JEE Journal of Ecological Engineering

Journal of Ecological Engineering 2024, 25(9), 218–225
https://doi.org/10.12911/22998993/191149
ISSN 2299–8993, License CC-BY 4.0

Received: 2024.06.12
Accepted: 2024.07.28
Published: 2024.08.01

Environmental Aspects of Using *Bacillus Subtilis* to Improve the Quality of Irrigation Water

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ABSTRACT

Currently, the scientific research for methods to restore the quality of superficial water, including irrigation water, is actively advancing. The use of preparations of microbiological for intensification of the water system process purification is becoming increasingly common. In this aspect, it is advisable to expand the scientific search for the environmentally safe means of superface water purification in order to get quality irrigation water through the use of *Bacillus subtilis* microorganisms. Therefore, there is a need to investigate the environmental aspects of using *Bacillus subtilis* microorganisms in order to improve the quality of water, in particular, irrigation water. The aim of the research is to investigate the possibility of using Bacillus subtilis microorganisms to diminish the phytotoxicity of irrigation water. The water samples were tested before and after treatment with microorganisms (*Bacillus subtilis)* according to chemical indicators at the first stage. The treatment effiectiveness was as follows: BOI_5 – 40%, suspended solids – 26%, COD – 37%, nitrite – 54%, ammonium nitrogen – 38%, manganese and lead – 56%, nitrate – 35%. After treatment with *Bacillus subtilis*, the water quality met the MPC norms for fisheries. At the next stage, the phytotoxic effect of the observed water samples before and after the treatment with *Bacillus subtilis* microorganisms (1:100 dilution) on germination of seeds, growth and root system of plants, was assessed. The effect of reducing toxicity was: root weight from 4% to 50%, with the least effect observed in the least polluted sample; root length from 48% to 65%, with the biggest effect observed in the most polluted water sample; length of aboveground part from 21% to 54%, without a connection to pollution in this case; weight of aboveground part from 25% to 46%, without a connection to pollution. The research found that after probiotic treatment, all water samples were classified as non-toxic by all biometric parameters. Definition the phytotoxicity of the roots, a clear correlation was observed between the increase in treatment effectiveness of *Bacillus subtilis* microorganisms and the increase in pollution levels. Thus, the effectiveness of using *Bacillus subtilis* microorganisms in order to reduce water phytotoxicity and improve irrigation water quality was found.

Keywords: *Bacillus subtilis* microorganisms, phytotoxicity, irrigation water, biometric parameters, microbiological treatment.

INTRODUCTION

The scientific search for methods to restore surface water quality, in particular water for further irrigation, is currently progressing well. Both domestic researches (DSTU 7286:2012; DSTU 2730:2015; Korchahin, 2020; Pysarenko et al., 2023 a; Pysarenko et al., 2023 b; Tomiltseva et al., 2017; Yatsyk et al., 2007) and foreign ones (Chelliapan et al., 2006; Cloern, 2001; Smith et al., 2006; Sannikova and Kovaleva, 2019; Taranenko et al., 2019; Pysarenko et al., 2022; Galytska et al., 2021) have been engaged in this activity. The existing methods to restore the quality of surface water can be divided into two groups: measures which carried out in the watershed and measures which carried out directly in the water reservoir (preventive measures). The effectiveness of the same methods differs in different water reservoirs. This is due to the differences in geography, climatic conditions, characteristics of the water reservoir and its economic use. In other words, purification methods for each particular water reservoir should be chosen according to its regional characteristics. However, more than one method should be used, but an optimal integrated system for the management of pollutants in the water reservoir should be formed.

It is important to note that it is difficult to use any method for surface water purification and the most effective method is to return it to its natural state and enhance the self-purification mechanism. However, it is impossible to eliminate uncontrolled pollution in modern conditions, so this task is very difficult. The use of chemical methods is also quite complicated in natural conditions, as their introduction into surface water bodies can lead to secondary pollution.

