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Efficiency of Probiotic Application for the Remediation of Contaminated Soils in Agrocenoses

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ABSTRACT

The problem of soil contamination is relevant today. Soil contamination is generally associated with intensive industrial activities, inadequate waste management, mining, military activities, or accidents. Pollutants (e.g. heavy metals) are accumulating in soil and have major indirect impacts on biodiversity, quality of groundwater resources, and food safety. Soil contamination of agricultural land is a particular threat. Due to the military action and other conditions, the numbers of sites where potentially polluting activities have taken place are increasing in Ukraine. The cultivation of agricultural crops on soils with a medium or high level of toxicity is only possible after the remediation of this area. The use of biological methods to intensify soil remediation processes, thereby reducing the additional burden on the environment, is becoming more widespread. The application of probiotics is an innovative and ecological method for the restoration of contaminated soils. This will enable to improve the conceptual approaches to the implementation of an ecologically safe model for the restoration of contaminated land under the conditions of military action in Ukraine. The aim of the research was to assess the phytotoxic effect of soil contaminated with heavy metals and petroleum products before and after probiotic application. The seedling method was used to determine the remediation potential of contaminated soils. An express test of *Triticum aestivum* was used to determine soil phytotoxicity. The research results show the negative impact of soil contamination with heavy metals and petroleum products on the biometric indices of *Triticum aestivum* plants and positive dynamics of biometric indices of *Triticum aestivum* in variants after probiotic treatment (86–92% compared to the control). The obtained data show that a significant phytotoxic effect is observed by all biometric indices of *Triticum aestivum* in all studied variants. The highest phytotoxic effect (33.56–42.70%) was observed in variants with combined contamination (PP+Zn+Pb). The results of probiotic application show a phytotoxic effect of less than 20% by all biometric indices of *Triticum aestivum* for all studied variants. Therefore, the results of the research can be used to develop recommendations for the remediation of land contaminated by military actions in Ukraine and the creation of sustainable agroecosystems.

Keywords: soil, probiotic, *Bacillus subtilis*, heavy metals, petroleum products, *Triticum aestivum,* phytotoxicity.

INTRODUCTION

After more than 200 years of industrialization, soil contamination is a widespread problem in Europe. The most common contaminants are heavy metals and mineral oils. Soil contamination is generally associated with intensive industrial activities, inadequate waste management, mining, military activities or accidents (Pysarenko et.al., 2022b). The management of these contaminated sites is a phased process, starting with a preliminary survey (searching for sites that are likely to be contaminated), followed by site investigations to determine the actual extent of contamination, its environmental impact, and finally remediation as well as aftercare measures (SWSR, 2015).

Due to the military action and other conditions, the numbers of sites where potentially polluting activities have taken place are increasing in Ukraine and in Europe now stand at approximately three million sites. Therefore, questions of soil contamination are relevant today because pollutants (e.g. heavy metals) are accumulating in the soil and have major indirect impacts on biodiversity as well as the quality of groundwater resources (SWSR, 2015).

The use of soils with medium and high toxicity for agricultural purposes is possible only after remediation of the territory. This is actualizing the issue of reducing technogenic pollution on agricultural land and restoring the soil from pollution.

The use of biological methods for intensification of soil remediation processes, which reduce the additional load on the environment, is becoming more and more widespread. One of the promising methods of cleaning various components of the environment corresponds to probiotic preparations. Research works of Baldi et al. (2007), Guo et al. (2010), Patyka et al. (2014), Cui et al.(2017), Pysarenko et al. (2021a,c, 2022a, 2022b), Mo et al. (2022), Devi et al. (2022), Xu et al (2023), Zhao et al. (2023) were dedicated to use microbial preparations (including probiotic and bacterial) for remediation of environment. Probiotics consist of probiotic bacteria and enzymes; they do not contain chemical or mineral contaminants. According to the method of application, probiotics can be classified as reagents, but due to their environmental friendliness, they do not have a negative impact on the water and soil quality compared to chemical reagents. By definition, probiotic bacteria are non-pathogenic, non-toxic, have a high adhesive and antagonistic ability to pathogenic microorganisms (Pysarenko et al., 2021a). The effectivity of probiotics application for wastewater treatment, decreasing of eutrophication processes of water bodies has been studied by (Pysarenko et al., 2020, 2021b).

