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Influence of microorganisms from the Arctic paleoecosystems on the morphophysiological characteristics of *Allium cepa*

Master's thesis

in the field of "Biology" 06.04.01

of the "Plant Biosecurity" Programme

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Tyumen, 2021

МИНИСТЕРСТВО НАУКИ И ВЫСШЕГО ОБРАЗОВАНИЯ
РОССИЙСКОЙ ФЕДЕРАЦИИ
Федеральное государственное автономное образовательное учреждение
высшего образования
«ТЮМЕНСКИЙ ГОСУДАРСТВЕННЫЙ УНИВЕРСИТЕТ»

ИНСТИТУТ ЭКОЛОГИЧЕСКОЙ И СЕЛЬСКОХОЗЯЙСТВЕННОЙ
БИОЛОГИИ (X-BIO)

РЕКОМЕНДОВАНО К ЗАЩИТЕ
В ГЭК
Заместитель директора
_____М.А. Жак
_____2021 г.

ВЫПУСКНАЯ КВАЛИФИКАЦИОННАЯ РАБОТА
магистра

Влияние микроорганизмов арктических палеоэкосистем на морфофизиологические характеристики *Allium cepa*

06.04.01 Биология

Магистерская программа «Биологическая безопасность растений / Plant Biosecurity»

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Acronyms and Abbreviations

ACIA – Arctic climate impact assessment

AMAP – Arctic Monitoring and Assessment Programme

ALT – active layer thickness

AP – accessory pigments

c.u. – conventional unit

CALM – circumpolar active layer monitoring

CAs – chromosomal aberrations

CFU – colony-forming unit

CH_4 – methane

Chl a – *chlorophyll a*

Chl b – *chlorophyll b*

CMIP5 - coupled model intercomparison project phase 5

CO_2 – carbon dioxide

DNA – deoxyribonucleic acid

DOC – dissolved organic carbon

et al. – et alia

FPH – Fish protein hydrolysate

i.e. - id est

IPCS – International Program on Chemical Safety

LHC – light harvesting complex

MI – Mitotic index

MN – micronucleus

MTOC – microtubule organizing center

Na_2SO_4 – Sodium sulfate

NAs – nuclear alterations

NB – Nota bene

PhM – photosynthetic microorganisms

PSCW – permafrost sensitivity to climate warming

RNA – Ribonucleic acid

RNCIM – Russian National Collection of Industrial Microorganisms

ROS – Reactive oxygen species

SOM – soil organic matter

WHO – World Health Organization

WTPs – Water Treatment Plants

Introduction

The current state of the cryosphere is characterized by the presence of atmospheric, hydrospheric, ground and underground ice and dispersed soils that have passed into a frozen state. It is known that dispersed soils that have passed into a frozen state contain a certain amount of water and microorganisms, which have internal energy – a measure of mechanical, thermal and biochemical processes.

Permafrost is widespread in the northern hemisphere, and their age reaches hundreds of thousands and millions of years. They contain live microorganisms, which, due to the relatively high temperature of the environment (-2 ...- 8 ° C), do not contain ice, but they are in an immobilized state and, thus, have an age, apparently, close to the age of the permafrost. The long-term viability of relict microbial cells is obviously due to their defense mechanism against thermal destruction and radiation, free radicals and other damaging factors. Thus, the *Bacillus sp*. strain was isolated from permafrost, the age of that is about 3 million years old and also the 16S rDNA sequence was identified, and preliminary testing was carried out on *Drosophila melanogaster*, as well as on laboratory mice. In the experiments, stimulation of the immune system and improvement of the physical condition of the latter were observed, which, in combination with the possible age of microbial cells, makes it possible to consider relict microorganisms as promising objects of study for gerontologists.

It is estimated that the average air temperature on our planet has increased by more than 1 degree since the beginning of the industrial era. The process of global warming is many times faster in the Arctic. The first change that we can already make is the destruction of permafrost.

Consequently, the study of microorganisms from the Arctic paleoecosystems, their behavior in the external environment, and their use in the economy are promising and topical issues. Nevertheless, the question of the influence of the relict microbiota on modern biological objects and, first of all, on their cellular apparatus, remains relevant.

The Allium test is used to study the influence of microorganisms on the cytogenetic apparatus in biology and ecology. The test is recommended by the experts of the World Health Organization as a standard in cytogenetic monitoring of the environment.

Aim: to assess the influence of microorganisms of the genus Bacillus from the Arctic paleoecosystems on the morphophysiological characteristics of *Allium cepa*.

To achieve the aim, the following tasks were solved:

1) To study the influence of microorganisms of the genus Bacillus on the morphometric parameters of Allium cepa.
2) To reveal the influence of microorganisms of the genus Bacillus on the photosynthetic activity of Allium cepa.
3) To study the influence of microorganisms of the genus Bacillus on the cell differentiation of Allium cepa.

Hypothesis for defense:

1) All studied microorganisms of the genus *Bacillus* (strains 948P-1 TS, 312 TS, 875TS and 948P-1 TS, 2-06-TS1) from dispersed soils that have passed into a frozen state of paleoecosystems affect the morphometric parameters of Allium cepa, which says about their toxicity in varying degrees of manifestation.
2) Some bacteria of the genus *Bacillus* (strains 948P-1 TS and 312 TS) are actively involved in the complex chemical process of converting the visible light energy into the chemical bonds energy of organic substances with the participation of the photosynthetic pigment *chlorophyll a*, which is contained both in the reaction centers of the photosystem and in the light-harvesting complex.
3) The influence of the studied strains from frozen rocks is manifested at the cellular level, which is expressed both in the mitosis-stimulating effect (*Bacillus* strain 312 TS) and in restraining the proliferative ability of *Allium cepa* cells (*Bacillus* strain 875TS and 948P-1 TS).

The originality of the work:

1) For the first time, it was established that the toxic effect of *Bacillus* from dispersed soils, which passed into a frozen state in the Holocene period, manifests itself through the effect on the chemical process of converting the visible light energy into the chemical bonds energy of organic substances with the participation of the photosynthetic pigment *chlorophyll a*, which is contained both in reaction centers photosystems and in the light-collecting complex.
2) For the first time, the toxic effect of *Bacillus* from dispersed soils, which passed into a frozen state in the Holocene period, was established through the regulation of the reproductive mechanism of cells growth and development of the *Allium cepa* root system.

1 Literature review

1.1 Permafrost

Permafrost underlies 25% of the Northern Hemisphere and 20% of Earth's land surface Jansson, Taş, 2014; Schuur et al. 2015). Approximately 60% of the Russian territories are permafrost (Shcherbakova, Troshina, 2018). Depending on the geographic location, heat flow, and host deposit type, permafrost can extend several hundred meters into the Earth's surface (Gilichinsky, Rivkina, Bakermans et al. 2005). Permafrost is normally defined as subsurface material that remains continuously frozen for at least 2 years, underlying an annually thawed active layer. The near-surface layers of permafrost typically are Holocene in age, whereas much older and deeper permafrost deposits in Siberia, Yukon valley (Canada), and Antarctica have been reported to be continuously frozen since their formation in the Pleistocene and even Pliocene epochs (Liang, Li, Vetter et al. 2021)

Permafrost underlies the glaciers and soils of polar and alpine regions. Permafrost soils contain about 20–70% of ice and 1–7% of unfrozen water in the form of salt solutions with low water activity (a_W = 0.85) (Gilichinsky et al. 1993). Since life depends upon liquid water, permafrost is one of the most extreme environments on the Earth. In addition, permafrost is characterized by constant negative temperature, inaccessibility of nutrient supplies, and complete darkness. It is surprising to discover photoautotrophic microorganisms which need to use light energy to drive their metabolic reactions within permafrost sediments. Because of the difficulty of studying permafrost in an undisturbed form, interactions among the organisms that live in it are not yet well understood (Vishnivetskaya, 2009).

Permafrost plays an significant part in global climate change, in the balance of greenhouse gases, arctic environment ecosystems, and human activities in the Arctic Regions (ACIA, 2005; AMAP, 2011; Hinzman et al., 2005; Romanovsky et al., 2010a; Shakhova et al., 2010). Changes in climatic parameters, particularly the air temperature, snow depth, and duration of the warm period over the last 50 years have resulted in an increase of permafrost temperature and deepening of the

active layer in numerous locations across the Arctic. Several locations along the southern permafrost boundary have lost permafrost completely, whereas in others, upper portions of the permafrost have thawed to depths below that of seasonal freezing (Streletskiy, Vasiliev, Anisimov, 2015)

Western Siberia, lying mainly in a permafrost area, has been urbanized for two decades or thereabouts. Russia has had the most notable experience of urbanization within the circumpolar zone and the landscape bears the most consummate signs of this experience in the region.

Oil extraction constitutes the major determinant of this urbanization. The Ob' region is one of the biggest oil and gas basins in the world, and contributes up to 68 % of the national production of oil and 91 % of gas. Thanks to Western Siberia, Russia is the first gas producer in the world, and the second for oil. This exploitation started in 1964. During the Soviet era, the development of the oil/gas industry came with the spread of modern society northwards within the almost uninhabited taiga first, and the tundra later, as far as the Yamal peninsula along the coast of the Arctic Ocean (Vaguet, 2013).

Permafrost degradation poses serious impacts ranging from local changes in topographic and hydrologic conditions (Hinzman et al., 2005; Shiklomanov, Nelson, 2013; Shur et al., 2005; Woo, 2012), impacts on infrastructure and sustainability of northern communities (Anisimov, Reneva, 2006; Anisimov et al., 2010; Grebenets et al., 2012; Nelson et al., 2001), and changes to vegetation and wildlife dynamics (Jorgenson et al., 2013), and to global impacts on changes in greenhouse gas emissions (Tarnocai et al., 2009; Wisser et al., 2011; Zimov et al., 2006). Permafrost degradation is a spatially heterogeneous process, meaning that permafrost characteristics, such as temperature, thickness, or extent, may react differently in different climatic zones.

1.2 Climate warming

Climate warming is more pronounced in cold regions of high altitudes (Pepin et al. 2015) and high latitudes (Cohen et al. 2014; Huang et al. 2018), leading to widespread permafrost degradation (Romanovsky et al. 2010; Schuur et al. 2015;).

Permafrost thaw has far-reaching influences on environments and human society in cold regions. The downward moving of permafrost table and the drainage of thawed water can lead to the subside of ground surface, undermining the stability of human infrastructure, and changing landscape and hydrologic processes of surface and sub-surface (Liljedahl et al. 2016, O'Neill et al. 2019). As a result, plant and soil microbial activities and consequent ecosystem processes can also be affected significantly (Wrona et al. 2016, Pelletier et al. 2019). The interaction of these physical, chemical, and biochemical processes in turn affect permafrost thermal regime (Loranty et al. 2018). It remains largerly uncertain whether these interactions will accelerate or decelerate permafrost degradation caused by climate warming.

Increasing air temperature (T_a) contributes to permafrost degradation particularly. During 1960–2009, changes in T_a contributed to over 80% of the change in permafrost area (Mcguire et al. 2016). Many model studies have examined the permafrost sensitivity to climate warming (PSCW). For instance, Koven et al. (2013) calculated permafrost degradation in the 21st century using monthly predicted soil temperature with 18 CMIP5 models. They found there is a wide range of permafrost degradation rate, varying from 0.2 to 3.5 million km^2 $^{\circ}C^{-1}$. Mcguire et al. (2016) also compared results of 15 models and found that permafrost area decreasing rate shows great differences among these models from 0.2 to 58.8 $\times 10^3$ km^2 yr^{-1}. The difference in complexity of the physical processes involved in these models and the lack of some critical processes such as thermokarst and ground ice dynamics, are the main reasons for these inconsistencies among models.

