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Improving the sanitary condition of fish pond bed by forage grass cultivation

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The main purpose of the complex of reclamation works for the preparation of ponds for further exploitation is to create conditions for accelerating the processes of mineralization of organic substances that have accumulated during the growing season, to increase the intensity of development of the natural forage base in the next season, to reduce the risk of fish diseases. The purpose of the work was to develop an environmentally friendly method of improving the sanitary condition of the soil of the pond bed during its casting by sowing agricultural plants having bactericidal properties. The studies were performed using conventional techniques. Bacteria of the group of *E. coli*, *Salmonella*, and *Enterococci* were isolated from the soil of the pond bed. The sanitary and bacteriological conditions of the soil of the bed of the pond during the cultivation of different crops after 140-150 days after the water was lowered showed that by the end of the growing season (after 150 days after the casting) there is a gradual decrease in microbial contamination of the soil, reduced the amount of coli-titer and titer of *Enterococci*. The intensity of microbial decontamination is directly dependent on the type of fodder grown. The results of cultivation of crops 90 days after the descent of water showed that the processes of self-purification of the soil of the botanical ground are influenced by their rhizosphere - a plot of soil directly adjacent to the roots of plants and to which the root secretions and soil microorganisms act. 90 days after the descent of water, the canary grass and white turmeric were the most active in the process of remediation of contaminated soil. Compared to baseline, microbial soil contamination during this period decreased by 79.6% ($p \leq 0.001$) and 78.3% ($p \leq 0.001$, respectively). The coli-titer and *Enterococci* titer was 0.01. The annual activity of amaranth and rapeseed was the least active in self-purification processes. After 90 days of soil experiment, number of mesophilic aerobic and facultative-anaerobic microorganisms decreased by 61.3% ($p \leq 0.001$) and 55.7% ($p \leq 0.001$), respectively, and the coli-titer and *Enterococci* titer decreased to 0.01. At the end of the growing season, from the beginning of the season, a further decrease in soil microbial contamination was observed, and the intensity of decontamination was directly dependent on the type of forage grasses.

Keywords: Soil; Bed; Pond; Casting; Botanical ground; White clover; *E. coli; S. dublin*

Introduction

In recent decades, there has been a rapid increase in the production and marketing of fishery products, and the organization of multi-directional use of fish ponds (production, fishing, recreation, education, etc.) provides significant additional economic benefits (Popp et al., 2019). Along with increasing production, the likelihood of biological problems that directly affect the quality and safety of products has increased (Føre et al., 2018, Jankowski et al., 20181, 2018b).

Among the diversity of abiotic and biotic environmental factors that affect the fish body and cause its disease the most important are the temperature, gas and salt regimes of water, fluctuations in their levels, current, age and species composition of fish community, polyculture, fish population density, as well as the presence of infectious or invasive pathogens in the aquatic environment (Bostock et al., 2010; Opiyo et al., 2018). Together with the soil of the reservoir bed other things such as bacteria, inferior plants, invertebrate forage and the water influence the fish, in particular the processes of their respiration, digestion, hematopoiesis and circulation, nervous system, reproduction, growth and development. Therefore, for providing normal life conditions to fish and maintaining its vitality at a certain level in reservoirs, it is necessary to create optimal zoo hygienic conditions that would ensure its normal physiological and immunological status (Cooke et al., 2004). The fish industry as a whole has a significant epizootiological status, which is determined by the presence or absence of infectious and parasitic fish diseases (Atkins, 2011; Hossain et al., 2011). Many diseases do not have vaccination protocols, and overuse of antibiotics and other chemicals is not economically justifiable and environmentally hazardous (Pulkkinen et al., 2010). A more sustainable strategy for the protection of fish against infection is the application of scientifically sound and well-conducted veterinary measures (de Bruijn et al., 2018; Mon-On et al., 2018).

Fishery intensification methods involve the introduction of organic matter into the ponds (in the form of fertilizer, animal feed, etc.) (Hossain et al., 2013). In addition, a significant amount of organic matter comes from the environment. Accumulation at the bottom

sediments of the remains of dead aquatic vegetation, invertebrates, fish excrement, lack of oxygen in the soil of a pond bed lead to the accumulation of pathogens of contagious fish diseases, siltation of reservoirs, deterioration of their sanitary status, and hygiene conditions resulting in a decrease in fish productivity (Rosa Rdos et al., 2013).

