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## The analysis of sludge's microbial make-up during *Chironomus* larvae cultivation

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### Abstract

Many microorganisms that thrive in wet and dry environments call silt (a nutrient medium) home. It is partly recovered from the sewer by prolonged aeration. The composition of such a substrate, and hence its leading indications, are dependent on the kind of organic impurities present. These nutrient-rich circumstances are ideal for the development of *Chironomus* larvae and a wide variety of microflora. In order to determine the microbiological make-up of the nutritive medium under different pasteurization procedures, the paper gives the results of research of bacteriological crops of the substrate for *Chironomus* larvae. *Pseudomonas sp.* bacteria make about 80% of the colony-forming organisms in native sludge, followed by *Bacillus subtilis* at 15% and *Micrococcus luteus* at 5%. The quantity of bacteria in the growth media was also observed to be reduced after being subjected to various pasteurization techniques. The quantity of bacteria was reduced by 16 times after long-term pasteurization at 65 °C. The number of colony-forming organisms was reduced by a factor of 22 after short-term pasteurization at 75 °C and by a factor of 358 after rapid pasteurization at 95 °C compared to the original nutritional medium.

**Keywords:** sludge; bacteria; pasteurization modes; colony growth.

### 1. Introduction

Numerous types of bacteria call silt home. The composition and many other properties of these bodies of water might vary depending on their source—river, marsh, pond, or lake (Osadchy, 2013; Bordiuh, 2013; Kummu et al., 2016). It's possible that sludge in pond water bodies has a high concentration of chemicals and other toxins. It is valuable and rich in rare components found in river and lake sediment. Lake silt is rich in organic materials and lime, making it a valuable organic fertilizer. There are many beneficial and nourishing components in river silt. Microorganisms play an important role in the formation of this sludge, as they aid in the removal of different contaminants and animal leftovers from the water. Silt is more prevalent in areas with poor water flow or stagnant water. Its composition gives off the impression of a jellylike blob. For an organic substance of animal and plant origin, bog silt is incredibly diverse in its composition and nutritional value (Kunst et al., 1997; Protasov, 2017; Abdelli et al., 2019; Merzlov et al., 2019; Pysarenko et al., 2022).

There are many distinct kinds of bacteria both in the silt and in the reservoir, each with its own unique

morphological characteristics and three main forms: cocci, rods, and spirilla. *Bacillus*, *Actinomyces*, *Corynebacterium*, *Micrococcus*, *Desulfotomaculum*, *Arthrobacter*, *Sarcina*, *Bacterium*, *Pseudomonas*, and other representatives of these and other genera are the primary organisms referred to in this species composition (Hilgren et al., 2009; Kryvytska et al., 2010; Kumar et al., 2012; Adler et al.). The water column and bottom sediments of the reservoirs provide the most suitable environment for these bacteria. In addition, a large number of bacteria may grow in environments with both water and air, as well as water and solid substrates. To thrive, bacteria need a reservoir's specific combination of factors, including a high concentration of organic substances, the presence of biogenic elements, and a variety of climatic conditions (including gas, temperature, hydrology, salinity, etc.) (Fedonenko et al., 2014; Fialko et al., 2018). One of the variables that supports a dynamic equilibrium in the biosphere is the abundance of microorganisms.

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Microorganisms are essential to any ecosystem, especially in aquatic environments; bacteria occupy one of these essential roles. Because due to their active participation, complex organic substances are changed into simple compounds directly suitable for better assimilation by hydrobionts of different levels (Furzikova et al., 2006; Gorshkova et al., 2018; Khilchevskiy & Karamushka, 2021). The vast and accelerated spread of various microorganisms due to the speed of reproduction, small size, resistance, and adaptation to various unfavorable and detrimental factors to life, as well as their diversity.

Due to their high adaptability potential, microorganisms can coexist in various conditions and environments. Waters of different origins are the natural environment for the development and existence of various microorganisms. In water bodies (salt, fresh), different taxonomic groups of bacteria, algae, and protozoa enter with different organic residues, dust, and soil. The contamination and microflora of water depend on the composition of the primary medium and the microorganisms' origin (Fotina et al., 2019).

## 2. Materials and methods

The study was conducted in the microbiological research methods laboratory of the Department of Microbiology and Virology of the Belotserkovsky National Agrarian University. Nutrient medium from the river Ros is the material of research. Samples of river silt were taken from a sampling depth of 0.9–1 m and a thickness of 9–10 cm.

