



Current state of soil microbiota in the Zaporizhzhia region of Ukraine

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The destruction of the Kakhovka HPP led to large-scale changes in the natural ecosystems of the Zaporizhzhia and Kherson regions of Ukraine, including changes in the soil microbiome. The research in the territory of Zaporizhzhia Oblast revealed natural, intensive phenomena of soil cover purification from organic matter contamination, including feces, occurring in the drainage zone formed as a result of the destruction of the dam. Sanitary state control allows us to assess the safety of soils for the environment and determine the possible impact on humans. Thus, a local contamination was revealed. The sanitary state of the soil was characterized by the presence of sanitary indicator microorganisms (*Escherichia coli*, *Enterococcus* spp., *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., and *Clostridium perfringens*), which indicated fresh or old fecal contamination. The detection of thermophilic bacteria, *Bacillus* spp., and saprophytic fungi was correlated with the degree of organic matter transformation processes and stages of soil self-purification. In the soils in unsatisfactory sanitary condition, the coli titer the perfringens titer measured ≤ 0.9 and ≤ 0.009 , respectively. Pathogenic *Salmonella* spp. were not detected in any of the soil samples, yet *Mycobacterium* spp. were isolated. In the soils of the drainage zone of most of the studied transects, the presence of *E. coli* and sulfite-reducing bacteria was established. The results obtained can be used for further monitoring and control of the sanitary condition of soils affected by the destruction of the Kakhovka HPP in order to prevent the spread of pathogenic microorganisms in natural ecosystems.

Keywords: fecal indicator bacteria; coli titer; perfringens titer; thermophilic bacteria; bacterial biodiversity; soil use.

Introduction

The territory of Ukraine is endowed with extremely fertile black soil. About 40,378.2 thousand ha (~ 70.0%) of the total area of the state land resources are allocated for agricultural lands. The latter are used to meet the most important needs of society (Boichenko et al., 2015; Ponomarenko et al., 2022; Yakovenko et al., 2025). As Ukrainian scientists note, the geographical location of the state, its natural and climatic conditions and soil fertility allow for the effective use of agricultural lands for growing a large number of crops, including grains, tubers, and fodder, as well as berries, fruits, and even rare and valuable medicinal plants (Vashenko, 2018; Shandrivska & Pyzh, 2024; Zinchuk & Tkachuk, 2024). The actual volumes of crops grown can meet domestic needs of the state and even have export potential (Poruchynska & Slashchuk, 2025; Pospelov et al., 2025).

Soil is an extremely complex and diverse bio-organomineral system (Yakovenko & Zhukov, 2025), which, while maintaining balance in the biocenosis, is capable of ensuring active growth and development of plants (Preston, 1995; Chi et al., 2022; Niu et al., 2022). In the process of soil formation, the soil microbiota and its proper functioning play an important role, directly affecting the qualitative and quantitative indicators, including soil fertility. Accordingly, the amount and nature of organic substances depend on the species composition of the microbiota and biochemical transformations occurring in the soil at a specific point in time, and are characteristic only for a specific area with appropriate climatic conditions. Scientists distinguish four main groups of microorganisms (except viruses), which are considered the basis of the soil microbiota: bacteria, fungi, actinomycetes, and algae. These microorganisms can be represented by dozens of genera and hundreds of species in the soil (Jha et al., 1992; Lumibao & Liu, 2024). According to Tate (1997), the number of species of microorganisms that can be simultaneously present in 1 g of soil is close to 4,000. Since microorganisms that inhabit soils are alive, they actively respond to biotic and abiotic factors. Starting from February 24,

2022, as a result of the full-scale invasion of Ukraine by the Russian Federation and the active military actions, many war crimes against the environment have been recorded in Ukraine (Marych & Pohorlets, 2023; Plotnikov, 2024; Michuda et al., 2025), including Kherson Oblast. For instance, the war has led to a surge in the number of detected cases of animals with rabies due to the lack of opportunity for veterinary specialists to carry out anti-epizootic measures, including vaccination of domestic and wild carnivores against rabies (Melnichuk et al., 2024). Moreover, the use of weapons during military operations is causing chemical pollution of the environment. As a result of missile strikes, oil depots have been damaged or completely destroyed. Large-scale fires have led to a release of over 499 thousand tons of toxic compounds into the atmosphere. At the same time, considerable pollution of water resources with oil products, heavy metals, and specific military pollutants was recorded, as well as a deterioration in the sanitary condition of drinking water resulting from the destruction of infrastructure associated with the water supply and sewage system. Explosions at hydraulic structures have led to catastrophic changes in the hydrological regime and large-scale environmental disasters (Chvaliuk et al., 2023; Tsyhanenko-Dziubenko et al., 2024; Bondar et al., 2025).

Soils actively respond to all changes, both natural and anthropogenic, which occur in the environment. The soil microbiota is particularly sensitive to changes, reacting both qualitatively and quantitatively. In particular, it has been established that fungicide and pesticide treatments of crops lead to qualitative and quantitative shifts in the soil microbiota (Isha et al., 2022; Streletskii et al., 2022). The effect of high temperatures, as a result of fires or burning of plant residues in the fields, is particularly harmful to the soil microbiota (Certini, 2005; Pérez-Valera et al., 2020). Slight warming of soils leads to increased growth of fungi and certain microorganisms, which require warming to become active. However, under conditions of critical (shock) temperatures, the quantitative indicators of both fungal microbiota and other microorganisms, as well as their species composition, tend to

significantly decrease (Dunn et al., 1985). The cause of negative shifts in the soil microbiota is both the effect of high temperatures and the effect of excess moisture. Soils that have been flooded naturally or artificially respond to new conditions of increased moisture by changing the microbial composition. One of the key factors that affects the qualitative and quantitative composition of soil microorganisms is access to O₂ (Burns & Ryder, 2001). It has been established that during natural flooding in the soil cover, the number of Gram-negative bacteria decreases, while Gram-positive bacteria are more resistant to the negative effects of the lack of O₂. At the same time, such conditions are optimal for the development of microscopic fungi and soil nematodes (Söderberg et al., 2004; Wagner et al., 2015; Wright et al., 2015).