The casting (leaving the reservoir without water for at least a year) - the process which is rather complicated and does not give a proper effect. During the summer, ponds with heavy sludge deposits are thoroughly drained in the first summer, and the following reclamation measures are taken during the next summer. The main purpose of the complex of reclamation works for the preparation of ponds for further exploitation is to create conditions for accelerating the processes of mineralization of organic substances accumulated during the growing season, increasing the intensity of the development of the natural forage in the next season reducing the risk of fish diseases (Suo et al., 2015). The bed of ponds is ploughed and limed. Depending on the capacity of the sludge, the casting may take several years. If a pond became devoid of water for only one summer, the following year there is an increased overgrowth of the reservoir (Sieben et al., 2016).

For the destruction of pathogens of contagious fish diseases in the environment of fisheries preventive and, if necessary (in connection with the occurrence of the disease), forced disinfection are regularly carried out (disinfestations of ponds and their hydraulic structures, swimming pools, aquariums, gardens, fishing gear, incubation shops and work wear (Verner-Jeffreys et al., 2009; Gonçalves & Gagnon, 2011; Jussila et al., 2014). For this purpose a number of disinfectants, that belongs to different chemical groups and have different physicochemical and biocidal properties, are used (Paliy et al., 2015, 2016, 2018; Rahman et al., 2017; Jaemwimol et al., 2019). However, the use of compounds is not always an effective and safe measure in fisheries (Straus et al., 2012). The most acceptable in modern aquaculture are environmentally friendly methods of water and soil disinfection (Céréghino et al., 2014).

During the casting, the ponds are sown with different crops. Their root systems maintain the soil of the bed in a loose state, and the crop removes excess minerals. Crop yields compensate for the lack of fish products in the ponds during the unproductive period (Marshall et al., 2016). One of the pressing problems today is the study of plant properties such as phytoncidal activity, which is the ability of plants to emit particularly specific substances - phytoncides that have bactericidal, fungicidal and antiparasitic properties (Dimkpa et al., 2009). The biological feature of plants to accumulate a variety of microorganisms in the soil around the roots and to isolate the bactericidal compound has become a prerequisite for attempts to use plants against pathogens of contagious animal and human diseases. Soil is a major defining natural self-healing resource, an environment in which the biological diversity of living organisms is formed (Berg & Smalla, 2009). Interaction between plants and microorganisms is a dynamic process in which the root system of plants plays an important role (Broeckling et al., 2008).

Plant secretions are known to influence the formation of soil microbiome and its function, the number of different ecological-trophic and physiological groups of microorganisms and their species diversity (Buée el al., 2009).The use of agro-aquaculture and aquaculture is becoming widespread in fisheries today. The sowing of herbs such as clover, turmeric and other species results in a decrease in the number of *E. coli* bacteria in the soil. Bacterial soil contamination was reduced 18-fold compared to control sample under sowing *Dactylis glomerata* (cock's foot) after 60 days. When growing oatmeal, ryegrass, clover, the death of *E. coli* and *B. aerogenes* in the soil occurred much earlier than in the breeding plant. In black soil cultivated with pea plants, timothy, carrots, and oatmeal, paratyphoid bacteria perished in 44-79 days, and in control soil bacteria survived up to 140-174 days (Nunan et al., 2005; Berg et al., 2005; Barret et al., 2011).

The aim of our work was to study the bacterial background of soil of the pond bed and sanitizing effect of forage plants.

Materials and Methods

Experimental studies were performed in the laboratory for monitoring the water and soils of the Sumy Hydrogeologic Land improvement station, which is the laboratory for ecological safety of land, the environment and the quality of products of the Sumy Branch of the State Agency for Public Administration.