Field observations also showed that the influence of warming on permafrost state is uneven around the world. Observations from the circumpolar active layer monitoring (CALM) program showed there is a great spatial heterogeneity of the changing rate of active layer thickness (ALT) (Luo et al. 2016a). ALT decreased slightly at five sites, while the other 12 sites experienced distinct increasing trends, varying from 0.05 cm a^{-1} at Site U1 (Barrow) to 8.4 cm a^{-1} at Site K0

(Kazakhstan). Even under the same climate in a small basin, Sun et al. (2019) concluded that permafrost is more sensitive to warming at low elevations and sunny slopes. The different responses of permafrost (or ALT) to warming indicate that the PSCW varies from sites to sites and regions to regions.

Predicting future carbon fluxes is complicated by the diversity of permafrost environments, ranging from high mountains, southern boreal forests, frozen peatlands and Pleistocene ice complexes (yedoma) up to several hundred meters deep, which vary widely in soil composition, soil organic matter (SOM) quality, hydrology and thermal regimes. Permafrost degradation can occur in many forms: thaw can progress downward from seasonally-thawed 'active layer' soils in warming climates or laterally because of changes in surface or groundwater flow paths (Grosse et al., 2011). Permafrost degradation can sometimes lead to dramatic changes in ecosystem structure and function, such as the formation of thermokarst bogs. Wildfires and other disturbances that remove vegetation and organic matter warm the ground, hastening permafrost degradation. The complexity of the Northern Arctic and Subarctic environments in terms of geology, vegetation, paleohistory and climate, suggests that understanding the microbial ecology in permafrost regions will require numerous studies throughout the Pan-Arctic (Graham, Wallenstein, Vishnivetskaya et al., 2011).

Considering that 25% of Earth's terrestrial surface is underlain by permafrost (ground that has been continuously frozen for at least 2 years), our understanding of the diversity of microbial life in this extreme habitat is surprisingly limited. Taking into account the total mass of perennially frozen sediment (up to several hundred meters deep), permafrost contains a huge amount of buried, ancient organic carbon (Tarnocai et al., 2009). In addition, permafrost is warming rapidly in response to global climate change (Romanovsky et al., 2010), potentially leading to widespread thaw and a larger, seasonally thawed soil active layer. This concern has prompted the question: will permafrost thawing lead to the release of massive amounts of carbon dioxide (CO_2) and methane (CH_4) into the atmosphere? This question can only be answered by understanding how the microbes residing in

permafrost will respond to thaw, through processes such as respiration, fermentation, methanogenesis and CH_4 oxidation (Schuur et al., 2009).

For most of human history, permafrost has been Earth's largest terrestrial carbon sink, trapping plant and animal material in its frozen layers for centuries. It currently stores about 1,600 billion tonnes of carbon – more than twice the amount in the atmosphere today. But thanks to rising temperatures, permafrost is fracturing and disappearing, leaving behind dramatic changes in the landscape.

Scientists are becoming increasingly worried that the thaw will lead to an epic feast for bacteria and archaea that produce carbon dioxide and methane. And although climate models have long accounted for the carbon-emitting capacity of Arctic permafrost and Arctic lakes, the microbial activity within has largely been treated as a black box, changing in sync with the physical properties of the ecosystem, including temperature and moisture (Brouillette, 2021).

In sunlit waters, photochemical alteration of dissolved organic carbon (DOC) impacts the microbial respiration of DOC to CO_2. This coupled photochemical and biological degradation of DOC is especially critical for carbon budgets in the Arctic, where thawing permafrost soils increase opportunities for DOC oxidation to CO_2 in surface waters, thereby reinforcing global warming. Sunlight significantly increases or decreases microbial respiration of DOC depending on whether photo-alteration produces or removes molecules that native microbial communities used prior to light exposure. Using high-resolution chemical and microbial approaches, we show that rates of DOC processing by microbes are likely governed by a combination of the abundance and lability of DOC exported from land to water and produced by photochemical processes, and the capacity and timescale that microbial communities have to adapt to metabolize photo-altered DOC (Ward, Nalven, Crump et al., 2017).

Furthermore, release of CO_2 from frozen soils could be the result of a release in trapped CO_2, or caused by basal microbial metabolism of bacteria, archaea and fungi. While knowing fine-scale microbial community structure may not be important in understanding overall ecosystem function, community structure can

explain process differences in intraseasonal variation and in experimental microcosms (Graham et al. 2014; Bier et al. 2015). Examining gene expression changes of microbes in frozen soils via metatranscriptomics and more targeted gene analysis enables an understanding of their response under various physical conditions. While 'meta-omics' studies provide clues to the active metabolic processes of microbial cells in subzero soils, the knowledge gleaned from these studies is still limited by poorly annotated or unannotated genes in the available databases. Microbial function and growth can be examined by more direct methods such as enzyme activity measurements and substrate incorporation (Nikrad, Kerkhof, Häggblom, 2016).

1.3 Permafrost microorganisms

The earth is inhabited by a vast quantity of diverse microorganisms. The relationship between them is determined by the fundamental drive of each species and strain to promote its own survival. For instance, some strains may live in tightly associated up to symbiotic relationships, thus heavily relying on their allies. Conversely, others may engage in ferocious competition, resulting in a relentless war to win over finite resources such as nutrients, light or territory (Bauer, Kainz, Carmona-Gutierrez, Madeo, 2018).

The Arctic is warming – fast. Microbes in the Arctic play pivotal roles in feedbacks that magnify the impacts of Arctic change. Understanding the genome evolution, diversity and dynamics of Arctic microbes can provide insights relevant for both fundamental microbiology and interdisciplinary Arctic science (Edwards, Cameron, Cook et al. 2020; Blaud, Lerch, Phoenix et al. 2015)

There is an increasing interest in studies of the microbial ecology of cold ecosystems, especially frozen ecosystems such as glacial habitats and frozen ground. Such an interest in understanding the role and behavior of microorganisms in these habitats is mainly related to the sensitivity of these environments to climate changes. The rapid development of emerging new technologies for the analysis and characterization of microorganisms, the incorporation of these technologies in studies of microbial life in cold habitats, and their

complementation with well-established traditional methods have enhanced our knowledge of these environments significantly, especially with regard to gene presence, functional gene potential, gene expression, and in situ identification of active microorganisms. Reduced sequencing costs and the availability of further genomes from different taxa will soon also give better insights into the evolution of cold adaptive characteristics and allow for the identification of taxa-specific cold adaptations (Goordial et al. 2016b; Millán-Aguiñaga, Soldatou, Brozio et al. 2019). Indeed, this should also result in an enhanced application of cold-adapted microorganisms and their cellular components in biotechnology (Collins and Margesin, 2019). More fundamental ecological research is still however urgently needed. The more we know, the better, earlier, and more appropriately we can react to microbial responses to climate changes in cold ecosystems. However, this is associated with a number of challenges and outstanding questions, as indicated below, and future studies should be focused on addressing these.

Microorganisms in cold ecosystems play a key ecological role in their natural habitats. Since these ecosystems are especially sensitive to climate changes, as indicated by the worldwide retreat of glaciers and ice sheets as well as permafrost thawing, an understanding of the role and potential of microbial life in these habitats has become crucial. Emerging technologies have added significantly to our knowledge of abundance, functional activity, and lifestyles of microbial communities in cold environments (Margesin, Collins, 2019; Petrov, Enoktaeva, Subbotin, 2015).

Permafrost also contains various other geomorphological structures including massive ground ice, cryopegs, and ice wedges (Steven et al. 2006) that harbor microbial populations. The description of the abundance, diversity, activity and distribution of microorganisms in permafrost and associated environments will be fundamental to our understanding of how microorganisms survive in permafrost, and how they will respond to future climatic warming and permafrost thawing. Lastly, permafrost microorganisms and microbial ecosystems are considered significant terrestrial analogs for similar organisms that may inhabit permafrost

environments that exist beyond the Earth, especially in light of the recent evidence of massive amounts of shallow ground ice near the surface of Mars (Gilichinsky 2002a; Gilichinsky et al. 2007).

Microorganisms in permafrost survive in an extreme environment characterized by constant subzero temperatures, low water and nutrient availability, and prolonged exposure to background radiation. Despite the harsh conditions, considerable abundance and diversity of microorganisms inhabit permafrost. Pioneering studies focusing on permafrost microbiology simply attempted to determine if permafrost harbored viable microorganisms. For example, microorganisms cultured from Canadian (James, Sutherland, 1942), Alaskan (Boyd and Boyd 1964) and Antarctic (Cameron, Morelli, 1974) permafrost samples were generally poorly characterized, and the studies were hampered by an inability to demonstrate that drilling and sample handling were performed aseptically. Recent developments using fluidless drilling (Gilichinsky et al. 1989; Khlebnikova et al. 1990; Juck et al. 2005), tracer microorganisms (Christner et al. 2005; Juck et al. 2005), nucleic acid stains (Christner et al. 2005) and fluorescent microspheres as microbial surrogates (Juck et al. 2005) have greatly improved our ability to recover intact permafrost samples and to monitor exogenous microbiological contamination of pristine permafrost samples.

Microbiology of permafrost and frozen soils is at its infancy. Until recently, permafrost has been addressed by microbiologists primarily as a natural depository of ancient forms of life. However, the recent finding of measurable winter gas emission to the atmosphere demonstrated that subzero microbial activity is an important driver of the observed global changes. This activity may significantly accelerate permafrost degradation under global warming; and this acceleration should be detected well before the visible signs of permafrost thawing appear (Panikov, 2009).

The study of existing collections of microorganisms isolated from permanently cold habitats, improved methods of sampling and enrichment will

increase the potential biotechnological applications of permafrost bacteria and archaea producing unique biomolecules (Shcherbakova, Troshina, 2018).

Mackelprang et al. (2011) observed that nitrogen fixation genes were abundant in permafrost despite the presence of biologically available nitrogen. After short-term thaw, nitrogen fixation genes decreased and denitrification genes increased (Mackelprang et al., 2011). These data have implications for predicting greenhouse gas emissions from Pleistocene permafrost that thaws due to climate warming. After thaw, temperature no longer protects organic matter making it susceptible to microbial degradation. Labile permafrost carbon may be protected by freezing conditions – even over geologic time – and thus particularly vulnerable to degradation during thaw.

1.4 Allium test method

The Allium test is often used in environmental studies to determine the effect of toxicants on plant objects, to study their toxic effects. At the same time, morphometric, macroscopic, microscopic indicators, photosynthesis, the level of genotoxicity, chromosomal aberrations are determined.

Cytogenetic studies are of great importance, in particular, the Allium test is now recognized all over the world, which is used in various studies (studies of water, bacteria, toxicants). The Allium test is less laborious, fast and inexpensive, and quite informative.

Allium cepa test is widely used to evaluate the effects of water pollution based on dividing cells since it is a very sensitive tool for prediction and recognition of environmental stresses (Farizan, Norfatimah, Aili et al. 2021).

Higher plants, including the *Allium cepa*, are indeed adequate organisms for studying toxicity because of their sensitivity, convenient in vivo test system for monitoring genotoxicity of environmental samples and biological agents since decades, preferred by International Program on Chemical Safety IPCS and World Health Organization WHO (Souza, Guedes, Fontanetti, 2016; Basu, Tripura, 2021).

The *Allium cepa* test is an easy, fast and very sensitive assay to detect environmental genotoxicity/antigenotoxicity of chemicals or natural plant products. This assay is related to the study of effect of chemicals at the genetic level which includes both microscopic and macroscopic parameters. Thus, this test provides an important method for the screening of environmental toxicity caused bytoxicants (Khanna, Sharma, 2013)

The *Allium cepa* test has been used by many researchers mainly as a bioindicator of environmental pollution (Bagatini et al. 2009; Leme, Marin-Morales, 2009), testing crude extracts of cyanobacteria (Laughinghouse, 2007), as well as to evaluate the genotoxic potential of medicinal plants (Camparoto et al. 2003; Knoll et al. 2006; Fachinetto et al. 2007; Lubini et al. 2008; Fachinetto et al. 2009; Fachinetto, Tedesco, 2009; Dalla Nora et al. 2010), because this test uses a model that is adequately sensitive to detect innumerous substances that cause chromosomal alterations (Tedesco, Laughinghouse IV, 2012).