Today, the scientific literature presents a significant number of methods for the restoration of contaminated lands, but the problems of using biological methods, in particular probiotic preparations, to clean the soil from heavy metals, oil products, and microbiological pollution are currently insufficiently studied.

The effectivity of probiotic application as innovative ecological methods of restoration of contaminated soils needs to be justified by experimental studies. This will enable to improve conceptual approaches to the implementation of an ecologically safe model of restoration contaminated lands in the conditions of military action in Ukraine.

The aim of this work was scientific justification of biological methods for restoration of contaminated soils in agrocenoses. The task of the research was to assess the phytotoxicity effect of contaminated soil with heavy metals and petroleum products, before and after probiotic application.

MATERIALS AND METHODS

The seedling method (ISO 11269-1:2004; ISO 11269-2: 2002) was used to determine the remediation potential of soils contaminated with heavy metals and petroleum products. An express test of *Triticum aestivum* was used to determine soil phytotoxicity. The response of this culture to different concentrations of pollutants is the basis of this method. This enables to determine the toxic or stimulatory effect of the substances tested. The phytotoxicity of the soil is determined by the magnitude of the phytotoxic effect by the number of plants that have grown since sowing on the 7th day, the size and mass of the plants (ground and root parts) on the 14th day.

The calculation of the phytotoxic effect of soil environment on the biometric indices of *Triticum aestivum* was carried out using the formula (Hrytsaienko, 2003; Pysarenko et. al., 2021c).

$$ФЕ = [(Mo - Mк)/Mo] \times 100\% \tag{1}$$

where: Mo – weight or growth rates of plants in control sample;
$Mк$ – mass or growth indicators of the plants in the study samples.

With the aim of studying the methods of remediation of contaminated soil, the preliminary model contamination with Pb, Zn (in the form of $(CH_3COO)_2Zn$ and $(CH_3COO)_2Pb$) and petroleum products was carried out.

Pollutant concentrations were 64 mg kg^{-1} (gross content) and 12.0 mg kg^{-1} (mobile form) for Pb(II), and 200 mg kg^{-1} (gross content) and 46.0 mg kg^{-1} (mobile form) for Zn(II). Concentration of petroleum products was 2000 mg kg^{-1}.

Concentrations of heavy metals (according to OSCE data on the territory of eastern Ukraine) correspond to the average level of soil contamination in the areas where military actions took place (Vasyliuk et al., 2017).

The scheme of the experiment included:
- Variant 1: control soil sample (K);
- Variant 2: soil sample contaminated petroleum products (PP);
- Variant 3: soil sample contaminated Zn (Zn);
- Variant 4: soil sample contaminated Pb (Pb);
- Variant 5: soil sample contaminated Pb + Zn (Pb + Zn);
- Variant 6: soil sample contaminated petroleum products + Pb + Zn (PP + Pb + Zn).

The experiment included experimental plots with three replicates.

The remediation of the contaminated soil was carried out by biological methods using probiotic preparations based on *Bacillus subtilis* at a dilution of 1:100. *Triticum aestivum* seeds (100 seeds each) were planted in separate containers. During the experiment, the germination of the plant seeds was assessed, the height and mass of the ground part and the length and mass of the plant roots were measured.

The content of heavy metals (gross content and mobile forms) and petroleum products before and after biological remediation was carried out according to the methods of DSTU 4770.9:2007, DSTU 4770.2:2007, MVV 31-497058-009-2002 in the laboratory of agro-ecological monitoring of PDAU.

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

RESULTS AND DISCUSSION

The data of biometric indices of *Triticum aestivum* on soil samples before and after treatment with probiotic preparations are given in the Tables 1, 2.