For the rehabilitation of the soil of the botanical ground (BG) were used four types of forage grasses, which were sown in the soil of the bed pond at the beginning of the growing season. Two perennial species were used: honey clover (*Melilotus albus*), true canary grass (*Phalaris canariensis*) and two annuals: winter rapeseed (*Brassica napus*) and forage amaranth (*Amaranthu spabulum*) herbs. The use of these forage crops is due to their availability, cheapness and ease of cultivation.

To examine the sanitary condition of the bed, the soil was sampled one, five, and ten days after the water was lowered and every 30 days thereafter for 4 months. A sterile, fluted knife with a wide blade was used to take a 200-300 g sample. The soil type was sod-podzolic, pH 6.2. Samples were taken at 5 points diagonally and then mixed into an average sample, which was placed in a sterile flask with a cotton-gauze plug. Before the experiment, the soil was dispersed. To account for the sanitary microorganisms, a sample of 30 ± 1 g was taken. In a sterile vessel, the sample was diluted with sterile distilled water in 1:10 ratio. The soil suspension of the first dilution was shaken vertically for 10 minutes, followed by treatment with a mechanical dispersant (RT-2) stirrer for three minutes. The soil suspension after the first treatment was used to prepare 10-fold dilutions.

To determine the microbial number, each dilution of soil suspension was introduced 1 cm^3 into Petri dishes, poured with meat-peptone agar (MPA) at 45ºC. The samples were kept at 28.5-30.5ºC for 48 hours. Colonies were counted in 20 squares of 1 cm^2 using a grid plate and a magnifying glass. The bacterial count in one gram of soil was determined by multiplying the average number of the colonies (30 to 300) by the cup area.

The soil research for *E. coli* bacteria was conducted by planting soil suspension (1:10) in Kessler medium. Samples were cultivated for 48 hours at 37ºC. As a positive result (turbidity and gas formation), bacteria were grown on Endo medium, Salmonella differential agar (HiMedia, India) and rose agar, which were cultivated for 24 hours at 37ºC. The result of the study was measured by coli-titer.

Detection of pathogenic *Enterobacteria* was carried out by the method of accumulation of microorganisms on special media (Müller-Kaufman and selenite broth), followed by transplantation on differential diagnostic media ("Salmonella Differential Agar" M1078, (HiMedia, India).

Determination of *Enterococci* in soil was carried out by the tetration method. The primary soil suspension in a volume of 10 cm^3 was sown in a liquid lactose-peptone medium and an alkaline polymyxin medium. The crops were cultivated at 37ºC for 24 hours. At the first signs of growth (diffuse turbidity of media), they were transplanted into MIS medium (milk inhibitory medium) and cultivated for 48 hours at 37ºC. The identification of isolated cultures was performed using an *Enterococci* reduction assay (adding 3.0% hydrogen peroxide to the colony under study) and the biochemical properties of the isolates.

The cultural properties of the microorganisms were studied on meat-peptone broth (MPB), meat-peptone agar (MPA) and xylose-lysine-deoxycholate agar (XLD), manufactured by HiMedia Laboratories, India. The cultivation was carried out at a temperature of 37 ± 0.57ºC. The incubation period ranged from 6-8 hours to 2-4 days. To study the morphological features of the studied cultures, two types of preparations were done: a) for conventional light microscopy, the sample was fixed on a flame and stained by Gram; b) "crushed drop", which was examined under a phase contrast device. From the biochemical indicators of cultures of microorganisms were determined: the ability to form hydrogen sulfide, indole, acetyl methyl carbinol (Voges-Proskauer test), to break down glucose, lactose, sorbitol and mannitol in Hiss medium, to absorb citrate salts (to grow on medium) below 6.0 (reaction with methylene red crops grown on Clark's medium), the nature of growth on XLD. The antigenic structure of the microbes was determined in the agglutination (RA) reaction on glass with agglutinating salmonellosis sera - polyvalent, O-complex and H-monovalent.

The virulent properties of bacterial isolates were determined in a biological sample in white mice with a live weight of 16-18 g, which were injected 0.5 cm^3 of suspension subcutaneously, with 2×10^9, 2×10^6 and 2×10^3 CFU respectively. 3 mice were taken per dose. The suspension with the least microbial cells that caused the death of the experimental animals was tested in 3 white mice, each of which were injected a lower dose of microbial cells of the test isolate. The suspension with the lowest concentration of microbial cells, which caused the death of the infected animal, further infected two white mice. However, if both animals were killed, this dose was considered virulent; if death occurred in only one animal, the concentration by one order of magnitude was considered virulent; observation of infected animals lasted 7 days.