The *Allium cepa* test is necessary since it is an excellent model *in vivo*, where the roots grow in direct contact with the substance of interest (i.e. effluent or complex medicinal mix being tested) enabling possible damage to the DNA of eukaryotes to be predicted. Therefore, the data can be extrapolated for all animal and plant biodiversity. The analysis of chromosomal alterations can be equal to the test of mutagenicity mainly for the detection of structural alterations; however, it is possible to find numerical chromosomal alterations, as well. The *Allium cepa* test is one of the few direct and unique methods for measuring damage in systems that are exposed to mutagens or potential carcinogens, and enables the evaluation of the effects of these damages through the observation of chromosomal alterations. For this undertaking, it is necessary that the sample remain in constant mitotic division, seeking to identify the toxic effects and alterations over a cell cycle; and the *Allium cepa* test is widely used for this purpose. It is excellent to use the *Allium cepa* test system since its principal unit is a vascular plant, making it an leading genetic model for evaluating environmental pollutants, detecting mutagens in various

environments and evaluating many genetic endpoints (point mutations to chromosomal alterations). *Allium cepa* is characteristic concerning its efficiency in detecting genetic damage. It was initiated by Levan, in 1938, for helping observe disturbances in the mitotic fuse due to colchicine action.

Relevant research by Fiskesjö (1985), demonstrated the significance of the *Allium cepa* test system for evaluating genotoxicity, showing that *Allium cepa* cells contain an oxidase enzyme system capable of metabolizing polycyclic hydrocarbonates. Even though other test systems have been shown to be sensitive for this detection, the results of the *Allium cepa* test should be considered as an alert for other organisms (i.e. bioindicators). Studies on sensibility and correlation among test systems are fundamental for a more accurate evaluation of environmental risks and for extrapolating the data to other groups of target organisms. A high sensitivity and good correlation with mammal tests and the same sensitivity as test systems of algae and human lymphocytes exist when compared with *Allium cepa*.

Furthermore, Rank and Nielsen (1993) performed adaptations for evaluating complex mixtures and Ma et al. (1995) adjusted the test for assessing mutagenicity and micronucleus analyses (MN) in F1 cells. Some researchers show certain restriction in regards to using plant test systems for evaluating certain classes of carcinogens, which require complex metabolization systems for the activation of its genotoxic action. However, Rank & Nielsen (1994) showed a correlation of 82% between the *A. cepa* test and the carcinogenicity test in rodents and concluded that the same was even more sensitive than the Ames test. Vincentini et al. (2001) reported that the *Allium cepa* test system is well accepted for the analysis of cytotoxicity and genotoxicity because the roots are in direct contact with the tested substance, allowing evaluation of different concentrations and times. The results by Camparoto et al. (2002) were similar when estimating the effects of infusions of *Maytenus ilicifolia* Mart. and *Bauhinia candicans* Benth with the test of *Allium cepa* and bone marrow cells of Wistar rats. Studies by Knoll et al.

(2006) used the plant model of *Allium cepa* to test several populations of *Pterocaulon polystachyum* DC (known as quitoco in the southern region of Brazil) at different concentrations and obtained precise results on cell division inhibition, whose effects were attributed to the presence of flavonoids in the infusions tested and the authors demonstrated that with an increase in the concentration of the infusions of *P. polystachyum*, lower mitotic index values were recorded.

Allium cepa test system signified general toxicity by growth retardation (root growth and root morphology), cytotoxicity by decreasing trends of mitotic index and elevation of cell apoptosis/necrosis, and clastogenic effects by producing chromosomal aberrations, such as stickiness, laggards c-mitosis and bridges (Jayawardena, Wickramasinghe, Udagama, 2021).

The use of medicinal plants for treating illnesses is an exploratory practice that is widely diffused in Brazil (Rosa, Ferreira, 2011) and due to this intense medicinal use, studies using bioindicators of toxicity and mutagenicity, such as the *in vivo* test of *Allium cepa* are necessary for contributing to their safe and efficient use. The plant test system of *Allium cepa* is as an ideal bioindicator for the first *screening* of genotoxicity, helping with studies that prevent damages to human health (Bagatini et al., 2007). Lubini et al. (2008) studied two species of the genus *Psychotria, Psychotria leiocarpa* Cham. & Schltdl. and *Psychotria myriantha* Mull. Arg. Using *Allium cepa* to test infusions at two concentrations of these species it was possible to verify the antiproliferative activity of the species *P. leiocarpa* and *P. myriantha*, and the results indicated that both species possessed the capacity to inhibit cell division and *P. myriantha* possessed genotoxic activity. We can indicate the use of *P. leiocarpa* in high concentrations as potentially therapeutic for inhibiting the cell cycle in eukaryotic organisms.

Studies by Souza et al. (2010) demonstrated that the species *Artemisia verlotorum* Lamotte (known in Brazil as infalivina) has antiproliferative and genotoxic capacity on the *Allium cepa* cell cycle. The authors found 32.2% of

the chromosomal alterations in the highest concentration of the tested aqueous extract, 48 g/L. It was found that with an increase in the aqueous extract of *A. verlotorum*, there was higher inhibition of cell division, and consequently lower values of MI.

Rank and Nielsen (2003) explain in their study the anaphase-telophase method of *Allium cepa* and made important considerations, such as genotoxic chemicals used for many purposes in manufacturing processes that can be found in environmental compartments such as air, water, soil, and sediments. The chemical can enter the environment from discharged wastewater, air emissions, during product consumption and from domestic and industrial waste sites. The important advantage of the *Allium cepa* test is that it is a "low budget" method, which besides being fast and easy to handle also gives reliable results (Tedesco, Laughinghouse IV, 2012).

Leme and Marin-Morales (2009) carried out an extensive review on the *Allium cepa* test and its use in environmental contamination, where they reported that vascular plants are recognized as excellent genetic models for detecting environmental mutagens and are frequently used in monitoring studies. *Allium cepa* is among the plant species used to evaluate DNA damages, chromosomal alterations and disturbances in the mitotic cycle. Furthermore, they reported that the test has been used to evaluate a large number of chemical agents, increasing its environmental application and it is a test characterized by being cheap. They also commented how the *Allium cepa* has advantages over other tests by the short preparation time for testing samples and although plants have low concentrations of oxidase enzymes, their results are consistent and can serve as a warning for other biological systems, since the target is DNA, which is common in all. In this review, they demonstrate that all types of effluents are also considered as complex mixtures and that the main results are of cytotoxicity, genotoxicity, and mutagenicity. In their review, Leme and Marin-Morales (2009) summed up figure from several scientists on a broad range of environmental contaminants and their genotoxic,

cytotoxic or mutagenic effects on *Allium cepa*, such as pesticides, herbicides, metals and heavy metals.

Hospital effluents can cause drastic problems to live organisms when not properly treated, and in developing countries, such as Brazil, environmental contamination by these effluents is common. This contamination is due to mutagenic compounds found within the effluent. Biomonitors (i.e. *Allium cepa*) can be used to alert the surrounding population of environmental contamination and genotoxic substances that have been released into the water. In a study by Bagatini et al. (2009), the *Allium cepa* test was used to evaluate the genotoxicity of a hospital effluent in Santa Maria, Rio Grande do Sul State, Brazil. During the study, chromosomal disruptions, anaphasic bridges, and micronuclei during telophase were observed, indicating environmental toxicity risk.

Laughinghouse (2007) studied the cytotoxic effects of crude extracts of cyanobacteria (blue-green algae), which can cause water pollution and the damages of direct toxin-producing strains can be tested using the *Allium cepa* test. The comparison of the toxic and genotoxic effects among species is fundamental for evaluating the biological risk of pollutants, particularly for compounds persistent in the environment (Bolognesi et al., 1999). The occurrence of blooms in continental waters used for human consumption causes essentially two issue for treatment. On one hand, being very small organisms, they can pass through filters at Water Treatment Plants (WTPs), reaching high densities in the distribution network. On the other, their toxins are not removed by the usual treatments (coagulation, flocculation, filtration, and disinfection), being even resistant to boiling. Besides these aspects, the traditional treatments can increase the risk of forming organochlorine compounds from the group of trihalomethanes, which act as carcinogenic compounds when water rich in organic matter is treated with chlorine. It is important when there is a higher density of toxic cyanobacteria, not to resort to using pre-chlorination, but to use activated charcoal filters and ozone, which

remove the toxins in the water more efficiently (Schmidt et al., 2002; Antoniou et al., 2005; Azevedo, 2006). The accidental ingestion of waters with high levels of toxins (acute ingestion) can cause intoxications, characterized by gastroenteritis with diarrhea, vomiting, nausea, abdominal cramps and fever, hepatitis with anorexia, asthenia and vomiting, or death (Jochimsen et al., 1998). The continued ingestion of low doses of toxins (chronic ingestion) can lead to chronic liver disorders. In fact, there should be me more studies showing what are the risks of chronic exposure are since there are many factors that can lead a person to be subjected to low doses of these toxins. These situations can also be triggered by the ingestion of mollusks, as filter feeders that accumulate non-lethal doses of these products in their tissues, which are passed along the food chain, finally to humans (Kuiper-Goodman et al., 1999; Azevedo, 2006; Carvalho, 2006).

The decrease in water quality, especially of environments used for public water supply, irrigation and recreation, is of concern. The increase in eutrophication in these systems by higher nutrient loads (especially phosphorus and nitrogen) have favored the predominance of toxigenic cyanobacteria threatening human and animal health, aside from elevating the cost of water treatment. Thus, as a result of eutrophication, many countries, have suffered from an increase of toxic blooms, which is a severe problem to public health (Werner, Laughinghouse IV, 2006).

Allium cepa test is an excellent bioindicator of chromosomal alterations that serve as an alert for the population that uses medicinal teas indiscriminately, and that its constant use in the analysis of the treatment of industrial and hospital effluents is extremely adequate. Currently, due to major concern with environmental pollution, the *Allium cepa* test has occupied an important place for the prevention and prediction of environmental impact that will be caused by the use and disposal of substances including drugs and herbicides (Bruna de Campos Ventura-Camargo, Dejanira de Franceschi de Angelis, Maria Aparecida Marin-Morales, 2016).

Although the test is merely a first assessment of genotoxicity, it always shows important scientific discoveries, and new adaptations of the test might reveal innumerous possibilities of its use, avoiding the use of animals for testing. More increments and analysis, as the sophistication of the method progresses, will lead us to get the most use for the benefit of the planet (Tedesco, Laughinghouse IV, 2012).

Allium cepa test shows higher sensitivity for reduction in mitotic index, metaphase clumping as abundant chromosomal aberrations indicative of toxicity and cell death, and appearance of micronuclei in F_1 cells. (Basu, Tripura, 2021).

1.5 Photosynthesis and chlorophyll

Photosynthetic microorganisms have a wide variety of secondary pigments, the most characteristic of which are spirilloxanthin, okenone, spheroidene in anoxigenic phototrophic bacteria, phycoerythrin and phycocyanin in cyano-bacteria and red algae, *chlorophylls b* and *c*, as well as a variety of eukaryotic xanthophylls. Spectrophotometric analysis of secondary pigments provides important information in environmental studies, such as describing the composition of communities using chemotaxonomy, establishing the physiological status of photosynthetic microorganisms, predicting and assessing the mass development of phototrophs in water bodies (Namsaraev, Sergeeva, 2020).