The composition of soil microorganisms largely depends on the proximity to facilities related to intensive livestock farming and places of crowded animal housing, landfills, sewage and sedimentation tanks, railway tracks, highways, oil and gas production industries, burial grounds, etc. (Kukurudziak et al., 2016; Pepko, 2019; Ablicieva et al., 2021). The composition of soil microbiota in Ukraine is significantly affected by military operations, and their direct and indirect consequences (Dovhanenko et al., 2024a; Romashchenko et al., 2025). As a result of the disaster at the Kakhovka HPP, large territories of not only Kherson, but also adjacent regions (Mykolaiv, Zaporizhzhia, and Dnipropetrovsk) were flooded, which caused the anomalous spread of various microbiota with water (from fields, landfills, settling tanks, sewers, cemeteries, cattle cemeteries, livestock facilities, processing industries, etc.) (Dovhanenko et al., 2024b; Hapich et al., 2024). Therefore, analyzing the sanitary condition of the soil in relation to the actual contamination with microorganisms is a relevant issue that requires further study. Accordingly, the objective of our study was to identify dangerous microorganisms and assess the level of microbiological contamination of alluvial soils of Zaporizhzhia after the destruction of the Kakhovka hydroelectric power station dam.

Materials and methods

The study was conducted within the city of Zaporizhzhia, the administrative center of Zaporizhzhia Oblast of Ukraine (Fig. 1). The climate of the study area is classified as humid continental, with hot

summers (Beck et al., 2023). The average annual temperature is about +10.4 °C, and the annual precipitation is 449 mm/year (Karamushka et al., 2022). The relief is mainly a gentle, hilly plain with absolute heights from 16 to 50 m ASL, with valleys 1–2 km wide and 30–50 m deep (Dacenko et al., 2014). The Dnipro riverbed within Zaporizhzhia Oblast is mostly located in a narrow valley with a high right bank (up to ~50 m), composed of crystalline rocks, and a low, gentle left bank with alluvial deposits (sand, loess, clay), which form several terraces. The studied soils are formed on layered sandy alluvial deposits.

Soil sampling for microbiological analysis was carried out in the summer of 2024. Six transects were set on the left bank of the Dnipro River (transects A, B, C) and the eastern shore of Khortytsia Island (transects D, E, F), which covered the floodplain biotopes, the drainage zone (which appeared after the destruction), and the shallow water of the water-edge zone (Table 1). The study plots (with an area of 25 m²) in the floodplain were located 1 m above the former waterline, which existed before the destruction of the Kakhovka Dam. In the drainage zone between the former and current waterlines, depending on the length of the transect, two or three study plots were established. In the shallow water-edge zone, the study plots were established 1 m below the existing waterline. Soil samples from each plot were collected using the “envelope” method (at four points in the corners and in the center of the plot) from a depth of 0–10 cm. The samples were placed in sterile containers and delivered to the laboratory. The studies were carried out within 24 hours from the moment of sample delivery, according to the current regulatory and technical documentation. Depending on the groundwater level and the degree of moisture, the soils of the floodplain were classified as Fluvisols, the soils of the drained zone as Gleysols, and those of the water-edge zone as Subaquatic Gleysols. Soil profiles are described and classified according to the World Reference Base for Soil Resources 2022 (IUSS Working Group of World Reference Base for Soil Resources, 2022).

Sterile tools, utensils, and paper were used to work with the soil. The soil samples were thoroughly mixed with a spatula, ground, and then stones and other solid objects were removed. An average sample of about 0.5 kg was prepared from each sample of one plot. The soil was dispersed before sowing.

