine (NE) on the pigment content of *L. platensis.*

Day of cultivation	Photosynthetic pigment content, mg/g of dry biomass								
	Day 0	Control		NE, 0.1 μM		NE, 1 μM		NE, 10 μM	
		7	14	7	14	7	14	7	14
Chlorophyll *a*	2.62± 0.02	20.96 ±1.2	1.09± 0.15	21.18 ±3.34	0.36± 0.06	20.3± 0.27	1.03± 0.04	21.64 ±0.97	0.89± 0.08
Carotenoids	2.53± 0.02	10.27 ±0.35	9.9± 1.24	10.65 ±1.68	9.51± 0.8	10.36 ±0.34	9.89± 0.54	10.93 ±0.66	10.04 ± 0.47

The Kruskal-Wallis test results with independent samples for the experimental and control groups. Chl *a*-day 7: Sig.=0.045 < 0.05, ruling the null hypothesis out, Chl *a*-day 14 : Sig.=0.033 < 0.05, ruling the null hypothesis out. Carotenoids-day 7: **Sig.=0.579 > 0.05, conforming with the null hypothesis**, Carotenoids-day 14: **Sig.=0.789 > 0.05, conforming with the null hypothesis**. Therefore, the data on carotenoids are **not valid** statistically.

Table 52. Impact of acetylcholine (ACh) on the pigment content of *L. platensis*

Day of cultivation	Photosynthetic pigment content, mg/g of dry biomass								
	Day 0	Control		ACh, 0.1 μM		ACh, 1 μM		ACh, 10 μM	
		7	14	7	14	7	14	7	14
Chlorophyll *a*	2.62± 0.02	21.72 ±1.41	0.58± 0.29	19.01 ±0.9	0.81± 0.07	16.6 ±0.91	0.86 ±0.13	22.86 ±0.65	0.92 ±0.28
Carotenoids	2.53± 0.02	8.16± 2.79	7.83± 0.87	10.25 ±0.25	7.47± 0.7	8.16± 0.66	7.02± 0.76	11.39 ±0.26	7.98± 0.11

The Kruskal-Wallis test results with independent samples for the experimental and control groups. Chl *a*-day 7 : Sig.=0.022 < 0.05, ruling the null hypothesis out, Chl *a*-day 14 : **Sig.=0.424 > 0.05, conforming with the null hypothesis**. Carotenoids-day 7: Sig.=0.05, ruling the null hypothesis out, Carotenoids-day14 : **Sig.=0.838 > 0.05 conforming with the null hypothesis**. Therefore, the data on Chl *a* (day 14) and carotenoids (day 7) are **not verifiable** statistically.

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5.3. CONCLUSIONS

5.3.1. Summing up the impact of neurotransmitters on the photosynthetic pigment content of microalgae. In *C. vulgaris,* ACh (Fig. 32), 5-HT (Fig. 28), histamine (Fig. 29), and NE (Fig. 31) increased the total chlorophyll and carotenoid content, whereas DA (Fig. 30) had no appreciable effect on photosynthetic pigments. *In S. quadricauda,* ACh (Fig. 37) brought about an increase in total chlorophyll and carotenoid content. which apparently reflected an increase in photosynthetic unit number. Histamine (Fig. 34) produced no effect on the photosynthetic pigment content, and an increase in the chlorophyll *a*/chlorophyll *b* and chlorophyll/carotenoid ratios was apparently due to a histamine-induced stress response. NE (Fig. 36) caused an increased in total chlorophyll and carotenoid content but only at the lowest tested concentration (1 μM). Presumably, an inhibitory (toxic) influence of higher NE concentrations overrode the stimulatory effect of NE. DA (Fig. 35) decreased the pigment content in *S. quadricauda* cells. It should be noted that toxic oxidation products of DA such as dopaminechrome (dopachrome) could contribute to the inhibitory effect. 5-HT virtually produced no effect on *S. quadricauda* pigments (Fig. 33).

The main trends documented in the present work are summarized in Table 53.

The above classification (see 4.3) of BAs based on their impact on the fatty acid composition of microalgal lipids seems to be, for the most part, consistent with the data on their influence on microalgal pigments. For instance, ACh and histamine, while increasing the share of unsaturated fatty acids in both tested microalgae, also augment the chlorophyll *a* and carotenoid content. Therefore, they are likely to increase photosynthetic activity, and high photosynthetic activity is characteristic of the early stages of the life-cycle of microalgal cultures.