Accessory pigments (AP) (carotenoids, phycobiliproteins, and a number of chlorophylls), as a rule, perform a light-harvesting or light-protecting function in the cells of microorganisms. Photosynthetic microorganisms (PhM) possess a wide variety of auxiliary pigments, the most characteristic of which are spirilloxanthin, okenone; spheroidene in anoxygenic phototrophic bacteria; phycoerythrin and phycocyanin in cyanobacteria and red algae; *chlorophylls b* and *c*, as well as various xanthophilic microorganisms of eukaryoles (Takaichi, 2011).

The intensity of plant photosynthesis also plays an important role in achieving high plant productivity. A decrease in the rate of photosynthesis occurs

due to the main components of the chloroplast, which can directly limit the photosynthetic potential of a plant (Muhamad, Chozin, Lubis et al 2014).

Chlorophyll is one of the main chloroplast components, and the pigments of *chlorophyll a* and *b* are involved in photosynthesis and affect the growth and development of plants (Taiz, Zeiger, 2006).

The amount of *chlorophyll a* in green leaves is 20-40% higher than the amount of *chlorophyll b* and plays a key role in photosynthesis. Carotenoids, in its turn, absorb the blue and violet rays of the sun and transfer them to *chlorophyll a*. It also protects chlorophylls from bright (strong) light (Beknazarov, 2009).

Chlorophylls and carotenoids are the main photosynthetic pigments that provide absorption of light quanta and photosensitization in plants (Kislichenko, Protska, Zhuravel, 2019).

Chlorophyll a (Chl a) is a universal pigment that converts light energy into charge separation energy, i.e., the first stage of energy conversion in the process of oxygenic photosynthesis. *Chlorophyll b* (Chl b) is a special chlorophyll of light-harvesting antenna complexes, which contributes to an increase in light collection at low light and dissipation of excess absorbed energy at high light (Tyutereva, Ivanova, Voitsekhovskaya, 2014). Carotenoids perform light-harvesting and light-protective functions, removing excess excitation energy (Demmig-Adams, Gilmore, Adams, 1996). The amount of chlorophyll is a factor that determines the intensity of photosynthesis and biological productivity of plants (Lakhanov, Kolomeichenko, Fesenko, 2004).

The studies carried out by now have shown the stability of the qualitative composition of pigments in higher plants. These are two types of chlorophylls and six main types of carotenoids (Young, Phillip, Savill, 1997). Each of these forms of pigments has a specific role and place in the plant's photosynthetic system (Esteban, Barrutia, Artetxe et al. 2015). At present, the structure and functioning of the pigment complex are well studied, which can serve as a basis for modeling various aspects of photosynthesis (Antal, Kovalenko, Rubin et al. 2013). Since pigments are integrated into chloroplast membranes and they are associated with

proteins, their quantitative content and ratio in the leaf may reflect the adaptation features of the photosynthetic apparatus as a whole and provide its functional diagnostics (Ivanov, Ronzhina, Yudina et al. 2020).

2 Materials and methods

The object of the study was the roots of onion seedlings *Allium cepa*, grade Stuttgarten Riesen. The sample of bulbs was homogeneous both in the control and in the experimental variants of the experiment. The average weight of the seed is 5-7 g, with a diameter of 1.5-2 cm.

Petri dishes, measuring cylinders (50 ml), 20 ml penicillin vials, slides and coverslips, measuring pipettes (10.0), an alcohol lamp, a Zeiss Primo Star microscope, and a Zeiss Axiocam 105 color digital camera were used as equipment and materials.

In order to study the effect of microorganisms from permafrost on the cytogenetic apparatus, samples were taken from wells in the region Tarko-Sale town (north of Western Siberia) and exposed the Upper and Middle Pleistocene sediments of the IV marine terrace (mIII1, mII2-4), repeatedly freezing and thawing and overlapping Holocene peat (bIV). The modern epicryogenic strata was formed after the thermal optimum of the Holocene about 5 thousand years ago. We used bacteria of the genus *Bacillus* (strains 2-06-TS1, 875TS, 948P-1 TS, 1257 TS and 312 TS) registered in the Russian National Collection of Industrial Microorganisms (registration number RNCIM, respectively: B-12402, B-12242, B-12245, B-12243 and B-12244). Cultivation of pure cultured strains of bacteria operating in slant nutrient agar (FPH – agar, Obolensk. TU 9398-020-780956-2006) in a thermostat at t = 36 ° C for 48 hours. Then, microorganisms were washed out from each tube with 5 ml of distilled water. The concentration of microorganisms was measured by the Koch method according to the number of colony-forming unit (CFU) on agar nutrient medium in Petri dishes. After determining the number of bacterial cells in the initial suspension, the density of the cultures was brought to a working concentration of $1x10^9$ microbial cells in 1 ml of distilled water (MC / ml).

The sample in each experimental group consisted of 3 bulbs. Onions were grown at room temperature in appropriate solutions for 7 days. The roots of seedlings grown on distilled water served as control 1. The roots of seedlings

grown on nutrient medium No. 1 of the FPH served as control 2. All roots of different lengths were used in the experiments. A 1.5–2.0 mm segment was cut from the root tip, and the cell fixation procedure (Clark's fixative) was carried out for two days. Then the material was washed twice from the fixative in 70% alcohol, and placed in containers with 70% alcohol for long-term storage.

For cytogenetic studies, the roots were dyed with a 2% acetoorcein solution. The roots were washed from alcohol in water in Petri dishes. The material was transferred into penicillin vials, which were 2/3 filled with dye. The vial was covered with a glass slide and heated over a flame of alcohols until a secret boil (fogging of the cover glass). The vial with the material was left for some time to stain the chromosomes (1 day). After that, preparations were prepared for microscopy.

Temporary squashed preparations of root meristems were prepared. For this, the tip of a meristem 2-3 mm long was cut off from the stained root with a blade, placed on a glass slide in a drop of 45% acetic acid, covered with a cover glass and gently crushed with a match until a monolayer of cells was obtained.

The prepared squashed preparations were analyzed under a Zeiss Primo Star microscope using Plan-Achromat 40x / 0.65 pp: 0.48 mm objectives. Image fixation was obtained using a Zeiss Axiocam 105 color digital camera. On the preparations we examined small, rounded-square cells with well-stained nuclei and intact cell walls. There were from 6468 to 8725 cells at all stages of the life cycle. During cytological analysis we calculated the mitotic index and recorded chromosomal aberrations: micronuclei, bridges, fragments, lagging and overshooting of chromosomes. Cells with an indeterminate type of aberration were recorded as cells with pathologies of the nuclear apparatus. The mitotic index and the frequency of occurrence of aberrant cells were determined according to B.A. Iwalokun (2011). We used the method of ana-telophase analysis of the frequency of chromosomal aberrations. The mitotic index was calculated by the formula:

MI = (P+M+A+T)/N * 100%

where (P+M+A+T) – the sum of cells at the stage of prophase, metaphase, ana- and telophase, and N is the total number of analyzed cells.

Morphophysiological evaluation of *Allium cepa* was carried out. The following parameters were investigated: the shape and length of the roots for each bulb. At the end of the experiment, the roots were cut under the base and the length of each root was measured, and the average value for each bulb was calculated. Then, the average value of the root length was established for the entire sample of bulbs. When calculating the parameter of root growth, the average root length for each bulb was calculated in the experimental and control series of experiments. Then the average value of the length was calculated for both the experimental series and the control. The changes in root length in the Allium test are indicative of toxicity. This is a very sensitive indicator, which is easily recorded visually and does not require any special reagents and equipment, correlates well with microscopic parameters and therefore is proposed as a short-term screening test. If there is a significant inhibition of root growth in comparison with the control, then the toxic effect of the influencing factor is noted. In the case of significant root growth, it can be said of a stimulating effect.

The index of the ratio of root length to feather length was calculated.

Photosynthesis pigments concentration estimation in the green part of *Allium cepa* seedlings by absorption spectrophotometry.

Ethanol 96% was used as a polar solvent, weighed in approximately 100 mg. The green part of each plant was ground in a porcelain mortar with the addition of a small amount of anhydrous sodium sulfate (Na_2SO_4) until a uniform colored powder was formed. Using a metal putty knife, the powder from the mortar was placed in a test tube, and 8 ml of 96% ethanol was added. The tube was shaken and the sediment was allowed to settle. The supernatant liquid was removed with a mechanical dispenser, carefully, without touching the sediment, and placed into spectrophotometer cuvettes with an absorbing layer 10 mm thick. The optical density of the extract was recorded at a wavelength of 665, 649 and 440 nm.

Optical density was determined using a PE-5400UF spectrophotometer. Control - pure solvent (96% alcohol), lcuvette = 2 cm.

The concentration of *chlorophyll a* and *b* in the volume of the spectrophotometer cuvette was calculated using the formulas (Wintermans, Mots):

$Chl.a = 13,70*A_{665} - 5,76* A_{649}$;

$Chl.b = 25,80*A_{649} - 7,60* A_{665}$;

$Chl._{a+b} = 6,10*A_{665} - 20,04* A_{649}$;

where A – optical density at a given wavelength

The concentration of carotenoids in the total extract of pigments was calculated using the formula (D. Wettstein):

$$Car. = 4,69* A_{440} – 0,27* Chl._{a+b};$$

Chl.a – *chlorophyll a* concentration, (mg/l)

Chl.b – *chlorophyll b* concentration, (mg/l)

Car. – the concentration of carotenoids in the total extract, (mg/l).

Based on the obtained concentrations, the content of photosynthetic pigments in the test material was determined:

$$F=(C*V) / (1000*m_i);$$

F – pigment content in plant material, (mg/l)

V – volume of the studied extract, (ml)

m_i – sample weight, (g)

C – pigment concentration in the volume of the studied extract, (mg/l)

The obtained values of the pigment content were recalculated per 100 g of plant material (Gavrilenko, Zhigatova, Ermakov).

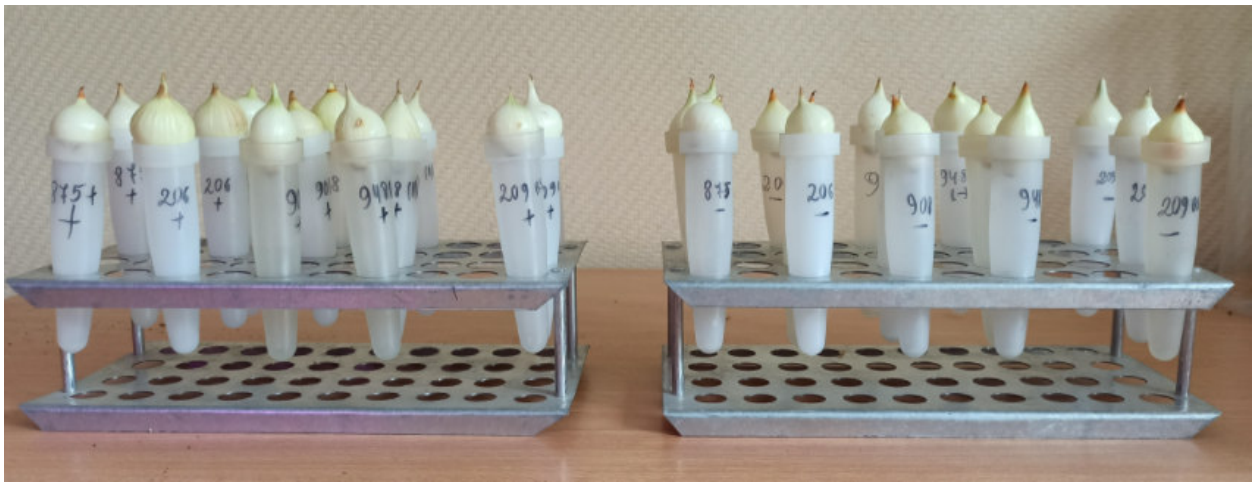
We subject the studied qualitative and quantitative characteristics to statistical processing. We used the integrated software package "SPSS Statistics 21 for Windows". To select the type of criteria (parametric or nonparametric) analysis, we studied the nature of the distribution of the studied features. For a normal (Gaussian) distribution, the following statistical parameters were used: mean (arithmetic mean, median, mode), variance and its derivative (standard deviation), which can also serve as additional criteria characterizing the

distribution of the studied features. We made a comparison of the statistical significance of differences or similarities between the statistical characteristics obtained in the study of the compared samples (according to the Student's t-test). The standard error of the arithmetic mean for evaluating the statistical significance between the mean values was calculated. We calculated the standard error of the arithmetic mean value for evaluating the statistical significance of arithmetic mean value.