Table 1
Soil profile data transects A, B, C, D, E, and F

Site	Location	Landform	Parent material	Soil water status	Soil classification
Transect A					
A1	47.75287° N, 35.20238° E	floodplain	sandy alluvium	slightly moist	Fluvisol
A2	47.75287° N, 35.20227° E	drainage zone	sandy loam alluvium	moist	Gleysols
A3	47.75286° N, 35.20215° E	drainage zone	sandy loam alluvium	wet	Gleysols
A4	47.75287° N, 35.20193° E	water-edge zone	sandy alluvium	permanently submerged	Subaquatic Gleysols
Transect B					
B1	47.82001° N, 35.14711° E	floodplain	sandy alluvium	dry	Fluvisol
B2	47.81986° N, 35.14712° E	drainage zone	sandy alluvium	moist	Gleysols
B3	47.81971° N, 35.14712° E	drainage zone	sandy alluvium	moist	Gleysols
B4	47.81959° N, 35.14712° E	drainage zone	sandy alluvium	moist	Gleysols
B5	47.81956° N, 35.14712° E	water-edge zone	sandy alluvium	permanently submerged	Subaquatic Gleysols
Transect C					
C1	47.82896° N, 35.12355° E	floodplain	sandy alluvium	dry	Fluvisol
C2	47.82874° N, 35.12327° E	drainage zone	sandy alluvium	moist	Gleysols
C3	47.82853° N, 35.12299° E	drainage zone	sandy alluvium	moist	Gleysols
C4	47.82834° N, 35.12269° E	water-edge zone	sandy alluvium	permanently submerged	Subaquatic Gleysols
Transect D					
D1	47.74777° N, 35.17041° E	floodplain	sandy alluvium	slightly moist	Fluvisol
D2	47.74774° N, 35.17006° E	drainage zone	sandy alluvium	moist	Gleysols
D3	47.74774° N, 35.17006° E	drainage zone	sandy alluvium	moist	Gleysols
D4	47.74767° N, 35.16940° E	drainage zone	sandy alluvium	moist	Gleysols
D5	47.74764° N, 35.16908° E	water-edge zone	sandy alluvium	permanently submerged	Subaquatic Gleysols
Transect E					
E1	47.81218° N, 35.14224° E	floodplain	sandy alluvium	slightly moist	Fluvisol
E2	47.81228° N, 35.14247° E	drainage zone	sandy alluvium	moist	Gleysols
E3	47.81240° N, 35.14270° E	drainage zone	sandy alluvium	moist	Gleysols
E4	47.81251° N, 35.14296° E	drainage zone	sandy alluvium	moist	Gleysols
E5	47.81264° N, 35.14320° E	water-edge zone	sandy alluvium	permanently submerged	Subaquatic Gleysols
Transect F					
F1	47.82081° N, 35.11624° E	floodplain	sandy loam alluvium	dry	Fluvisol
F2	47.82094° N, 35.11635° E	drainage zone	sandy loam alluvium	moist	Gleysols
F3	47.82111° N, 35.11645° E	drainage zone	sandy alluvium	wet	Gleysols
F4	47.82111° N, 35.11645° E	drainage zone	sandy alluvium	wet	Gleysols
F5	47.82145° N, 35.11656° E	water-edge zone	sandy alluvium	permanently submerged	Subaquatic Gleysols



Fig. 1. General view of sampling sites (transects): *a* – Balabynska Bay (village Balabyn, Zaporizhzhia Oblast); *b, c, d* – Zaporizhzhia city (left bank of the Dnipro River); *e, f* – Zaporizhzhia city, Khortytsia Island (right bank of the Dnipro River)

In order to detect soil and sanitary indicator microorganisms, ten-fold dilutions were prepared from a soil sample (10 g) and sterile tap water, diluting to 1×10^{-7} g/mL. The soil was treated by 3-minute mixing on an IKA C-MAG MS 4 magnetic stirrer (IKA Werke, Germany). Separate pipettes were used to prepare each dilution. Then, soil cultures were grown on different nutrient media. The number of microorganisms detected was calculated per 1 g of absolutely dry soil: a soil sample (10–20 g) was placed in a preweighed glass jar and dried in a drying oven at 105 °C to constant weight (the first control weighing was carried out after 3 hours, the following ones every 2 hours).

Sanitary-bacteriological study of the soil suspensions was carried out using methods generally accepted in microbiological practice. Pollution indicators were determined using universal and special nutrient agars (nutrient, enterococcus, bismuth sulfite, Wilson-Blair, Kessler, and magnesium [LLC Pharmaktyv, Ukraine], Endo, Czapek-Dox [HiMedia Laboratories Pvt. Ltd, India]). The isolated cultures of microorganisms from characteristic colonies were identified and differentiated using Gram staining, and their cultural and enzymatic properties were studied.

The total microbial count (TMC) of each soil sample was determined to assess the biological activity of the soil medium. For this purpose, 1 mL of the suspension was inoculated into molten peptone agar and cultured at 37 °C for 24 hours in a thermostat (TSO-80/1, Micromed, China, 2018). Inoculation from each dilution was performed on two parallel Petri dishes. After incubation, the number of colony-forming units was counted and converted to 1 g of absolutely dry soil (CFU/g soil).

To detect *E. coli*, the titration method was used. For this purpose, the soil suspension from the first and subsequent dilutions was inoculated into bottles with Kessler's medium and cultured at 37 °C for 48 hours. From the bottles in which gas formation and turbidity or only turbidity were observed, the cultures were transferred to Endo medium and cultured for 24 hours at a temperature of 37 °C.

Sulfate-reducing bacteria and *C. perfringens* were quantified from prepared soil dilutions (up to 1:1,000,000) in two parallel rows of tubes with Wilson-Blair medium, one row of which was heated (at 80 °C for 15 minutes), and the second row was not heated. Incubation of the cultures was carried out at 37 °C for 24 hours. The degree of fecal contamination was determined by detecting thermophilic bacteria on meat-peptone agar after 24 hours of incubation. Soil suspensions in dilutions of 1:10 – 1:1,000,000 were plated on two parallel Petri dishes and cultured at 60 ± 2 °C.

Enterococci were isolated using the surface method by plating a 1:10 – 1:100 dilution of the suspension onto enterococcus agar. Cultivation was carried out at 37 °C for 48 hours.

The total number and percentage of soil bacilli, which is an indicator of the depth of mineralization of the organic substrate, were determined on nutrient agar after preliminary heating of soil sample dilutions at 80 °C (for 15 minutes). In contaminated soils, bacilli were isolated in the range of up to 20%.

Saprophytic fungi were isolated in soil suspension dilutions 1:10–1:100 by surface inoculation, seeded on Czapek agar, and cultivated at 22 ± 2 °C for 48–72 hours.

Acid-fast mycobacteria were detected by flotation. For this purpose, caustic soda, distilled water, and xylene were added to the filtrate of the soil suspension, kept until a flotation ring was formed, treated with sulfuric acid and inoculated onto a Stonebrink medium (Graso Zenon Sobiecki, Poland). Cultivation was carried out for 90 days at a temperature of 36 ± 2 °C. The isolated mycobacteria were identified and differentiated with smear staining using the Ziehl-Neelsen method, and their cultural and enzymatic properties were studied.