The antigenic properties of the isolates were studied in Guinea pigs weighing 350 ± 30 g. The experimental animals were injected 0.5 cm^3 of suspension subcutaneously with a concentration of 2×10^9 CFU in sterile phosphate-buffered saline (PBS) with a pH of 7.2, washed twice with PBS and killed with formalin 8-hour cultures of the tested strains on the IPB samples. On the 14th day after immunization 1.5-2 cm^3 of blood were taken from Guinea pigs. Blood serum was tested for antibodies in the agglutination (RA) reaction with homologous Salmonella antigens. The reaction was carried out in a volume of 1 cm^3 in polystyrene tablets. In a row of wells, 0.5 cm^3 of 2-fold dilutions of the test sera were made on a carbolized solution (0.85%) of sodium chloride solution with a pH of 7.0, starting with a dilution of 1:5 and ending with a dilution of 1:640, and 0.5 cm^3 of antigen. We used 2-billion suspensions on sterile FSB with a pH of 7.2 as the antigen, twice washed with PBS formalized 8-hour cultures of the studied strains. After connecting the components, they were thoroughly mixed in a circular motion on a flat table surface. The plates were covered with clean lids, preventing evaporation of the liquid, placed in a thermostat and kept at 37 ± 0.3ºC for 4 hours, and then the first (preliminary) reading of the reaction was performed. After 18-20 hours of keeping at room temperature, a second (final) reading of the reaction was recorded. A stereoscopic microscope was used to better evaluate the reaction. RA was evaluated in crosses according to the conventional procedure (++++ (4+) - complete enlightenment of the fluid and formation at the bottom of the well of agglutinate in the form of an umbrella, which when shaken broke into large tubercles, the liquid remained transparent; +++ (3+) - at the bottom of the test tube can be seen clearly formed precipitate of microbial cells in the form of an umbrella with a slightly compacted center; when shaking the precipitate was broken into much smaller lumps, a slight opalescence is noticeable; ++ (2+) well visible precipitate not glued microbial cells in the form of a button, in which shaking formed very small lumps of agglutinate, when shaking the liquid becomes cloudy; + - at the bottom of the test tube a well-expressed precipitate of microbial cells in the form of a button, at the edges of which barely visible lumps of microbial cells, - microbial mass is deposited by a dense precipitate, which, when shaken, turns into a continuous uniform mud. The reaction was considered to be no less than two crosses.

During the period of economic maturity of the plants (haymaking), the yield of green mass was recorded in the areas of 40 m^2 with 3-fold repetition. Crops were harvested by mowing and weighing the resulting mass. The nutrient content of the green mass was determined. The data were processed using a Microsoft Excel 7.0 software.

Results and Discussion

According to the results of the research, a number of microorganisms were isolated. Upon identification, it was found that on the Endo medium, coliform bacteria colonies were red in color with metallic luster, on differential Salmonella agar (HiMedia, India) - colorless, and on brine agar - yellow-orange. Salmonella Differential Agar (HiMedia, India) *Salmonella* colonies were red in color; black colonies with metallic luster were registered on bismuth sulfite agar; colorless, slightly pink transparent colonies were recorded on Endo medium; colorless, slightly pink colonies were recorded on Ploskirev's medium, sometimes with black center. On MIS medium, growth of *Enterococci* (*E. fecalis, E. faecium*) was recorded as dot-like, round, convex black colonies with metallic luster. All isolated enterococcal strains were catalase negative, which fermented sucrose, sorbitol, and mannitol.