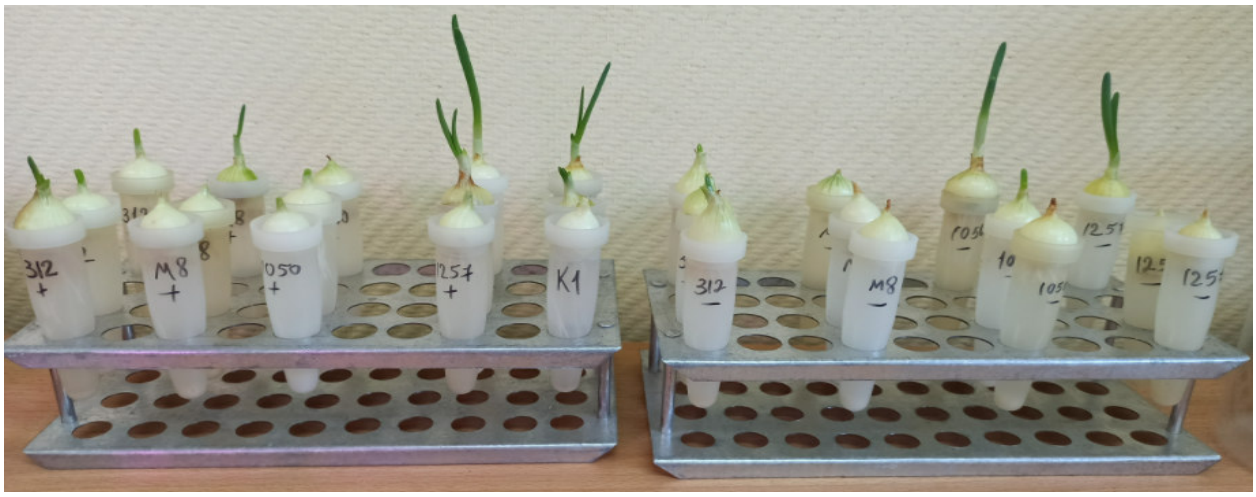
We used nonparametric methods for comparing two samples with the calculation of the Wilcoxon signed-rank test, the Spearman's ranks correlation coefficient in the lack of a normal distribution.

3 Results and Discussion

Studies were carried out using the *Allium cepa* onion, grade Stuttgarten Riesen in order to study the toxicity of microorganisms from permafrost and the processes caused by their vital activity, on modern living objects (Fig. 1).



A



B

Fig. 1. - *Allium cepa* onion, grade Stuttgarten Riesen: A – 1 day; B – 5 days [Image by the author]

Analysis of the growth and development of onions when treated with strains of microorganisms from permafrost rock showed that strains of microorganisms delay the development of the root system of onions in comparison with neutral control 1 (Tables 1 and 2).

Table 1

The characteristics of the influence of microorganisms on the development of the root system of *Allium cepa*, cm [Compiled by the author]

	Control 1	Control 2	8-75	2-06	9-48	3-12	12-57
N	93	59	74	81	45	75	63
Mean	4.87	0.40*	1.34*/#	1.66*/#	0.35*	3.36*	2.51*
Standard error of the mean	0.19	0.03	0.14	0.09	0.03	0.10	0.16
Standard deviation	1.80	0.24	1.23	0.83	0.18	0.89	1.31
Minimum	0.80	0.10	0.20	0.40	0.10	0.90	0.20
Maximum	7.20	1.20	5.50	3.30	0.80	4.80	4.90

NB: * - significance of difference with control 1 ($p < 0.001$), # - significance of difference with control 1 ($p < 0.001$).

At the same time, this is most observed in relation to *Bacillus*, strains 2-06-TS1, 875TS, 948P-1 TS (1.66 $\pm$ 0.09; 1.34 $\pm$ 0.14 and 0.35 $\pm$ 0.03 cm, respectively). Table 2 also shows that under the influence of the *Bacillus* 948P-1 TS strain, the number of roots was 2 times less.

When compared with control 2, we found a significant (in 3.35 – 8.4 times) stimulation of the growth of onion root system under the influence of the microbiota of permafrost rocks, except for *Bacillus* strain 948P-1 TS (Figures 2 and 3).

Fig. 2. - Onion root system *Allium cepa,* grade Stuttgarten Riesen [Image by the author]

It is known that the ratio of the average length of the ground part to the average length of the root system is the coefficient of seedling symmetry (Zhirnova D.F., Pantyukhov I.V., Goldman I.V., 2008), the optimal number is considered to be 0,8-1,1.

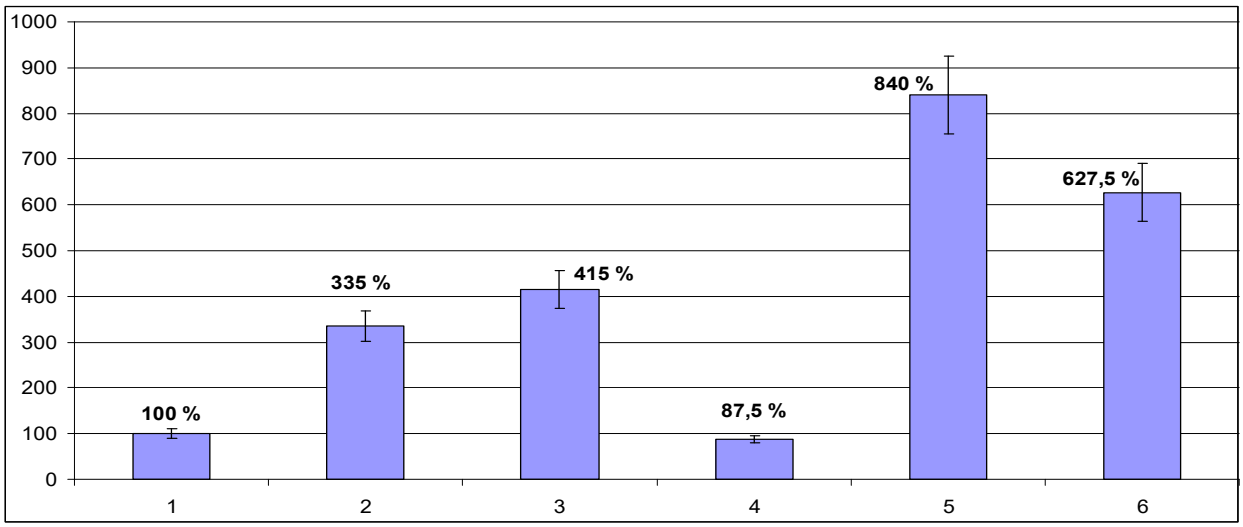


Fig. 3. - Characteristics of the influence of microorganisms on the development of the root system of *Allium cepa* in relation to control 2 (NB: 1 - control 2; 2 - strain 875TS; 3 - strain 2-06-TS1; 4 - strain 948P-1TS; 5 - strain 312TS ; 6 - strain 1257 TS) [Image by the author]

Values above or below this limit indicate that one of the vegetative organs of the seedling significantly prevails over the other and, as a result, the conditions in which the plant develops are not comfortable for it. In the variant with the use of the 2-06-TS1 strain, the symmetry coefficient is at the border of the optimum (Table 2, Figure 4).

Table 2

Influence of microorganisms on vegetative parameters of *Allium cepa*, cm [Compiled by the author].

	Control 1	Control 2	8-75	2-06	9-48	3-12	12-57
Number of roots, %	100	63.44 ± 6.27 **	79.57 ± 4.69 **/#	87.1 + 3.72 */##	48.39 ± 7.45 **/#	80.64 ± 4.56 **/#	67.74 ± 5.89 **/#
The ratio of roots to feathers, c.u.	0.59 ± 0.2	2.01 ± 1.25 *	0.32 ± 0.08 */#	1.33 + 0.73 */#	0.4 ± 0.21 */#	4.09 ± 0.62 **/#	4.06 ± 3.37 */#

NB: * - significance of difference with control 1 (* - $p < 0.01$; ** - $p < 0.001$), # - significance of difference with control 2 (# - $p < 0.01$; ## - $p < 0.001$).

Fig. 4. - Vegetative indicators of *Allium cepa* [Image by the author]

Analyzing leaf parameters, it's possible judge the vegetative activity of onion seedlings (table 3)

Table 3

Influence of microorganisms on shoots of *Allium cepa*, cm [Compiled by the author]

	Control 1	Control 2	8-75	2-06	9-48	3-12	12-57
N	10	5	10	9	7	7	7
Mean	8.43	1.28 **	3.62 */#	2.52 **	1.57 **	0.83 ***	3.16 *
Standard error of the mean	1.76	0.66	0.87	0.71	0.62	0.22	1.02
Standard deviation	5.56	1.48	2.74	2.13	1.64	0.59	2.69
Minimum	1.10	0.10	0.30	0.20	0.20	0.20	0.00
Maximum	17.30	3.50	7.60	6.50	4.50	1.90	6.70

NB: * - significance of difference with control 1 (* - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$), # - significance of difference with control 2 (# - $p < 0.05$; ## - $p < 0.01$; ## - $p < 0.001$).

It was found that the studied strains have a negative effect on onion shoots. This is especially evident in relation to *Bacillus* strains 312TS, 2-06-TS1 and 948P-1TS (0.83 ± 0.22; 2.52 ± 0.71 and 1.57 ± 0.62, respectively). When compared with control 2, it was established a significant (in 2 – 2.8 times) stimulation of the growth of the leaf part (feathers) of onion under the influence of the microbiota of permafrost rocks, except for *Bacillus* strains 948P-1 TS and 312TS (Figure 5). As was shown earlier, the ratio of the average length of the ground part to the average length of the root system of onions (the coefficient of symmetry of seedlings) under the influence of *Bacillus* strain 948P-1 TS was in 2 times less than the standard indicators (0.4 ± 0.21).