The use of microbiological preparations, based on the use of *Bacillus subtilis* microorganisms, in order to intensify the purification of water systems is becoming increasingly common (Oliferchuk et al., 2008; Pysarenko et al., 2021 b; Anjaneyulu et al., 2005; Ferreira et al., 2011; Yang et al., 2008; Kulyk et al., 2020; Taranenko et al., 2021). However, at present, the use of *Bacillus subtilis* microorganisms in the operation of water systems purification to produce high-quality irrigation water, the impact of irrigation water on soil microorganisms, is not enough investigated. Despite the whole range of methods for surface water quality restoration, including irrigation water, presented in scientific publications, the use of bacteria, in particular the microorganism *Bacillus subtilis*, for surface water treatment has remained understudied. Therefore, there is a need to study the aspects of using probiotics to diminish the phytotoxicity of irrigation water.

The aim of the research is to investigate the aspects of using *Bacillus subtilis* microorganisms to diminish the phytotoxicity of irrigation water.

MATERIALS AND METHODS

Phytotoxicity was determined using the seedling method (ISO 11269-2:2012; Pysarenko et al., 2021; Milenko et al., 2022)), which is based on the reaction of the experimental crop to various pollutants. This identifies the toxic or stimulating effects of certain substances (Hrytsaienko et al., 2003; Pisarenko et al., 2019; Shevnikov et al., 2022). The phytotoxicity of water was determined by the phytotoxic effect on the number of plants that grew from the sowing date on day 7, the weight and size of plants (aboveground and root parts) on day 14 (ISO 11269-2:2012).

The determination of the phytotoxic effect of the soil environment on the biometric parameters of *Triticum aestivum* plants was carried out on the basis of the calculation according to the following formula (Hrytsaienko et al., 2003).

$$PE = \frac{M_0 - M_k}{M_0} \cdot 100\% \quad (1)$$

where: M_o – growth or weight parameters of the control sample plants; M_k – growth or weight parameters of the plants under study.

All experiments were conducted in four replications. The water samples were taken in accordance with ISO 5667-11:1993. The measurement was carried out in accordance with the measurement procedures (DSTU; GOST; MVV) approved for use (List of regulatory documents, 2007). The samples were analysed for the following indicators: biological oxygen demand BOI_5 (ISO 5815-1:2019), nitrate (ISO 7890-3:1988), ammonium nitrogen (ISO 5664:1984), nitrite (ISO 6777:1984), chemical oxygen demand COD (ISO 6060:1989), suspended solids (ISO 7888:1985), and lead (ISO 8288:1986).

To assess the quality of irrigation water, samples were taken at a depth of 0.2–0.5 m from the surface of the reservoir in the Psel River (Shyshaky District, Poltava Region), which is used for irrigation. Water samples were taken between 10.00 p.m. and 6.00 p.m. in spring, summer and autumn for the period of 4 years (2019–2022) at four sampling points, in three repetitions. The criterion for choosing sampling sites was the degree of anthropogenic load on the water system in place of intake of water for irrigation:

- Level 1 – there is no anthropogenic load at the sampling site;
- Level 2 – anthropogenic load due to the influence of agricultural land (inflow of biogenic substances);
- Level 3 – anthropogenic load from sewage treatment facilities;
- Level 4 – anthropogenic loading of technogenically polluted territories (solid waste landfill). The sampling sites are shown in Figure 1.

The mathematical processing of the experimental data was carried out according to the generally accepted methods using the MS Excel.

RESULTS AND DISCUSSION

In order to assess the effectiveness of using *Bacillus subtilis* microorganisms to regulate the quality of irrigation water, the first stage involved the examination of water samples before and after treatment by chemical indicators.

In the comparative studies, *Bacillus subtilis* microorganisms were tested: 1:10 dilution; 1:100 dilution; 1:1000 dilution) The experiments were carried out in the static (laboratory) conditions. The most polluted water sample collected from the Psel River was used for the experiment. The temperature was maintained at the level of 20 °C. The purification period was 14 days.

Since agricultural irrigation is carried out only during the summer period, therefore the research was carried out only during this season. The research results (Table 1) showed that the following indicators changed: BOI_5, COD, suspended solids, nitrite and nitrate ions, ammonium nitrogen, heavy metals (manganese, lead). There is no impact on the phosphorus and iron content.