Salmonella were isolated from soil suspension (55.5 g of soil in 500 mL of sterile tap water) by diluting it in magnesium medium to 0.45, 4.5, 45, and 450 mL, which corresponded to 0.05, 0.5, 5, and 50 g of soil, respectively. After 5 and 20 hours of incubation, subculturing onto bismuth-sulfite agar was performed. The isolated bacteria were identified according to the generally accepted method.

Results

It was found that the qualitative and quantitative characteristics of the soil microbiota depended on both the sampling point and its proximity to the Dnipro River. In 75.0% of the soil samples from Transect A, the coli titer was higher than acceptable for the soil classified as clean and safe (Table 2), which characterizes it as polluted with feces.

Table 2

Assessment of the level of microbiological contamination of soils of Transect A

Parameters	Sampling study plots			
	A1	A2	A3	A4
TMC, CFU/g	2.10×10^7	1.30×10^6	2.52×10^7	1.52×10^6
Coli titer, g	0.9	–	0.67	0.01
Coliform bacteria, CFU/g				
<i>E. coli</i> , CFU/g	1.10×10^0	–	1.50×10^0	1.00×10^2
<i>Klebsiella</i> spp., CFU/g	1.30×10^2	1.00×10^1	2.00×10^2	1.50×10^2
<i>Enterobacter</i> spp., CFU/g	–	–	–	–
<i>Salmonella</i> spp., CFU/g	–	–	–	–
<i>Proteus</i> spp., CFU/g	–	–	–	–
<i>Pseudomonas</i> spp., CFU/g	–	–	–	1.5×10^3
<i>Perfringens</i> titer, g	–	–	–	0.00101
<i>C. perfringens</i> , CFU/g	–	–	–	9.90×10^2
Sulfate-reducing bacteria, CFU/g	–	–	1.83×10^2	5.50×10^2
Thermophilic bacteria, CFU/g	1.50×10^4	1.29×10^6	2.63×10^3	5.00×10^2
<i>Bacillus</i> spp., CFU/g	5.00×10^5	3.10×10^3	4.30×10^5	–
<i>Enterococcus</i> spp., CFU/g	–	–	–	–
Saprophytic fungi, CFU/g	3.42×10^3	3.40×10^3	2.00×10^2	1.00×10^2

Note. “–” – no representatives of the corresponding microorganisms were detected in the sample of the indicated plot.

From the Transect A study plots, bacteria of the *Escherichia coli* group were isolated: *Klebsiella* spp. 1.00×10^1 – 2.00×10^2 CFU/g, as well as sulfate-reducing bacteria and high levels of thermophilic bacteria, which indicates environmental pollution with manure and decomposing remains. Therefore, according to the degree of contamination, the soil from such plots is characterized as highly polluted and as sanitary unsuitable according to the degree of danger. At the same time, from the Transect A soil samples, microorganisms from the genus *Bacillus* spp. were isolated, which indicates incomplete mineralization processes, that is, decomposition of organic substances and compounds that have been contained in plant or animal remains.

The composition of the soil microbiota of Transect B (the city of Zaporizhzhia, left bank of the Dnipro, sampling plot 180–200 m downstream from the source of the Sukha Moskovka River) exhibited

a greater diversity of microbial cenosis, in particular according to the indicators of coliform bacteria and *Pseudomonas* spp., compared with the indicators of the soil sampled at the Transect A plots (Table 3). Only in one soil sample plot (B3) of this transect, among the total microbial number, *Escherichia coli* was isolated and its coli titer was equal to 100 g. The number of thermophilic bacteria was within the acceptable level, however the perfringens titer was 0.001 g. Other samples with high TMCs contained bacteria of the *E. coli* group, as well as decomposer bacteria such as thermophilic bacteria, the number of which was above the acceptable level, and also sulfate-reducing bacteria. In addition, soil bacilli were detected, accounting for up to 20% of the total microbial contamination. Such results indicate incomplete mineralization processes in the soil, as well as contamination with organic fertilizers. Thus, along Transect B, a different qualitative and quantitative composition of the soil microbiota was determined, which characterizes the site as unevenly contaminated with organic residues, in which mineralization processes continue.

Table 3
Assessment of the level of microbiological contamination of soils of Transect B

Parameters	Sampling study plots				
	B1	B2	B3	B4	B5
TMC, CFU/g	3.43×10 ⁶	1.30×10 ⁶	3.51×10 ⁴	3.18×10 ⁸	3.21×10 ⁴
Coli titer, g	–	–	100.0	–	–
Coliform bacteria, CFU/g:					
<i>E. coli</i>	–	–	1.0×10 ²	–	–
<i>Klebsiella</i> spp.	1.1×10 ¹	2.0×10 ¹	–	6.0×10 ¹	–
<i>Enterobacter</i> spp.	–	1.0×10 ¹	–	2.0×10 ¹	1.0×10 ¹
<i>Citrobacter</i> spp.	–	–	–	3.0×10 ¹	2.0×10 ¹
<i>Salmonella</i> spp., CFU/g:	–	–	–	–	–
<i>Proteus</i> spp., CFU/g:	–	–	–	–	–
<i>Pseudomonas</i> spp., CFU/g:	–	–	–	–	–
<i>P. fluorescens</i> , CFU/g:	–	–	–	–	1.00×10 ¹
Perfringens titer, g	–	0.00067	0.001	–	0.00067
<i>C. perfringens</i> , CFU/g	–	1.5×10 ³	1.5×10 ³	–	1.5×10 ³
Sulfate-reducing bacteria, CFU/g	–	1.5×10 ³	–	1.5×10 ³	1.5×10 ³
Thermophilic bacteria, CFU/g	9.88×10 ²	1.04×10 ³	7.00×10 ²	6.33×10 ³	5.05×10 ³
<i>Bacillus</i> spp., CFU/g	–	4.11×10 ³	–	–	–
<i>Enterococcus</i> spp., CFU/g	–	–	–	–	–
Saprophytic fungi, CFU/g	1.03×10 ⁵	1.22×10 ⁴	1.42×10 ⁴	1.53×10 ⁴	1.35×10 ⁴

Note: see Table 2.