Characterizing the morphological features, tinctorial and cultural properties of *Salmonella*, it is established that they are well stained with aniline, Gram negative. Morphologically, these are sticks with rounded ends, small, polymorphic, mobile. Culture has shown rapid growth on the IPB. Even at the fourth hour of incubation at 37 ± 0.3ºC, a steady opacity of broth was clearly visible, which became more intense with each subsequent hour; after 6-8 hours of incubation, a barely visible precipitate began to form. At MPA, all Salmonella isolates after 24 hours of incubation formed smooth, translucent, with a blue tinge slightly convex with a flat surface and smooth edges of slightly mucous colonies, 2-4 mm in size, which were easily removed by a bacteriological loop. At XLD, all induced Salmonella formed round convex smooth, black edges with smooth edges, under which the medium was also colored black. The culture grew well on the differential diagnostic environment of XLD, forming smooth, slightly convex, shiny, black colonies on its surface. Selected cultures had typical Salmonella biochemical properties. Yes, they fermented glucose with the formation of acid and gas, digested with acid maltose and mannitol, did not ferment lactose and sucrose, did not hydrolyze gelatin, did not form hydrogen sulfide and did not form indole, gave a positive reaction with methyl red and negative reaction for Voges-Proskauer test. *S. dublin* culture isolated from the soil of the bed pond was pathogenic for white mice (lethal dose 1×10^3 CFU). The number of mesophilic aerobic and facultative-anaerobic microorganisms (total microbial number) (MAFAnM) was also determined.

The study of the nature of the change of the sanitary condition of the soil of the pond bed after the descent of water and its wintering was performed during the entire period of casting and research, which lasted from May to October (Table 1).

The data of Table 1 indicate that in the first day of the study the amount of number of MAFAnM soil became $2.3 \times 10^6 \pm 0.06$ CFU/g, which is 2.3 times higher than the MPC for contaminated soil, and the coli-titer and *Enterococci* titer was 0.001. In all soil samples tested, *E. coli* serovariants O4 and O8 were isolated. 10 days after the start of the study in the bed soil, a decrease of number of MAFAnM of 4.5% was observed, but the coli-titer and the *Enterococci* titer remained unchanged. In soil samples, along with *E. coli* (O4, O8), bacteria from the genus *Salmonella (S. dublin)* were also isolated. 30 days after the start of the study in the soil there was a more significant decrease in bacterial contamination: number of MAFAnM decreased by 1.35 times to 1.7 ± 0.04

million CFU/g, and coli-titer and titer of *Enterococci* was 0.001, were also isolated pathogenic isolates of *E. coli.* After 60 days in the surface layer of soil (0-20 cm), despite further reduction of bacterial contamination to 1.3 ± 0.05 million CFU/g, pathogenic *E. coli* (serovariant O8) were isolated. After 90 days in the soil, there was a further decrease in the BMD to 1.1 ± 0.04 million CFU/g. Coli-titer and *Enterococci* titer was 0.001, pathogenic *E. coli* strains (O8) were isolated. At the end of the harvesting period, less microbial contamination was recorded on 120 and 150 days after the start of the observation, compared to the first day. Thus, the total bacterial soil contamination decreased by 2.87 and 3.29 times, respectively, to 0.8 ± 0.02 and 0.7 ± 0.05 million CFU/g, respectively, and the coli-titer and *Enterococci* titer was 0.01. In comparison with MPC for contaminated soil, these figures were 1.43 times lower, but compared to MPC for slightly contaminated soil they were more than 700 times higher. As before, pathogenic *E. coli* (serovar O8) was isolated. Thus, casting a pond for 150 days duration is not sufficient for complete self-cleaning and rehabilitation of the soil bed from pathogenic microflora. In order to intensify these processes, it is necessary to find effective and environmentally friendly methods of repairing the soil of the bed during casting of ponds. For the study of bactericidal properties of agricultural plants, 4 types of fodder grasses were selected, which can be used not only for soil sanitation of the pond bed, but also for feeding farm animals. To perform the experiment, forage grasses were sown in the soil of the botanical ground (BM) of the pond bed at the beginning of its casting.

Table 1. Sanitary and bacteriological indicators of soil bed pond (M ± m, n=10).