Their “youth” period is presumably prolonged by these BAs. Conversely, DA increases the percentage of saturated fatty acids in the lipid fraction, hypothetically promoting the “aging” of microalgal cultures. In line with this, DA decreases the photosynthetic pigment content, albeit only in *S. quadricauda.* A discrepancy was

observed with NE that increased the share of some saturated fatty acids and, nonetheless, augmented the photosynthetic pigment content at the same concentration of 1 μM. However. the effect of NE was ambiguous, similar to that of 5-HT.

Table 53. Summarized data on the influence of BA on photosynthetic pigment content in the tested microalgae (based on the data of Tables 38-52, Fig. 28-37).

Characteristics	Species/strain of microalgae	Biogenic amines				
		5-HT	His	DA	NE	ACh
Chlorophyll *a* content	*C. vulgaris*	↗	↗	—	↗	↗
	S.quadricauda	—	↗	↘	↗	↗
	IPPAS H-239	↘	↘	↗	↗	↗
	BM-1	—	↗	↗	↗	↗
	L. platensis	↗	↘	↗	—	↘
Chlorophyll *b* content	*C. vulgaris*	↗	↗	—	↗	—
	S.quadricauda	—	↘	↘	↗	↗
	IPPAS H-239	↘	↘	↗	↗	↗
	BM-1	—	↗	↗	↗	↗
	L. platensis	Chlorophyll *b* lacking				
Carotenoid content	*C. vulgaris*	↗	—	—	↗	↗
	S.quadricauda	—	↗	↘	↗	↗
	IPPAS H-239	↘	↘	↘	↗	↗
	BM-1	—	↗	↗	↗	↗
	L. platensis	↗	↗	↗	—	↗

Designations: ↗, upward trends; ↘, downward trends; —, insignificant or ambiguous effects. IPPAS H-239 and BM-1, *H. lacustris* strains

The following is concerned with another subject of the present work, *Haematococcus lacustris*. It should be re-emphasized that its culture is characterized by a quasi-aging process involving the transition *from the "green" vegetative (chlorophyll-rich) via the "brown" palmelloid (combining chlorophylls and carotenoids) to the "reddish" encysted (carotenoid-accumulating) form.*

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Among the neurotransmitters tested in the present work, NE and ACh stimulated the synthesis of both chlorophylls and carotenoids in the two strains of *H. lacustris* on day 7 and 14 of cultivation; histamine and DA promoted these processes in strain BM-1 only. In distinction from BM-1, histamine, 5-HT, and DA decreased the chlorophyll and carotenoid content of strain IPPAS H-239.

5.3.2. Possible mechanisms of action: modifying the culture's developmental dynamics. The results presented in this work seem to be somewhat paradoxical in light of the literature data on the competition between (1) the synthesis of chlorophyll forming a part of the photosystems and (2) the formation of secondary carotenoids, especially astaxanthin, in *H. lacustris* (reviewed, Solovchenko, 2015; Mularczyk et al., 2020). However, are these two process inevitably antagonistic (according to the zero-sum principle)?

The data obtained can be interpreted in terms of the available information on the intermediate developmental stage (the "middle-aged" culture) that combines chlorophyll and carotenoid production. Such a culture chiefly contains non-motile palmelloid cells featuring a sizeable amount of astaxanthin against the background of a significant chlorophyll content (Solovchenko, 2015).

As pointed out above, the palmelloid cell numbers significantly increased in the presence of NE and, in strain IPPAS B-239, also of ACh.

Presumably, ACh and NE in both tested strains as well as histamine and DA in strain BM-1 lock the culture's development at the "middle-aged" palmelloid stage. To re-emphasize, ACh and histamine prolonged the relatively early developmental stage with a high PUFA and chlorophyll content in *S. quadricauda* belonging to the same class (Chlorophyceae) as *H. lacustris* (see above). Therefore, the hypothetic mode of action of neurotransmitters is based on regulating the carotenoid and chlorophyll synthesis pathways so as to achieve an equilibrium between them and maintain sufficiently high concentrations of both pigment types.

On the contrary, such neurotransmitters as 5-HT in strain IPPAS H-239

presumably prevent the cultures from staying in the palmelloid stage, which can account for the neurotransmitters' inhibitory effects on both carotenoid and chlorophyll synthesis.