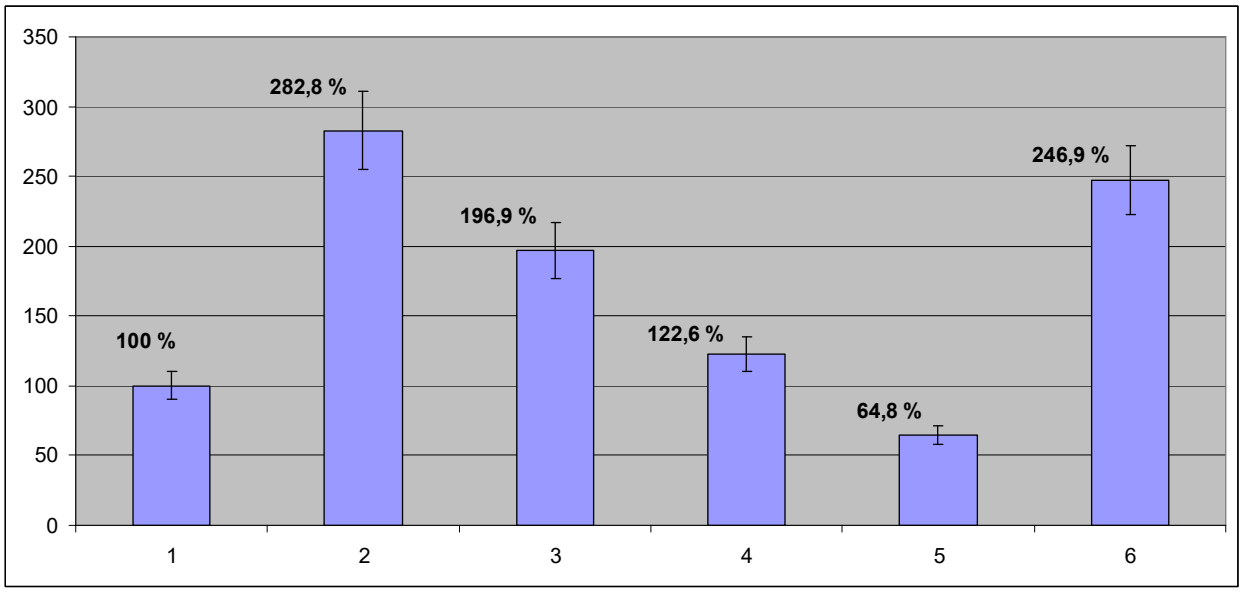


Fig. 5. - Characteristics of the influence of microorganisms on the shoots development of *Allium cepa* in relation to control 2 (Note: 1 - control 2; 2 - strain 875TS; 3 - strain 2-06-TS1; 4 - strain 948P-1TS; 5 - strain 312TS; 6 - strain 1257 TS) [Image by the author]

Analysis of variance was taken to search for the effect of the microbiota of frozen soils on the morpho-physiological parameters of onions by studying the significance of differences in the mean values (Table 4).

When analyzing the data obtained, we proceeded from the fact that if the actual Fisher ratio (F) was greater than the critical one, then the mean grades of gradation differ from each other and the investigated independent factor(s) significantly affects the change in the dependent data with a significance level of α

<0.05. If less, then the average grades of gradation do not differ from each other and the factor(s) does not have a significant impact.

Table 4

Results of dispersion and correlation analyses of the influence of the microbiota of frozen soils on the morpho-physiological parameters of *Allium cepa*, c.u. [Compiled by the author]

Independent factors / Dependent factors	8-75		2-06		9-48		3-12		12-57	
	F / α	r / p	F / α	r / p	F / α	r / p	F / α	r / p	F / α	r / p
Root length	11.39 0.004	-0.65 0.004	2.73 0.118	-0.38 0.118	31.59 <0.001	-0.82 <0.001	0.89 0.359	-0.23 0.359	4.19 0.057	-0.46 0.057
Number of roots	1.45 0.246	-0.29 0.246	0.35 0.562	-0.15 0.562	10.65 0.005	-0.63 0.005	1.27 0.277	-0.27 0.277	3.39 0.084	-0.42 0.084
Feather length	6.01 0.026	-0.52 0.026	13.82 0.002	-0.68 0.002	17.49 0.001	-0.72 0.001	28.33 <0.001	-0.80 <0.001	10.74 0.005	-0.63 0.005
Number of feathers	0.00 1.0	0.00 1.0	0.271 0.61	-0.13 0.61	3.57 0.077	-0.43 0.077	3.57 0.077	-0.43 0.077	2.75 0.117	-0.38 0.117

NB: F - Fisher's correlation coefficient; α - the significance level of the Fisher coefficient; r - the correlation coefficient; p - level of significance of the correlation coefficient.

Thus, among the analyzed microorganisms of dispersed soils that have passed into a frozen state, all bacteria have a significant negative effect to one degree or another on the morpho-physiological parameters of *Allium cepa*. Thus, *Bacillus* strain 875TS with a significance level of α = 0.004 causes a retardation of root growth (F = 11.39) and, to a lesser extent, shoots of *Allium cepa* (F = 6.01 at α = 0.026). At the same time, the *Bacillus* strain 2-06-TS1 also significantly retards the growth of shoots of *Allium cepa* (F = 13.82 at α = 0.002). The most pronounced negative effect on the analyzed morphophysiological parameters is exerted by *Bacillus* strain 948P-1 TS. Under its influence, the length (F = 31.59 at α <0.001) and the number of roots (F = 10.65 at α = 0.005) decrease, as well as the length of the shoots (F = 17.49 at α = 0.001). It was also found that with a significance level of α <0.001 and a significance level of α <0.005, there is a significant delay in the development of shoots in length when exposed to *Bacillus* strains 312 TS and 1257 TS (F = 28.33 and F = 10.74, respectively).

Modern research makes it possible to use the ratio of photosynthetic pigments to analyze the resistance of plants to unfavorable environmental conditions (Davison P.A., Hunter C. N., Horton P., 2002). *Chlorophyll a* is contained both in the reaction centers of the photosystem and in the light-harvesting complex (LHC), while *chlorophyll b* is considered as an additional pigment located mainly in the LHC (Figure 6).

Fig. 6. - Sample preparation for researching the photosynthetic activity of the *Allium cepa* onion [Image by the author]

Table 5 shows the obtained results of the influence of the microbiota of dispersed aqueous soils, which have passed into a frozen state, on the photosynthetic activity of *Allium cepa* onions.

It was found that practically all researched *Bacillus* strains produce photosynthetic pigments (*chlorophyll a* and *b*) except for *Bacillus* strain 875TS, which indicates a decrease in plant resistance to the influence of permafrost microbiota. In this case, photosynthesis suffers both in the reaction centers of the photosystem and in the LHC.

Table 5

Influence of microorganisms on the photosynthetic activity of *Allium cepa*, cm [Compiled by the author]

	chlorophyll α	chlorophyll β	chlorophyll α + β	carotenoids	coefficients	
					α/β	(α+β)/ carotenoids
Control 1	4.13 ± 0.16	13.48 ± 0.69	17.61 ± 0.6	8.13±0.42	0.32±0.02	2.18 ± 0.03
Control 2	3.02 ± 0.1 ***	7.22 ± 0.31 ***	10.25± 0.24 ***	4.55±0.17 ***	0.43±0.03 *	2.26 ± 0.03 *
8-75	4.16 ± 0.39	13.09 ± 1.0 ###	17.25± 1.37 ###	7.79±0.66 ###	0.32±0.01 ##	2.22 ± 0.04
2-06	3.84 ± 0.21 ***/#	12.08 ± 0.05 */###	15.92± 0.24 */###	7.14±0.03 */##	0.32±0.02 ##	2.23 ± 0.04
9-48	2.98 ± 0.42 *	7.29 ± 0.82 ***	10.27± 1.21 ***	4.43±0.44 ***	0.4 ± 0.03	2.29 ± 0.05 **
3-12	2.97 ± 0.14 **	8.65 ± 0.26 ***	11.62± 0.28 ***/##	5.66±0.06 ***/###	0.35±0.02 #	2.05 ± 0.06 ##
12-57	3.64 ± 0.21 ***/#	11.52 ± 0.54 */##	15.16± 0.75 */###	6.88±0.3 **/###	0.32±0.04 ##	2.2 ± 0.01

NB: * - significance of difference with control 1 (* - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$), # - significance of difference with control 2 (# - $p < 0.05$; ## - $p < 0.01$; ## - $p < 0.001$).

Figure 7 shows the characteristics of the effect of microorganisms on onion photosynthesis in relation to control 2.

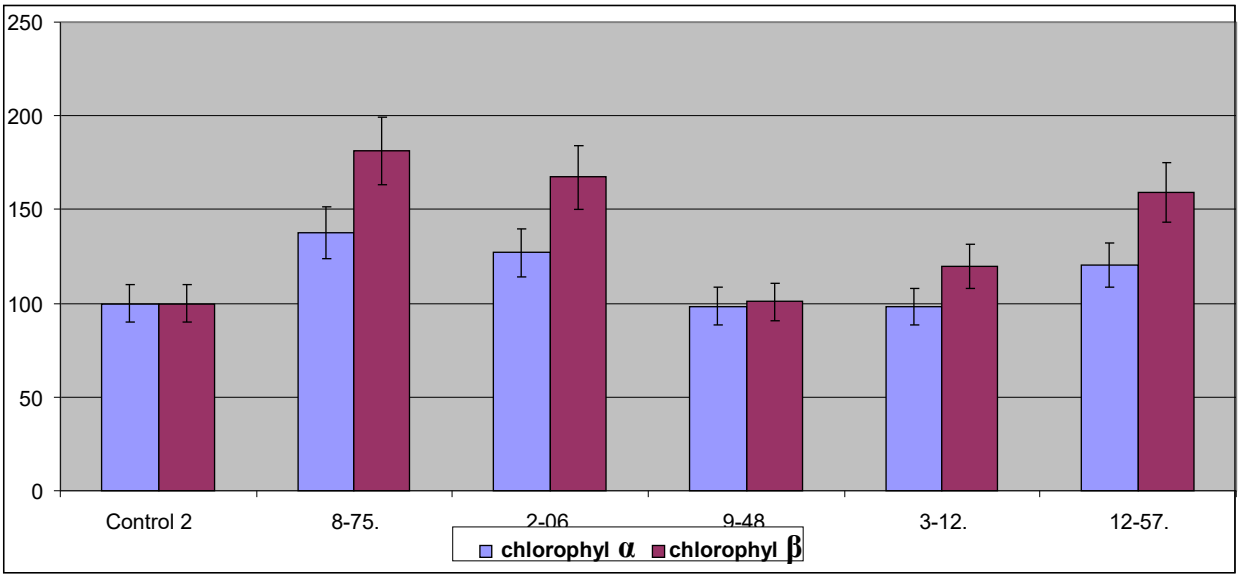


Figure 7. - Characteristics of the effect of microorganisms on photosynthesis of *Allium cepa* in relation to control 2 [Image by the author]

It was found that *Bacillus* strains 2-06-TS1 and 1257 TS with a significant difference $p < 0.05$ stimulate the photosynthetic activity of onions in the reaction centers of the photosystem and in the LHC.

When analyzing the *chlorophyll a/b* ratio, it wasn't found significant differences with respect to the neutral control (control 1), which indicates the balance of the photosynthetic system and the level of plant adaptability to unfavorable environmental conditions.

A low value of the *chlorophyll a/b* ratio indicates an increase in the content of chlorophyll *b* and the "switching on" of its synthesis to increase plant resistance and an increase in the amount of LHC photosystems, being an auxiliary pigment of photosynthesis, increases in concentration only in a state of ecological trouble, an increase in its concentration may indicate a decrease plant sensitivity to bright light.

Based on the literature data, a decrease in the *chlorophyll a/b* ratio may indicate an increase in plant productivity and plant resistance to unfavorable environmental conditions (Derendovskaya A., Zhosan S., 2008; Ryktor I.A., Zubkova Yu.N., Butyugin A. V., Pogromskaya Ya.A., 2012). It is known that carotenoids are low-molecular antioxidants and are able to quench the formation of reactive oxygen species (ROS), thereby protecting lipid fatty acids from peroxidation and allowing the integrity of membranes to be preserved. Carotenoids also have an antenna function and are part of the LHC (Maslova T.G., Markovskaya E.F., Slemnev N.N., 2020). They transfer electrons in an excited state to photochemical reaction centers, absorbing light in the spectral region in which chlorophyll cannot absorb, and protect pigments and lipid unsaturated fatty acids from oxidative damage by eliminating excess reactive oxygen species. The increased content of carotenoids relative to chlorophylls may indicate an increase in the level of stress resistance of plants.

The content of carotenoids was the lowest in variants with *Bacillus* strains, strains 948P-1TS and 312TS; in all the others, the value was also reduced except for *Bacillus* strain 875TS. Therefore, an indicator of plant stress resistance can be

the ratio of the sum of the amount of *chlorophyll a* and *chlorophyll b* to the amount of carotenoids, the highest value of which among the obtained is the variant with the use of the 948P-1TS strain compared to control 1 (2.29 ± 0.05 and 2.18 ± 0.03, respectively at $p < 0.001$).