In the soil cover samples of Transect C, located in the city of Zaporizhzhia on the banks of the Dnipro River (250–270 m below the Voznesenskyi Beach), certain differences were found compared with the data obtained from the study areas described above. In particular, the soil samples of this transect exhibited high TMCs. In 50% of the samples (C2–C3) of the total samples studied, a low coliform titer (<0.9) was determined, and also sulfate-reducing bacteria (C1 and C4) were isolated (Table 4).

In three soil samples (C2–C4), the number of thermophilic bacteria exceeded 1,000 CFU/g. The obtained data on the contamination of the dry land areas with thermophilic bacteria indicate a natural self-cleaning of the soil cover in the area from organic residues. In all the studied samples of Transect C, the growth of saprophytic fungi was established. Organic soil contamination was also indicated by the detection of bacilli, the highest indicators of which were recorded at point C3. This characterizes incomplete mineralization processes. The detection of thermophilic bacteria, *C. perfringens*, and *Escherichia* is a sign of contamination of the site with manure or compost at different stages of decomposition.

When analyzing the composition of the soil microbiota of Transect D (Zaporizhzhya city, left bank of the Dnipro River, Domakha), indicators characterizing the type of vegetation and soil were taken into account. Alluvial sandy soils were dominant in this area, and the vegetation of individual plots was close to forest. It was established that the TMC was quite high (Table 5). Studies of the soils of this area revealed no *E. coli*. However, *Klebsiella* spp., *Enterobacter* spp., and *Citrobacter* spp., as well as *Pseudomonas* spp. were isolated from the samples. The latter are considered indicators of organic matter de-

composition. It should be noted that from sample D2, acid-fast *Mycobacterium* spp. were isolated, which, by growth rate and ability to form pigment, were attributed to group IV of atypical mycobacteria (Runyon, 1959). The diverse qualitative composition of the soil microbiota of Transect D characterizes its high microbiological activity. Sufficiently high TMCc in samples taken from points D1, D3, D4, and D5 and the isolation of *C. perfringens* and sulfate-reducing bacteria indicate long-term soil contamination. Thermophilic bacteria, *Bacillus* spp., and saprophytic fungi indicate the processes of soil purification from organic contamination.

Table 4
Assessment of the level of microbiological contamination of soils of Transect C

Parameters	Sampling study plots			
	C1	C2	C3	C4
TMC, CFU/g	4.00×10 ⁷	8.74×10 ⁹	6.31×10 ⁷	2.04×10 ⁸
Coli titer, g	–	0.0277	0.0106	4.16
Coliform bacteria, CFU/g:				
<i>E. coli</i>	–	3.60×10 ¹	9.40×10 ¹	2.40×10 ¹
<i>Klebsiella</i> spp.	–	–	–	–
<i>Enterobacter</i> spp.	2.30×10 ¹	–	7.40×10 ¹	2.50×10 ¹
<i>Salmonella</i> spp., CFU/g:	–	–	–	–
<i>Proteus</i> spp., CFU/g:	–	–	–	–
<i>Pseudomonas</i> spp., CFU/g:	–	4.77×10 ¹	8.00×10 ¹	1.5×10 ²
<i>P. fluorescens</i> , CFU/g:	–	–	–	0.00018
Perfringens titer, g	–	–	–	5.50×10 ³
<i>C. perfringens</i> , CFU/g	2.75×10 ³	–	–	2.90×10 ³
Sulfate-reducing bacteria, CFU/g	8.00×10 ²	7.50×10 ⁴	2.83×10 ⁴	5.00×10 ⁵
Thermophilic bacteria, CFU/g	–	1.00×10 ²	3.70×10 ³	3.00×10 ³
<i>Bacillus</i> spp., CFU/g	–	–	–	–
<i>Enterococcus</i> spp., CFU/g	3.18×10 ⁵	5.69×10 ³	5.85×10 ⁵	5.00×10 ⁵

Note: see Table 2.

The composition of the soil microbiota of Transect E, despite the remoteness of the site from the densely populated residential area of the city of Zaporizhzhia and the uniqueness of the historical complex of Khortytsia Island, still had signs of contamination. The soil cover sampled from all the studied plots of Transect E, according to the conducted studies, had varying degrees of fecal contamination, as evidenced by the fact of detection of *Enterococcus* spp., *E. coli*, *Enterobacter* spp., *Citrobacter* spp., *C. perfringens*, and sulfate-reducing bacteria. Low perfringens titer values (<0.0009) point to a significant and long-standing soil contamination with manure and sewage wastes. The obtained values of TMC in the soil cover may indicate ongoing intensive processes of self-purification from organic pollution. This assumption is confirmed by the presence of at least a small number of thermophilic bacteria in the samples from plots E2 and E3, and their above-threshold values at plot E1, indicating the processes of decomposition of organic residues with the release of a large amount of heat. In addition, bacilli (≤20 %) were found in some samples, which further confirms the fact of natural self-purification of the soil at these points from organic pollution and incomplete processes of soil mineralization. Isolation of *Proteus* spp. confirmed the processes of decomposition of organic materials. It should be noted that soil samples from this site, similarly to those from Transect A, contained a low number of saprophytic fungi. Therefore, the TMC of the soil taken from this transect was low among the six sites studied. The isolated soil microbiota indicate the duration of the processes of soil self-purification.