All research samples of the substrate were weighed into test tubes of 10 g, pre-sterilized. The total number of test tubes is 12, of which: the first three have native sludge; in the next three, the nutrient medium was subjected to long-term pasteurization (65 °C); and in three test tubes,

Also, the bacterial composition of the environment is formed and depends on environmental conditions, except for some groups of bacteria that can develop and coexist regardless of environmental conditions (Klymenko et al., 2014).

A different number of microorganisms is located not only in the thickness of the medium but also in the surface silt layer while forming a thin bacterial film. The zone of this film contains many bacteria of different origins, namely, iron and sulfur bacteria, which act as transforming substances in the water body (Ye et al., 2014; Kassich & Nechiporenko, 2020).

Among the bacteria, some are found in the aquatic environment and soils of different origins, air, terrestrial plants, and animals of different shapes and origins (Collins et al., 2016).

*The aim of the study.* These studies aim to establish the microbiological composition of the nutrient medium under various pasteurization regimes and identify bacterial colonies that can affect the quantitative and qualitative composition of the microflora of the substrate for *Chironomus* larvae.

there was sludge, which was pasteurized during short-term pasteurization (75 °C). The final stage of the pasteurization of the nutrient medium was sludge, which was acted upon at a temperature of 95 °C, flash pasteurization.

The study was carried out in a microbiological box sterilized by ultraviolet light. According to the serial dilution method (L. Pasteur), serial dilutions of the material were prepared in a sterile liquid nutrient medium ( $10^{-3}$ ..... $10^{-7}$ ) (Fig. 1). Then, using a 3-fold and 7-fold dilution, our native and pasteurized substrate was inoculated on sterile meat-peptone agar (MPA), into the surface and thickness.



Fig. 1. Preparation of serial dilutions before inoculation



The material for inoculation into the medium was in a liquid state; it was taken with a sterile graduated pipette, in the amount of 1 ml of a diluted microbial suspension was poured into a sterile Petri dish and poured into the medium melted and cooled to 45–50 °C by MPA. In a circular motion and

shaking the Petri dish, the material was mixed to be evenly distributed in the medium. After the complete thickening of the last cup, our samples were placed in a thermostat at a temperature of 37 °C, which is optimal for the growth of various microorganisms in the sludge (Fig. 2).



Fig. 2. Inoculation on the surface of the MPA medium

Microbiological cultures were performed to obtain and identify individual bacterial colonies. The description of our crops and the study of cultural properties were carried out on the third day of research.

### 1. Results and discussion

The microbiological characteristics showed that the nutrient media contained colonies of various microbes. A colony is an accumulation of bacteria visible to the naked eye on the surface or in the thickness of a nutrient medium. To determine the

type of microorganisms grown on the surface of the agar, each Petri dish was carefully examined, and isolated colonies were studied. According to the general indicators of cultural properties, attention was paid to color, size, shape, consistency, the surface of the colonies, the nature of the edges, gloss, transparency, and other features.

After sowing, we saw the growth of such colonies as *Pseudomonas spessialis*, *Bacillus subtilis*, and *Micrococcus luteus* at various serial dilutions (Fig. 3).

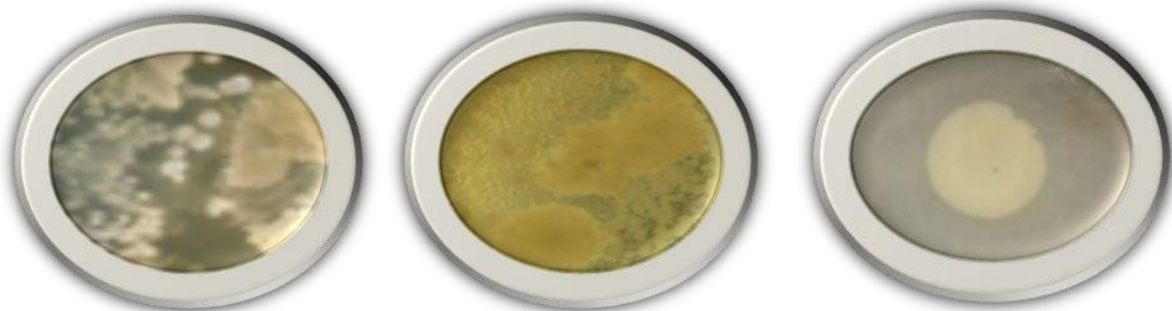


Fig. 3. Growth of the colony

*Pseudomonas spessialis*, identified in a nutrient medium, can metabolize various nutrients; they belong to rod-shaped bacteria and are the least picky about growth factors. Aerobes are about 0.5–1.0×1.5–5.0 microns in size. According to some indicators of cultural properties, the colonies of *Pseudomonas spessialis* were of different