Thus, the research established that the highest effect on most substances was observed at a 1:10 dilution of *Bacillus subtilis*. The purification effectiveness was as follows: BOI_5 – 40%, COD – 37%, suspended solids – 26%, ammonium nitrogen – 38%, nitrite – 54%, nitrate – 35%, manganese and lead – 56%. After treatment with *Bacillus subtilis*, the water quality met the fishery MPC norms.

Today, the use of *Bacillus subtilis* microorganisms for the treatment of environmental

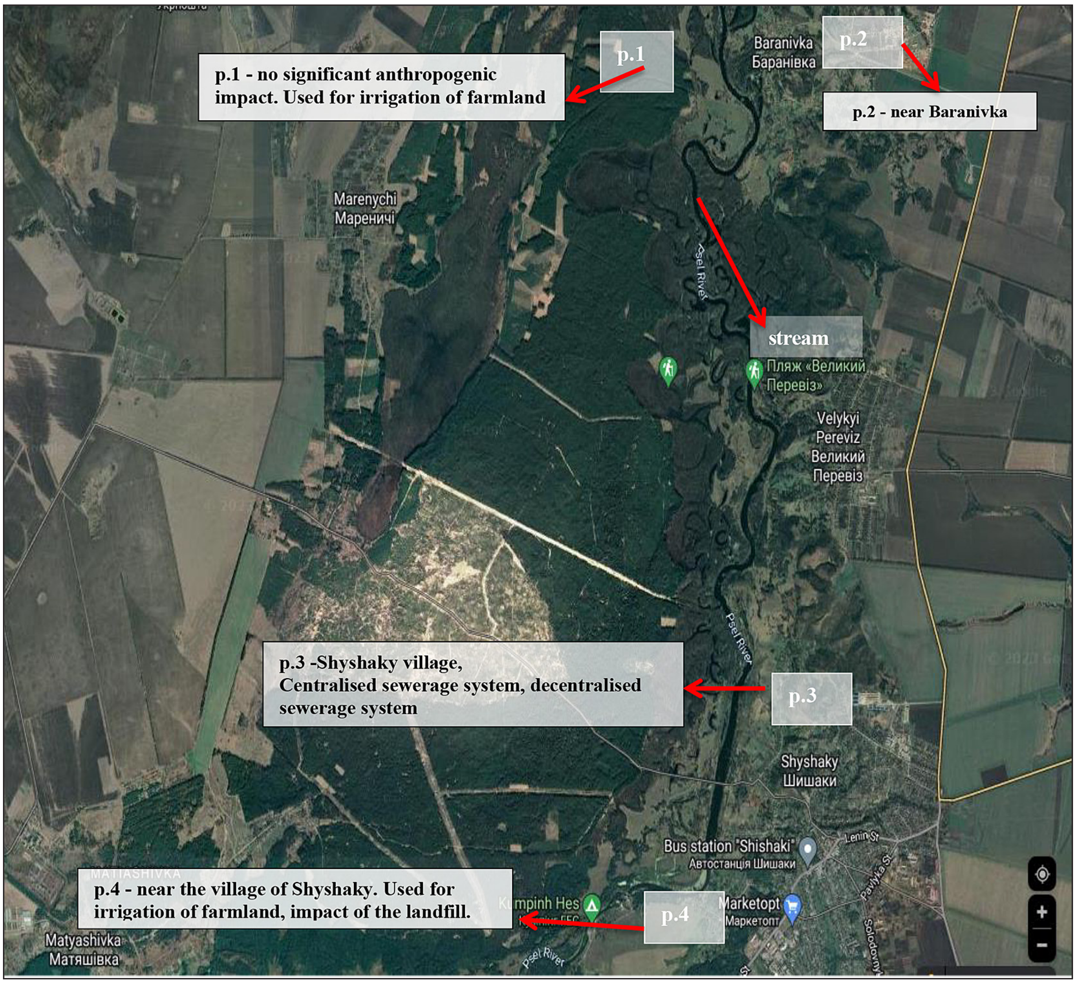

Figure 1. Water sampling sites (Poltava region. Ukraine: p.1 – outside of populated areas; p.2 – 0.5 km from Baranivka village, Myrhorod district (downstream); p.3 – Shyshaky village, Myrhorod district; p.4 – 1 km from Shyshaky village, Myrhorod ditrict (downstream)