Analysis of variance was applied to search for the effect of the microbiota of frozen soils on the photosynthetic activity of onions by studying the significance of differences in mean values (Table 6).

Table 6

Results of dispersion and correlation analyses of the influence of the microbiota of frozen soils on the photosynthetic activity of *Allium cepa*, c.u. [Compiled by the author]

Independent factors / Dependent factors	8-75		2-06		9-48		3-12		12-57	
	F / α	r / p	F / α	r / p	F / α	r / p	F / α	r / p	F / α	r / p
chlorophyll α	0.27 0.61	-0.13 0.61	0.59 0.455	-0.19 0.455	18.58 0.001	-0.73 0.001	10.29 0.005	-0.63 0.005	0.55 0.47	-0.18 0.47
chlorophyll β	0.21 0.65	-0.12 0.65	0.19 0.666	-0.11 0.666	0.92 0.352	-0.23 0.352	2.04 0.172	-0.34 0.172	0.61 0.45	-0.19 0.45
chlorophyll α+β	0.39 0.54	-0.15 0.54	0.61 0.45	-0.19 0.45	7.59 0.014	-0.57 0.014	10.29 0.005	-0.63 0.005	1.07 0.317	-0.25 0.317
carotenoids	0.08 0.78	-0.07 0.78	1.25 0.28	-0.27 0.28	1.32 0.268	0.28 0.268	0.07 0.792	0.07 0.792	0.49 0.496	-0.17 0.496
α/β	0.07 0.79	-0.07 0.79	0.14 0.72	-0.09 0.716	3.15 0.095	-0.41 0.095	1.05 0.321	-0.25 0.321	0.23 0.638	-0.12 0.638
(α+β)/ carotenoids	0.10 0.76	-0.08 0.76	3.24 0.091	0.41 0.091	0.22 0.643	0.12 0.643	0.71 0.412	-0.21 0.412	0.26 0.619	-0.13 0.619

NB: F - Fisher's correlation coefficient; α - the significance level of the Fisher coefficient; r - the correlation coefficient; p - level of significance of the correlation coefficient.

It was found that only two *Bacillus* strains 948P-1 TS and 312 TS have a significant effect on the photosynthesis of *Allium cepa*. Moreover, both strains cause a decrease in the production of chlorophyll a, which is contained both in the reaction centers of the photosystem and in the light-harvesting complex ($F = 18.58$ at $\alpha = 0.001$ and $F = 10.29$ at $\alpha = 0.005$, respectively).

Thus, in our experiments, we can note the lack of uniformity of the action of permafrost strains on the *Allium cepa* onion. That is why it is important to study the influence of permafrost microbiota on the cell life cycle, which is subdivided

into two main stages: interphase and mitosis. Interphase is the period of cell preparation for division.

It was revealed that *Bacillus* strains 875TS ($p<0.05$) and 948P-1TS ($p<0.001$) increase this period (Table 7).

Table 7

Influence of microorganisms on the cytogenetic system of *Allium cepa* [Compiled by the author]

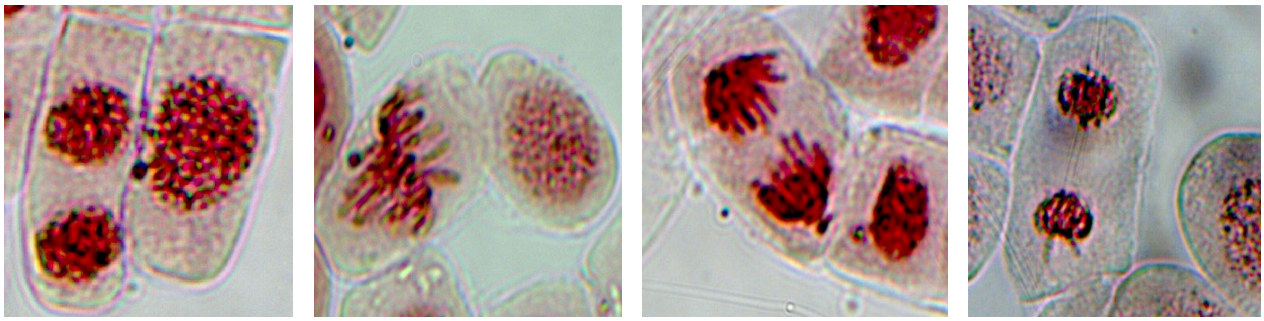
	Control 1	Control 2	8-75	2-06	9-48	3-12	12-57
Total number of cells, items	6841	7642	8602	6690	6468	8725	7529
Prophase, %	2.97 ± 0.41	1.25 ± 0.11 ***	2.0 ± 0.16 */##	2.03 ± 0.13 */###	0.98 ± 0.08 ***	2.79 ± 0.15 ###	2.49 ± 0.43 #
Metaphase, %	2.28 ± 0.47	1.03 ± 0.09 *	1.24 ± 0.11 *	1.85 ± 0.15 ###	0.84 ± 0.08 **	1.88 ± 0.15 ###	2.12 ± 0.51 #
Anaphase, %	1.87 ± 0.39	1.06 ± 0.13	1.25 ± 0.1	2.01 ± 0.17 ###	0.86 ± 0.06 *	1.8 ± 0.13 ##	1.91 ± 0.28 #
Telophase, %	1.69 ± 0.28	0.89 ± 0.11 *	1.01 ± 0.08 *	1.35 ± 0.14 #	0.89 ± 0.08 *	1.3 ± 0.08 #	1.49 ± 0.16 #
Interphase, %	91.18 ± 1.37	95.77 ± 0.27 **	94.48 ± 0.27 */##	92.76 ± 0.37 ###	96.43 ± 0.13 **/#	92.23 ± 0.31 ###	92.0 ± 1.22 ##
Mitotic index, c.u.	8.82 ± 1.37	4.22 ± 0.27 *	5.52 ± 0.27	7.24 ± 0.37 ###	3.57 ± 0.13 **/#	7.76 ± 0.31 ###	8.0 ± 1.22 ##

NB: * - significance of difference with control 1 (* - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$), # - significance of difference with control 2 (* - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$).

Moreover, the activity of enzymes involved in energy metabolism increases, replication of DNA molecules and the synthesis of proteins - histones occur, with which each DNA strand is connected. RNA synthesis increases according to the amount of DNA.

Mitosis consists of four phases: prophase, metaphase, anaphase, telophase (Figure 8). In prophase, the volume of the nucleus increases. Chromosomes spiralize, become visible, shorten, thicken. It can be seen that they consist of two chromatids connected by a centromere. The centrioles diverge to the poles of the cell. A spindle apparatus is formed. By the end of prophase, the nucleoli and nuclear envelope dissolve, and the chromosomes end up in the cytoplasm.

Prophase is the longest phase of mitosis. When exposed to *Bacillus* strains 875TS, 2-06-TS1 and 948P-1 TS, this phase is shortened, especially in the latter variant (in 3 times).



prophase metaphase anaphase telophase

Fig. 8. - Phases of mitosis of the *Allium cepa* onion [Image by the author]

In metaphase, spiralization reaches its maximum, chromosomes are located in the equatorial plane of the spindle, forming a metaphase plate. Sister centromeres and chromatids face opposite poles. The mitotic spindle is fully formed and consists of filaments connecting the poles to the centromeres of the chromosomes. It is clearly seen that chromosomes consist of two chromatids connected in the centromere region. The number and shape of chromosomes are clearly visible, which allows them to be counted and studied. The metaphase is usually very short. However, in the studies carried out in controls 1 and 2, in this phase of mitosis there were 2.28 $\pm$ 0.47 and 1.03 $\pm$ 0.09 percent of cells, respectively. When exposed to *Bacillus* strains 875TS and 948P-1 TS, the metaphase was shortened, and in the latter variant it was very short.

In anaphase, centromeres are separated, chromatids (daughter chromosomes) become independent. The spindle filaments attached to centromeres pull daughter chromosomes to the poles of the cell. The movement of chromosomes is provided by the interaction of centromeric regions of chromosomes with microtubules of the spindle of division. The cell contains two diploid sets of chromosomes. Anaphase is usually very short. It was noted that the studied *Bacillus* strains do not affect this phase of mitosis, except for the 948P-1 TS strain, which shortens it by 2.2 times (0.86 $\pm$ 0.06 compared to the control 1.87 $\pm$ 0.39).

Mitosis ends in telophase. Chromosomes, consisting of one chromatid, are located at the poles of the cell. They despiralize and become invisible. A nuclear envelope is formed, the threads of the achromatin spindle disintegrate. The nucleolus is formed in the nucleus. The division of the cytoplasm (cytotomy and cytokinesis) and the formation of two daughter cells occurs. In plant cells, a membrane septum forms in the center, which grows towards the cell walls. A cellular wall is formed in plants after the formation of a transverse cytoplasmic membrane.

It was found that *Bacillus* strains 875TS and 948P-1 TS affect telophase, shortening it by 1.7 and 1.9 times, respectively.

Thus, as a result of mitosis, the genetic material is precisely distributed between the two daughter cells. Both daughter cells receive a diploid set of chromosomes. Mitosis maintains the constancy of the number of chromosomes in a number of generations and serves as a cellular mechanism for the processes of growth, development of the organism, regeneration, and asexual reproduction. For a group of onion cells that rarely complete a cell cycle, a significant part is in the resting stage of the cell cycle.

Under the influence of the studied *Bacillus* from permafrost rocks, it occurs an ambiguous violation of the course of mitosis. One of the correct ways to quantitatively analyze the intensity of division is the mitotic index. It was found that the proliferative capacity of cells decreases in 2.5 times when exposed to only one *Bacillus* 948P-1 TS strain (3.57 ± 0.13 compared to the control 8.82 ± 1.37 at $p < 0.01$). In all other variants, the intensity of onion cell division does not change. Based on this, a conclusion is made about the absence of the mitotic or mitosis-stimulating effect of the studied microorganisms from dispersed aqueous soils that have passed into a frozen state.

Variance and correlation analyses were applied to search for the influence of frozen soils microbiota at the stage of the cell cycle of onion development by investigating the significance of differences in mean values (Table 8).

Table 8

Results of dispersion and correlation analyses of the influence of the microbiota of frozen soils at the stage of the cell cycle of development of *Allium cepa*, c.u. [Compiled by the author]

Independent factors / Dependent factors	8-75		2-06		9-48		3-12		12-57	
	F / α	r / p	F / α	r / p	F / α	r / p	F / α	r / p	F / α	r / p
Prophase	3.93 0.065	-0.44 0.065	5.1 0.038	-0.49 0.038	14.92 0.001	-0.69 0.001	0.81 0.383	-0.22 0.383	4.95 0.041	-0.49 0.041
Metaphase	14.64 0.001	-0.69 0.001	5.47 0.033	-0.51 0.033	40.07 <0.001	-0.85 <0.001	2.80 0.113	-0.39 0.113	7.14 0.017	-0.55 0.017
Anaphase	8.07 0.012	-0.58 0.012	0.58 0.456	-0.19 0.456	25.75 <0.001	-0.79 <0.001	0.41 0.53	-0.16 0.53	3.0 0.103	-0.40 0.103
Telophase	20.59 <0.001	-0.75 <0.001	8.64 0.01	-0.59 0.01	28.77 <0.001	-0.80 <0.001	6.47 0.022	-0.54 0.022	6.89 0.018	-0.55 0.018
Interphase	37.18 <0.001	0.84 <0.001	10.58 0.005	0.63 0.005	51.72 <0.001	0.87 <0.001	33.11 <0.001	0.82 <0.001	5.65 0.03	0.51 0.03
Mitosis	0.01 0.921	0.25 0.921	1.14 0.301	0.26 0.301	11.64 0.004	-0.65 0.004	5.42 0.033	0.50 0.033	0.42 0.526	0.16 0.526
Mitotic index, c.u.	10.85 0.005	-0.64 0.005	2.13 0.164	-0.34 0.164	60.55 <0.001	-0.89 <0.001	0.71 0.411	-0.21 0.411	0.39 0.54	-0.15 0.54

NB: F - Fisher's correlation coefficient; α is the significance level of the Fisher coefficient; r is the correlation coefficient; p - level of significance of the correlation coefficient.