shapes (large, convex, shiny, flat, irregularly shaped, punctate, slimy, dwarf, and folded). In terms of color, they were yellowish, gray, and yellowish-gray and had an oily consistency. *Bacillus subtilis* is a rod-shaped bacterium that produces endospores that do not exceed the size of the cell itself. According to its properties, the



chemoorganoheterotroph breaks down starch and glycogen and ammonifies proteins. Reaches the size of 3–5×0.6 microns, each cell is mobile and has several peritrichous. Colonies can grow on different media: synthetic nutrients, vegetables, MPA, and MPB. The peculiarities of such bacteria are that they are colorless, velvety, pink, finely wrinkled, and dry. Also, according to the description of the edges of the colonies, one can note a characteristic feature for the species of these bacteria (coral and waviness).

*Micrococcus luteus* is a saprophytic bacterium, non-motile, Gram-positive, and widely distributed in various environments. It is unpretentious to different nutrient media. Therefore, it is well With the help of calculations of the results obtained, it was seen that the native sludge contained the most

cultivated. It has a spherical regular shape, convex, smooth, shiny, and opaque, not exceeding 0.5–1.5 microns in size. The color of such bacteria can be different depending on the color of the pigment: bright yellow, golden, lemon yellow, fawn, and white. In the studying smears it was single or in the form of irregular clusters that did not form spores.

At the stage of further studies, a pure culture was obtained to have a good view of the form; preparation smears were prepared, which were stained using the Gram method. After performing preparation smears, microscopy was performed using an immersion system.

significant number of microorganisms (Table 1).

Temperature regime of pasteurized sludge	Bacterial colony count
1 native silt	$4,3 \times 10^7$ CFU/1g
2 lasted 65 °C	$2,6 \times 10^6$ CFU/1g
3 short-term 75 °C	$1,9 \times 10^6$ CFU/1g
4 instant 95 °C	$1,2 \times 10^5$ CFU/1g

The data obtained indicate that, according to the number of counts of bacterial colonies in native and pasteurized sludge, the most significant number was in non-pasteurized sludge (native)  $4.3 \times 10^7$  CFU/1g.

In crops where the nutrient medium was pasteurized at a temperature of 65 °C (long-term)

and 75 °C (short-term), the growth of colony-forming organisms had a negligible amount of bacteria.

In crops of pasteurized sludge during instant pasteurization (95 °C), there was the least amount of bacteria, which was  $1.2 \times 10^5$  CFU/1g.

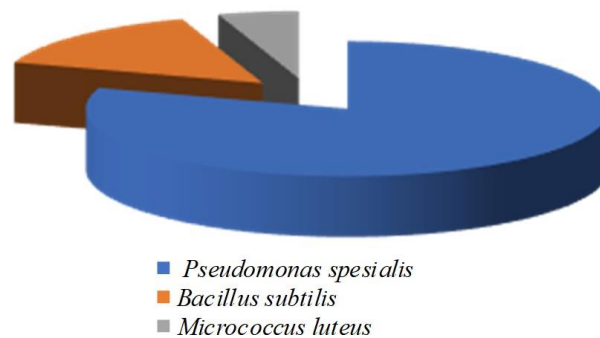


Fig. 4. Colonies of bacteria in native sludge, %

Also, according to the results of calculations (Fig. 4), it was found that native sludge has the most colonies of bacteria from the entire colonies: *Pseudomonas spsialis* 80%, *Bacillus subtilis* 15%, *Micrococcus luteus* 5%.

### 3. Conclusions

1. It was revealed that in native sludge colonies of bacteria of the family *Pseudomonas spsialis*, 80%, *Bacillus subtilis*, 15%, and *Micrococcus luteus*, 5%. Bacteriological inoculations on the MPA medium on the surface of the medium and in the

thickness of the medium were introduced per 1 ml, showing that microorganisms affecting the growth and development of *Chironomus* larvae live in unpasteurized sludge.

The number of colony-forming organisms under various pasteurization regimes has decreased. During pasteurization at 65 °C (long-term) by 16 times, during pasteurization at 75 °C (short-term) by 22 times, during pasteurization at 95 °C (instant), the number of colony-forming organisms from the total number of colonies decreased by 358 times.