Transect F was located on the right bank of the Dnipro River and also on Khortytsia Island, approximately 2,000 m upstream. Its soil microbiota showed a heterogeneous composition (Table 7). Thus, the analysis of soil microbiota indicates a heterogeneous sanitary and microbiological state of the soils in Transect F. Isolation of *E. coli*, *Enterococcus* spp., as well as bacteria of the genera *Klebsiella*, *Enterobacter*, *Citrobacter*, *Proteus*, and *C. perfringens*, indicates organic and fecal contamination of the soils. The most contaminated plot was F4, which demonstrated high TMC, above-threshold limits of coli titer, and the presence of enterococci, confirming a fresh fecal contamination. Isolation of thermophilic bacteria and saprophytic fungi indicates the processes of decomposition and mineralization of organic substances in the soil.

Table 5

Assessment of the level of microbiological contamination of soils of Transect D

Parameters	Sampling study plots				
	D1	D2	D3	D4	D5
TMC, CFU/g	2.00×10 ⁸	6.00×10 ⁷	1.75×10 ⁸	4.14×10 ⁸	3.42×10 ⁸
Coli titer, g	–	–	–	–	–
Coliform bacteria, CFU/g:					
<i>E. coli</i>	–	–	–	–	–
<i>Klebsiella</i> spp.	–	2.00×10 ¹	6.00×10 ²	4.00×10 ²	–
<i>Enterobacter</i> spp.	–	–	1.00×10 ⁰	1.00×10 ¹	8.00×10 ¹
<i>Citrobacter</i> spp.	1.00×10 ²	3.00×10 ²	–	–	–
<i>Salmonella</i> spp., CFU/g:	–	–	–	–	–
<i>Proteus</i> spp., CFU/g:	–	–	1.00×10 ¹	–	–
<i>Pseudomonas</i> spp., CFU/g:	–	4.00×10 ²	2.90×10 ²	–	5.00×10 ¹
Perfringens titer, g	0.00018	0.001	–	0.00018	0.00018
<i>C. perfringens</i> , CFU/g	5.50×10 ³	1.00×10 ³	–	5.50×10 ³	5.50×10 ³
Sulfate-reducing bacteria, CFU/g	1.00×10 ³	1.00×10 ³	–	5.00×10 ³	5.58×10 ³
Thermophilic bacteria, CFU/g	–	–	2.00×10 ³	–	–
<i>Bacillus</i> spp., CFU/g	–	5.00×10 ³	3.00×10 ³	–	–
<i>Mycobacterium</i> spp. (acid-fast)	–	2.00×10 ⁰	–	–	–
<i>Enterococcus</i> spp., CFU/g	–	–	–	–	–
Saprophytic fungi, CFU/g	3.70×10 ³	1.37×10 ³	9.70×10 ³	6.44×10 ⁴	–

Note: see Table 2.

Table 6

Assessment of the level of microbiological contamination of soils of Transect E

Parameters	Sampling study plots				
	E1	E2	E3	E4	E5
TMC, CFU/g	2.00×10 ⁵	5.71×10 ⁵	7.80×10 ⁷	1.50×10 ⁶	2.01×10 ⁵
Coli titer, g	0.0025	–	0.0022	–	–
Coliform bacteria, CFU/g:					
<i>E. coli</i>	4.00×10 ²	–	4.50×10 ²	–	–
<i>Klebsiella</i> spp.	–	–	–	–	–
<i>Enterobacter</i> spp.	1.40×10 ⁻¹	8.00×10 ⁻²	4.30×10 ¹	5.40×10 ⁻¹	–
<i>Citrobacter</i> spp.	2.80×10 ¹	2.00×10 ¹	–	4.80×10 ¹	3.00×10 ²
<i>Salmonella</i> spp., CFU/g:	–	–	–	–	–
<i>Proteus</i> spp., CFU/g:	2.00×10 ¹	–	–	–	1.14×10 ²
<i>Pseudomonas</i> spp., CFU/g:	–	–	–	–	–
Perfringens titer, g	0.00007	0.00099	0.00099	0.00099	0.00099
<i>C. perfringens</i> , CFU/g	1.50×10 ⁴	1.01×10 ³	1.01×10 ³	1.01×10 ³	1.01×10 ³
Sulfate-reducing bacteria, CFU/g	–	1.00×10 ³	1.00×10 ³	1.00×10 ³	5.50×10 ³
Thermophilic bacteria, CFU/g	4.00×10 ³	7.40×10 ²	2.20×10 ²	–	–
<i>Bacillus</i> spp., CFU/g	–	1.00×10 ³	1.00×10 ⁴	1.00×10 ²	0
<i>Enterococcus</i> spp., CFU/g	2.70×10 ²	–	2.00×10 ¹	–	–
Saprophytic fungi, CFU/g	3.45×10 ²	5.15×10 ²	1.53×10 ³	1.43×10 ³	2.81×10 ³

Note: see Table 2.

Discussion

Soil is a biologically active environment inhabited by various bacteria (mainly saprophytes and opportunistic pathogens, and sometimes pathogenic bacteria), as well as fungi, actinomycetes, and other organisms that participate in the decomposition of organic matter, and processing of nutrients. Some representatives of soil microbiota can serve as indicators of environmental pollution (Borie et al., 2008). The data from Tugel et al. (2000) indicate that soil can contain 8–15 tons of bacteria, fungi, protozoa, nematodes, earthworms, and arthropods.