5.3.3. Potential biotechnological implications. The data discussed in this subsection seem to hold no biotechnological value at first glance. The production of astaxanthin with *H. lacustris* is only commercially viable against the background of its chemical synthesis if the astaxanthin content in *H. lacustris* cells exceeds 5% of the biomass' dry weight (Del Campo et al., 2007). Such a high astaxanthin yield is only possible with the final "reddish" encysted culture stage of *H. lacustris* and not the palmelloid stage. Should neurotransmitters be not included in the list of potential biotechnologically applicable promoters of carotenoid synthesis in *H. lacustris*. even though neurotransmitters are quite inexpensive if applied at very low (submicromolar) concentrations?

Apparently, dismissing neurotransmitters as biotechnologically useless substances is premature. The accumulation of palmelloid cells in the culture is of biotechnological interest per se since palmelloid cells are much easier to convert to astaxanthin-filled "reddish" cells than vegetative "green" cells (Wang et al., 2014). Palmelloid cells require comparatively low doses of supplementary factors (high light, high salinity, etc.) for this purpose.

As pointed out in one of the preceding subsections, DA enhanced the yield of the biotechnologically valuable carotenoid astaxanthin if applied in combination with NaCl-induced stress; DA also promoted biomass accumulation by *H. pluvialis* and lipid formation in it (Zhao et al., 2025a).

The data obtained in the present work concerning the concomitant stimulation of carotenoid and chlorophyll synthesis by some neurotransmitters in *H. lacustris* can potentially be used to boost the astaxanthin yield by combining the neurotransmitters with low doses of other factors that promote astaxanthin production and facilitate the synthesis of additional microalgal products such as

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PUFA-enriched lipids. They can be isolated from the biomass after extracting carotenoids from it (Solovchenko, 2015).

It was already noted that 5-HT and NE also extend the early stages of the development of the *L. platensis* culture. At least, the effect of 5-HT resulting in increasing the photosynthetic pigment content is in compliance with the suggestion concerning the "youth prolongation" in the *L. platensis* culture. This implies photosynthesis intensification and an increase in the desaturation degree of the fatty acids.

In the cyanobacterium *L. platensis,* the effects of exogenous BAs are to be considered against the background of the dynamics of photosynthetic pigments in the control culture that reached the maximum chlorophyll *a* and carotenoid levels by day 7 of cultivation, and, subsequently, the chlorophyll concentration decreased whereas the carotenoid content remained at the plateau level. In contrast to the eukaryotic microalgae, ACh and histamine lowered the chorophyll level on day 7 while augmenting the content of carotenoids as light-harvesting molecules, which could account for photosynthesis stimulation and securing an increase in biomass and fatty acid content, as mentioned above. The content of all photosynthetic pigments was elevated in the presence of 5-HT and DA (Table 48-52).

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6. ENDOGENOUS NEUROTRANSMITTERS IN REPRESENTATIVES OF THE PHYTO- AND ZOOPLANKTON AND IN COASTAL PLANTS[10]

The data obtained on the effects of some neurotransmitters (BAs) on microalgae raise the issue concerning their possible contribution to the chemical interaction among the components of aquatic ecosystems. If the predominantly stimulatory effects of low (micromolar) BA doses reflect the results of coevolution between microalgae and other inhabitants of water bodies (and other natural habitats). what ecosystem components produce the BAs and use them to interact with microalgae? This section of the experimental part of the present work deals with three possible sources of BAs in aquatic ecosystems: (1) microalgae per se; (2) representatives of the zooplankton; (3) coastal plants capable of releasing secretable BAs into the aquatic environment. The whole naturally produced pool of neurotransmitters in aquatic ecosystems is expected to coexist with human-produced polluting substances.

6.1. TESTING MICROALGAE FOR ENDOGENOUS NEUROTRANSMITTERS

High performance liquid chromatography (HPLC) analysis with amperometric detection (see Materials and Methods above) failed to reveal any detectable amounts of 5-HT, DA, NE, or DOPA (the DA precursor) in the samples of the culture liquid and the disintegrated biomass of *S. quadricauda* and *C. vulgaris* cultures taken during the stationary growth phase (day 7 and 4 of cultivation for *S. quadricauda* and *C. vulgaris*, respectively). These negative data are not presented in the form of a table in this work.