## References

- Abdelli, F., Jardak, M., Elloumi, J., Stien, D., Cherif, S., Mnif, S., Aifa, S. (2019). Antibacterial, anti-adherent and cytotoxic activities of surfactin(s) from a lipolytic strain *Bacillus safensis* F4. *Biodegradation*, 30, 287–300. [[Crossref](#)] [[Google Scholar](#)]
- Adler, C., Corbalan, N. S., & Seyedsayamdost, M. R. (2012). Catecholate Siderophores Protect Bacteria from Pyochelin Toxicity. *PLOS ONE*, 7(10), e46754 [[Crossref](#)] [[Google Scholar](#)]
- Bordiu, N. S. (2013). The analysis of sanitary quality of outplant drinking water. *Technology Audit and Production Reserves*, 5(4(13)), 49–51. [[Crossref](#)] [[Google Scholar](#)]
- Collins, C.P., Carlsson, J., Rowcroft, P., & Tibbles, B. (2016). Ecosystem status of the deep Black Sea, soft sediment, benthic community. *Marine Policy*, 73, 216–223. [[Crossref](#)] [[Google Scholar](#)]
- Desloover, J., Roobroeck, D., Heylen, K., Puig, S., Boeckx, P., Verstraete, W., & Boon, N. (2014). Pathway of nitrous oxide consumption in isolated *Pseudomonas stutzeri* strains under anoxic and oxic conditions. *Environmental Microbiology*, 16(10), 3143–3152. [[Crossref](#)] [[Google Scholar](#)]
- Fedonenko, O. V., Sharamok, T. S., & Marenkov, O. M. (2014). Osnovy akvakultury: kultyvuvannya mikrovodorostei ta bezkhubetnykh: Navchalnyi posibnyk. Dnipropetrovsk (in Ukrainian). [[Article](#)] [[Google Scholar](#)]
- Fialko, N., Nosovskyi, V., Sherenkovskiy, J., Meranova, N., Sharaevskiy, I., & Pioro, I. (2018). Specifics of the flow of supercritical water under conditions of mixed convection. *Thermophysics and Thermal Power Engineering*, 40(3), 12–19. [[Crossref](#)] [[Google Scholar](#)]
- Fotina, T., Nazarenko, S., & Fotin, A. (2019). Sanitary and micro-biological indicators of drinking water of livestock farms. *Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies. Series: Veterinary Sciences*, 21(95), 112–116. [[Crossref](#)] [[Google Scholar](#)]
- Furzikova, T. M., Serhiichu, M. H., Vlasenko, V. V., Shvets, Yu. V., & Pozur, V. K. (2006). Mikrobiolohiia. Praktykum: pidruchnyk. Kyiv: Fitosotsiotsentr (in Ukrainian). [[Google Scholar](#)]
- Gorshkova, O. G., Gudzenko, T. V., Voljuvach, O. V., Konup, I.P., & Beljaeva, T. O. (2018). Purification of water from phenol and heavy metal ions by association of bacteria of the genus *Pseudomonas*. *Microbiology & Biotechnology*, 2, 70–80 (in Ukrainian). [[Crossref](#)] [[Google Scholar](#)]
- Hilgren, J., Swanson, K. M. J., Diez-Gonzalez, F., & Cords, B. (2009). Susceptibilities of *Bacillus subtilis*, *Bacillus cereus*, and Avirulent *Bacillus anthracis* Spores to Liquid Biocides. *Journal of Food Protection*, 72(2), 360–364. [[Crossref](#)] [[Google Scholar](#)]
- Kassich, V., & Nechiporenko, O. (2020). Effect of probiotics on rumen microorganisms. *Bulletin of Sumy National Agrarian University. The Series: Veterinary Medicine*, 2(49), 3–8. [[Crossref](#)] [[Google Scholar](#)]
- Khilchevskiy, V., & Karamushka, V. (2021). Global Water Resources: Distribution and Demand. In: Leal Filho, W., Azul, A.M., Brandli, L., Lange Salvia, A., Wall, T. (eds). *Clean Water and Sanitation. Encyclopedia of the UN Sustainable Development Goals*. Springer. [[Crossref](#)] [[Google Scholar](#)]
- Klymenko, M. O., Pryshchepa, A. M., Klymenko, O. M., & Stetsiuk, L. M. (2014). Otsiniuvannya stanu vodnykh ekosystem za pokaznykamy biotestuvannya: monohrafiia. Rivne: NUVHP (in Ukrainian). [[Monohrafiia](#)] [[Google Scholar](#)]
- Kryvytska, T. M., Bahaieva, O. S., Uzhevskaya, S. P., Nepomiashcha, N. M., & Ivanytsia, V. O. (2010). Kharakterystykashtamiv bakterii rodu *Bacillus* z lavitsydneyu aktyvnistiu do hrybnykh komarykiv *Bradysia pilistriata* frey (Sciariidae). *Mikrobiolohiia i biotekhnolohiia*, 3, 86–94 (in Ukrainian). [[Crossref](#)] [[Google Scholar](#)]
- Kumar, P., Khare, S., & Dubey, R. (2012). Diversity of *Bacillus* from Disease Suppressive Soil and their Role in Plant Growth Promotion and Yield Enhancement. *New York Science Journal*, 5(1), 90–111. [[Article](#)] [[Google Scholar](#)]
- Kummu, M., Guillaume, J., de Moel, H., Eisner, S., Flörke, M., Porkka, M., Siebert, S., Veldkamp, T., & Ward, P. (2016) The world's road to water scarcity: shortage and stress in the 20th century and pathways towards sustainability. *Scientific Reports*, 6, 38495. [[Crossref](#)] [[Google Scholar](#)]
- Kunst, F., Ogasawara, N., Moszer, I. et al. (1997). The complete genome sequence of the Gram-positive bacterium *Bacillus subtilis*. *Nature*, 390, 249–256. [[Crossref](#)] [[Google Scholar](#)]
- Merzlov, S. V., Bezpalyi, I. F., & Korol-Bezpalala, L. P. (2019). Vstanovlennia optymalnykh biotekhnolohichnykh umov rozvedennia i rozvytku lychynok Chironomus. *Tekhnolohiivyrobnystva i pererobky produktii tvarynnystva: zb. nauk.prats. Bila Tserkva: BNAU*, 1(147), 135–147 (in Ukrainian). [[Crossref](#)] [[Google Scholar](#)]
- Osadchyi, V. I. (2013). Hidrolohichni chynnyky formuvannya khimichnoho skladu poverkhnivykh vod. *Naukovi pratsi UkrNDHMI*, 265, 54–65 (in Ukrainian). [[Article](#)] [[Google Scholar](#)]