Thus, among the analyzed microorganisms of dispersed soils that have passed into a frozen state, only *Bacillus* 948P-1 TS and 312 TS strains have a significant effect on mitosis.

Moreover, their impact is multidirectional. So if *Bacillus* strain 948P-1 TS inhibits cell division ($F = 11.64$ at $\alpha = 0.004$), then *Bacillus* strain 312 TS, on the contrary, stimulates mitosis ($F = 5.42$ at $\alpha = 0.033$).

Noteworthy is the fact that all studied bacteria of the genus *Bacillus* with a significance level of $\alpha < 0.03$ cause a temporary delay in cell division, which allows the cell to grow and the ability to assess the suitability of external and internal

conditions for DNA duplication and subsequent division. At the same time, in the interphase phase, the activity of enzymes participating in energy metabolism increases, replication of DNA molecules and the synthesis of proteins - histones occur, with which each DNA strand is connected. RNA synthesis increases according to the amount of DNA.

In addition, it was found that *Bacillus* 875TS and 948P-1 TS strains demonstrate the proliferative ability of *Allium cepa* cells ($F = 10.85$ at $\alpha = 0.005$ and $F = 60.55$ at $\alpha < 0.001$, respectively), which indicates the absence of mitotic or mitosis-stimulating action.

Plant systems had a major part in early investigations of the genetic changes caused by mutagenic chemicals and radiation. One of the most suitable plants for detecting different types of xenobiotics is *Allium cepa*. This plant is useful for evaluating DNA damages that express such as chromosomal aberrations (CAs), disturbances in the mitotic cycle, nuclear alterations (NAs) and presence of micronuclei (MNi) in meristem cells.

The decrease in the mitotic index (MI) of *Allium cepa* meristem cells can be considered as a reliable method to determine the presence of cytotoxic agents in the environment. CAs are including changes in either chromosomal structure or in the total number of chromosomes. Structural chromosomal alterations may be induced by DNA breaks, inhibition of DNA synthesis and replication of altered DNA. The CAs, such as chromosome bridges and breaks, are indicators of a clastogenic action. The numerical CAs (aneuploidy and polyploidy) are consequences of abnormal segregation of chromosomes (chromosome losses, delays, adherence, multipolarity and C-metaphases) which can occur either spontaneously or by the action of aneugenic agents. Chromosome without telomeres become “sticky” and may fuse with other broken chromosome ends (Fig. 9.).

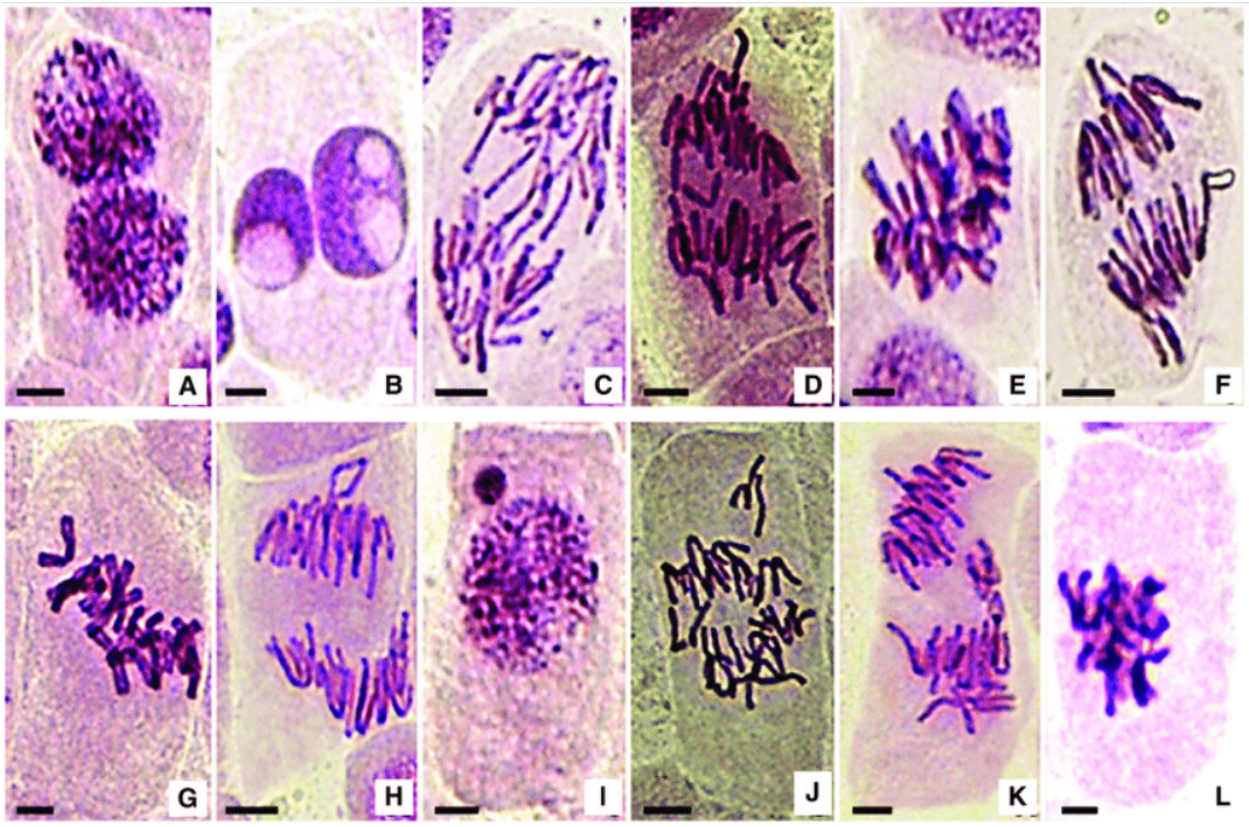


Fig. 9. - Chromosome aberrations in bleaching powder-treated *Allium cepa* root tip cells. (A) Binucleate cell, (B) binucleate cell showing nuclear lesions, (C) chromosome bridge and laggards, (D) chromosome laggard, (E) C-metaphase, (F) diagonal anaphase, (G) diagonal metaphase and vagrant chromosome, (H) early movement in disturbed anaphase, (I) micronucleus, (J) scattered polyploid cell, (K) shift in MTOC, (L) sticky metaphase. Bar represents 5 µm [Image by Neelamkavil, Thoppil, 2015].

The result of these chromosomal rearrangements are acentric fragments, dicentric bridges that can be observed in mitotic cells of the first cell cycle after mutagenic treatment or MNi in the interphase cell in the next cell cycle (Nefic, Musanovic, Metovic, Kurteshi, 2013).

At the stage of anaphase and telophase, chromosomal material lagging behind the poles (acentric fragments and rings, lagging chromosomes) and bridges are counted. All of them are visually easily seen and distinguishable. Frequently, lags and overshoots, specifying changes in the achromatin spindle, are counted and included in a separate category.

There is no universal standard for accounting for aberrations. Practically, it happens different types of aberrations in the same cell. In theoretically, any

combination of aberrations can be expected. The foundation for this is the asynchronous replication of hereditary material. Moreover, many types of aberrations can be recorded, such as multipolar mitoses and other types of aberrations.

Ana-telophase analysis of chromosomal aberrations on onion cells (*Allium cepa*) is recommended as a tool for environmental cytomonitoring. By measuring genotoxicity, it helps to assess the degree of environmental risk from household and industrial products, which are constantly introduced and increasingly become important in global community.

4 Conclusion

When assessing the influence of microorganisms of the genus *Bacillus* from the Arctic paleoecosystems on the morphophysiological characteristics of *Allium cepa*, differences were revealed between the studied strains in terms of their effect

on the morphophysiological parameters of plants, which is explained by the different mechanisms of action of these strains. It can be assumed that bacteria, when ingested with water, rather actively begin to break down reserve nutrients and thereby exert a direct toxic effect on the germination energy of *Allium cepa*, which is observed when using *Bacillus* strains, strain 875TS, which causes a delay in the growth of roots and shoots of *Allium cepa*. At the same time, the *Bacillus* strain 2-06-TS1 also significantly inhibits the growth of shoots of *Allium cepa*. The most pronounced negative effect practically on the analyzed morphophysiological parameters is exerted by *Bacillus* strain 948P-1 TS. Under its influence, the length and number of roots decreases, as well as the length of the shoots. Also, strains *Bacillus* 312 TS and 1257 TS delay in the development of shoots in length which is a negative effect.

It is known that soil microorganisms are capable of synthesizing a very large amount of various biologically active substances, including cytokinins, gibberellins, and microbial phytohormones. It is quite expected that bacteria producing such substances can affect the morphophysiological and biochemical parameters of plants, including the activation of the photosynthetic system (carotenoids, azomethine, diene conjugates). The selected strains of *Bacillus* bacteria affect the concentration of these substances in *Allium cepa*.

It was found that only two *Bacillus* strains 948P-1 TS and 312 TS have a significant effect on the photosynthesis of *Allium cepa*. Moreover, both strains cause a decrease in the production of chlorophyll, which is contained in the reaction centers of the photosystem and in the light-harvesting complex.

Taking into account the influence of bacterial strains, isolated from permafrost, on the morphophysiological and biochemical parameters of plants, it is possible to develop biopreparations of targeted or complex action on their basis using a combination of strains. For this, it is necessary to assess their cytotoxic effect.

According to the results of the analyzed microorganisms of dispersed soils that have passed into a frozen state, only *Bacillus* 875TS, 948P-1 TS, and 312 TS

strains have a significant effect on the stage of the cell cycle of onion development. At the same time, *Bacillus* strain 948P-1 TS inhibits mitosis, and *Bacillus* strain 312 TS, on the contrary, stimulates it. In addition, it was found that *Bacillus* strains 875TS and 948P-1 TS significantly inhibit the proliferative ability of *Allium cepa* cells, which indicates the absence of mitotic or mitosis-stimulating effects in them.

Apparently, the studied bacteria of the genus *Bacillus*, causing a temporary delay in cell division, allow the cell to grow and give the ability to assess the suitability of external and internal conditions for DNA duplication and subsequent division. At the same time, in the interphase phase, the activity of enzymes participating in energy metabolism increases, replication of DNA molecules occurs, the synthesis of proteins - histones, with which each DNA strand is connected. RNA synthesis increases according to the amount of DNA.

Summaries

1. All investigated microorganisms of the genus *Bacillus* cause a negative effect on the morphometric parameters of Allium cepa. According to the degree of

manifestation of the negative influence, the bacteria are arranged in the following order:

• strain 2-06-TS1;

• strains 875TS, 312 TS and 1257 TS;

• strain 948P-1 TS.

2. Strains *Bacillus* 948P-1 TS and 312 TS have a significant decrease in the production of *chlorophyll a*, which is contained both in the reaction centers of the photosystem and in the light-harvesting complex.

3. Bacillus strains 875TS, 948P-1 TS, and 312 TS have an impact on the cell cycle stages of onion development. At the same time, Bacillus strain 875TS and 948P-1 TS inhibit the proliferative ability of Allium cepa cells, which indicates that they have no mitotic or mitosis-stimulating effect. *Bacillus* strain 312 TS, on the contrary, stimulates mitosis.

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