[10] The results presented in this section have been published in the following supplementary works: Oleskin A. V., Postnov A. L., **Cao B.** Impact of biogenic amines on the growth of green microalgae // J. Pharm. Nutr. Sci. 2021. V. 11. P. 144–150. DOI: 10.29169/1927-5951.2021.11.17. SNIP: 0.193 (0.8 p.s./0.5 p.s.); **Cao B.,** Oleskin A. V., Vlasova T.A. Detecting biogenic amines in food and drug plants with HPLC: medical and nutritional implications // J. Pharm. Nutr. Sci. 2020. V. 10(3). P. 88–91. DOI: 10.29169/1927-5951.2020.10.03.2. SNIP: 0.193 (0.7 p.s. /0.4 p.s.).

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6.2. DETECTING ENDOGENOUS NEUROTRANSMITTERS IN THE WATER FLEA *Daphnia magna*

The microalga-consuming zooplanktonic crustacean *D. magna* was tested chromatographically for BAs (1) in its tissues and (2) in the incubation medium (see Materials and Methods for the protocol). The supernatant obtained by disintegration (followed by centrifugation) of 30-35 adult *D. magna* individuals was revealed to contain DA at submicromolar concentrations (Table 54) close to those that caused growth stimulation in *C. vulgaris* and *S. quadricauda.* These microalgae form a part of the water flea's diet.

Table 54. Endogenous BAs concentrations in the disintegrated biomass (μmol/kg) and incubation medium (mmol/L) of *D. magna*

Data	NE	DOPA	DA	5-HT
Biomass	N/D	N/D	**0.255±0.05**	N/D
Incubation medium	N/D	N/D	N/D	N/D

The Table contains the averaged results of four independent repeats.

6.3. DETECTING ENDOGENOUS NEUROTRANSMITTERS IN HIGHER PLANTS

The leaves of the following shrubs and trees growing around the ponds and lakes of the Dayun Park in Shenzhen were sampled for HPLC analysis (Table 55): *Plumeria rubra* L. cv. *acutifolia*. *Syzigium jambos* (L.) Alston. *Buxus megistophylla* (*Euonymus japonicas* cv. *aureoma*. boxwood). and *Cinnamomum bodinieri* Levl. The samples were revealed to contain micromolar amounts of 5-HT and submicromolar or micromolar (in *C. bodinieri*) amounts of NE and DA. The leaves of the tested plants, except *B. megistophylla,* also contained 3-methyltryptamine (3-MT) that is chemically related to 5-HT. The leaf samples lacked epinephrine and DOPA, the DA precursor. To sum up, the leaves of the tested plant species were established to contain BA concentrations that are sufficient for producing specific effects on aquatic ecosystem components if released into the water environment.

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Table 55. Concentrations (means ± standard deviations) of 5-HT, epinephrine (E), NE, DA, 2.3-dihydrophenylalanine (DOPA), and 3-methyltryptamine (3-MT) in the total fraction obtained by disintegrating plant leaves

Plant species	E	NE	DA	5-HT	DOPA	3-MT
Plumeria rubra L. cv. *Acutifolia*	0	0.43±0.03	0.11±0.05	1.41±0.07	0	2.24±0.09
Syzigium jambos(L.) Alston	0	0.34±0.01	0.09±0.04	1.18±0.06	0	1.87±0.07
Buxus megistophylla	0	0.27±0.02	0.06±0.01	0.25±0.02	0	0
Cinnamomum bodinieri Levl.	0	1.21±0.14	3.09±0.12	1.21±0.09	0	0.33±0.03

All concentrations are expressed in micromoles per 1 kg of wet biomass.

6.4. CONCLUSIONS

6.4.1. Endogenous neurotransmitter data: possible implications. Based on HPLC data, no detectable amounts of BAs and DOPA. the DA precursor were contained in the cells and the culture liquid of *C. vulgaris* and *S. quadricauda.* Submicromolar DA concentrations were revealed in the disintegrated (mortar-ground) biomass of *Daphnia magna* (Table 54), and this was interpreted in terms of the suggestion that *D. magna* stimulates the growth of its prey (microalgae).

It was established in the present work (Table 55) that waterfront plants of city park ponds and lakes in Shenzhen produce BAs at sufficient concentrations to exert an effect on aquatic ecosystem components. including phytoplanktonic microalgae. provided that the BAs are excreted into the water bodies.