As the organic matter content of the soil increases, the qualitative composition of the soil microbiota also changes (Ausec et al., 2009). Soil microbial diversity can vary depending on different forms of anthropogenic activity: urbanization, agricultural intensification, pesticide use, and environmental pollution. However, the consequences of these changes for underground and aboveground ecosystems remain poorly understood (Kirk et al., 2004). Yoon et al. (2024), when studying soil from seven different locations in South Korea (rice fields, highland fields, forest areas, areas contaminated with hydrocarbons and heavy metals, greenhouse soils, and reclaimed tidal soils), established the dependence of microbial diversity and composition of the biocenosis on land use methods and soil chemical properties: Polluted soils were observed to have reduced bacterial biodiversity.

Table 7

Assessment of the level of microbiological contamination of soils of Transect F

Parameters	Sampling study plots				
	F1	F2	F3	F4	F5
TMC, CFU/g	1.33×10 ⁷	4.85×10 ⁶	4.51×10 ⁵	4.64×10 ⁸	1.58×10 ⁸
Coli titer, g	100	–	–	0.9	–
Coliform bacteria, CFU/g:					
<i>E. coli</i>	1.00×10 ¹	–	–	1.10×10 ⁰	–
<i>Klebsiella</i> spp.	–	–	3.00×10 ⁻¹	1.00×10 ⁻¹	–
<i>Enterobacter</i> spp.	1.00×10 ²	1.00×10 ¹	–	1.00×10 ²	4.00×10 ²
<i>Citrobacter</i> spp.	3.00×10 ²	–	–	4.10×10 ²	1.20×10 ⁰
<i>Salmonella</i> spp., CFU/g:	–	–	–	–	–
<i>Proteus</i> spp., CFU/g:	–	1.00×10 ¹	–	4.00×10 ¹	7.00×10 ⁰
<i>Pseudomonas</i> spp., CFU/g:	2.00×10 ²	–	2.00×10 ⁻¹	–	–
Perfringens titer, g	–	0.00067	–	0.001	–
<i>C. perfringens</i> , CFU/g	–	1.50×10 ³	–	1.00×10 ³	–
Sulfate-reducing bacteria, CFU/g	–	5.50×10 ³	1.00×10 ³	9.00×10 ²	4.30×10 ³
Thermophilic bacteria, CFU/g	–	4.00×10 ²	–	–	–
<i>Bacillus</i> spp., CFU/g	–	1.00×10 ⁴	–	–	–
<i>Enterococcus</i> spp., CFU/g	4.80×10 ²	–	–	1.20×10 ⁵	–
Saprophytic fungi, CFU/g	9.92×10 ³	1.06×10 ⁴	3.32×10 ³	1.01×10 ⁴	1.15×10 ³

Note: see Table 2.

Nacke et al. (2008) demonstrated a higher bacterial diversity in pasture soils than in forest soils near the Swabian Jura mountain range (Germany). Seasonal differences in bacterial and fungal communities were reported, with more bacteria isolated in May and July, and more fungi in September and October (Sun et al., 2017). According to Boer et al. (2005), fungi are able to decompose organic matter (cellulose and lignin), the amount of which increases in autumn when plants die. Kong et al. (2023) observed a predominance of Proteobacteria (33.94% to 52.09%), Acidobacteriota (4.94% to 15.88%), Bacteroidota (6.52% to 11.15%), Actinobacteriota (7.18% to 9.61%), and Firmicutes (4.52% to 16.80%) during soil remediation and mine rehabilitation. The authors claim that during the melioration, which was carried out in the second year after the start of reclamation, a decrease in the number of dominant microorganisms (representatives of the genus *Bacillus*) was noted, while the use of solid fertilizers contributed to the development of microbial biocenosis. Fierer et al. (2007) emphasize that a better understanding of the structure and functions of soil bacterial communities is possible through the differentiation of copiotrophic and oligotrophic categories of bacteria.

The sanitary condition of the soil (fecal contamination) of a certain territory is assessed by determining its microbiological composition. Isolation of sanitary indicator microorganisms (such as *E. coli*, *C. perfringens*, *Enterococcus* spp., and other bacteria) indicates the origin, degree, and duration of the contamination. Valério et al. (2022) isolated numerous microorganisms, including fecal indicator bacteria, from the beach sand of Terceira Island (Portugal), which contained marker genes of dogs, seagulls, ruminants, and humans. Without a doubt, some of the isolated pathogens could pose a threat to the population. Since the soil microbiota is crucial for the health of the planet, clear standards for its identification are needed, which will allow combating environmental problems caused by human activity.

Research revealed that the survival and persistence of *E. coli* strains is subject to soil type: In loamy soil, the survival of *E. coli* was higher ($P \leq 0.05$) than in sandy soil (Alegbeleye & Sant'Ana, 2023). Premsuriya et al. (2007) found that grazing cattle on perlite-rich soil improved the soil texture and increased the nutrient content, leading to a greater number of Firmicutes and Chloroflexi and a lower number of Actinobacteria. However, in such areas, regardless of livestock grazing, the level of enteric pathogens of the Enterobacteriaceae family has not increased.

Coliform bacteria contaminate environmental objects, including agricultural lands, through the application of animal manure as fertilizer to the soil (Fatoba et al., 2021). According to Staley et al. (2014), *E. coli* and *Enterococcus* spp. are common indicators of fecal contamination of environmental objects. Cools et al. (2001) found that the survival rates of bacteria inhabiting the gastrointestinal tract of both animals and humans varied in different soil types, with *E. coli* surviving better in sandy soil, and *Enterococcus* spp. surviving longer

in loamy soil. The survival time of these bacteria in the soil was 68–80 days. In addition, their survival is affected by lower temperature (5 °C) and higher moisture content. We found that low coli titers (≤ 0.9) in some soil samples from transects A, C, E, and F indicated fresh fecal contamination. Furthermore, coliform bacteria were isolated: *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., and *Enterococcus* spp., suggesting fecal contamination and unsatisfactory sanitary condition of the soil.