The results of the study show the negative impact of soil contamination with heavy metals and petroleum products on the biometric indices of *Triticum aestivum* plants. The variants with the combined use of pollutants (Zn+Pb) and (PP+Zn+Pb) had the greatest negative effect. The reduction in biometric indices of Triticum aestivum on contaminated soil was 33–43% in comparison with the control.

Research results show the positive dynamics of biometric indexes of *Triticum aestivum* in the variants after probiotic treatment (86–92% compared to the control). The highest positive effect of probiotic application was in variant (Zn+Pb+PP). The part of seed germination increased by 61%; the length of the of ground part of plant and the length of the roots increased by 50–55%; by 28–30% the weight of the ground part of the plants and the root system increased compared to the control.

The results of the phytotoxicity assessment before and after the probiotic application are shown in Tables 3, 4.

The obtained data show that a significant phytotoxic effect is observed by all biometric indices

Table 1. Biometric indices of *Triticum aestivum* before treatment with probiotic

Variants	Seed germinating, %	Average length of ground part of plant, cm	Average root length, cm	Weight of ground parts of plant, g	The weight of the root system, g
K	89	15.1	11.2	2.98	1.1
PP	69	10.9	7.6	2.18	0.81
Zn	74	12.5	8.9	2.17	0.84
Pb	70	11.8	8.6	2.29	0.95
Zn+Pb	60	10.3	7.9	2.15	0.8
PP+Zn+Pb	51	9.2	6.5	1.98	0.72

Table 2. Biometric indices of *Triticum aestivum* after treatment with probiotic

Variants	Seed germinating, %	Average length of ground part of plant, cm	Average root length, cm	Weight of ground parts of plant, g	The weight of the root system, g
K	95	16.8	11.8	3.12	1.3
PP	90	15.5	10.8	3.02	1.08
Zn	84	14.5	10.1	2.71	1.02
Pb	80	14.1	10	2.64	0.98
Zn+Pb	77	13.1	9.6	2.5	0.93
PP+Zn+Pb	82	13.8	10.1	2.54	0.97

Note: All biometric indices *of Triticum aestivum* were measured on the 7th day after treatment with probiotic.

Table 3. Phytotoxic effect before treatment with probiotic, %

Variants	By seed germinating	By average length of ground part of plant	By average root length	By weight of ground parts of plant	By weight of the root system
PP	22.47[2*]	27.81[2*]	32.14[2*]	26.85[2*]	26.36[2*]
Zn	16.85[1*]	17.22[1*]	20.54[2*]	27.18[2*]	23.64[2*]
Pb	21.35[2*]	21.85[2*]	23.21[2*]	23.15[2*]	13.64[1*]
Zn+Pb	32.58[2*]	31.79[2*]	29.46[2*]	27.85[2*]	27.27[2*]
PP+Zn+Pb	42.70[3*]	39.07[2*]	41.96[3*]	33.56[2*]	34.55[2*]

Note: The phytotoxic effect more than 20% is considered as significant. 1* – phytotoxic effect is insignificant; 2* – medium phytotoxic effect; 3* – higher than average phytotoxic effect; 4* – negative phytotoxic effect. Negative values of the phytotoxic effect indicate the stimulation of plant growth and development under the influence of the studied factors.

Table 4. Phytotoxic effect after treatment with probiotic, %

Variants	By seed germinating	By average length of ground part of plant	By average root length	By weight of ground parts of plant	By weight of the root system
K	-6.74[4*]	-11.26	-5.36	-4.70	-18.18
PP	-1.12	-2.65	3.57	-1.34	1.82
Zn	5.62	3.97	9.82	9.06	7.27
Pb	10.11	6.62	10.71	11.41	10.91
Zn+Pb	13.48	13.25	14.29	16.11	15.45
PP+Zn+Pb	7.87	8.61	9.82	14.77	11.82

Note: The phytotoxic effect more than 20% is considered as significant. 1* – phytotoxic effect is insignificant; 2* – medium phytotoxic effect; 3* – higher than average phytotoxic effect; 4*– negative phytotoxic effect. Negative values of the phytotoxic effect indicate the stimulation of plant growth and development under the influence of the studied factors.

of *Triticum aestivum* in all studied variants. The highest phytotoxic effect (33.56–42.70%) was observed in the variant with combined contamination (PP+Zn+Pb).