In addition to the putative hydroecological role, the tested plants include species that are used to produce food. medicines. and cosmetic. *Syzigium jambos,* the "rose apple" and *Cinnamomum bodinieri,* a source of oils utilized in cosmetics, are of considerable practical importance. The presence of neurotransmitters in their tissues points to possible neurochemical effects of pharmaceutical. nutraceutical. and cosmetic products obtained from these plants.

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6.3.2 Biogenic amines as ecomones. Taken together, the data on the BA effects on microalgae as well as the fact that BAs are produced by representatives of the zooplankton and by coastal plants are in agreement with the hypothesis that neurotransmitters form a part of the ecosystem-level pool of signals that mediate interactions among many different components of aquatic ecosystems. «Apart from influencing the functioning of separate components of natural ecosystems, these neurotransmitters are likely to exert regulatory effects on such ecosystems as coherent entities. This is the reason why these chemical factors can be denoted as ecomones, in addition to biomediators» (Олескин, Постнов, 2022, С.10).

The term *ecomones* was used by a number of ecologists in the 1960s-1980s (Florkin, Schoffenfels, 1969; Pastels, 1973, 1982). They emphasized the endogenous synthesis of ecomones by the components of ecosystems regulated by them as well as their possible entry into an ecosystem from outside.

Apart from neurotransmitters, such ecosystem-level signals certainly include a plethora of other chemicals. For instance, dimethylsufide, as mentioned in the Review section, is released by microalgae upon contacting the zooplankton and attracts marine birds and other organisms that predate on it (Hay, 2009).

Currently, neurotransmitters and their derivatives, agonists, and antagonists enter aquatic ecosystems because they form a part of human-produced pollutants. Estimating the concentrations of neurochemicals in natural and artificial water bodies is of paramount importance for the present-day world for two reasons. First, monitoring neurotransmitter concentrations in aquatic ecosystems is expected to help us elucidate their regulatory and communicative functions at the ecosystem level. Second, conducting such research will enable us to evaluate the detrimental influence of humankind on the functioning of natural ecosystems. including those of various water bodies ranging from shallow ponds and lakes to the World Ocean *in toto*.

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CONCLUDING REMARKS

To sum up, the present work is concerned with the action of neuroactive biogenic amines as human-produced new-generation exotoxicants on biomass accumulation, fatty-acid composition, and photosynthetic pigment content of some eukaryotic microalgae and cyanobacteria. It was established that the tested substances such as dopamine, norepinephrine, serotonin, histamine, and acetylcholine significantly promote the growth of the cultures of microalgae under study at sufficiently low (micromolar or submicromolar) concentrations that are close to their physiological concentrations in the plant or animal organism. For instance, ca. 0.1 μM norepinephrine is contained in the human blood (Eldrup, 2004). The tested neurotransmitters when applied within the same concentration range, affected (increased or, conversely, decreased) the fatty acid content in microalgal cells. Acetylcholine proved to facilitate fatty acid synthesis in all research subjects of the present work; as for the rest of the neurotransmitters, their statistically verifiable effects were different with different representativies of microalgae.

In light of the results of this work, it seems plausible that microalgae can start uncontrollably growing even in the presence of trace amounts of neurotransmitters in the parts of water bodies that are relatively distant from the points at which neurotransmitter-containg wastewater is discharged into them by food- or drug-producing plants. Due to the very low concentrations involved, neurotransmitters can exert their effects even though they are naturally degraded thanks to abiotic (e.g, photooxidation) and biotic (enxyme-dependent) processes. The data obtained give grounds for the suggestion that “neuroactive” wastewater should be much better purified in the future in order to prevent water blooming and progressive eutrophication of rivers, lakes, and oceans.

Neurotransmitters change the ratio between saturated and unsaturated fatty acids (see Table 37 above), and the influence of neurotransmitters is also different with different microalgal species. To reiterate, acetylcholine and histamine

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increased the polyunstaturated fatty acid percentage at the expense of decreasing the saturated and/or monounsaturated fatty acid content in *C. vulgaris* cells; serotonin significantly lowered the contributions of saturated and monounsaturated acids, even though it failed to statistically verifiably elevate the PUFA percentage. All tested neurotransmitters also influenced (at micromiolar concentrations) the photosynthetic pigment contents, acetylcholine behaving as a quasi-universal biosynthesis promoter with the tested microalgae, although it only raised the carotenoid content in the cyanobacterium *L. platensis* and even reduced, at a concentration of 1 μM, the chlorophyll level on day 7 of cultivation (see Table 53).