Casteel et al. (2006) found total coliform bacteria and high levels of *C. perfringens* spores ($P < 0.001$) in soil samples from flooded eastern North Carolina after Hurricane Floyd and other storms in 1999. Environmental sites have been contaminated by fecal waste from sewage and livestock farms. In our study, high levels of isolated *C. perfringens* indicated long-standing fecal contamination of certain soil sites. Certain species of decomposer bacteria play an important role in soil purification, as they are able to break down pesticides and other pollutants (Tugel et al., 2000). Lawal et al. (2020) in their studies conducted in the Northern Guinea Savannah (Nigeria), found a significantly higher content of bacteria of the genera *Bacillus* and *Micrococcus* in the silt and clay fraction of the soil, while the bulk of the soil was much richer in the population of fungi. The authors claim that in soils where cultivation is carried out, there is an accumulation of a larger amount of organic matter and, accordingly, a higher microbial population, since they are more resistant than fungi. The isolated species of bacilli play the role of active decomposers of organic matter in the soil. In general, representatives of the *Bacillus* genus are display notable resistance to environmental factors (Tugel et al., 2000). The results of our studies revealed the presence of *Bacillus* spp. in samples from the areas where soil self-purification processes were incomplete.

Ueda et al. (2001) isolated mixed cultures of thermophilic bacteria from compost, soil, animal feces, and intestinal contents, as well as feed. López et al. (2021) conducted a study of thermophilic bacteria and fungi that inhabited compost material and participated in the decomposition of lignocellulose, and established their ability to grow in a wide range of temperatures. This property allows them to survive and remain metabolically active at all stages of composting. In an experiment, on the 20th day of the composting process of municipal solid waste in the city of Isfahan (Iran), the researchers established the presence of only *G. stearothermophilus*, *A. acidocaldarius* and *B. schleglii*. The thermotolerant fungi *Cladosporium*, *Aspergillus*, *Mucor*, *Rhizopus*, and *Absidia* spp. were isolated on the 15th day (Ghazifard et al., 2001). Therefore, our isolation of thermophilic bacteria in quantities exceeding the acceptable norm (10^2 – 10^3) from the samples of transects A, B, C, and E indicated contamination of soil plots with manure or compost.

Mycorrhizal fungi are more efficient at processing carbon to form new cells and less nitrogen (carbon to nitrogen ratio 10:1), and unlike bacteria, require a constant source of nutrition and grow better in no-till soils (Tugel et al., 2000). Saprophytic fungi are aerobic. Garcha et al. (2016) isolated populations of bacteria, fungi, and actinomycetes in the foothill zone of Punjab in the amount of 6.8×10^3 to 1.4×10^5 CFU/g, from 2.9×10^3 to 3.6×10^4 CFU/g, and from 2.0×10^3 to 5.3×10^4 CFU/g according to different land use systems. In soils with a low content of molecular oxygen, which is necessary for the metabolism of fungi, their number gradually decreases, up to their complete disappearance (Tugel et al., 2000). Our studies revealed a low number and the absence of saprophytic fungi in the soil samples that were waterlogged and compacted, namely transects A, D, and F, especially samples that were under water (A5, D5, and F5 – $< 1.15 \times 10^3$ CFU/g).

Representatives of the genus *Pseudomonas* in healthy soils indicate a well-established microflora. In soils, *P. fluorescens* is able to promote plant growth by producing growth factors, exhibiting anti-fungal activity, and secreting a compound that can inhibit the growth of pathogens (Tugel et al., 2000). However, high levels of *Pseudomonas* spp. indicate fecal contamination of the soil. Cools et al. (2001) reported that the use of manure as a fertilizer can promote the transfer of antibiotic resistance genes to the inhabitants of soil ecosystems *Proteus* spp. and *Pseudomonas* spp. The small number of *Proteus* spp. and *Pseudomonas* spp. that we isolated indicated the biological activity of the soil environment.

Conclusions

At all the sampling points, there are completely natural, intensive phenomena of soil cover purification from organic contamination, including feces. Monitoring the sanitary condition of areas A, B, C, D, E, and F allows us to assess the safety of soils for the environment and determine the possible impact on humans. The results of the studies revealed a local contamination of the plots of the studied areas. The lowest indicators of the intensity of vital activity of microorganisms were found in samples of transects A, E, and partly Transect B (mainly from 10^4 to 10^7 CFU/g). The TMCs at points of transects C and D and partly F were $\geq 10^7$ CFU/g. The sanitary condition of the soil was characterized by the release of sanitary indicator microorganisms (*E. coli*, *Enterococcus* spp., *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., and *C. perfringens*), indicating fresh or old fecal contamination. The detection of thermophilic bacteria, *Bacillus* spp., and saprophytic fungi explained the degree of organic matter transformation processes and the stages of soil self-purification. The degree of contamination of soil samples was characterized by coli titer and perfringens titer. For soils in an unsatisfactory sanitary condition, the coli titer was ≤ 0.9 , and the perfringens titer was ≤ 0.009 . Pathogenic *Salmonella* spp. were not detected in any of the soil samples, but *Mycobacterium* spp. were isolated. The presence of *E. coli* and sulfate-reducing bacteria was established in the soils of the drainage zone of most of the studied transects. The results obtained are important and can be used when assessing the epizootic situation that has developed in Zaporizhzhia Oblast as a result of the destruction of the Kakhovka HPP. At the same time, regular monitoring of the sanitary condition of the soils of the drainage zone formed after the destruction of the dam is necessary in order to prevent the spread of pathogenic microorganisms in natural ecosystems.

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