The results of probiotic application with the aim of soil remediation from studied pollution (heavy metals and oil products) show phytotoxic effect less than 20% by all biometric indices of *Triticum aestivum* for all studied variants. This indicates an insignificant level of phytotoxicity of the soil.

The increase in the biometric indices of *Triticum aestivum* can be explained by the activation of the microbiological biome of the soil, since petroleum products act as nutrients for probiotic microorganisms, indicating the effectiveness of probiotic treatment.

Table 5. Soil contaminant levels before and after probiotic treatment

Variants		Content of pollutants before probiotic treatment, mg kg^{-1}			Content of pollutants after probiotic treatment*, mg kg^{-1}		
		PP	Pb	Zn	PP	Pb	Zn
PP	-	2000±8.7	-	-	850±2.8	-	-
Zn	Gross content	-	-	220.0±4.5	-	-	218.8±2.8
	Mobile form	-	-	46.2±1.8	-	-	17.8±0.6
Pb	Gross content	-	64.0±4.2		-	63.5±1.3	-
	Mobile forms	-	12.1±0.7		-	4.2±0.1	-
Zn+Pb	Gross content	-	62.9±3.8	218.1±5.8	-	62.8±3.5	216.8±6.3
	Mobile forms	-	13.2±0.5	44.9±1.1	-	5.1±0.7	16.4±0.8
PP+Zn+Pb	Gross content	1950±10.7	64.8±2.5	222.1±3.6	680±5.7	64.1±3.1	220.8±4.2
	Mobile forms		12.5±0.8	47.2±1.0		5.3±0.4	18.1±0.7

Note: – statistically significant difference, $p < 0.05$. *Contents of pollutants were measured on the 7th day after treatment with probiotic.

In order to understand the mechanism of action of probiotic preparations on pollutants in the soil, the determination of the content of heavy metals (gross content and mobile forms) and petroleum products before and after probiotic treatment was carried out in the agro-ecological monitoring laboratory. The results of the research are presented in Table 5.

According to the results of chemical analysis of soil before and after treatment with probiotic (1:100 dilution), it was established that:

- the content of petroleum products after probiotic treatment was in 2–3 times lower compared to the variants before probiotic treatment and lower than the maximum permissible concentration.
- the gross content of Zn, Pb does not decrease after probiotic treatment. However, at same time, the mobile form decreases by 2–3 times and becomes lower than the maximum permissible concentration. Since plants can only absorb mobile forms of heavy metals with their food, this explains the decrease in soil phytotoxicity after probiotic treatment. It can therefore be concluded that probiotics immobilize heavy metals. This reduces soil phytotoxicity and ensures food safety.

CONCLUSIONS

The results of studies of the application of probiotic preparations on the most polluted soils (Zn+Pb+NP) show an improvement of 86–92% in biometric indices compared to the control: 61% in germinated seeds; 50–55% in the length of shoots and roots; 28–30% in the weight of the soil part and the root system.

The results of the probiotic treatment (Bacillus subtilis, dilution 1:100) in all studied variants with contaminants (heavy metals and petroleum products) show an insignificant level of soil phytotoxicity by biometric indicators of *Triticum aestivum*, less than 20%.

A significant improvement in the biometric indices of *Triticum aestivum* is observed in the variants of probiotic treatment on the control sample and on the sample contaminated with petroleum products.

The results of chemical analyses of the soil show a decrease in the gross content of petroleum products by 2–3 times and stimulate the growth and development of *Triticum aestivum* in the variants with probiotic treatment.

In this way, the effectiveness of probiotic application to reduce soil phytotoxicity of agrocenoses contaminated with heavy metals and petroleum products as a result of military operations in Ukraine was established. The results of the research can be used to develop recommendations for the remediation of land contaminated by military actions in Ukraine and its return to economic circulation in the context of ensuring the ecological and food security of the region and the creation of sustainable agro-ecosystems.

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