Time period (days)	Coli-titer	Pathogenic microorganisms	Enterococci titre	MAFAnM CFU/year
1	0.001	*E. coli* O4, O8; *S. dublin*	0.001	$2.3 \pm 0.06 \times 10^6$
5	0.001	*E. coli* O4, O8; *S. dublin*	0.001	$2.3 \pm 0.03 \times 10^6$
10	0.001	*E. coli* O4, O8; *S. dublin*	0.001	$2.2 \pm 0.03 \times 10^6$
30	0.001	*E. coli* O4, O8	0.001	$1.7 \pm 0.04 \times 10^6$
60	0.001	*E. coli* O8	0.001	$1.3 \pm 0.05 \times 10^6$***
90	0.001	*E. coli* O8	0.001	$1.1 \pm 0.04 \times 10^6$
120	0.01	*E. coli* O8	0.01	$0.8 \pm 0.02 \times 10^6$***
150	0.01	*E. coli* O8	0.01	$0.7 \pm 0.05 \times 10^6$***
Maximum Permissible Concentrations (MPC)				
Slightly Contaminated	1.0-0.01	Missing	1.0	1.0×10^3
Soil Contaminated	0.01-0.001	Available	0.1-0.01	1.0×10^6

Note: *** - p≤0.001 compared to control.

The results of an experiment to study the effect of fodder grasses on the sanitary and microbiological condition of BM soil before and after sowing of annual and perennial fodder grasses are given in Table 2.

Table 2. Indicators of the sanitary-bacteriological condition of the soil of the bed of the pond for the cultivation of fodder grasses (M ± m, n=10).

MAFAnM to baseline, %	Microbiological indicators: million CFU/g, $\times 10^6$	Coli-titer	Enterococci titer	Pathogenic microorganisms
Honey Clover				
30	1.11 ± 0.03***	48.26	0.001	0.001	*E. coli* O4, O8
90	0.5 ± 0.02***	21.74	0.01	0.01	
150	0.083 ± 0.005***	3.61	0.1	0.1	absent
Winter Rapeseed				
30	1.45 ± 0.08	63.04	0.001	0.001	
90	1.02 ± 0.04***	44.34	0.01	0.01	*E. coli* O4, O8
150	0.28 ± 0.008***	12.17	0.1	0.1	
Amaranth				
30	1.27 ± 0.04***	55.22	0.001	0.001	*E. coli* O4, O8
90	0.89 ± 0.03**	38.70	0.01	0.01	
150	0.11 ± 0.006***	4.78	0.1	0.1	absent
Canary Grass				
30	0.92 ± 0.04***	40.00	0.001	0.001	*E. coli* O4, O8
90	0.47 ± 0.03***	20.43	0.01	0.01	
150	0.12 ± 0.005***	5.22	0.1	0.1	absent
Soil After Water Descent (Control)				
0	2.3 ± 0.06	0	0.001	0.001	*E. coli* O4, O8 *S. dublin*

Note: *** - p≤0.001 compared to control.

The results of microbiological studies show that, within 30 days after planting, the level of microbial contamination of the soil sections of the botanical site decreased. Intensity of contamination of soil microflora during this period in areas with perennial grasses was less than with annuals. Thus, number of MAFAnM in the soil areas during cultivation of canary grass and honey clover

decreased by 60.0 and 51.7%, respectively, and 44.8% and 37% respectively when growing amaranth and rapeseed. Higher active sanitizing capacity of the soil in the initial growing season (after 30 days when growing perennial grasses) can be explained by the better formed network of the root system of these plants - the rhizosphere and the features of its influence on the microbiocenosis of the adjacent soil. However, it should be noted that, despite the characteristic decrease in the degree of microbial contamination of the soil during the initial period of vegetation, the coli-titer and titer of *Enterococci* remained high at 0.001. Pathogenic *E. coli* (serovariants O4, O8) were isolated in soil samples in all areas of plant cultivation during this growing season.

The results of cultivation of crops 90 days after the descent of water showed that the processes of self-purification of the soil of the botanical ground are influenced by their rhizosphere - a plot of soil directly adjacent to the roots of plants and to which the root secretions and soil microorganisms act. The intensity of the number of MAFAnM decrease in the soil depends on the type of crop. 90 days after the descent of water, the canary grass and honey clover were the most active in the process of remediation of contaminated soil. Compared to baseline, microbial soil contamination during this period decreased by 79.6% ($p \leq 0.001$) and 78.3% ($p \leq 0.001$, respectively). The coli-titer and *Enterococci* titer was 0.01.