The data on the impact of neurotransmitters on fatty acid and pigment contents in microalgae including a cyanobacterium at low (micromolar) concentrations give grounds for the suggestion that these organisms possesss an analog of population density-gauging (QS) systems that are widely spread in the microbial realm. Presumably, such microalgal quasi-QS systems bind neurotransmitters as specific signals. Interaction between some of the tested neurotransmitters and the receptors may result in promoting or inhibiting fatty acid desaturase activity. This should phenomenologically manifest itself in prolonging or shortening the earlier developmental stages that are characterized by a high fatty acid desaturation degree.

Of significant importance may be the antioxidant activity of neurotransmitters exemplified by serotonin that can prevent unsaturated lipid peroxidation, in an analogy to the stimulatory effects of phytohormones (including auxins) on the enzymatic and nonenzymatic systems of ROS quenching in microalgae (Qiu et al., 2025).

Of special interest is research on the effects of neurotransmitters on genomic systems that control the synthesis and metabolism of fatty acids and photosynthetic pigments, similar to studies on the mechanism of action of phytohormones on the microalgal genome (Alsenani et al., 2019; Zhao et al., 2022; Qiu et al., 2025). In this context, a promising area of research – to be addressed in the author's further studies – is based on using published databases for screening for the reference

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genomic sequences of the receptors, signal transmission pathway components, biosynthetic enzymes, and transporters of neurotransmitters in model organisms. These data can lay the foundations for the quest for homologous sequences in published microalgal genomes, to be followed by phyloge-netic analysis aimed at estimating the degree of evolutionary conservation of relevant genes.

In ecotoxicological terms, a promising idea is to edit microalgal genomes, supplementing them with operons for enzymatic neurotransmitter degradation in order to prevent their possible eutrophicating effects on water bodies.

The data obtained on the stimulatory effects of micromolar neurotransmitter concentrations on biomass accumulation, fatty acid composition, and photosynthetic pigment content is of potential relevance to biotechnology, forasmuch as such neurotransmitter amounts are relatively inexpensive. Developments aimed at boosting the biomass yield by adding, e.g., histamine or acetylcholine (depending on the species involved) to microalgal cultures hold some promise in terms of promoting the production of drug preparations, food additives, or biofuel from microalgae. From the biotechnological viewpoint, the neurotransmitters acetylcholine, histamine, and norepinephrine are of considerable interest as fatty acid synthesis-promoting agents in respective microalgal species. They work at low concentrations, which may provide for the commercial competitiveness of relevant biotechnological developments. An exemplary calculation concerning acetylcholine is contained in section 3.3 above. Interestingly, the fatty acid content of *H. lacustris* is likely to be correlated with the secondary carotenoid level because secondary carotenoids, especially astaxanthin, are compartmentalized in cytoplasmic lipid droplets (Solovchenko, 2015).

The neurotransmitters that increase the percentage of PUFAs, including ω-3 and ω-6 fatty acids are of potential interest as sources of drugs and cosmetics with antioxidant properties. Microalgal lipids are also convertible to biodiesel via esterification. To reiterate, the neurotransmitters (like dopamine and serotonin with

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S. quadricauda and *C. vulgaris* or acetylcholine with *L. platensis*) that increase the total fatty acid content without augmenting the PUFA share are recommendable for relevant R & D activities in the future.

From the ecological and hydrobiological viewpoint, the neurotransmitter effects on the growth-related and biochemical characteristics of *C. vulgaris, S. quadricauda, H. lacustris,* and *L. platensis* as widespread inhabitants of freshwater and brackish water bodies is of much interest in terms of the neurotransmitter role in multidirectional interactions among the components of natural or artificial aquatic ecosystems. This work briefly deals with such neurotransmitter producers in aquatic ecosystems as (i) representatives of the zooplankton exemplified by the dopamine-forming *D. magna* (Table 54) and (ii) coastal plants with underwater roots that were shown to produce endogenous neurotransmitters in this work (Table 55), drawing on similar studies conducted by other researchers, especially on the fundamental research that was carried out by Victoria Roshchina (Рощина, 1991, 2010, 2011; Roshchina et al., 2019, 2022).