- Protasov, O. O. (2017). Bioheomika. Ekosystemy svitu v strukturi biosfery. Instytut hidrobiolohii NAN Ukrainy. Kyiv: Akadempriodyk (in Ukrainian).  
[\[Abstract\]](#)
- Pysarenko, P., Samoilik, M., Dychenko, O., Taranenko, A., Galyt-ska, M., & Nimets, O. (2022). Agroecological peculiarities of natural brines and minerals' impact on soil microorganisms. *Bulletin of Poltava State Agrarian Academy*, 2(2), 157–164.  
[\[Crossref\]](#) [\[Google Scholar\]](#)
- Sass, G., Miller Conrad, L. C., Nguyen, T. H., & Stevens, D. A. (2020). The *Pseudomonas aeruginosa* product pyochelin interferes with *Trypanosoma cruzi* infection and multiplication in vitro. *Transactions of The Royal Society of Tropical Medicine and Hygiene*, 114(7), 492–498.  
[\[Crossref\]](#) [\[Google Scholar\]](#)
- Shafique, M., Jawaid, A., & Rehman, Y. (2016). As(V) Reduction, As(III) Oxidation, and Cr(VI) Reduction by Multi-metal-resistant *Bacillus subtilis*, *Bacillus safensis*, and *Bacillus cereus* Species Isolated from Wastewater Treatment Plant. *Geomicrobiology Journal*, 34(8), 687–694.  
[\[Crossref\]](#) [\[Google Scholar\]](#)
- Smirnov, O. I., Zelena, P. P., Yumyna, Y. M., Kalynovskyi, V. I., Taran, N. I., & Shvartau, V. V. (2022). Biosyntezy nanochastynok sribla z antybakterialnym efektom proty *Micrococcus luteus* – zbudnyka nozokomialnykh infektsii. *Dopovidi Natsion-alnoi akademii nauk Ukrainy*, 5, 94–101 (in Ukrainian). [\[Crossref\]](#) [\[Google Scholar\]](#)
- Stein, T. (2005). *Bacillus subtilis* antibiotics: structures, syntheses and specific functions. *Molecular Microbiology*, 56(4), 845–857.  
[\[Crossref\]](#) [\[Google Scholar\]](#)
- Ye, L., Hildebrand, F., Dingemans, J., Ballet, S., Laus, G., Matthijs, S. (2014). Draft genome sequence analysis of a *Pseudomonas putida* W15Oct28 strain with antagonistic activity to Gram-positive and *Pseudomonas* sp. Pathogens. *PLOS ONE* 9(11), e110038.  
[\[Crossref\]](#) [\[Google Scholar\]](#)
- Zeigler, D. R., & Nicholson, W. L. (2017). Experimental evolution of *Bacillus subtilis*. *Environmental Microbiology* 19(9), 3415–3422.  
[\[Crossref\]](#) [\[Google Scholar\]](#)