The least active activity in the processes of self-purification was made by annual plants - amaranth and rapeseed. After 90 days of soil experiment, number of MAFAnM decreased by 61.3% ($p \leq 0.001$) and 55.7% ($p \leq 0.001$), respectively, and the coli-titer and *Enterococci* titer decreased to 0.01.

At the end of the growing season, there was a further decrease in soil microbial contamination from the beginning of the season. Moreover, the intensity of decontamination was directly dependent on the type of forage grasses. Compared to the initial level, after 150 days after planting a plant from a pond in the soil of its bed, where kelp and amaranth were grown, number of MAFAnM decreased by 96.4% ($p \leq 0.001$) and 95.3% ($p \leq 0.001$), respectively, and coli-titer and titer of *Enterococci* - from 0.001 to 0.1. No pathogenic microorganisms were isolated. According to the indicators of the sanitary condition, contaminated soil is considered to contain more than 1 million bacteria in 1 g, and less contaminated is less than 1 thousand bacteria. According to the indicators of the coli-titer it is estimated: contaminated - 0.01-0.001; slightly contaminated - 1.0-0.01 and unpolluted - 1.0 and above. The results of the study of the impact of forage crops on the sanitary condition of the soil BM bed is presented in Table 3.

Table 3. The degree of soil contamination of the botanical site at the end of the growing season.

Type of plant	Sanitary evaluation	Pathogenic microorganisms
Amaranth	Slightly Contaminated	not registered
Winter Rapeseed	Contaminated	*E. coli* (O4, O8)
Canary Grass	Slightly Contaminated	not registered
Honey Clover	Slightly Contaminated	not registered
Control	Contaminated	*E. coli* (O4, O8); *S. dublin*

Table 3 shows that the soil BM bed rate from the category "contaminated" under the condition of cultivation of agricultural herbs (amaranth and honey clover) at the end of the growing season (after 150 days) was restored to the category "slightly contaminated", indicating a high remedial capacity of agricultural crops, which improves the health of the soil. The results of the main indicators of fodder crops when harvested for green fodder are presented in Table 4.

Table 4. The yield and nutrition of the green mass of forage crops.

Type of plant	Yield of green mass, t/ha	Feed units per 1 kg of green mass	Digestive protein, g per 1 feed unit
Amaranth	172.1	0.14	129
Winter Rapeseed	75.4	0.15	180
Canary Grass	51.2	0.16	80
Honey Clover	94.2	0.19	190

Analyzing the results of research on the productivity of fodder crops harvested for green fodder, we can note the following, fodder amaranth exceeded the yield of green mass: canary grass (120.9 t/ha when harvested for green fodder), winter rapeseed (96.7 t/ha when harvested for green fodder) and honey clover (77.9 t/ha for green fodder). According to the data obtained in one feed unit of green mass of winter rapeseed contains 180 g of digestible protein in the diet 100-110 g. Green amaranth feed, under similar conditions, contained 129 g of digestible protein, and honey clover and canary grass, respectively 190 and 80. It should be noted that when harvesting these crops for green fodder providing digestible protein feed unit of honey clover exceeds winter rapeseed, amaranth feed and canary grass, respectively, 1, 1.5 and 2.4 times when harvesting for green feed.

Conclusion

Growing of fodder grasses in the soil of a pond bed during the casting is an eco-friendly and highly effective remedial action, which significantly reduces its microbial contamination. The results of the experiment demonstrated the antimicrobial effect of the plant rhizosphere on the number of mesophilic aerobic and facultative anaerobic soil microorganisms (MAFAnM). During 150 days of the growing season in the bed of the pond, the MAFAnM of honey clover *(Melilotus albus)*, canary grass *(Phalaris canariensis)*, winter rapeseed *(Brassica)* and amaranth *(Amaranthus pabulum)* were lower by 8.43 ($p \leq 0.001$), 3.37 ($p \leq 0.001$), 1.33 ($p \leq 0.001$), and 1.45 ($p \leq 0.001$) times respectively compared with the control area where the plants were not planted.

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