Taking account of literature data and the author's own findings concerning the important role of neurotransmitters as ecosystem-level signals, it seems reasonable to denote neurotransmitters as *ecomones*; this term was used in the literature with reference to such versatile signal molecules (Florkin, Schoffenfels, 1969; Pastels, 1973, 1982; Олескин, Постнов, 2022). Nonetheless, ecosystem-level regulatory molecules were within the scope of classical works that were produced by Russian researchers. They preferred the term *allelochemicals* with reference to active exometabolites in multispecies associations including aquatic communities (Тамбиев, 1984, quoted according to: Олескин, Постнов, 2022j). A flourishing subfield of ecology is *biochemical ecology* centered around the notion of chemical regulators; they operate at various organization levels within ecosystems (Остроумов, 1986, 2006; Теличенко, Остроумов, 1990; Ostroumov, 2006, 2023, 2024).

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As far as my further research is concerned, I plan to directly measure neurotransmitter concentrations in natural water bodies exemplified by the Glubokoe Lake in the Moscow Region and the lakes and ponds of the city parks of Shenzhen and in artificial (aquacultural) water bodies for the purpose of evaluating the impact of industrial facilities that are located in their vicinity and can cause the neurochemical pollution of aquatic ecosystems.

CONCLUSIONS:

1. Neurotransmitters (DA, NE, 5-HT, histamine, and ACh), potentially important new-generation ecotoxicants, stimulate the growth of the following test subjects: the green microalgae *Chlorella vulgaris* Beijer, *Scenedesmus quadricauda (Turp.)* Breb. K-1149, *Haematococcus lacustris* IPPAS H-239 and BM-1 (5-HT and AChonly) and the *cyanobacterium Limnospora platensis* IPPAS B-256 (5-HT, ACh, and histamine) if added at micromolar concentrations. Histamine proved to be the most efficient growth-stimulating agent with *C. vulgaris* and *S. quadricauda* and 5-HTwith *L. platensis*.

2. Gas chromatography with mass spectrometry revealed the ACh and DA increased the total fatty acid content in *C. vulgaris* cells, whereas 5-HT, histamine, and NE decreased it. As for *S. quadricauda* and *H. lacustris*, ACh, histamine, and NE (in *H. lacustris* only) increased, and DA and 5-HT (in *H. lacustris* only) decreased the total fatty acid content. ACh and 5-HT increased and NE decreased the fatty acid content in *L. platensis*.

3. Based on their effects on FA contents and ratios, the tested neurotransmitters can be classified into two subgroups: (1) those that increase the share of unsaturated fatty acids, such as AChand histamine (in *C. vulgaris* and *S. quadricauda*), 5-HT (in *C. vulgaris* only), and NE (in both tested *H. lacustris strains*) and (2) those that increase the share of saturated fatty acids; these are DA (in all tested eukaryotic microalgal species except *H. lacustris*), 5-HT and NE (in *S. quadricauda* only). As for *L. platensis*, it was 5-HT and NE that increased the PUFA percentage in the

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lipids.

4. All tested neurotransmitters except DA increased the total chlorophyll and carotenoid content in *C. vulgaris*. ACh and NE augmented the chlorophyll and carotenoid content in *S. quadricauda*; 5-HT did not affect it. Histamine increased the chlorophyll *a* and carotenoid content while decreasing the chlorophyll *b* content; DA decreased the chlorophyll and carotenoid content of *S. quadricauda*. NE and ACh stimulated chlorophyll and carotenoid synthesis in *H. lacustris* (strains IPPAS H-239 and BM-1); histamine and DA promoted these processes in strain BM-1 only. ACh and histamine increased the carotenoid content and decreased the chlorophyll concentration in *L. platensis* cells; 5-HT and DA increased the content of both chlorophyll and carotenoids.

5. The results of the present work testify to a plausible ecotoxic action of human-produced neuroactive biogenic amines that can enhance phytoplankton proliferation, posing the threat of water blooming. As for endogenous neurotransmitters, the data of the thesis point to their probable involvement in the interaction between the phytoplankton exemplified by microalgae and other components of aquatic ecosystems that include zooplankton representatives such as *Daphnia magna* and waterfront plants that contain HPLC-detectable neurotransmitter concentrations.

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