Table 1. Water quality indicators before and after treatment with *Bacillus subtilis* microorganisms

Indicators	Before treatment (average)**	Dillution *Bacillus subtilis* 1:10**	Dillution *Bacillus subtilis* 1:100**	Dillution *Bacillus subtilis* 1:1000**	MPC*/ standard
$BOI_{5,}$ mgO_2 dm^{-3}	4.2	3.3	3.0	2.5	3.0
Suspended solids, mg dm^{-3}	26.41	21.68	20.85	19.55	25.0
COD, mgO_2 dm^{-3}	34.05	23.45	23.38	21.53	25.0
Ammonium nitrogen, mg dm^3	1.441	1.009	0.997	0.897	0.5-1.0
Nitrate ions, mg dm^{-3}	11.02	8.15	8.15	7.22	40.0
Nitrite ions, mg dm^{-3}	0.15	0.08	0.08	0.07	0.08
pH	8.0	7.9	7.9	7.8	8.0
Manganese, mg dm^{-3}	0.03	0.02	0.02	0.01	0.01
Lead, mg dm^{-3}	0.055	0.03	0.03	0.02	0.03

Note: *MPC generalized list of maximum permissible concentrations (MPC) and tentatively safe levels (TSL) of harmful substances for the water of water management reservoirs, 1995; ** – statistically significant difference, $p < 0.05$. *Contents of pollutants were measured on the 14th day after treatment with probiotic.

components, including water systems, is a poorly investigated area. Thus, the next stage was to assess the phytotoxic effect of the water samples before and after processing with *Bacillus subtilis* microorganisms (at a dilution of 1:10) on root system, growth and germination plants.

Comparison of the number of germinated winter wheat seeds and the phytotoxic effect (PE) on water samples before and after water treatment with *Bacillus subtilis* microorganisms is shown in Figures 2–3.

Existing biological methods of water purification are mainly used for wastewater treatment and require the installation of additional equipment (aeration tanks, septic tanks, etc.). The installation of these structures is economically impractical, and they will not provide the required amount of water purification (taking into account the capacity of the required water for irrigation). The use of chemical methods of water purification creates risks of secondary soil pollution. Previous studies (Pysarenko et al, 2023 a) have established that the maximum effect of probiotics lasts 7–14 days, and after settling, purified water can be used for irrigation. Based on the author's research (Pysarenko et al, 2023 b), it was established that probiotics, once in the soil, also improve the vital activity of the soil microflora and their effect lasts up to 60 days.

As a result of water treatment with *Bacillus subtilis*, the phytotoxicity of water by the number

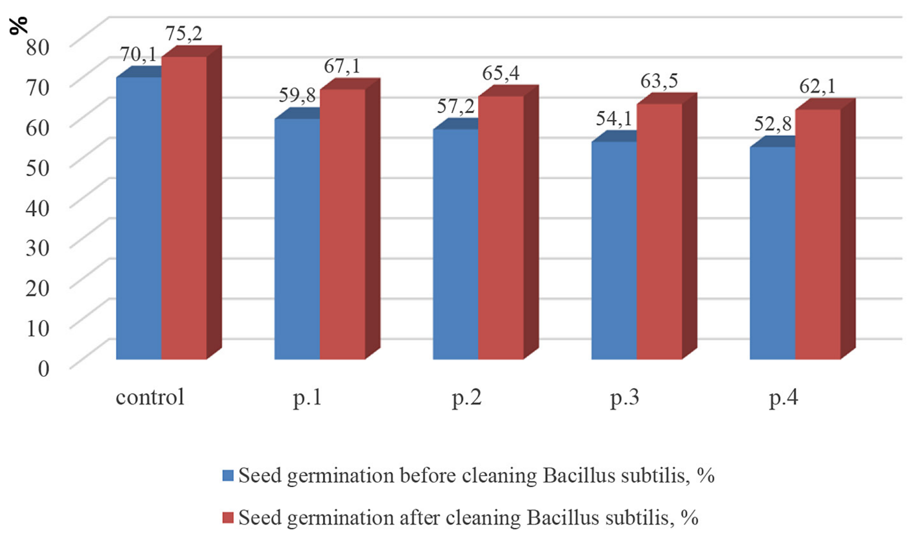

Figure 2. The number of germinated winter wheat seeds in water samples before and after water treatment with *Bacillus subtilis* microorganisms, %

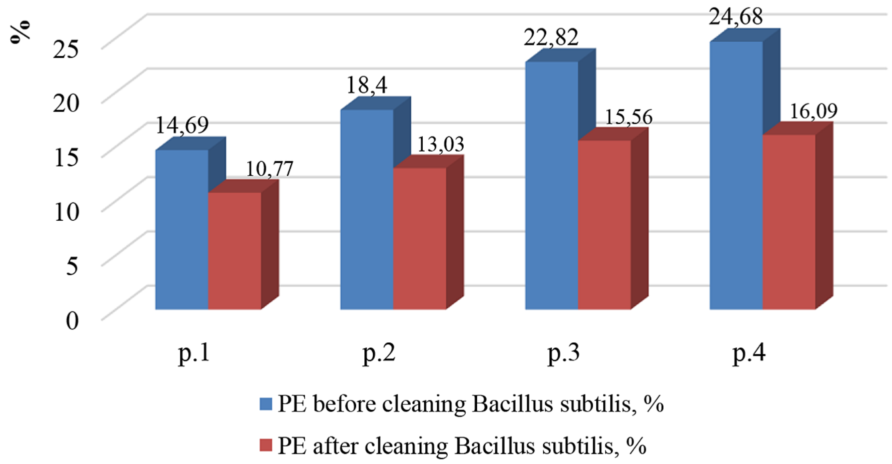

Figure 3. Phytotoxic effect on water samples (by the number of germinated seeds of winter wheat) before and after water treatment with *Bacillus subtilis* microorganisms, % (0–20% – no phytotoxicity; 20–40% – medium phytotoxicity; 40–60% – above medium phytotoxicity; 60–80% – high phytotoxicity)

Table 2. Phytotoxicity of water before and after biological treatment with *Bacillus subtilis* microorganisms

Variants	Length of seedlings, %	Average root length, %	Weight of aboveground parts, %	Weight of root system, %.
	Before biological treatment with *Bacillus subtili* microorganisms			
p.1	5.52[1*]	5.71[1*]	10.14[1*]	1.67[1*]
p.2	9.66[1*]	**24.12**[2*]	17.39[1*]	8.33[1*]
p.3	**23.45**[2*]	**25.57**[2*]	**26.81**[2*]	15.83[1*]
p.4	**20.31**[2*]	**40.09**[3*]	**22.46**[2*]	18.33[1*]
	After biological treatment with *Bacillus subtili* microorganisms			
p.1	4.11[1*]	2.60[1*]	7.25[1*]	1.63[1*]
p.2	7.53[1*]	10.39[1*]	12.32[1*]	4.07[1*]
p.3	10.96[1*]	12.99[1*]	14.49[1*]	8.13[1*]
p.4	12.33[1*]	14.29[1*]	16.67[1*]	10.57[1*]

Note: [1*] – no phytotoxicity; [2*] – medium phytotoxicity; [3*] – phytotoxicity is above medium.

of germinated seeds (winter wheat) in all samples decreased by 5-9%, and water samples from T.3 and T.4 turned from medium toxic to non-toxic ones (no toxicity). Similar research on other biometric parameters before and after water treatment with probiotics is presented in Table 2.

CONCLUSIONS

As a result of the research, it was found that after treatment with *Bacillus subtilis* microorganisms, all samples of irrigation water were classified as non-toxic (no toxicity) by all biometric parameters. The effect of toxicity reduction was:

- by the root length from 48% to 65%, with the biggest effect observed in the most polluted water sample;
- by the root weight from 4% to 50%, with the lowest effect observed in the least polluted sample;
- by the length of the aboveground part from 21% to 54%, there is no connection with pollution in this case;
- by the weight of the aboveground part from 25% to 46%, and there is no connection with pollution.

Definition the phytotoxicity of the roots, a clear correlation was observed between the increase in treatment effectiveness of *Bacillus subtilis* microorganisms and the increase in pollution levels. Thus, the research proved the effectiveness of using *Bacillus subtilis* microorganisms to reduce water phytotoxicity, suggesting that the quality of irrigation water quality can be restored.

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